A New *Bemisia tabaci* Biotype in the Southwestern United States and its Role in Silverleaf of Squash and Transmission of Lettuce Infectious Yellows Virus

S. Cohen, J. E. Duffus, and H. Y. Liu

Visiting scientist, Volcani Institute of Agricultural Research, Bet Dagan, Israel; research plant pathologists, USDA-ARS, U.S. Agricultural Research Station, Salinas, CA 93905.
The work was conducted at the U.S. Agricultural Research Station, Salinas, CA 93905.
Accepted for publication 23 July 1991 (submitted for electronic processing).

ABSTRACT

Cohen, S., Duffus, J. E., and Liu, H. Y. 1992. A new *Bemisia tabaci* biotype in the southwestern United States and its role in silverleaf of squash and transmission of lettuce infectious yelllows virus. Phytopathology 82:86-90.

Collections of *Bemisia tabaci* from California desert regions have been shown to be a mixture of biotypes. These whitefly biotypes differ in a number of ways including their ability to induce silverleaf of squash. The physiological differences of the newly found whitefly biotype, including host preference, larval development, transmission of lettuce infectious yellows virus, and the induction of silverleaf symptoms, clearly distinguish it from the previously occurring biotype. Silverleaf of squash was induced by nymphal feeding activity; however, the physiological condition of the host as influenced by light intensity, quality, and duration are important factors in silverleaf expression. Differences between the whitefly biotypes in induction of silverleaf are quantitative and qualitative. Double-stranded RNA bands were not detected from nymph-infested leaves or from silverleaf symptomatic tissue, suggesting that whitefly-induced silverleaf in California is similar to a systemic phytotoxemia.

Bemisia tabaci (Gennadius) whitefly transmitted virus diseases have caused staggering losses to desert southwest agriculture since 1981 (2,4,5). The vectors and the diseases they transmit appear to be increasing in importance throughout the world.

B. tabaci in Florida was not an economic problem until 1986, when nurseries reported outbreaks on ornamentals (9). Florida squash growers have suffered significant economic damage since 1987, from a disease (silverleaf syndrome), induced by the feeding of *B. tabaci*. A similar and probably identical disorder (thought to be partially related to drought stress) occurs in Israel (8). Bharathan et al (1) suggested that a double-stranded (ds) RNA is, or is associated with, the causal agent of the *B. tabaci*-induced silverleaf of squash. Yokomi et al (13) on the other hand, suggested that a toxicogenic factor associated with nymphal feeding may be the cause of silverleaf.

Silverleaf of squash has not occurred in the southwestern United States, and populations of *B. tabaci* from this region have previously not been capable of inducing typical silverleaf (1).

During the fall of 1990, collections of *B. tabaci* were made from melons to reevaluate the possibility that southwest desert whiteflies could induce squash silverleaf. Later in the fall, some growers and agriculturalists, along with farm advisor Frank Laemmlen, noticed apparent differences in *Bemisia* host preferences in the desert and sent a whitefly culture on broccoli. *B. tabaci* colonies resulting from these isolations from the

California desert region have been shown to be a mixture of biotypes differing in a number of ways, including their ability to induce silverleaf of squash; this has been reported (3). The present report presents experimental evidence that a whitefly biotype new to the Southwest is occurring in the region and is capable of inducing silverleaf.

MATERIALS AND METHODS

***Bemisia* colonies. Original Imperial Valley (IV-81).** Approximately 300–500 *B. tabaci* collected in 1981 from field cotton (*Gossypium hirsutum* L.) in the Imperial Valley, California, were transferred to virus-free sweetpotato, *Ipomoea batatas* (L.) Lam. and maintained for 10 yr in muslin-covered cages. The cages were maintained in growth rooms at temperatures that ranged from 26 to 32 C or in an insectary greenhouse. Over this period of time, the population was used in numerous transmission experiments with several *Bemisia*-transmitted viruses with no indication of variability in regard to insect transmission or the induction of silverleaf. In experiments that involved rearing the populations on squash (*Cucurbita pepo* L.), at least four generations were passed on this host before using the individuals for experiments.

