A Collection and Marking System Suitable for Epidemiological Studies on Whitefly-Borne Viruses

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ABSTRACT

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A simple system designed to monitor the movement and infectivity of whiteflies in epidemiological studies of whitefly-borne viruses is described. A vacuum collector, based on a small, cordless, rechargeable vacuum cleaner, was modified to collect insects directly into clear plastic sleeve cages. The cages can be used directly as inoculation chambers in the field, as a direct measure of population density, and as a means of monitoring insect movement with tracer fluorescent dyes. Seedlings inoculated in the collection cages are carried or shipped to the greenhouse and observed for symptoms, eliminating the need for growing bait plants in the field.

The major parameters in epidemiological studies of insect-borne viruses are vector population dynamics and distribution, the percentage of inoculative insects, takeoff and landing behavior of the vector, virus-vector relationships, and the virus hosts.

Only a few large-scale epidemiological studies have been carried out so far on whitefly-borne viruses (1,2,4,7,9,10). In most of the studies, the information gathered is far from complete. This may be due, in part, to the lack of appropriate systems for insect collection and monitoring. Two main techniques have been used for trapping adult whiteflies: sticky (yellow) traps (2) and high-powered suction samplers (8). The large suction sampler, besides being heavy and difficult to work with, sucks the insects into a net from which they have to be transferred into transparent cages for classification. Moreover, the chances of the whiteflies surviving the high suction treatment are markedly low (12; *unpublished results*). This method cannot be readily used for an evaluation of the inoculativity rate of the whitefly population where captured insects must be fed on test plants. In a comparison of the large suction device with bait plants for determining inoculativity, bait plants were vastly superior (4). However, the technical difficulties involved in bait plant experiments, such as watering the plants in the field, sunburn, other pests and diseases, and transportation, limit the use of the bait plant technique to a small scale. Sticky traps have been used in studies on whitefly distribution and to trap whiteflies previously marked with fluorescent dust (2,4,8). In these experiments, it was difficult to identify the types of glowing objects on the sticky traps under ultraviolet illumination.

In efforts to obtain a more thorough understanding of the epidemiology of lettuce infectious yellows virus (LIYV) (6) vectored by *Bemisia tabaci* Genn., a system to collect and monitor whitefly populations was developed and tested. The results of these studies are presented.

MATERIALS AND METHODS

Maintenance of LIYV, whiteflies, and test plants. LIYV was maintained in lettuce (*Lactuca sativa* L.) and cheeseweed (*Malva parviflora* L.), and the virus was transferred using the vector of *B. tabaci*.

B. tabaci were kept on sweet potato (*Ipomoea batatas* (L.) Lam.) or cotton (*Gossypium hirsutum* L.) plants grown in muslin-covered cages. The cages were maintained in an insectary greenhouse, and the whiteflies were collected by aspirator. Five- to six-leaf stage lettuce seedlings (cultivar Summer Bibb) grown by the plug method in Styrofoam trays were used as test plants. The root plugs were wrapped with a thin layer of Parafilm before inoculation in collector cylinders (sleeve cages).

Collection device. The vacuum collector was based on a cordless, rechargeable vacuum cleaner (Dustbuster Plus, Black and Decker, Inc., Shelton, CT) that was modified to collect insects directly into clear, cylindrical, plastic sleeve cages fitted with removable plastic caps (Figs. 1 and 2). Cages were made from 50-dram plastic vials (Thornton Plastics, Salt Lake City, UT). During the collection process, the edge of the sleeve cage was brushed against the foliage suspected of having whiteflies. After insect collection, a lettuce test plant plug was introduced directly into the cage with the vacuum on, and then the cage was capped.

