Association Between *Bemisia tabaci* Density and Reduced Growth, Yellowing, and Stem Blanching of Lettuce and Kai Choy

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ABSTRACT

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Colonization by high densities of sweetpotato whitefly, *Bemisia tabaci*, B-biotype, reduced growth of lettuce (*Lactuca sativa*) and kai choy (*Brassica campestris*). A 41% reduction in the weight of lettuce plants resulted when they were colonized by 200 adult whiteflies and their offspring for 3 wk. The weight of kai choy plants was reduced 14 and 21% when they were colonized by 100 and 200 adult whiteflies and their offspring, respectively, for 3 wk. In contrast, colonization by 10, 50, and 100 whiteflies and their nymphs per lettuce plant, or 10 and 50 whiteflies plus their nymphs per kai choy plant, did not reduce growth significantly. Exposure to 50 or more whiteflies plus nymphs caused leaf yellowing and distortion in lettuce, while exposure to 10 or more whiteflies plus nymphs caused stem and midvein blanching and leaf curling in kai choy. Weight loss increased as immature density increased on both plants. Exposure to 200 adults for 48 hr with no nymphs did not induce weight loss or symptom development in kai choy. If immature offspring were allowed to feed, however, symptoms developed in all cases. Removal of immatures from symptomatic plants with insecticide treatment resulted in nonsymptomatic new growth. The increase in symptoms with increasing numbers of whiteflies and the recovery of plants following whitefly removal support the hypothesis that these syndromes are phytotoxic disorders caused by a toxin or toxins and not by a pathogen. The importance of this information to growers and to sweetpotato whitefly management is discussed.

The importance of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), as a direct pest and as a vector of plant viruses (3,5,6,8,12,16) has increased worldwide in the past decade. Since 1987, in the United States, increases in field populations of *B. tabaci*, B-biotype (9), also referred to as the poinsettia strain (1,18) or the silverleaf whitefly (19), have been associated with a variety of phytotoxic disorders including stem whitening in *Brassica* spp. (4) and pumpkin (9), chlorosis and distortion of new growth of *Crossandra infundibularis* (13,17), squash silverleaf of *Cucurbita* spp. (2,7, 9,10,14,20,22,24), and irregular ripening of tomato (14,21). Although these disorders are associated with the B-biotype of *B. tabaci*, their etiology remains unknown. Symptoms increase in severity with increasing whiteflies, and removal of whiteflies after silverleaf development results in nonsymptomatic new growth, suggesting that this disorder is toxicogenic in nature (20,24).

In Hawaii, weight loss and symptoms of yellowing and stem blanching in lettuce (*Lactuca sativa* L.), several *Brassica* spp., and other green leafy vegetables have been observed following colonization of these crops by *B. tabaci*. Poor soil, nutrient deficiencies, bad seed, and whitefly feeding were considered as possible causes of such symptoms; however, the similarity of these symptoms to white streaking previously associated with *B. tabaci*-infested cabbage (4) suggested that these disorders were primarily associated with whitefly feeding.

The objectives of this study were to determine if 1) sweetpotato whitefly feeding induces weight loss and symptoms of yellowing and stem blanching in green leafy vegetables, 2) a relationship exits between the density of whiteflies and the severity of symptoms, 3) plants recover after removal of whiteflies, and 4) adult feeding contributes significantly to the induction of symptoms in lettuce and kai choy.

MATERIALS AND METHODS

Insect colony. *B. tabaci* adults used in these experiments were of mixed age and from a population originally collected from squash (*Cucurbita* sp.) at the University of Hawaii Agricultural Experiment Station at Poamoho on Oahu, in August 1991. The colony was 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 (9), and by its ability to induce symptoms of squash silverleaf in zucchini (10).

Plants. All tests were done using 2-wk-old lettuce (*Lactuca sativa* cv. Green Mignonette) and kai choy (green mustard cabbage, *Brassica campestris* L. = *B. rapa* L. cv. Waianae) in 10-cm pots, one plant per pot. A commercially prepared standard potting soil mixture was used with Osmocote controlled-release fertilizer added to the mixture at recommended rates.