New Imperial Valley (IV-90). Approximately 1,200 *B. tabaci* collected in the fall of 1990 from field melon (*Cucumis melo* L.) and broccoli (*Brassica oleracea* var. *botrytis* L.) were transferred to sweetpotato and squash, and grown in muslin-covered cages as above. These isolations were not selected in any way from other naturally occurring *B. tabaci* in the region.

Silverleaf bioassays and nymphal development. Squash cultivar Black Magic was used for bioassays. Seeds were planted in 10-cm pots, and the plants were used at about the first true leaf stage.

Individual female whiteflies were caged on squash leaves for 4 days and maintained in growth rooms at 26–28 C at 2,000 lx in continuous light provided by cool white fluorescent tubes. After 4 days the cage was removed, the female whitefly was removed, and nymphs were allowed to develop. Development rate was measured by counting the larvae, pupae, and emerging adults. The sex of the adults was also determined.

In some instances, light and temperature conditions were altered, as were whitefly populations. Under normal greenhouse conditions in January in Salinas, light intensities of between 13,000 and 80,000 lx were measured with a 10-h day. In some tests additional light of 24 h duration was added to greenhouse grown colonies (supplied by cool, white fluorescent tubes about 1 m above the plants). In other tests, growth rooms at 26–28 C with light intensities of 2,000 lx and 3,000 lx (measured at plant canopy height and supplied by continuous, cool, white fluorescent tubes) were used for silverleaf development. An impression that glass over the plants reduced silvering prompted experiments of placing glass sheets over plants in the 3,000 lx rooms. Light was maintained under the glass at 3,000 lx by lowering the lights. Silverleaf symptom expression was rated using a five-interval scale: 0 = asymptomatic; 1 = slight vein silvering; 2 = silvering of primary veins; 3 = silvering extends between veins, but veins still prominent; 4 = silvering of entire leaf. Light measurements were made by using a type LI-170 Quantum/Radiometer/Photometer (Lambda Instrument Corp., Corpus Christi, TX)

In cases in which virgin whiteflies were used, the pupae were removed from the leaf with a needle. The pupae were attached to the underside of cucumber (*Cucumis sativa* L.) leaves (because of easier manipulation) with a moistened brush and covered with a leaf cage. After 3–4 days, the emerged adults were sexed and used in the various experiments.

Host plant suitability. The suitability of various hosts for *B. tabaci* was determined in growth rooms at 26–28 C with 3,000 lx continuous light provided by cool white fluorescent tubes. The nymphal development time, survival, and number of developing immatures from a 4-day oviposition period was determined. Females in groups of 10 were caged on half leaves of various host species for 4 days. The cages were removed and the number of surviving females was determined. The number of developing larvae (first determined 13 days after egg deposition) and the developmental time from egg deposition to adult eclosion was determined. These tests were replicated 10 times.

Transmission of lettuce infectious yellows virus (LIYV). The LIYV isolate was obtained from commercial lettuce (*Lactuca sativa* L.) plants collected in the Imperial Valley of California during the fall of 1981. The virus was maintained in lettuce and cheeseweed (*Malva parviflora* L.) and transferred from plant to plant via inoculation with the IV-81 colony.

Individual *B. tabaci* females from the IV-81 and IV-90 colonies were given 24-hr acquisition feeding intervals in leaf cages on opposite half-leaves of LIYV-infected Summer Bibb lettuce. The individual whiteflies were then transferred serially at 24-h intervals to two- to three-leaf stage Summer Bibb lettuce.

