Squash Silverleaf Symptoms Induced by Immature, but Not Adult, *Bemisia tabaci*

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ABSTRACT

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The causes of plant disorders such as squash silverleaf and tomato irregular ripening associated with feeding of the whitefly, *Bemisia tabaci* (B-biotype), are not well understood. Whether immatures, adults, or both cause symptoms is important to management because it determines the target of control and selection of appropriate management strategies. We measured the relative ability of adult and immature whiteflies to induce symptoms of squash silverleaf in zucchini. Plants exposed to 20 adult whiteflies for 48 h and subsequently dipped in insecticidal soap to remove immature offspring did not develop silverleaf symptoms. In contrast, all plants exposed to adults, but on which immatures were allowed to develop, showed symptoms of silverleaf. Furthermore, a significant regression was found (SYMPTOM = 1.37 + 0.064 IMMATURE, $R^2 = 0.68$) indicating a positive relationship between the severity of silverleaf and the number of immatures present on a plant. In no case were silverleaf symptoms present unless immatures were also present on the plants. When this experiment was repeated under more stringent conditions in which plants were exposed to 20 adults per day for each of 10 consecutive days, but not to immatures, plants did not develop silverleaf symptoms. These findings support previous hypotheses that adult *B. tabaci* do not contribute significantly to induction of squash silverleaf and directly contrast recent suggestions that adult *B. tabaci* at relatively low levels can induce silverleaf symptoms.

Additional keywords: plant disorders, sweetpotato whitefly.

Since 1987, increases in densities of what has been called the "B-biotype" (7) or "poinsettia strain" (1,16) of the whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), in the United States have been associated with a variety of phytotoxic disorders of unknown etiology. These disorders include squash silverleaf of *Cucurbita* spp. (2,3,12,21), chlorosis of new growth of *Crossandra infundibularis* (10,15), irregular ripening of tomato (12,18), and stem whitening and blanching in *Brassica* spp. (4,9). Of these disorders, squash silverleaf has been the most studied because obvious symptoms develop in less than 14 days, appear at low levels of whitefly infestation, and are relatively easy to rate. The association between sweetpotato whitefly feeding and symptoms of squash silverleaf has been well documented repeatedly at many locations in the continental United States (3,5,7,10,12,17,21), Hawaii (8), and Puerto Rico (19). The severity of silverleaf symptoms is related to the density of whiteflies feeding on the plant (17,21).

There have been conflicting reports about the stages of *B. tabaci* that are capable of inducing silverleaf symptoms. Three studies have reported that immatures, but not adults, induce symptoms (10,17,21), whereas recently one study reported that adults can induce symptoms (3). Schuster et al (17) reported that symptom expression appeared to be related more to nymphal density than adult density. Hoelmer et al (10) and Yokomi et al (21) found that exposure of squash plants for 15 days to as many as 200 *B. tabaci* adults, but not immatures, did not produce symptoms. Immature feeding was prevented in these experiments by exposing groups of adult whiteflies to a single leaf in a clip cage for 5 days, removing this leaf from the plant before eggs hatched, and placing another group of adult whiteflies on another leaf of the same plant. This process was repeated for three 5-day periods. One concern, with regard to these results, is that removal of leaves after adult feeding could interfere with symptom induction if translocation of a causal agent from source leaves was required and if such translocation was a slow or delayed process (i.e., longer than 5 days). Bharathan et al (3) questioned the results of Yokomi et al (21) and suggested that squash silverleaf could be caused by adult whiteflies, in the absence of immatures, with densities as low as 20 adults per plant for 48 h. In these experiments, oviposited eggs were removed using cellophane tape, and plants subsequently were sprayed once with insecticide. Although all plants in Bharathan et al (3) trials developed silverleaf, it was not noted if the plants were inspected at the end of the test period for the presence of immature whiteflies that may have escaped treatment. This is a critical point, because as few as three immature whiteflies per plant can induce silverleaf symptoms (21).