Damage induced by adults and immatures. Adult whiteflies at densities of 0, 10, 50, or 100 per plant were allowed to colonize lettuce or kai choy plants. Clear plastic cups (0.5 L) with organdy-covered ventilation holes were inverted over the plants to cage the whiteflies. Five plants of each host were used for each density. Plants and caged whiteflies were maintained for 2 days at 28 C ± 3 C, 12:12 D:L, and then moved to outdoor cages (0.6 m^2) with plants of each host–density relationship in a separate cage (five plants per cage). Plastic cup cages were then removed to allow adult whiteflies free access to any of the enclosed five plants. Adults and offspring were allowed to develop undisturbed for 4 wk under ambient conditions (mean high and low temperatures ± standard deviation [SD] = 24 C ± 2 C and 17 C ± 1.7 C, respectively). Plants were cut at soil level and weighed to measure the wet weight of above-ground tissue. This procedure was repeated for lettuce with whitefly densities of 0, 50, and 100 adults per each of 10 plants for each density. To test the hypothesis that sweetpotato whitefly feeding induces weight loss, the mean weights of plants were compared by analysis of variance (Minitab statistical software) (15), followed by an LSD comparison of means (23).

Relationship between immature density and weight loss. This experiment was done to determine the density of immature whiteflies required to induce symptoms of weight loss in lettuce and kai choy. The procedure used was the same as above except that adult densities of 0, 100, and 200 whiteflies were used to colonize plants. Plants remained in the cages for 3 wk under ambient conditions

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(mean high and low temperatures for lettuce were 27 C ± 1.4 C and 18 C ± 0.9 C, and for kai choy were 28 C ± 1.5 C and 20 C ± 1.0 C, respectively), at which time they were harvested and weighed. The total number of immature whiteflies older than the crawler stage were counted on the entire plant at the time of harvest. To test if a relationship exists between number of immatures per plant and weight loss, a regression (15) of plant weight on the number of immatures per plant was performed. To test the hypothesis that sweetpotato whitefly feeding induces weight loss, mean weight of plants and the number of immatures were compared by analysis of variance (Minitab statistical software) (15), followed by an LSD comparison of means (23).

Plant recovery after whitefly removal. Kai choy plants were colonized by 0 (6 plants) or 100 (18 plants) adult whiteflies in cup cages as described above. After 2 days, all adults were removed with an aspirator; however, immatures were allowed to develop. Plants were moved into two outdoor cages as described in previous sections. Two weeks after initial whitefly exposure, the presence or absence of stem blanching and leaf mottling and curling symptoms were recorded by leaf on each plant. Plants with symptoms were divided into two groups. The first group (nine plants) was dipped in a solution of diazinon insecticide at the labeled rate, to kill all whiteflies. The remaining symptomatic plants (nine) were not dipped, and whitefly populations were allowed to continue feeding. Five days later, symptoms were again recorded by leaf on each plant in both groups.

Adult contribution to symptom induction on kai choy. To determine whether adult feeding contributed significantly to symptom induction in kai choy, two treatments were compared. In the first treatment, 200 adult whiteflies were allowed to feed on kai choy plants in small cages as described. After 2 days, adults were removed. To prevent feeding by immature whiteflies, plants were fully immersed in an insecticidal soap solution (M-pede, Mycogen Corp., 200 ml/L) 7 and 10 days following initial whitefly exposure. Plants not exposed to whiteflies were also dipped on days 7 and 10 to serve as a control for effects due to insecticidal soaps. Plants not exposed to whiteflies or insecticidal soaps were also included. In the second treatment, 200 adult whiteflies were allowed to feed on kai choy plants for 2 days. Adults were removed and immatures were allowed to develop. This procedure was performed twice, for a total of 20 replicates of each treatment. Plants were maintained in outdoor cages (1.2 m^2) under ambient conditions (mean high and low temperatures: 1st experiment, 26 C ± 1.2 C and 20 C ± 3.4 C; 2nd experiment, 27 C ± 1.9 C and 22 C ± 0.8 C, respectively). Four weeks after exposure, the mean weight of plants and the number of immature whiteflies present beyond the crawler stage on each plant were recorded. To test if adult feeding contributes significantly to weight loss and symptom development, comparisons were made between dipped and undipped plants using a two-way analysis of variance (15).

