Relationships Between the Sweetpotato Whitefly and the Squash Silverleaf Disorder

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ABSTRACT

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Squash silverleaf (SSL), a newly recognized disorder of many squash varieties in Florida, is of unknown etiology. Extracts from SSL-affected tissue did not react with antisera to several cucurbit and whitefly-transmitted viruses. Silverleaf was not graft or mechanically transmitted. Early SSL symptoms were induced in new foliar growth of squash (*Cucurbita pepo* 'Senator') after 3 days of feeding by nymphs of the sweetpotato whitefly (SPWF), *Bemisia tabaci* (average number = 25.4 nymphs per plant). The SPWF nymphs produced chlorotic spots on leaves at their feeding sites, which were clearly different from SSL symptoms. Silverleaf symptoms were always produced on foliage that developed after exposure to nymphs and increased in severity as long as the nymphs fed, indicating that the causal factor was translocated. New growth from SSL-affected plants was symptomless when nymphs were eliminated either by removing infested leaves or by insecticide treatment. Colony nymphs reared on five other host plant species all induced SSL symptoms on test squash plants. Adult SPWFs (up to 200 per plant), however, did not induce SSL symptoms even after 15 days of continuous feeding. Double-stranded RNA (dsRNA) bands of approximately 4.0 and 4.4 $\times$ 10^6 daltons were detected by polyacrylamide gel electrophoresis from nymph-infested leaves of SSL-affected plants; dsRNA was, however, not detected from symptomatic tissue free from SPWF contamination, or from cotyledon tissue or healthy controls. Identical size dsRNA bands were detected from both nymph and adult *B. tabaci*. The relationship between the SPWF and SSL suggested that a toxicogenic factor associated with nymphal feeding may be causing SSL. The role of the dsRNA in SSL etiology, if any, is not yet understood.

Additional keywords. insect-plant interactions, insect toxin, insect vectors, insect virus.

Squash silverleaf (SSL) has appeared sporadically in south Florida for the past few years (17). South Florida squash growers suffered substantial economic damage due to SSL in 1987–88. By spring 1989, SSL had become widespread throughout much of the state. Symptoms first appear as a lightening of the veins of new foliar growth. On subsequent new growth, this paleness intensifies and veins appear silver, particularly along the midrib and laterals. Finally, the interveinal area and the entire upper leaf surface become silver while the lower leaf surface remains normal (17,29). These symptoms are striking on individual plants and in entire fields (Fig. 1A, 1B, and 1C). Fruit color can be lighter than usual, and color breaks occur in dark-fruited varieties. Substantial economic losses in Florida have now been attributed to SSL due to poor fruit quality and yield reduction. These symptoms are distinct from those reported for genetic silvering of squash (4). Most cultivars of *Cucurbita pepo* L. are susceptible, as are some varieties of *C. moschata* (Duchesne) Duchesne ex Poiret and *C. maxima* Duchesne (17). Silverleaf symptoms have not been observed in other cucurbits such as cucumber, watermelon, and cantaloupe. The causal agent of SSL is unknown. While Florida vegetable growers were experiencing SSL, they also had severe outbreaks of the sweetpotato whitefly (SPWF), *Bemisia tabaci* (Gennadius). Every case of SSL that we have observed was associated with infestations of this whitefly (Yokomi, *unpublished data*).

The SPWF is an economically important pest in the tropics worldwide, primarily because it transmits a number of plant viral agents affecting a wide variety of crops (1,7,20). This whitefly has been reported in Florida on different host plants as early as 1894 (27), but it was not an economic problem until 1986, when many nurseries had SPWF outbreaks on ornamentals (24). The SPWF caused chlorotic spots on some ornamentals and induced distorted new growth. Osborne (23) suggested that this damage might be due to a toxicogenic factor produced by the whitefly. Conover et al (6) conducted a limited visual survey of whitefly-borne viruses near Homestead, FL, in September of 1981 and found a number of plant species, especially weeds, with symptoms typical of geminiviruses. The SPWF was closely associated with all diseased plants in their survey. The occurrence of bean golden mosaic in Florida has been confirmed by Hiebert et al (10).