TABLE 1. Squash silverleaf development following placement of individual *Bemisia tabaci* females on squash and sweetpotato and subsequent growth of progeny nymphs

Squash						Sweetpotato					
IV-81			IV-90			IV-81			IV-90		
Insect number	Silver[a] leaf	Nymph[b] number	Insect number	Silver leaf	Nymph number	Insect number	Silver leaf	Nymph number	Insect number	Silver leaf	Nymph number
1	2	27	15	4	114	28	1	37	42	4	14
2	2	48	16	4	21	29	1	49	43	4	45
3	2	69	17	4	66	30	1	64	44	4	46
4	1	54	18	4	39	31	1	40	45	4	35
5	1	55	19	4	110	32	1	60	46	4	33
6	1	62	20	4	62	33	1	45	47	2	37
7	1	44	21	4	69	34	1	49	48	3	7
8	1	50	22	4	62	35	1	40	49	3	12[c]
9	1	53	23	3	6	36	1	55	50	3	15
10	1	40	24	3	(60)	37	1	30	51	0	8[c]
11	1	61	25	3	(38)	38	1	44	52	0	(33)
12	1	48	26	2	62	39	1	31			
13	1	50	27	0	(45)	40	1	25			
14	1	45				41		9			
Average	1.2			3.3			1			2.8	

[a]Silverleaf rating: 0, asymptomatic; 1, slight vein silvering; 2, silvering of primary veins; 3, silvering extends between veins, but veins still prominent; 4, silvering of entire leaf.

[b]Number of nymphs of both sexes developing from a single female. Those nymphs in ( ) were all males indicating a virgin female was placed on the plants.

[c]F_2 populations developing from these nymphs produced silvering with a 4 rating.

In another experiment, *B. tabaci* (mixed sexes) were placed on cotton (immune to LIYV) following a 24-h acquisition period in groups of five. After 0, 1, 2, 3, and 4 days on cotton, 10 cages of each population were transferred to two- to three-leaf stage lettuce for a 48-h inoculation feeding period.

Extraction and analysis of double-stranded RNAs from plant tissue. Double-stranded (ds) RNAs were extracted from 7 g of plant tissue using the method of Morris and Dodds (7). dsRNA was precipitated with ethanol and resuspended in electrophoresis buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.8) and was analyzed by electrophoresis in a horizontal 1.5% agarose slab gel (5 × 65 × 100 mm).

Electrophoresis was for 2 h at 50 V constant voltage at room temperature. After electrophoresis, gels were stained with ethidium bromide (20 ng/ml), and nucleic acid bands were visualized on a UV transilluminator.

RESULTS

Effect of light on silverleaf development. Preliminary observations on silverleaf symptoms induced by the IV-81 and IV-90 colonies indicated that light intensity, quality, and duration play a role in the silverleaf syndrome. A few preliminary experiments were conducted in an effort to develop a uniform testing procedure.

Under normal greenhouse conditions silvering developed with the IV-90 colony, but not with the IV-81 colony. When additional light of 24 h duration was added to the IV-81 colony, complete silvering of about a 4.0 rating developed in the IV-81 colony.

In the 3,000-lx rooms, complete silvering developed with both colonies; however, the IV-81 colony developed the complete silvering a week later than the IV-90 colony. When glass was placed above both colonies but the light was maintained at 3,000 lx, only the IV-90 colony developed complete silvering. Also, when the duration of the light period was reduced to 12 h, only the IV-90 colony developed complete silvering. In the 2,000-lx rooms, complete silvering only developed in the IV-90 colonies.

Silverleaf development induced by different whitefly populations. Silverleaf bioassays on plants exposed to individual females from IV-81 and IV-90 colonies reared on squash and sweetpotato are shown in Table 1. Developing nymphs from females from the original colony, IV-81, whether reared on squash, cotton, melon, broccoli, or sweetpotato were unable to induce typical silverleaf under the conditions of the 2,000-lx growth room. The average silverleaf rating induced by the progenies of the 28 individual females from the IV-81 colony was 1.1 (slight vein silvering). The developing nymphs from the new IV-90 colonies induced typical silverleaf in most instances. The average silverleaf rating induced by the progenies of 24 individual females from the IV-90 colonies was 3.1. In instances that virgin females were placed on the squash plants and only male nymphs were produced (insects 24, 25, 27, and 52), silverleaf ratings were less than complete silvering or 0. The F_2 population of whiteflies developing from insects 49 and 51 induced complete silvering on test plants, indicating that the low number of developing nymphs 12 and 8, respectively, was the probable reason for the low silverleaf rating of these insects.