In developing strategies to manage *B. tabaci* and squash silverleaf, it is important to understand the relative contributions of adult and immature whiteflies in symptom induction. If low densities of adults feeding for a relatively short time can induce silverleaf, different control strategies would be needed from those needed if immature development and feeding must occur before silverleaf symptoms are induced. Therefore, it is critical that the conflicting results presented in the current literature be reexamined. The aim of this paper is to present research results that clarify the potential role of immature and adult *B. tabaci* in inducing silverleaf symptoms.

MATERIALS AND METHODS

Insect colony and plant material. *B. tabaci* used in these experiments were from a population originally collected from squash (*Cucurbita* sp.) at the University of Hawaii Agricultural Experiment Station at Poamoho (Oahu) in August 1991 and maintained on cotton (*Gossypium hirsutum* L.) in outdoor cages on the University of Hawaii campus at Manoa, under ambient conditions. This population was characterized as the B-biotype by polyacrylamide gel electrophoresis (PAGE) analysis of esterase banding patterns as described previously (7) and by its ability to induce symptoms of squash silverleaf in zucchini (8). Zucchini, *Cucurbita pepo* L. 'Ambassador,' and squash, *Cucurbita pepo* L. 'Dixie,' plants were grown under greenhouse conditions with natural lighting, in 10-cm pots using a commercially prepared standard potting soil mixture and watered daily as needed. Plants were used for experiments at the two-to-three-leaf stage of growth.

Symptom rating. The severity of symptoms on each plant leaf was rated by visual inspection on a scale from 0 to 5 as follows: 0 = no symptoms; 1 = mild mottling and vein clearing, with <10% of leaf surface silver; 2 = 10–30% of leaf surface silver; 3 = 30–60% of leaf surface silver; 4 = 60–90% of leaf surface silver; and 5 = entire leaf surface silver. Symptoms of silverleaf do not occur on leaves that are fully formed before whitefly immature feeding. For this reason and to standardize the number of leaves rated per plant, the seven youngest leaves were rated. These ratings were totaled for each plant and used as an overall plant rating.

Efficacy of mechanical removal of eggs. Groups of 20 adult whiteflies were collected from the colony and exposed to 20 potted zucchini plants (two-to-three-leaf stage) using clip cages (one per plant) attached to the lower leaf surface of the first (oldest) plant leaf. Adults were allowed to feed and oviposit for 48 h after which they were removed with an aspirator. On 10 plants, eggs were removed from exposed leaves using cellophane tape and a pin under a low-power microscope. On the remaining 10 plants exposed to adults, eggs were allowed to hatch and develop normally. Ten additional plants not exposed to whiteflies were used as controls. Plants were maintained in outdoor cages under ambient conditions (mean high and low temperature ± SD = 26.5 ± 1.7, 17.2 ± 0.9 C, respectively; Manoa). After 3 wk, the number of immatures present and symptom severity were recorded for each plant. To test the hypothesis that mechanical removal of eggs was effective in eliminating immatures and that immature removal affected symptom severity, mean number of immatures and mean symptom severity ratings were compared using one-way analysis of variance (13) followed by LSD comparison of means (20).

Contribution of immatures and adults to silverleaf induction. Experiment 1. Groups of 20 adult whiteflies per plant were exposed to 20 zucchini plants (two-to-three-leaf stage) using clip cages (one per plant) attached to the lower surface of the first (oldest) leaf. Adults were allowed to feed and oviposit for 48 h after which they were removed. Ten of these plants were subsequently dipped in a solution (200 ml/L) of insecticidal soap (Empede, Mycogen Corp., San Diego, CA) at 5, 7, 9, and 11 days after first exposure to adults to kill emerging offspring. The remaining ten plants exposed to adults for 48 h were not treated, and offspring were allowed to develop normally. Ten plants not exposed to whiteflies were dipped as described above and served as controls. Plants were maintained in outdoor cages under ambient conditions (mean high and low temperature 25.8 ± 2.1, 18.5 ± 1.2, respectively). After 3 wk, the number of immatures present and symptom severity for each plant were recorded as described above. To test the hypothesis that removal of immatures affected symptom development, the mean number of immatures and symptom severity ratings were compared using one-way analysis of variance (13) followed by LSD comparison of means (20). Only plants with the exposed leaf alive at the end of the study were used in the analyses.