Bharathan et al (2) found evidence of dsRNAs associated with SPFW-caged Dixie squash symptomatic for SSL in Homestead, FL, which suggested a viral etiology. A preliminary report by Yokomi et al (30) presented evidence that SSL symptom induction was caused by direct feeding of the SPWF, and they suggested that an insect toxin may be involved. The present report presents further evidence that SSL was caused by a translocatable factor produced in the plant by feeding of the SPWF, which did not persist in the plant in the absence of the whitefly. The dsRNAs were found to be directly associated with the insect and not the SSL-affected plant.

MATERIALS AND METHODS

Silverleaf. The SSL observed in this study was from plants that became naturally affected at the University of Florida, Central Florida Research and Education Center (CFREC), Apopka, and the U.S. Horticultural Research Laboratory (USHRL), Orlando, FL. Silverleaf symptoms in our experimental plants, pumpkin and squash (*C. pepo* L. 'Small Sugar' from D. V. Burrell Seed Growers Co., Rocky Ford, CO, and 'Senator' from Asgrow Seed Co., Kalamazoo, MI), appeared identical to those previously described (17,29).

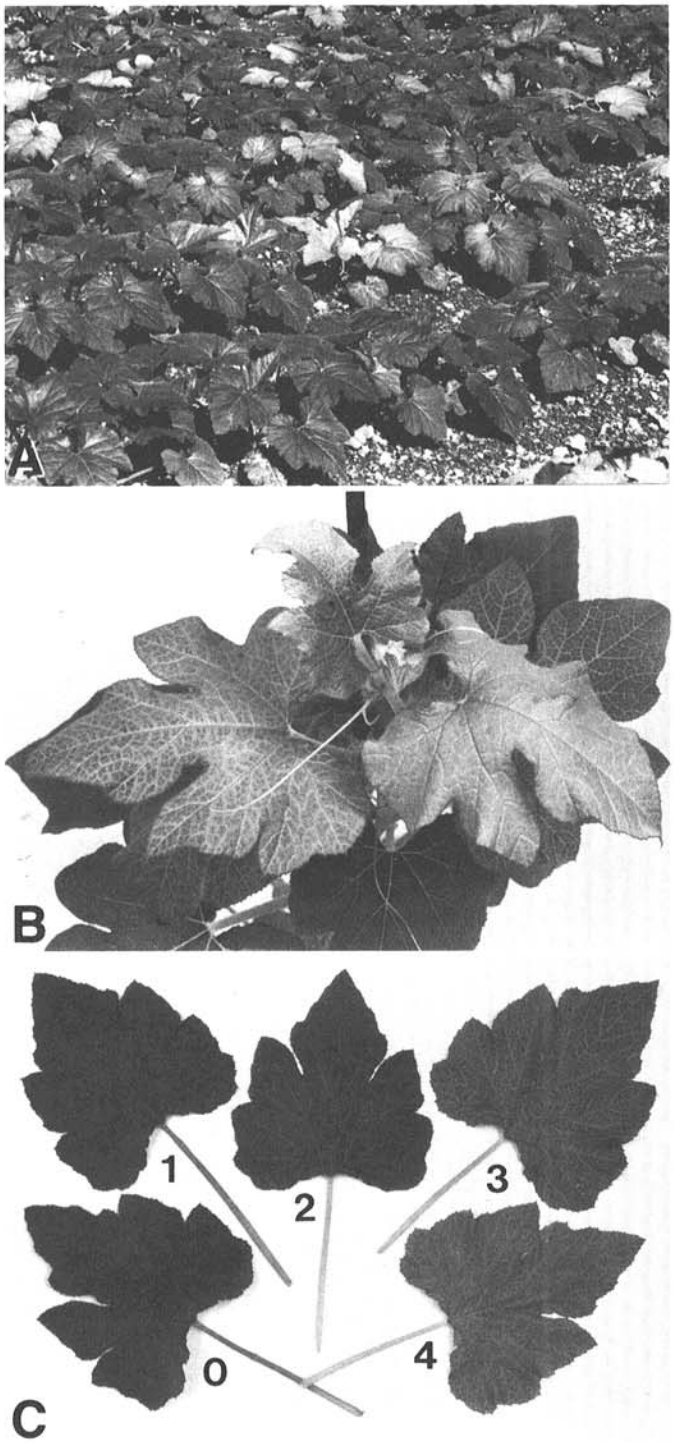

Fig. 1. Photographs of squash silverleaf. **A,** Dixie squash field showing silverleaf-affected plants. **B,** Pumpkin plant with silverleaf symptoms on new growth. *Bemisia tabaci* nymphs are on lower foliage which is non-symptomatic. **C,** Squash leaves showing the silverleaf rating scheme which ranged from 0 (healthy) to 5 (completely silver) (5 not shown). (Photograph 1A courtesy of G. W. Simone, University of Florida, Gainesville.)