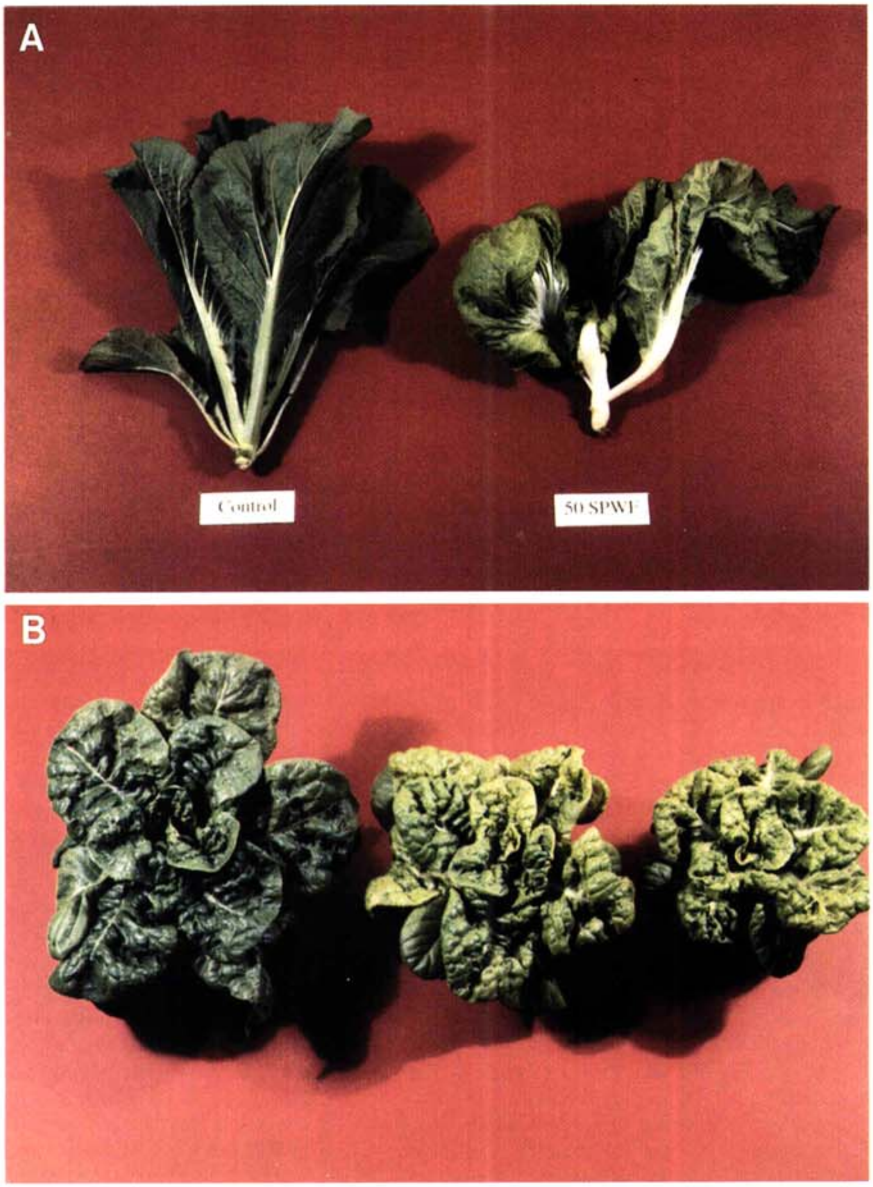

Fig. 1. Symptoms induced by colonization of *Bemisia tabaci:* **(A)** stem blanching and leaf curling in kai choy plants colonized by 0 (left) and 50 (right) whiteflies per plant; and **(B)** yellowing in lettuce colonized by, left to right, 0, 100, and 200 whiteflies per plant.