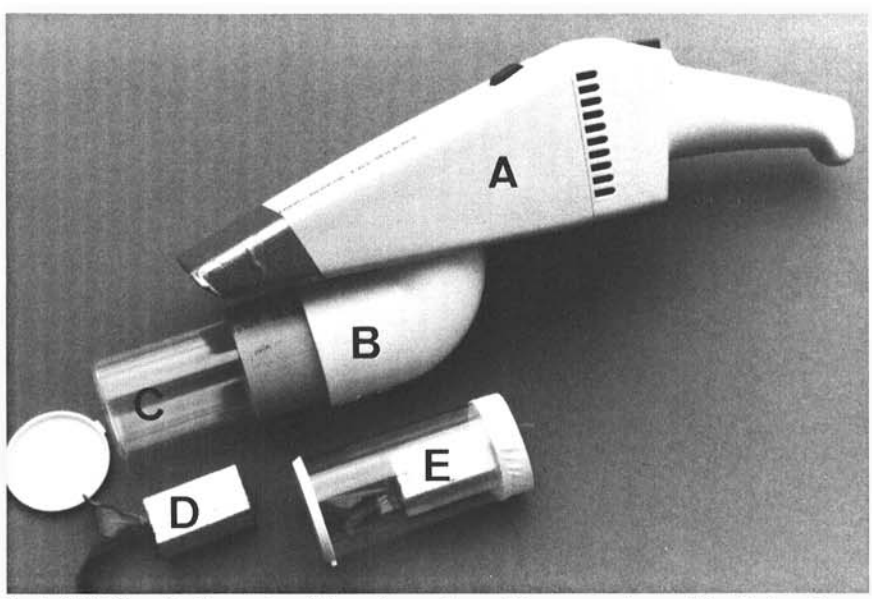

Fig. 1. Whitefly collection system: **(A)** cordless rechargeable vacuum cleaner, **(B)** cylinder adaptor, **(C)** clear plastic cylinder sleeve cage, **(D)** lettuce test plant, and **(E)** field inoculation chamber (test plant in sleeve cage).

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Following a 24-hr inoculation period, the plugs were removed from the cages, washed free of whiteflies with water, and taken to the greenhouse. The plugs were planted into 10-cm pots and monitored for symptoms of LIYV over a period of 6 wk.

Table 1. Effect of vacuuming on the survival of whiteflies (*Bemisia tabaci*)

	No. of living insects following treatment[a]	
Leaf cage	Vacuuming (1 min)	Without vacuuming
1	7	10
2	10	10
3	7	10
4	3	9
5	6	10
6	7	8
7	8	10
8	7	9
9	10	10
10	10	10
11	5	10
12	10	10
Total	90/120	116/120
Percent	75	97

[a]Ten whiteflies per leaf cage (collected from the same colony) placed after the treatments in pairs for 48 hr on the opposite half leaves of sweet potatoes. Difference between treatments is significant ($P = 0.004$).

Survival of whiteflies during the collection process. The survival rate of whiteflies subjected to 1 min of vacuuming was measured after 48 hr and compared with control insects that had not been subjected to the treatment. After the treatment, the whiteflies were kept in groups of 10 in leaf cages (3) positioned in pairs on the opposite half leaves of sweet potato.

Table 2. Effect of the vacuum collector and simulated shipping conditions on the transmission of lettuce infectious yellows virus by whiteflies (*Bemisia tabaci*)

	Transmission			
Test	Collection and simulation[a]		Control[b]	
1	(10/10)[c]	100	(6/8)	75
2	(10/10)	100	(8/8)	100
3	(4/5)	80	(6/8)	75
4	(4/5)	80	(9/9)	100
5	(10/10)	100	(7/8)	88
6	...		(7/8)	88
7	...		(6/8)	75
8	...		(9/9)	100
Total	(38/40)	95	(58/66)	88

[a]Inoculative whiteflies were vacuumed for 1 min. Lettuce seedlings were maintained for a total period of 5 days in the dark. Inoculation took place in the plastic collection cylinders (15 insects per plant).
[b]Inoculative whiteflies (15 insects per plant) were placed directly on young leaves of lettuce seedlings with leaf cages.
[c]Number of plants infected divided by total number of plants inoculated (in parentheses). Difference between treatments is not significant ($P = 0.004$).