Insect colony. *Bemisia tabaci* was obtained from local populations of SPWF that were infesting greenhouse-grown plants at the CFREC in 1986. The whiteflies were maintained on several host plants including *Hibiscus rosa-sinensis* L., poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch), *Crossandra infundibuliformis* (L.), lima bean (*Phaseolus limensis* Macfady 'Henderson' from Asgrow Seed Co.), and pumpkin. The ornamental plants were obtained from commercial nurseries in central Florida. The whiteflies at the CFREC were reared in an air-conditioned insectary with a 15-hr photoperiod at 28 C, whereas the insects at the USHRL were maintained in an evaporatively cooled greenhouse where temperatures sometimes reached 40 C inside the insect cage.

Serology. Leaves from greenhouse-grown pumpkin and squash that developed SSL symptoms were ground by mortar and pestle and diluted 1:10 and 1:20 in 0.1 M Tris buffer, pH 8.0. The crude extract was loaded into polystyrene microtiter plates (Immulon 2, Dynatech Laboratories, Inc., Alexandria, VA) for plate-trapped, indirect enzyme-linked immunoassay (15). Standard procedures were followed which included washing with phosphate-buffered saline with 0.5% Tween 20, pH 7.4, incubating at 37 C with the unlabeled virus-specific antibody, followed by incubation with goat anti-rabbit or goat anti-mouse antiserum conjugated with alkaline phosphatase, addition of *p*-nitrophenyl phosphate (0.6 mg/ml), and reading the reaction at OD_{405}. Polyclonal antisera to the following viruses were used: zucchini yellow mosaic virus (ZYMV) (1133-1); watermelon strain of papaya ringspot virus (1125); watermelon mosaic virus 2 (1132); tomato spotted wilt virus; lettuce infectious yellows virus; and squash leaf curl virus. A broad-spectrum monoclonal antibody to potyviruses (12) was also used. Homologous antigens to the antisera were included in these tests to verify test conditions.

Transmission. Leaves separated into different ages (i.e., young or mature) from SSL-affected plants were ground in buffer (50 mM borate, 10 mM ethylenediaminetetraacetic acid [EDTA], pH 8.0) using a chilled mortar and pestle. The extract (1:9 [part tissue/parts buffer]) was rubbed onto Carborundum-dusted pumpkin cotyledons. Test samples included SSL alone, ZYMV alone, a source plant infected with ZYMV (FC 1119) which was also showing SSL symptoms, and a healthy control which had not been exposed to whiteflies. Forty replications were used for the SSL-alone treatment, whereas 15 replications were used in all other treatments.

Apical growth from four SSL-affected and four healthy pumpkins was grafted into the stem at the cotyledon of healthy pumpkin plants in an attempt to graft transmit SSL. SSL regeneration experiments were conducted by making rootings from the terminal 10 cm of growth from the plant apex from 11 SSL-affected and 10 healthy pumpkin plants. The rootings were irrigated by a modified Hoagland's solution (19).

Silverleaf bioassays. Pumpkin and squash were used for the SSL bioassays. Seeds were planted in pasteurized soil mix (Vergro Container Mix A from Verlite Co., Tampa, FL), supplemented with Micromax and Osmocote 19-6-12 (both from Sierra Chemical, Milpitas, CA), and dolomite, and grown in a greenhouse under scheduled pesticide application. In Test 1, seedlings were transferred to a 0.72 m^3 cage covered with 70-mesh nylon chiffon (Collapsible Field Cage, BioQuip Products, Gardena, CA), and adult whiteflies were introduced by aspiration. The adults were allowed to oviposit and give rise to nymphs. Treatments included 40, 100, 200, and approximately 1,000 adults per plant. The first three treatments had six plants per cage, whereas the last treatment had 12 plants per cage. In Test 2, adult whiteflies were restricted to one leaf by enclosing an expanding leaf (8–9 cm wide) with the insects inside a cage (12 cm diam). The cage was made from sections of 1 cm (o.d.) Tygon tubing joined into a circle to make a rim on which organdy was glued by rubber cement. A hole was cut in the rim to allow the leaf petiole to exit the cage. After 5 days, the leaf with the whiteflies was removed before nymphs could hatch, and another leaf closer to the plant apex was exposed to new whitefly adults in another leaf cage. This was repeated three times in succession. Treatments included 0,