RESULTS

Damage induced by adults and immatures. Stem and midvein blanching occurred on all kai choy plants colonized by 10, 50, or 100 whiteflies and their nymphs (Fig. 1A). All lettuce plants colonized by 50 or 100 adult whiteflies and their nymphs showed yellowing (Fig 1B), but mild or no yellowing symptoms occurred in plants colonized by only 10 adults.

No significant weight loss occurred in kai choy plants colonized by 10 or 50 adults; however, kai choy plants colonized by 100 adult whiteflies had a mean reduction in plant weight of 62% (F = 16.55, df = 3,16, $P < 0.001$; LSD = 25.79) compared to the controls (Table 1). Lettuce plants colonized by up to 100 adult whiteflies had no significant weight loss compared to the controls (F = 1.22, df = 3,45, P = 0.315) (Table 1).

Relationship between immature density and weight loss. Weight loss increased as immature density increased for lettuce (weight = 66.2 − 0.02 immature; P = 0.024, R^2 = 0.17, df = 1,28) and kai choy (weight = 68.1 − 0.006 immature; $P < 0.001$, R^2 = 0.64, df = 1,13) (Fig. 2), indicating that the greater the number of immatures feeding, the more severe the damage. The presence of immatures on control plants (6 ± 4) was likely due to contamination by adults from adjacent colonized cages (Table 2). No symptoms appeared on plants with these low levels of contamination. All plants colonized by 100 or 200 adults and their immatures showed symptoms of yellowing in lettuce or stem blanching in kai choy. Lettuce plants colonized by 100 adults showed a mean of 631 immatures per plant and no significant reduction in weight. Lettuce colonized by 200 adults had a mean of 904 immatures and a 41% weight reduction ($F = 6.76$, df = 2,29, $P = 0.004$; LSD = 15.79) (Table 2). Weight of kai choy plants colonized by 100 adults, with a mean of 926 immatures per plant, was significantly reduced (14%) compared to controls; while those colonized by 200 adults, with a mean of 2,205 immatures, showed a significant reduction of 21% ($F = 7.52$, df = 2,12, $P = 0.008$; LSD = 8.58) (Table 2).

Plant recovery after whitefly removal. All plants exposed to adult whiteflies and their nymphs developed symptoms of stem blanching and leaf curling on new growth, while plants without whiteflies remained symptom free. Five days after the removal of immatures from symptomatic plants, however, eight out of nine plants recovered, with the youngest leaves being symptom free (Table 3). Symptoms did not disappear from leaves that were already symptomatic at the time of whitefly removal; however, in some cases symptoms of leaf curling diminished. One plant in this group continued to develop symptoms. It was noted that the diazinon treatment did not fully remove all whiteflies, and some immatures were still present on this plant at the time of the second symptom rating. In contrast, eight out of nine plants with immature populations of whiteflies continued to develop symptoms on new growth. One plant, on which the majority of immature whiteflies had pupated, showed some recovery on the youngest two leaves. Control plants not exposed to whiteflies did not develop symptoms of stem blanching and continued to produce symptomless growth throughout the course of the experiment.