The progenies of insects 26 and 47 induced less silvering in these tests than other progenies of the group. This silvering test was run early in regard to the collection date of the colony, and the assumption was made that these individuals may have represented mixtures of the old biotype in the predominantly new biotype. The progenies of insect 26 were lost, but populations derived from insect 47 have been shown to be similar in all ways tested (nonsilvering and development rate) to the IV-81 colony. The variation that occurred in the early colonies of the IV-90 population in regard to the induction of silverleaf has in a very few generations completely disappeared. Thus, it appears that the IV-90 population directly from the field was a mixture of biotypes, but that the new biotype has completely replaced the old one in our IV-90 cultures.

Silverleaf bioassay of 1991 field *Bemisia*. It appeared from the collections of field *Bemisia* in the fall of 1990 (the IV-90 colony) that populations were predominantly the new biotype. It was of interest to test the new field generations of *Bemisia* in the desert regions during the spring of 1991. Female *Bemisia* shipped from the Imperial and Palo Verde valleys of California and the Dome Valley and Yuma area of Arizona were caged individually on squash seedlings, and the squash plants subsequently were monitored for nymph development and silvering. Numbers of developing nymphs were not determined. The developing nymphs from 120 of 129 females collected in the region induced silverleaf ratings of 2 or greater with an average reading of 3.15. The progenies of only nine females induced silverleaf ratings of less than 2, with an average rate of 0.8.

Development rate of IV-81 and IV-90 populations. In a preliminary test, individual female *B. tabaci* reared on squash were