This experiment was repeated using another cultivar of squash, *Cucurbita pepo* 'Dixie,' with nine replicates of each treatment. Plants were maintained in outdoor cages under ambient conditions (mean high and low temperature 25.0 ± 2.1 and 18.7 ± 0.7 C, respectively). After 2 wk, the number of immatures and symptom severity for each plant were recorded and analyzed as described above.

Experiment 2. Experiment 1 was repeated with zucchini (*C. pepo* 'Ambassador') using longer exposure of plants to adult whitefly feeding to ensure a stringent testing of the hypothesis that adults do not induce symptoms of silverleaf at the densities tested. Five treatments were used to determine the effects of continuous exposure of zucchini plants to 20 adult whiteflies for a continuous period of 10 days without the removal of exposed leaves. The treatments were as follows: 1) A group of 20 adults were allowed to feed and oviposit in a clip cage on the underside of the oldest leaf (designated as leaf 1) for 48 h after which they were removed. Leaf 1 was subsequently dipped four times (at 5, 7, 9, and 11 days after exposure) in a solution of the insecticidal soap as described previously, to kill emerging offspring. Immediately after removal of adults from leaf 1, another group of 20 adults was allowed to feed in a clip cage on the underside of leaf 2 (second oldest leaf) for 48 h. The adults were removed, and this leaf was dipped four times as just described. This process was continued for 10 days until five leaves were exposed to adult whiteflies, and all immatures were subsequently killed by soap treatments; 2) Groups of 20 adults were allowed to feed and oviposit for 48 h on leaves 1–5 sequentially as described above.

Leaves were not dipped and immatures developed normally; 3) Ten plants not exposed to whiteflies were dipped as described above as controls; 4) Ten plants not exposed to whiteflies and not dipped were used as a second control; and 5) Groups of 25 male whiteflies were allowed to feed continuously on leaf 1 for 3 days, after which leaves were not dipped.

Plants were maintained in outdoor cages under ambient conditions (mean high and low temperature 26.9 ± 2.1 and 20.7 ± 0.7 C, respectively). After 3 wk, the number of immatures present and symptom severity for each plant were recorded as described above. To test the hypothesis that adults feeding for an extended period, in the absence of immatures, resulted in silverleaf symptom development, the mean number of immatures and mean symptom severity ratings were compared using one-way analysis of variance (13) followed by LSD comparison of means (20). Only plants with the exposed leaf alive at the end of the study were used in the analyses.

Relationship between immature density and symptom severity. A regression of silverleaf symptom severity on number of immatures per zucchini plant was performed using data from all experiments to determine if there was a relationship between immature density and symptom severity (13).

RESULTS

Efficacy of mechanical removal of offspring. Mechanical removal of offspring reduced the number of immatures significantly ($F = 301.8$, df = 2,27, $P < 0.001$; LSD = 17.58)(Table 1), but not completely. All plants exposed to adults and subsequently subjected to mechanical removal had at least one immature present in the area exposed to whiteflies (mean ± SE 5.6 ± 1.1), and seven out of ten plants developed silverleaf symptoms. Silverleaf symptom severity was significantly lower ($F = 91.36$, df = 2,27, $P < 0.001$; LSD = 4.43) on plants that had eggs removed mechanically than on those on which eggs were not removed (Table 1). External contamination on a leaf that was not clip caged occurred in one control plant found to have a population of 16 immatures, which subsequently developed symptoms of silverleaf, and in one exposed plant found to have a population of 23 immatures in addition to those found on the exposed leaf. These immatures were included in totals because they likely contributed to silverleaf symptoms.

TABLE 1. Mean number of *Bemisia tabaci* immatures and symptom severity of zucchini plants exposed to 20 adult whiteflies for 48 h with immatures allowed to develop or with immatures removed mechanically

Treatment	Plants with symptoms (no.)	Immatures (no.)	Symptom severity[y]
Adults + immatures	10/10	186 ± 10.3 a[z]	19.7 ± 0.6 a
Immatures removed	7/10	5.6 ± 1.1 b	4.1 ± 1.2 b
Controls	1/10	1.7 ± 0.3 b	1.2 ± 1.2 b

[y] Symptom severity was rated on each of the seven youngest leaves using a scale of 0–5 (with 0 meaning no symptoms, 5 the entire leaf surface silver) and totaled for a per plant rating.