Adult contribution to symptom induction on kai choy. Adult feeding did not contribute to symptom development. Plants exposed to 200 adults and dipped in insecticidal soap to kill immatures did not show significant weight loss compared to dipped controls ($F = 0.21$, df = 1,35, $P = 0.647$) (Table 4), nor did these plants show symptoms of stem blanching or leaf curling. In contrast, plants exposed to 200 adults for 48 hr and a mean of 436 nymphs (not dipped) showed 20% weight loss ($F = 5.32$, df = 1,34, $P = 0.027$) compared to undipped controls (Table 4). In addition, all plants exposed to immature whiteflies developed symptoms of stem blanching and leaf curling (Table 4), as in previous experiments. Insecticidal soap dips caused significant weight loss in plants ($F = 43.38$, df = 1, $P < 0.001$); thus, comparisons were made only between dipped or undipped plants.

DISCUSSION

Our results demonstrate that *B. tabaci* populations in Hawaii induce symptoms of weight loss, stem blanching, and yellowing in lettuce and kai choy. To our knowledge, this is the first report of weight loss in lettuce and kai choy due to whitefly feeding. We found a positive relationship between the number of im-

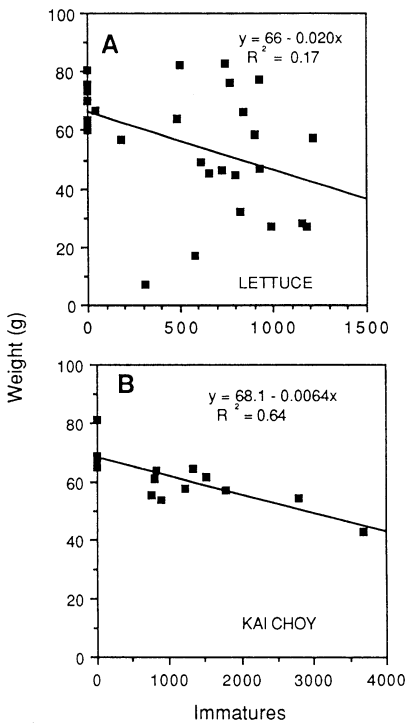

Fig. 2. Regression of plant weight on number of immature *Bemisia tabaci* feeding on each plant of **(A)** lettuce and **(B)** kai choy.

Table 1. Mean weights of kai choy and lettuce plants continuously colonized by various densities of adult whiteflies and their offspring for 4 wk

Density of adults	n[x]	Weight (g) $\bar{x} \pm$ SE	Symptoms[y]
Kai choy			
0	5	113 ± 8.5 a[z]	−
10	5	113 ± 5.2 a	+
50	5	112 ± 8.8 a	+
100	5	43 ± 10.9 b	+
Lettuce			
0	15	40 ± 5.8 a	−
10	5	60 ± 12.8 a	−/+
50	15	38 ± 5.7 a	+
100	15	29 ± 5.3 a	+

[x] Number of plants.
[y] Yellowing of lettuce or stem blanching of kai choy.
[z] Means in columns followed by the same letter are not significantly different, analysis of variance followed by LSD test, $P = 0.05$.

Table 2. Weights of kai choy and lettuce plants continuously colonized by various densities of adult whiteflies and their offspring for 3 wk

Density of adults	n[x]	Mean weight (g) ± SE	Mean ± SE No. immatures	Symptoms[y]
Kai choy				
0	5	70 ± 3 a[z]	0 ± 0 a	−
100	5	60 ± 2 b	926 ± 109 b	+
200	5	55 ± 3 b	2,205 ± 456 c	+
Lettuce				
0	10	69 ± 2 a	6 ± 4 a	−
100	10	58 ± 7 a	631 ± 66 b	+
200	10	41 ± 6 b	904 ± 84 c	+

[x] Number of plants.
[y] Yellowing of lettuce or stem blanching of kai choy.
[z] Means in columns followed by the same letter are not significantly different, analysis of variance followed by LSD test, $P = 0.05$.

Table 3. Number of kai choy plants showing symptoms of stem blanching, leaf mottling, and/or leaf curling; symptoms evaluated on the five youngest leaves of each plant 5 days after insecticide treatment to remove whiteflies

	Leaf				
Treatment	1[y]	2	3	4	5
No whiteflies	0/6	0/6	0/6	0/6	0/6
Whiteflies removed[z]	1/9	1/9	4/9	7/9	9/9
Whiteflies not removed	8/9	8/9	9/9	9/9	9/9

[y] 1 = Youngest leaf.
[z] Whiteflies were removed from symptomatic plants by dipping in an insecticide solution.