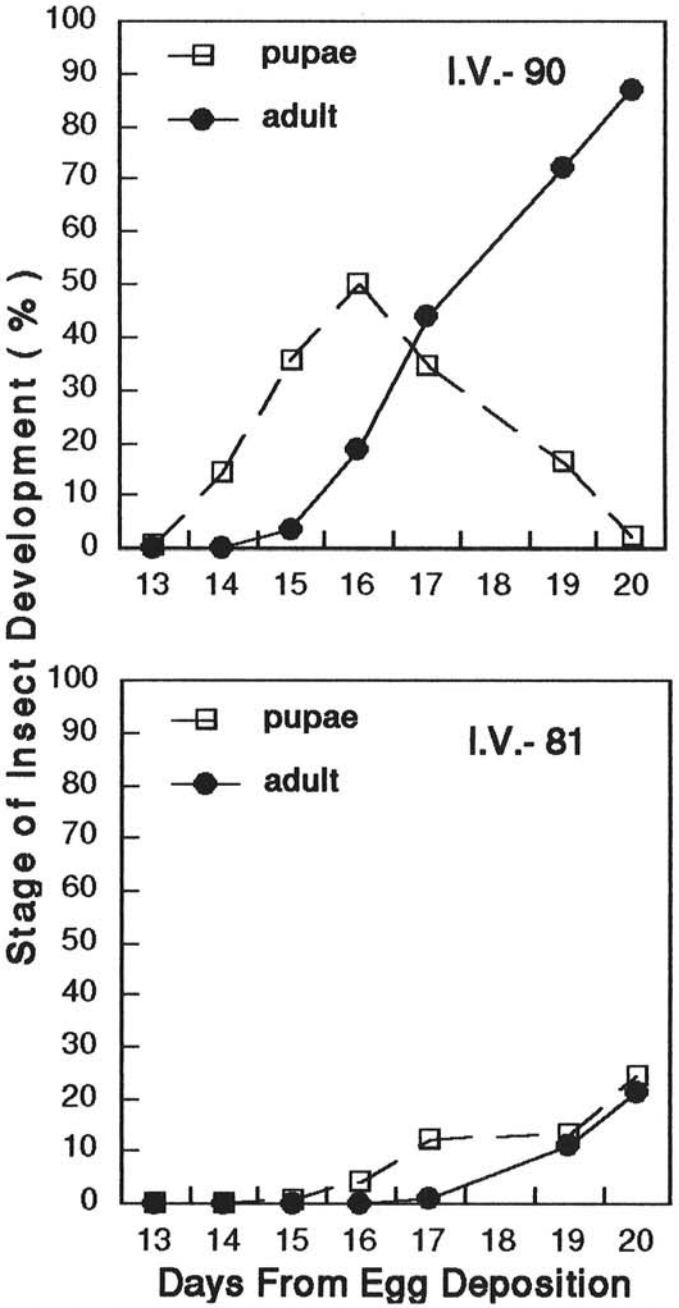

Fig. 1. The development rate of two *Bemisia tabaci* colonies. Both colonies were reared on squash. Individual female whiteflies were caged on single squash leaves of individual plants for 4 days and maintained at 26–28 C at 2,000 lx continuous light. The number of developing nymphs, pupae, and adults were counted. The percentage of nymphs reaching the pupal and adult stages are shown.

caged on squash leaves for 4 days and maintained at 26–28 C at 2,000 lx continuous light. The number and development rate of nymphs, pupae, and adult eclosion were determined (Fig. 1).

Nymphs of the IV-90 colony developed significantly faster (in the range of 25–30%) than the original IV-81 colony. After 20 days, nearly 90% of the IV-90 whiteflies were adults in comparison to only 21% of the IV-81 colony. Similar results were obtained using different colonies reared on sweetpotato (data not shown).

Host plant suitability. Major differences in the host suitability of the important crop species grown in the southwest desert to the original (IV-81) and newly found (IV-90) whitefly populations were observed (Table 2). A significantly higher number of nymphs of the IV-90 population survive to maturity on alfalfa, broccoli, cotton, lettuce, and melon. On the other hand, nymphal survival of the IV-81 colony was significantly higher on bean and sweetpotato than the IV-90 colony. The increased survival rates on these important hosts may be due to oviposition rates and/or decreased pre-adult mortality; however, these factors were not determined. The source of whiteflies for the lettuce, bean, and alfalfa tests were different, and it is possible that this difference could have affected nymphal survival rates even though adult survival was not affected.

Development rates from egg deposition to adult eclosion was significantly faster on most plant species tested with the IV-90 population, ranging in these tests from 7 to 16% faster.

LIYV transmission. Preliminary transmission tests comparing the efficiency of the new IV-90 population and the original IV-81 population showed major differences in these two groups of whiteflies in their ability to transmit LIYV. A total of 26 of 50 females of the IV-81 population that were transferred individually in daily serial transfers transmitted LIYV. The insects transmitted LIYV to 35 plants in the serial transfers and retained the virus for a maximum of 3 days. None of the 50 females from the IV-90 population transmitted LIYV.

In a separate test, groups of five mixed sex whiteflies were given an acquisition feeding on LIYV-infected lettuce and then placed on cotton. After 0, 1, 2, 3, and 4 days on cotton, the IV-81 population transmitted LIYV to 5, 4, 1, 0, 0, respectively, of 10 test plants fed upon, the IV-90 population transmitted LIYV to 1, 1, 0, 0, 0 test plants.

dsRNA analysis. dsRNA bands were not detected from nymph-infested leaves or from silverleaf symptomatic tissue grown in our facility. Under conditions in which tomato bushy stunt virus, cucumber mosaic virus, and silverleaf tissue from Florida (supplied by K. R. Narayanan) all showed dsRNA bands, no bands were detected from healthy squash leaves or from silvering leaves induced by either of the whitefly biotypes.

DISCUSSION

The sweetpotato whitefly has become increasingly more important in the United States since its outbreak occurrence in the southwest deserts in 1981. The whitefly caused economic losses to cotton by the contamination of the lint with honeydew and the transmission of the cotton leaf crumple virus. It has caused losses of more than 100 million dollars in vegetable production in the region in its role as a vector of lettuce infectious yellows virus and squash leaf curl virus. *Bemisia* has become an economic problem in Florida since 1986 because of its induction of feeding related diseases, such as silverleaf of squash, uneven ripening in tomatoes, and the transmission of geminiviruses. The whitefly has been increasing in incidence in Texas and has caused serious damage in that region as a vector of a new geminivirus (12). In addition to its roles as an inducer of feeding related diseases and a vector of plant viruses in agricultural environments, the sweetpotato whitefly is becoming a major factor in the nursery trade in protected environments throughout the United States and the world.