[z] Means in columns followed by the same letter are not significantly different, analysis of variance followed by LSD test, $P = 0.05$.

Contribution of immatures and adults to silverleaf induction. Experiment 1. Results were similar for both zucchini and 'Dixie' squash. No silverleaf symptoms developed on control plants or on plants exposed to adult whiteflies and subsequently dipped (not exposed to immatures), and immatures were not detected on any of these plants (Table 2). All plants on which immatures stages were allowed to develop showed severe silverleaf symptoms (zucchini: $F = 158.1$, df = 2,24, $P < 0.001$; LSD = 1.44; 'Dixie' squash: $F = 40.1$, df = 2,20, $P < 0.001$; LSD = 3.48) compared with undipped and dipped control plants. Symptoms were recorded on plants with as few as two immatures present at the end of the study. In no case did symptoms occur on plants unless immatures were present.

Experiment 2. In all cases in which squash silverleaf symptoms were observed, immature whiteflies were found on plants. Two out of nine plants that were exposed to adults and subsequently dipped four times had immature whiteflies on exposed leaves and showed symptoms of silverleaf (Table 3). Plants that had no immatures present, however, showed no silverleaf symptoms. Control plants, dipped or undipped, had no symptoms of silverleaf, and no immatures. All plants on which immatures were allowed to develop showed significant silverleaf symptoms ($F = 6.01$, df = 3,29, $P < 0.001$; LSD = 2.46). Plants exposed to 25 males feeding for 72 h showed no silverleaf symptoms and had no immatures.

Relationship between immature density and symptom severity. Symptom severity was positively associated with number of immatures per plant (SYMPTOM = 1.37 + 0.064 IMMATURE, $R^2 = 0.68$, $P < 0.001$, df = 1,89) (Fig. 1).

TABLE 2. Mean number of *Bemisia tabaci* immatures and symptom severity of zucchini plants exposed to 20 adult whiteflies for 48 h with immatures allowed to develop or with immatures removed by four treatments of insecticidal soap

Treatment	Plants with symptoms (no.)	Immatures (no.)	Symptom severity[x]
Zucchini			
Adults + immatures	10/10	153.8 ± 15.6 a[y]	11.1 ± 1.0 a
Immatures removed	0/10	0 ± 0 b	0 ± 0 b
Controls	0/9[z]	0 ± 0 b	0 ± 0 b
'Dixie' Squash			
Adults + immatures	8/8	50.5 ± 5.2 a[y]	12.8 ± 1.9 a
Immatures removed	0/8	0 ± 0 b	0 ± 0 b
Controls	0/7	0 ± 0 b	0 ± 0 b

[x] Symptom severity was rated on each of the seven youngest leaves using a scale of 0–5 (with 0 meaning no symptoms, 5 the entire leaf surface silver) and totaled for a per plant rating.

[y] Means in columns followed by the same letter are not significantly different, analysis of variance followed by LSD test, $P = 0.05$.

[z] Only plants with the exposed leaf alive at the end of the study were used.

DISCUSSION

Our results show that immature, but not adult, *B. tabaci* induced silverleaf symptoms in zucchini. Continuous feeding of up to 20 adult *B. tabaci* per plant per day for 10 days did not induce symptoms of silverleaf. In contrast, as few as two immatures per plant feeding for about 14 days could induce silverleaf symptoms. Symptom severity increased as the number of immatures per plant increased. These results provide support for those previously reported by Hoelmer et al (10), Schuster et al (17),

TABLE 3. Mean number of *Bemisia tabaci* immatures and symptom severity of zucchini plants exposed to 20 adults for 10 days with immatures allowed to develop or with immatures removed by 4 treatments of insecticidal soap

Treatment	Plants with symptoms (no.)	Immatures (no.)	Symptom severity[w]
Adults + immatures	6/6[x]	221 ± 67 a[y]	12.8 ± 2.2 a
Immatures removed (dipped)	2/9[z]	9 ± 6 b	0.8 ± 0.7 b[x]
Controls, no dips	0/9	0 ± 0 b	0 ± 0 b
Control, dipped	0/9	0 ± 0 b	0 ± 0 b

[w] Symptom severity was rated on each of the seven youngest leaves using a scale of 0–5 (with 0 meaning no symptoms, 5 the entire leaf surface silver) and totaled for a per plant rating.