Simulation of field studies. Whiteflies previously fed for 24 hr on LIYV-infected cheeseweed were caged in groups of 15 in collection cylinders and were exposed to 1 min of vacuuming. Lettuce seedling plugs previously kept for 3 days in a plastic bag in a dark room (simulating shipping conditions to the field) were introduced into the cylinders for a 24-hr inoculation feeding access period at room temperature. The plants were then thoroughly washed with a stream of tap water to remove the whiteflies before being repacked in plastic bags and stored for 2 additional days in the dark room (simulating shipment back to the laboratory). After that period, the seedlings were transplanted into pots in an insect-protected greenhouse that was fumigated weekly with dichlorvos and resmethrin. As a control, groups of 15 LIYV viruliferous whiteflies enclosed in leaf cages were placed for 24 hr on the young leaves of the lettuce test plants. The whiteflies were then washed off with water, and plants were transferred to the

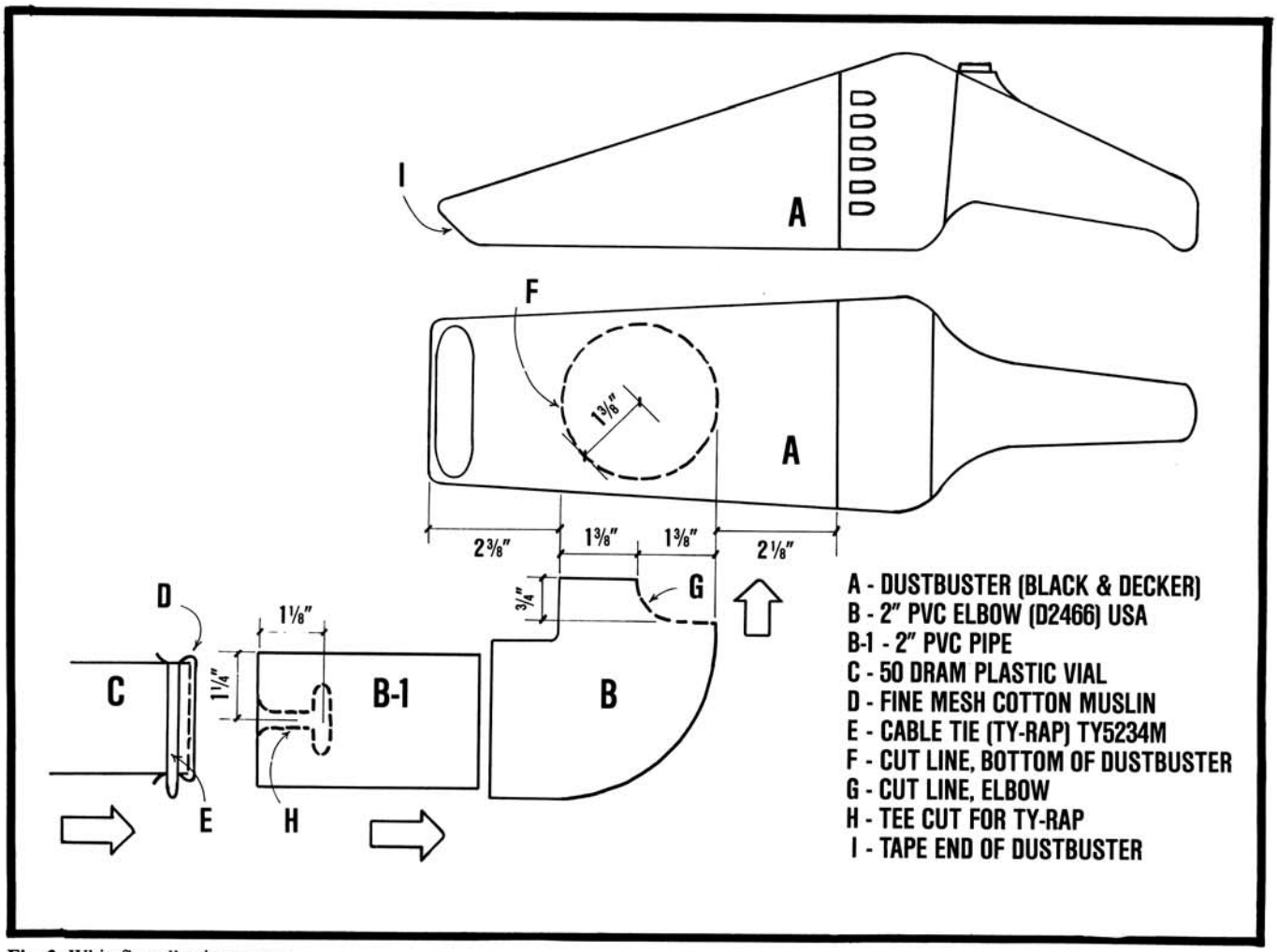

Fig. 2. Whitefly collection system.