Table 4. Mean number of offspring produced, weight of kai choy plants, and stem blanching symptoms 3 wk after exposure to 200 adult *Bemisia tabaci* for 48 hr with immatures allowed to develop or with immatures removed by two treatments with insecticidal soap dips

Treatment		n[v]	Mean no. imm. ± SE	Mean weight (g) ± SE	Symptoms
WF[w]	Dips[x]	19	0 ± 0	53.1 ± 2.7 a[y]	−
No WF	Dips	19	0 ± 0	54.6 ± 2.0 a	−
WF	No dips	17	436 ± 64	67.6 ± 3.6 a[z]	+
No WF	No dips	20	0 ± 0	77.9 ± 3.0 b	−

[v] Number of plants.
[w] WF = exposed to adult whiteflies.
[x] Dips = insecticidal soap dip (M-pede, Mycogen Corp. 200 ml/L) to kill immatures 7 and 10 days after initial whitefly exposure.
[y] Comparison of WF-Dips to No WF-Dips: not significantly different using two-way analysis of variance, Minitab statistical software, $F = 0.21$, df = 1, $P = 0.647$.
[z] Comparison of WF-No dips, to No WF-No dips: significantly different, two-way analysis of variance, Minitab statistical software, $F = 5.32$, df = 1, $P = 0.027$.

mature whiteflies and weight loss per plant, while removal of immatures resulted in nonsymptomatic new growth. Both results are suggestive of a toxicogenic response, similar to that proposed for silverleaf of squash (24), rather than a pathogenic relationship.

Our results support previous findings that it is primarily the nymphal stage, and not the adult stage, of *B. tabaci* that induces toxicogenic responses (11,13,20, 24). Because of the phenology of the leafy vegetables we used in these experiments, it is impossible to continuously remove all eggs produced by exposure to adults without damaging plants or without some offspring escaping treatment. Hence, the long-term effects of adult feeding alone could not be tested on kai choy or lettuce. Nevertheless, the results of our experiments show that exposure of plants to 200 adults for 48 hr with no nymphs feeding (WF, Dips) did not result in symptom development or weight loss when compared to dipped controls (No WF, Dips). In contrast, exposure to 200 adults plus their nymphs (WF, No dips) resulted in symptom development and significant weight loss compared to undipped controls (No WF, No dips) (Table 4). These results support the hypothesis that symptom development in plants on which adults were removed after 48 hr was a result of immature feeding alone. Comparison of weight loss among all treatments could not be made due to the effects of insecticidal dipping on plant weight loss; however, comparison of stem blanching symptom development among treatments shows that only plants exposed to immature whiteflies developed symptoms of stem blanching.

These results are important in terms of management of the sweetpotato whitefly in leafy vegetable crops, such as kai choy and lettuce. Short-term feeding by adults does not appear to contribute significantly to symptom development in these crops; hence, control measures should be aimed at reducing oviposition and subsequent larval development. Because plant weight loss and other symptoms ultimately are dependent on immature density, a reduction in larval numbers can result in higher plant weight and symptomless plants. Thus, techniques to reduce immature populations, such as oviposition repellents and selection of plant varieties unfavorable for immature growth, can benefit growers and should receive increased attention as control strategies. In addition, these findings suggest that the use of biological control could be successful, because there is a threshold of whitefly numbers colonizing a plant below which plant weight loss will not be significant. Thus, these crops may tolerate the low levels of whiteflies required to maintain natural enemy activity. The presence of stem blanching and leaf mottling symptoms at whitefly densities below those that cause weight loss will complicate damage threshold estimates because consumer acceptance thresholds must also be considered.

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