[x] Only plants with the exposed leaves alive at the end of the study were used.

[y] Means in columns followed by the same letter are not significantly different, analysis of variance followed by LSD test, $P = 0.05$.

[z] The 7/9 plants without symptoms had no immatures present.

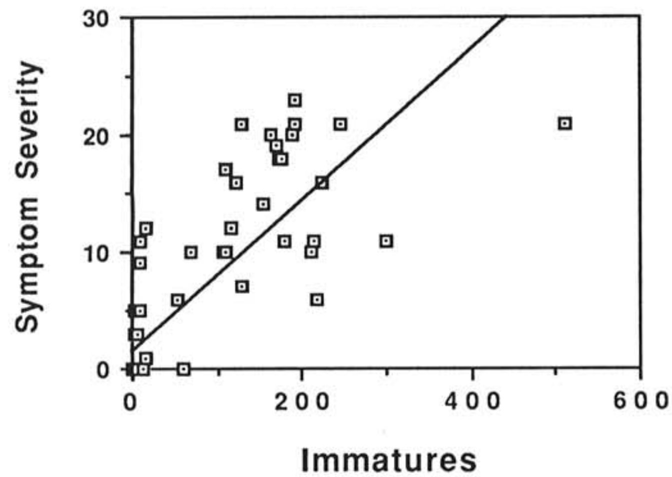

Fig 1. Relationship between the severity of silverleaf symptoms on zucchini and the number of immature *Bemisia tabaci* per plant at the time of rating. SYMPTOM = 1.37 + 0.064 IMMATURE, $R^2 = 0.68$, $P < 0.000$, df = 1,89. Symptom severity was rated on the seven youngest leaves using a scale of 0–5 (with 0 meaning no symptoms, 5 the entire leaf surface silver) and totaled over the entire plant.

and Yokomi et al (21). Indeed, our experiments provide a more stringent test of adult contribution because leaves exposed to whiteflies remained on the plant after adult feeding. In this study, the lack of silverleaf symptoms when plants were exposed to males alone adds further support for their results and eliminates the complication of removing offspring; however, differences between ability of male and female *B. tabaci* to transmit plant viruses have been reported (6), and such differences may be present in silverleaf disorder induction. Thus, conclusions regarding adult contribution to silverleaf induction using male feeding studies alone must be viewed with caution.

The inability of adult *B. tabaci* to induce silverleaf symptoms at the rate of 20 adults for 48 h on zucchini and 'Dixie' squash conflicts with findings of Bharathan et al (3), who found 100% induction of silverleaf in 'Dixie' squash at this rate of adult feeding. Although factors such as differences in environmental conditions (5) and biological differences between populations of whiteflies (7,16) could vary the levels of symptom severity induction, it is unlikely that these factors alone would result in the contrast between the findings of Bharathan et al (3) and those of three others (10,21, and ours). It is more likely that these differences resulted from contamination of plants by immature stages. This idea is supported by our results that show that mechanical removal, even combined with repeated insecticidal soap dips to remove immatures, was not 100% effective. Our findings strongly support the contention that plants must be inspected for the presence of immature contamination before conclusions about adult contribution to symptom induction can be made.

Targeting the appropriate insect stage is important in developing management programs. On the basis of our results with *B. tabaci* populations in Hawaii, controlling immatures or reducing oviposition by adults should be targeted to manage silverleaf rather than trying to drastically reduce the number of adults that may feed for relatively short times in crops. Control strategies such as row covers and barriers to prevent or delay adult entry, repellents to deter oviposition, and selection for crop plants that have characteristics unfavorable for immature development could provide suitable defense without attempting to completely eliminate low levels of adult feeding. Such an approach has the added benefit of reducing unnecessary pesticide applications to vegetable crops, thus enhancing the potential for biological control (11) and reducing the development of pesticide resistance (14).