40, 100, and 200 adults per plant and three replications per treatment. In Test 3, immature SPWF were transferred to 24 test plants on 3.5-mm bean leaf discs with 10–30 mature eggs and/or first instar crawlers. The discs were glued with water-thinned white glue to leaf one, a one-half to three-fourths expanded leaf, at the growing point of the test plant. New growth of all test plants was examined daily for symptoms of SSL. Recovery of SSL-affected plants was examined in Test 4 by either removing leaves with SPWF infestation or insecticide treatment. Each treatment in Test 4 had six replications. Efficacy of pesticide application for SPWF and SSL control was determined in Test 5 on uncaged squash in a SPWF-infested greenhouse. Insecticides (bifenthrin 10 WP [2.41 and 4.78 g a.i./L] mixed with acephate 75S [0.79 g a.i./L]) were applied either two or four times to runoff at 7-day intervals. Each treatment had 10 plants.

Whiteflies reared on poinsettia, pumpkin, crossandra, and hibiscus were also used to determine their capacity to induce SSL symptoms.

Temperatures ranged from 21 to 35 C in the test greenhouse, and no supplemental lighting was provided. Silverleaf symptom expression was rated using a six-interval scale: 0, asymptomatic; 1, secondary veins pale on a sector or entire young leaf; 2, veins pale and appear "netted"; 3, primary veins silver; 4, silvering extends between the veins and the leaf appears "etched"; and 5, leaf entirely silver on the upper surface (Fig. 1C).

DsRNA analysis. Pumpkin seedlings at the four true-leaf stage were exposed to approximately 20 SPWF adults per seedling in a cage (0.6 × 0.6 × 0.9 m) in the greenhouse. The adults were allowed to feed and oviposit freely on the plants. Eggs began to hatch after 5 days and gave rise to a large population of nymphs. Control plants were kept SPWF-free in identical cages but in an adjacent greenhouse with the same growing conditions and temperatures as those used for the SPWF-infested plants. Plant tissue was harvested daily from day 5. The tissue was partitioned into three groups: 1) younger tissue at the plant apex with no adults or nymphs; 2) older tissue which was infested with nymphs and had chlorotic feeding spots induced by the nymphs; and 3) cotyledons. Tissue (4–5 g fresh weight), frozen in liquid nitrogen, was triturated by mortar and pestle, and the nucleic acids were extracted and isolated by cellulose chromatography (13). Purified nucleic acids were resuspended in STE buffer (0.1 M NaCl, 50 mM Tris, 1 mM EDTA, pH 6.8) and analyzed by electrophoresis on 6% polyacrylamide gels (Mini-Protean II, Bio-Rad Laboratories, Richmond, CA) for 4 hr at 100 V per gel in TAE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.8). Gels were stained with 10 ng/ml ethidium bromide in electrophoresis buffer, destained with several changes of water over 10 min, viewed with a UV light transilluminator (302 nm), and photographed through an orange filter on Polaroid Type 55 film (Polaroid Corp., Cambridge, MA). Double-stranded RNA extraction and analysis were also conducted with nymphs and adults from the insectary. Approximately 50 mg of fresh weight of insects were ground in a glass homogenizer with the extraction buffer (2× STE, 0.5% 2-mercaptoethanol, 1% PVP-10, 0.4 mg/ml bentonite, 1.5% SDS, pH 8) (13); the isolation of dsRNA was the same as that for plant tissue. Confirmation of dsRNA was made by treating the extracted nucleic acids with 2 μg/ml DNase (DNase I, Sigma Chemical Co., St. Louis, MO) in TKM buffer (10 mM Tris, 10 mM KCl, 1 mM MgCl, pH 7.5) and 1 μg/ml RNase (RNase A, Sigma Chemical Co.). RNase treatment was conducted in both high salt (0.3 M NaCl) conditions and water on the dsRNAs in the gel (9). Molecular weight standards used for size estimation of the dsRNA included citrus tristeza virus (CTV), tobacco mosaic virus (TMV), and cucumber mosaic virus (CMV). Both TMV and CMV were provided by D. E. Purcifull, University of Florida, Gainesville.