Our data present clear evidence that distinct biotypes of *B. tabaci* are occurring in the southwest desert regions of the United States. These biotypes, which in preliminary examination cannot be distinguished by morphological means (R. J. Gill, *personal communication*), differ in a number of quantitative and qualitative factors, including their ability to induce silverleaf of squash, in the suitability of a number of major crop species as hosts, in their development rates, and in their ability or efficiency in transmitting LIYV. Other evidence (*unpublished data*) clearly separates these biotypes by isozyme differences.

The difference reported here between the IV-81 and IV-90 populations could not be related to natural variation within the populations of the region. There has been no evidence of variability in development rates, host range, virus transmissibility, or the occurrence of silverleaf.

TABLE 2. Host plant suitability of different *Bemisia tabaci* colonies as determined by adult survival, nymphal development rate and number, and survival of developing larvae and nymphs[a]

Plant host	Survival of 10 females after 4 days (average/cage)		Developing larvae (average/cage)		Minimum development time from egg to adult (days)		Development time for 50% adult eclosion (days)	
	IV-81	IV-90	IV-81	IV-90	IV-81	IV-90	IV-81	IV-90
Alfalfa *Medicago sativa*	2.9	2.7	13.6	67.3*	23	20	25	23*
Broccoli *Brassica oleracea* var. *botrytis*	6.4	6.9	17.9	180.0*	28	24	32	27*
Bean *Phaseolus vulgaris*	6.8	6.51	105.0	12.9*	22	21	26	26
Cotton *Gossypium hirsutum*	6.7	7.9	61.4	139.0*	22	20	24	22*
Lettuce *Lactuca sativa*	4.3	5.6	2.5	34.1*	24	21	25	22*
Melon *Cucumis melo*	5.1	7.5*	16.7	130.4*	23	21	27	25*
Sweetpotato *Impomoea batatas*	7.6	3.5*	81.1	39.4*	19	19	23	23
Sugarbeet *Beta vulgaris*	0	0	7.9	5.2	...[b]	...	...	...

[a] Female *Bemisia tabaci* in groups of 10 were caged on half leaves of the various host species for 4 days. After the 4-day period, the cages were removed and the number of surviving females was determined. The number of developing larvae were determined after 13 days, and the development time from the beginning of egg deposition to the adult eclosion was also determined. The source of the female whiteflies used in these tests were colonies grown on squash except for the IV-81 colony used on lettuce, bean, and alfalfa, which were reared on sweetpotato. The tests were replicated 10 times and were analyzed by the sign test. Significant differences $P < 0.05$ indicated by *.

[b] Did not mature.

There is little information on the natural variation within *B. tabaci* populations to induce silverleaf of squash. The evidence from Florida indicates that silverleaf symptoms were always associated with infestations of the whitefly (1,6,10,13). Populations from California, on the other hand, did not induce silverleaf, (the present work with the IV-81 population) and a completely separate population supplied by Dr. Tom Perring from the Imperial Valley (1).

The results presented in this study indicated that silverleaf of squash was induced by nymphal feeding activity, and this confirms the work of Florida workers (1,10,13). Preliminary observations reported here indicate, however, that the phenomena is more complex than simple feeding of nymphs, and that the physiological condition of the host plant as influenced by light intensity, quality, and duration are very important factors in silverleaf expression. For example, Imperial Valley whiteflies, as reported by Bharathan et al (1), did not induce silverleaf under the conditions used in Florida. However, under conditions of high light intensity and a long duration, silvering can be induced by the early Imperial Valley population.

The two biotypes discussed here differ markedly in their abilities to induce silverleaf. Larval density can be a factor in severity of silverleaf. However, under conditions where thousands of nymphs of the IV-81 biotype are developing on squash, silverleaf will not develop unless light conditions are right. This implies that the differences between the two biotypes in respect to silvering is not only quantitative but is also qualitative. Genetic silvering of cucurbita is a complex phenomenon that is also modified by a combination of nongenetic influences such as light and temperature (11).