LITERATURE CITED

1. Bethke, J. A., Paine, T. D., and Nuessly, G. 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. Ann. Entomol. Soc. Am. 84:407-411.
2. Bharathan, N., Graves, W. R., Narayanan, K. R., Schuster, D. J., Bryan, H. H., and McMillan, R. T., Jr. 1990. Association of double-stranded RNA with whitefly-mediated silvering in squash. Plant Pathol. 39:530-538.
3. Bharathan, N., Narayanan, K. R., and McMillan, R. T., Jr. 1992. Characteristics of sweetpotato whitefly-mediated silverleaf syndrome and associated double-stranded RNA in squash. Phytopathology 82:136-141.
4. Brown, J. K., and Costa, H. S. 1992. First report of whitefly-associated squash silverleaf disorder of *Cucurbita* in Arizona and of white streaking disorder of *Brassica* species in Arizona and California. Plant Dis. 76:426.
5. 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 yellows virus. Phytopathology 82:86-90.
6. Costa, A. S. 1976. Whitefly-transmitted plant diseases. Annu. Rev. Phytopathol. 14:429-449.
7. Costa, H. S., and Brown, J. K. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. Entomol. Exp. Appl. 61:211-219.
8. Costa, H. S., Johnson, M. W., Ullman, D. E., Omer, A. D., and Tabashnik, B. E. 1993. Sweetpotato whitefly (Homoptera: Aleyrodidae): Analysis of biotypes and distribution in Hawaii. Environ. Entomol. 22:16-20.
9. Costa, H. S., Ullman, D. E., Johnson, M. W., Tabashnik, B. E. 1993. Association between *Bemisia tabaci* density and reduced growth, yellowing and stem blanching of lettuce and kaichoy. Plant Dis. In Press.
10. Hoelmer, K. A., Osborne, L. S., and Yokomi, R. K. 1991. Foliage disorders in Florida associated with feeding by the sweetpotato whitefly, *Bemisia tabaci*. Fla. Entomol. 74:166-166.
11. Lynch, J. A., and Johnson, M. W. 1989. Mass rearing the greenhouse whitefly parasitoid *Encarsia formosa*, for augmentative releases in fresh market tomatoes in Hawaii. Pages 123-127 in: 1989 Agricultural Development in the American Pacific Crop Protection Conference Proceedings. M. W. Johnson, D. E. Ullman, and A. Vargo, eds. University of Hawaii Research Extension Series 134. Honolulu, HI.
12. Maynard, D. N., and Cantliffe, D. J. 1989. Squash silverleaf and tomato irregular ripening: New vegetable disorders in Florida. Fla. Coop. Ext. Ser., IFAS VC-37. 4 pp.
13. Minitab Reference Manual. 1991. Data Tech Industries. Valley Forge, PA.
14. Omer, A. D., Johnson, M. W., Tabashnik, B. E., Costa, H. S., and Ullman, D. E. 1993. Sweetpotato whitefly resistance to insecticides in Hawaii: Intra-island variation is related to insecticide use. Entomol. Exp. Appl. 67:173-182.
15. Osborne, L. S. 1988. The not so sweet sweetpotato whitefly. Florida Foliage 14:8-15.
16. Perring, T. M., Cooper, A., and Kazmer, D. 1992. Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera:Aleyrodidae) on broccoli by electrophoresis. J. Econ. Entomol. 85:1278-1284.
17. 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.
18. Schuster, D. J., Mueller, T. F., Kring, J. B., and Price, J. F. 1990. Relationship of the sweetpotato whitefly to a new tomato fruit disorder in Florida. HortScience 25:1618-1620.
19. Segarra-Carmona, A. E., Bird, J., and Escudero, E. 1990. Silvering of *Cucurbita moschata* (Duchesne) Poir associated with *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) in Puerto Rico. J. Agric. Univ. P. R. 74:477-478.
20. Sokal, R. R., and Rohlf, F. J. 1981. Biometry. Freeman & Company, New York. 859 pp.
21. Yokomi, R. K., Hoelmer, K. A., and Osborne, L. S.1990. Relationship between the sweetpotato whitefly and the squash silverleaf disorder. Phytopathology 80:895-900.