TABLE 1. Effect of exposure of squash (*Cucurbita pepo* 'Senator') to the sweetpotato whitefly (SPWF), *Bemisia tabaci*, on the development of squash silverleaf symptoms

Plants per cage (no.)	Adults per cage (no.)	First symptom appearance (days)
Test 1 (with nymphs)[a]		
6	40	9–11
6	100	9–11
6	200	9–11
12	1,000	9–11
Test 2 (without nymphs)[b]		
3	40	none[c]
3	100	none
3	200	none
3	0	none

[a] Adults allowed to oviposit freely on test plant. Eggs hatched in 5–6 days.

[b] Adults restricted by leaf cage to one leaf for feeding and oviposition. SPWFs and eggs were removed from plant before nymph hatch on day 5 and a new batch of SPWF adults were caged on another leaf on the same test plant. This was done three times in succession.

[c] No silverleaf symptoms developed after 15 days of continuous adult exposure. Final inspection for symptoms made on day 20.

RESULTS

Serology. Extracts from SSL-affected plants did not react at either dilution tested with any of the antisera used in the enzyme-linked immunosorbent assay tests. Homologous viral antigens included in the assays were all positive and verified test conditions. No reactions with heterologous antigens were observed with the antisera used.

Transmission. No mechanical transmission of SSL was obtained with any of the treatments including leaf age. ZYMV was transmitted to all 15 plants inoculated from the ZYMV-alone treatment, indicating that test conditions were appropriate for many mechanically transmissible plant viruses. There was a decrease in ZYMV transmission efficiency (7 of 15 plants infected) when the SSL-ZYMV dually infected plant was the donor inoculum. No transmissions were obtained from healthy controls.

Three of four SSL grafts were established. Silverleaf was not transmitted to any of the propagations. In addition, SSL was not regenerated in rooted propagations made from SSL-affected stock. New plant growth from both the SSL-grafted and the SSL-regenerated propagations along with the healthy controls was normal in appearance.

Silverleaf bioassays. In Test 1, SPWF nymphs began to emerge on day 5, and SSL symptoms developed after day 9 on new foliage (Table 1). The upper surface of leaves developed chlorotic spots where mature nymphs were attached. These leaves, however,

TABLE 2. Squash silverleaf symptom development on new leaves of squash (*Cucurbita pepo* 'Senator') following exposure to sweetpotato whitefly (SPWF) *Bemisia tabaci* nymphs

Leaf no.[b]	Exposure to nymphs[a] (days)								Plants with symptoms (%)
	2	3	4	5	6	7	8	9	
1	0	0	0	0	0	0	0	0	0
2	···	0	1.2	1.9	1.7	1.9	2.0	2.0	13
3	···	1.0	1.7	2.2	2.2	2.2	2.4	2.4	75
4	···	···	···	1.5	2.3	2.5	2.7	2.8	96
5	···	···	···	···	2.0	2.3	2.5	3.5	100

[a] Average silverleaf symptoms rated each day per leaf as follows: 0, asymptomatic; 1, secondary veins pale on a sector or entire young leaf; 2, veins pale and appearing "netted"; 3, primary veins silver; 4, silvering extends between the veins and the leaf appears "etched"; 5, leaf entirely silver on the upper surface. No rating because leaf had not formed indicated by ···. Mean number of nymphs per plant = 25.4 (range 14–37). There were 24 replications per leaf.

[b] Leaf number 1 was an expanding leaf near the plant apex upon which the nymphs were placed; leaves 2 through 5 were new leaves that developed during insect exposure and were closer to the apex but were kept SPWF-free.

did not develop SSL symptoms. All plants exposed to whiteflies developed SSL symptoms by day 14. Symptom severity in subsequent new growth increased through day 17. Symptom expression and chronology were identical when squash plants were exposed to the whitefly at the first or eighth true leaf stage and when the test whiteflies were reared on poinsettia, bean, pumpkin, hibiscus, or crossandra (data not shown). In Test 2, where plants were exposed to adults alone for 15 continuous days, no symptoms developed on the test plants (Table 1). These results indicated that nymphal feeding by *B. tabaci* was associated with SSL and that feeding by adults alone did not induce SSL symptoms.