Table 3. Effect of Fire Orange pigment on the life span of whiteflies (*Bemisia tabaci*)[a]

Days after treatment	Method	Cage number 1	2	3	4	5	6	7	8	9	10	Total
1	Treatment	10/10[b]	10/10	8/10	10/11	11/11	11/11	11/11	11/11	10/10	9/10	101/105
	Control	11/11	11/11	10/10	8/10	10/10	10/10	10/10	8/10	5/10	8/10	91/102
2	Treatment	9/10	10/10	8/9	10/10	11/11	11/11	11/11	11/11	10/10	9/10	100/103
	Control	11/11	11/11	10/10	8/10	10/10	9/9	9/10	8/10	5/20	8/10	89/101
3	Treatment	8/10	10/10	5/8	10/10	11/11	11/11	11/11	11/11	9/10	9/10	95/102
	Control	11/11	11/11	10/10	8/10	10/10	9/9	9/10	8/10	5/10	8/10	89/101
4	Treatment	8/10	10/10	5/8	10/10	11/11	11/11	11/11	11/11	9/10	9/10	95/102
	Control	10/11	11/11	8/10	7/10	9/10	9/9	9/10	8/10	5/10	8/10	84/101
5	Treatment	8/10	10/10	5/8	10/10	11/11	11/11	11/11	11/11	9/10	9/10	95/102
	Control	10/11	11/11	8/10	7/10	9/10	8/9	8/10	8/10	5/10	8/10	82/101
6	Treatment	8/10	10/10	5/8	10/10	11/11	9/11	11/11	11/11	9/10	9/10	94/102
	Control	10/11	11/11	8/10	7/10	9/10	8/9	7/10	8/10	5/10	8/10	81/101
7	Treatment	8/10	10/10	4/8	escape	11/11	8/11	11/11	10/11	8/10	8/10	78/92
	Control	10/11	11/11	8/10	7/10	7/10	8/9	6/10	8/10	5/10	8/10	78/101

[a]Whitefly females collected from the same colony were divided into two groups and placed in leaf cages. Fire Orange powder was forced into one cage using a small atomizer. Clean air was blown into the second cage, which was used as a control. The insects were then released onto cotton plants located in large muslin cages. The whiteflies were collected 24 hr later in groups of 10 per leaf cage, and these cages were placed on new cotton plants. Number of living and dead insects in each cage was recorded daily.
[b]Numerator is the number of living whiteflies and the denominator is the total per cage (living divided by total no.). Differences between treatment and control were not significant ($P = 0.004$).

greenhouse adjacent to the treated group.

Studies with fluorescent pigments. Daylight fluorescent pigment, Fire Orange (Day-Glo Color Corp., Cleveland, OH), was used in experiments to track the flight of whiteflies. A small atomizer was used to apply the pigment to whiteflies enclosed in leaf cages with screened covers and bottoms. Air was blown on whiteflies in control cages. The whiteflies were then released onto sweet potato or cotton plants in large muslin cages. The whiteflies were collected 24 hr or more later and were used for different tests. In the experiments designed to test the effect of the vacuum collector, the whiteflies were placed into the cylinder and vacuumed for 1 min before being used. The effect of Fire Orange on the life span of *B. tabaci* was tested with whitefly females collected from the same colony and divided into two groups. One group was treated, as mentioned above, with Fire Orange and the second group was used as a control and treated with air. Whiteflies were collected after 24 hr in groups of 10 per leaf cage, and the cages were placed in pairs on the opposite half leaves of cotton plants. The numbers of living and dead insects were recorded daily.

The persistence of the pigment on the whiteflies under field conditions after they were trapped on yellow sticky traps was tested. Whiteflies (in groups of 20) were placed, at different intervals after marking, on the traps, which were then placed outdoors.

The chance of the newly hatching adults from a leaf previously covered with dust to become contaminated was tested as follows. A detached sweet potato leaf with larvae was dusted with Fire Orange. The leaf was then placed in a tall glass cylinder with a petroleum jelly coated cover to capture the emerged whitefly adults. This cover was placed under ultraviolet illumination to determine the number of adults contaminated with the pigment.