We have not detected dsRNA from silverleaf-affected plants grown in our facility or from larvae or adult whiteflies.

We have induced silverleaf with the IV-81 population, which did not produce dsRNA in Florida (1). These results suggest that the whitefly-induced silverleaf in California is similar to a systemic phytotoxemia.

Preliminary transmission tests indicate major differences in the biotypes in their ability to transmit LIYV. These differences appear not to be related to host plant suitability, but to other not defined transmission characteristics. The epidemiological consequences of the transmission differences, the mechanisms involved, and their implications are under study.

The colony IV-90, first collected in the fall of 1990 from squash and broccoli, has been maintained on squash and sweetpotato for approximately 10 mo. Early tests with this population indicated that individuals (for example insects 26 and 47, Table 1) produced less silvering than other individuals in the group. Evidence indicates that those individuals are closer physiologically to the IV-81 population than to IV-90 population. The apparent aggressive development of the new biotype in the laboratory (the complete loss of individuals of the old biotype in the cultures) and the shift in the population in desert regions this spring could lead to the virtual disappearance of the old biotype with resulting significant changes in virus epidemiology.

The virtual replacement of the old biotype in the laboratory (IV-90 colony) and in the field by the new biotype along with the great number of differences between the two are strong evidence for the introduction of this new biotype into the southwest desert.

The occurrence of silverleaf-inducing whitefly biotypes on nursery stock, including poinsettia and hibiscus in various parts of California (3), and the movement of such nursery stock throughout the country and world indicates that this type of movement is the probable vehicle for the apparent changes in *Bemisia*.

LITERATURE CITED

1. Bharathan, N., Graves, W. R., Narayanan, K. R., Schuster, D. J., Bryan, H. H., and McMillan, Jr., R. T. 1990. Association of double stranded RNA with whitefly-mediated silvering in squash. Plant Pathol. 39:530-538.
2. Cohen, S., Duffus, J. E., Larsen, R. C., Liu, H. Y., and Flock, R. A. 1983. Purification, serology, and vector relationships of squash leaf curl virus, a whitefly transmitted geminivirus. Phytopathology 73:1669-1673.
3. Cohen, S., Duffus, J. E., Liu, H. Y., and Perry, R. 1991. Induction of silverleaf of squash by *Bemisia* whitefly from California desert whitefly populations. Plant Dis. 75:862.
4. Duffus, J. E., and Flock, R. A. 1982. Whitefly transmitted disease complex of the desert southwest. Calif. Agric. 36:4-6.
5. Duffus, J. E., Larsen, R. C., and Liu, H. Y. 1986. Lettuce infectious yellow virus–a new type of whitefly transmitted virus. Phytopathology 76:97-100.
6. Maynard, D. N., and Cantliffe, D. J. 1989. Squash silverleaf and tomato irregular ripening: new vegetable disorders in Florida. Fla. Coop. Ext. Ser. VC-37.
7. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854-858.
8. Paris, H. S., Nerson, H., and Burger, Y. 1987. Leaf silvering of Cucurbita. Can. J. Plant Sci. 67:593-598.
9. Price, J. F., Schuster, D. J., and Short, D. E. 1987. Managing sweet potato whitefly. Greenhouse Grower (December):55-57.
10. Schuster, D. J., Kring, J. B., and Price, J. F. 1991. Association of the sweetpotato whitefly with a silverleaf disorder of squash. HortScience 26:155-156.
11. Shifriss, O. 1984. Further notes on the silvery-leaf trait in Cucurbita. Cucurbit Genetics Coop. Rpt. 7:81-82.
12. Stenger, D. C., Duffus, J. E., Villalon, B. 1990. Biological and genomic properties of a geminivirus isolated from pepper. Phytopathology 80:704-709.
13. Yokomi, R. K., Osborne, L. S., and Hoelmer, K. A. 1990. Relationship between the sweetpotato whitefly and the squash silverleaf. Phytopathology 80:895-900.