Table 4. Persistence of Fire Orange pigment on whiteflies (*Bemisia tabaci*) on traps coated with petroleum jelly in the field at different days after dusting[a]

Days after dusting	Days on traps 0	1	2	3	4	5	6	7	8	9	10	11	12	13
1	+[b]	+	+	+	+	+	+	+	+	0	+	+	+	+*
2	+	+	+	+	+	+	+	+	0	+*	...	...	...	...
3	+	+	+	+	+	+	+	0	+*	...	...	...	...	...
4	+	+	+	+	+	+	0	+*	...	...	...	...	...	...
5	+	+	+	+	+	0	+*	...	...	...	...	...	...	...

[a]Dusted whiteflies collected at different intervals after the marking date were stuck to traps outdoors. Traps were examined daily under ultraviolet light.
[b] + = Still glowing, 0 = not examined, * = test terminated.

The effect of the vacuum collector on the persistence of the pigment on the whiteflies was determined as follows. Dusted whiteflies were collected into cylinders and cooled in a refrigerator. The number of insects fluorescing under ultraviolet light was counted through the cylinder walls. The whiteflies were then held at room temperature until they became active and were then vacuumed for 1 min. The insects were again cooled and counted. The whiteflies were then blown out of the cylinder onto yellow sticky traps and the number of fluorescing insects was observed.

In all the above-mentioned tests the detection of the pigment was done in a dark room using a compact 4-W ultraviolet VL-21 lamp (UVP, Inc., San Gabriel, CA). The results were analyzed by the sign test or the Mann-Whitney *U* test (11).

RESULTS

Effect of the vacuuming on the survival of the whiteflies. A significant reduction ($P = 0.004$) of about 23% in the survival of whiteflies 48 hr after the 1-min treatment with the vacuum collector was noted (Table 1). The amount of reduction does not seriously hamper most types of field studies in which the technique is useful. The fact that a 48-hr inoculation feeding access period is long enough for successful transmission of all of the known whitefly-borne viruses (5) ensures that the survivers can be effective vectors.

Effect of the vacuum collector and simulated shipping conditions on the transmission of LIYV by the whiteflies. A total of 95% transmission was achieved by the groups of whiteflies in the simulation of collection and shipping tests (Table 2).

This percent does not differ significantly from that established by the control groups (88%). Therefore, the combined effect, if any, of the collecting process and shipping conditions on the transmission rate is minor.

Effect of Fire Orange pigment on the life span of *B. tabaci*. The results (Table 3) indicate no effect of the Fire Orange pigment on the life span of *B. tabaci* through 7 days. Thus, the pigment did not appear to affect the whiteflies during the period of LIYV retention (3 days) (6).

Persistence of Fire Orange on the insects on yellow sticky traps. Yellow sticky traps are one of the tools used in epidemiological studies of whitefly-borne viruses. It was important to test the persistence of the Fire Orange

Table 5. Effect of the vacuum collector on the persistence of Fire Orange pigment on *Bemisia tabaci*

Treatment	Days after dusting[a]						
	1	2	3	4	5	6	Total
Before vacuuming[b]	(18/22)[c] 82	(18/24) 75	(20/20) 100	(26/26) 100	(11/11) 100	(25/25) 100	(118/128) 92
After 1 min of vacuuming	(18/22) 82	(18/24) 75	(20/20) 100	(26/26) 100	(11/11) 100	(25/25) 100	(118/128) 92
On yellow sticky traps[d]	(22/22) 100	(24/24) 100	(20/20) 100	(26/26) 100	(11/11) 100	(25/25) 100	(128/128) 100

[a]Number (in parentheses) and percent of fluorescing insects at indicated days after dusting.
[b]Whiteflies enclosed in plastic collection cylinders.
[c]Number of fluorescing insects dividied by total number of dusted insects (in parentheses). Difference between treatments is not significant (Mann-Whitney U test, $P = 0.53$).
[d]Whiteflies were released from the cylinders onto yellow sticky traps.

pigment on the trapped whiteflies in the field. The results show that all whiteflies tested leaving the site (source) through 5 days after dusting still retained detectable pigments for at least 6 additional days (Table 4).

Treatment of whitefly larvae with Fire Orange. All of the adults (17 out of 17) that emerged 2 days after dusting were coated and 86% (30 out of 35) after 3 days were coated. The results indicate that heavy dusting with Fire Orange can effectively coat newly emerging adults.

Effect of the vacuum collector on the persistence of the pigment on the whiteflies. The possibility that the action of the vacuum collector may clean the dust from the whiteflies was tested. Also, it was important to compare the detection rate of the living whiteflies enclosed in the collection cylinders with that of the whiteflies on the sticky traps. The results in Table 5 show that the fluorescent pigment was still detectable on the whiteflies after a period of 6 days and that no significant reduction in the detection of marked insects is caused by the vacuuming or by the walls of the collection cylinders.

DISCUSSION

A simple system designed to monitor the movement and infectivity of whiteflies in epidemiological studies of whitefly-borne viruses is suggested. The collection device and shipping procedures are currently being used in studies of the epidemiology of LIYV in the desert southwest of the United States. The collection device captures living insects in small cages. The cages with insects can be used directly as inoculation chambers, as a measure of population densities, and as a means of monitoring movement (with the fluorescent pigment). Under field conditions, whiteflies could be collected at very low population levels that were virtually impossible to detect by visual methods.

The vacuum collector method offers, perhaps, a better alternative than placing bait plants into the field for monitoring insect infectivity levels and would be applicable to epidemiological studies with other types of insect-borne viruses.

LITERATURE CITED

1. Bock, K. R. 1983. Epidemiology of Cassava mosaic virus in Kenya. Pages 337-347 in: Plant Virus Epidemiology. R. T. Plumb and J. M. Thresh, eds. Blackwell Scientific Publications, Oxford.
2. Cohen, S., Berlinger, M., Lehman-Sigura, N., Kern, J., Harpaz, I., and Ben-Joseph, R. 1986. The epidemiology of a whitefly-borne virus in Israel. Proc. Workshop Epidemiol. Plant Virus Dis., Orlando, Fla. 1986. VII:35-36.
3. Cohen, S., and Harpaz, I. 1964. Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Genn.). Entomol. Exp. Appl. 7:155-166.
4. Cohen, S., Keren, J., Harpaz, I., and Ben-Joseph, R. 1986. Studies on the epidemiology of a whitefly-borne virus, tomato yellow leaf curl virus, in the Jordan Valley. (Abstr.) Phytoparasitica 14:158.
5. Duffus, J. E. 1987. Whitefly transmission of plant viruses. Pages 73-91 in: Current Topics in Vector Research. K. F. Harris, ed. Springer-Verlag, New York.
6. Duffus, J. E., Larsen, R. C., and Liu, H. Y. 1986. Lettuce infectious yellows virus—A new type of whitefly-transmitted virus. Phytopathology 76:97-100.
7. Fauquet, C., and Fargette, D. 1986. A summary of the epidemiology of African cassava mosaic virus. Proc. Workshop Epidemiol. Plant Virus Dis., Orlando, Fla. 1986. VII:1-30.
8. Meyerdirk, D. E., Hart, W. G., and Burnside, J. 1979. Marking and dispersal study of adults of the citrus blackfly *Aleurocanthus woglumi*. South. Entomol. 4:325-329.
9. Muniyappa, V. 1983. Epidemiology of yellow mosaic disease of horsegram (*Macrotyloma uniflorum*) in south India. Pages 331-335 in: Plant Virus Epidemiology. R. T. Plumb and J. M. Thresh, eds. Blackwell Scientific Publications, Oxford.
10. Shivanathan, P. 1983. The epidemiology of three diseases caused by whitefly-borne pathogens. Pages 323-330 in: Plant Virus Epidemiology. R. T. Plumb and J. M. Thresh, eds. Blackwell Scientific Publications, Oxford.
11. Siegal, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York. 312 pp.
12. Zalom, F. G., Natwick, E. T., and Toscano, N. C. 1985. Temperature regulation of *Bemisia tabaci* (Homoptera: Aleyrodidae) populations in Imperial Valley cotton. J. Econ. Entomol. 78:61-64.