In Test 3, nymphs that developed from eggs settled and fed on the same leaf (leaf one) upon which the leaf disc was attached. There was an average of 25.4 nymphs per plant in this test. The infested leaf developed chlorotic spots where older nymphs were feeding as in Test 1. Silverleaf symptoms began to appear on the new foliar growth after 3 days of nymphal exposure (Table 2). Symptom severity increased with time in each new leaf that developed within 9 days of nymphal exposure indicating that the SSL factor was translocatable (Fig. 1B). In three cases, symptoms were induced by only three nymphs. Control plants without whitefly nymphs remained symptomless.

In Test 4, SSL began to appear on day 4 on new growth (Fig. 2), and symptom severity increased through day 10 of nymphal exposure. When nymphs were removed on day 10, no SSL symptoms were seen on new foliage formed after removal. The plants were kept nymphfree, and they remained symptomless throughout the remainder of the experiment. When nymphs were allowed to complete their life cycle and emerge as adults, there was a sharp decline in symptoms on new growth (Fig. 2). As the maturing nymphs reached the nonfeeding "prepupal" stage on days 11–17, symptoms continued to decline until day 20, when all new growth was asymptomatic. From days 11–20, the F_1 adults oviposited; and after day 21, the F_2 generation began to emerge. Silverleaf reappeared on new growth from day 24 and continued to increase until the end of the experiment on day 30. This was additional evidence that only nymphs induced SSL symptoms. When nymphs were absent, no new SSL symptoms were observed.

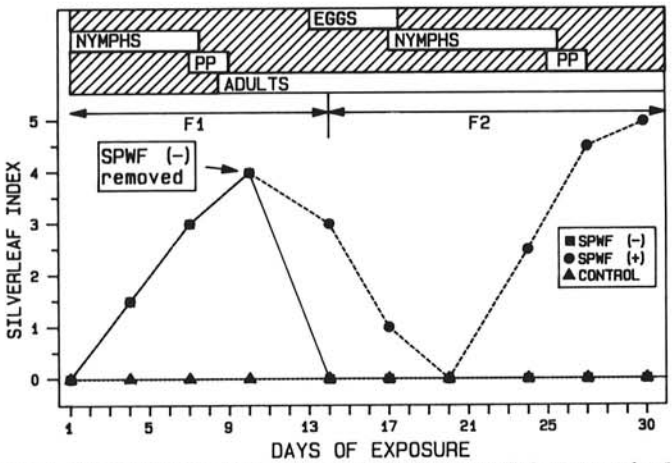

Fig. 2. Effect of removal of sweetpotato whitefly (SPWF) (*Bemisia tabaci*) nymphs from squash silverleaf (SSL)-affected squash (*Cucurbita pepo* 'Senator'). Each treatment had six replications. Silverleaf symptoms were rated daily on new foliar growth as follows: 0, asymptomatic; 1, secondary veins pale on a sector or entire young leaf; 2, veins pale and appearing "netted"; 3, primary veins silver; 4, silvering extends between the veins and the leaf appears "etched"; 5, leaf entirely silver on the upper surface. Life stages of SPWF during the experiment are depicted as bars above the SSL index. The F_1 is the insects that developed from mature eggs and first instar crawlers that were introduced on the test plant. The F_2 is the generation that developed from mature F_1 insects. PP = "prepupal" or mid-late fourth instar stage which is nonfeeding; □ nymphs (-) = nymphs removed; ○ nymphs (+) = nymphs allowed to remain on plant, emerged as adults, and gave rise to the F_2 generation; △ control = no adult or nymphal SPWF.

Similar "recovery" was observed by chemical control of the SPWF on SSL-affected plants.

In Test 5, chemical control of the SPWF prevented the plant from developing SSL symptoms (Table 3). Although SPWF adults were continuously present in the greenhouse, the insecticides had enough residual activity to control the nymphal stage. The few nymphs that were observed in this test were early instars which did not survive to the late instar stage.

Detection of dsRNA. Double-stranded RNAs were detected in SSL-affected pumpkins from the asymptomatic foliage which was contaminated with nymphs (Fig. 3A, lane 4), whereas no dsRNA was detected from symptomatic tissue which was free of SPWF nymphs (Fig. 3A, lane 3) or from healthy control pumpkin leaves (Fig. 3A, lanes 1 and 2). The dsRNAs consisted of two bands estimated to be 4.0 and 4.4 $\times$ 10^6 daltons in size based on comparisons with the dsRNAs from CTV (Fig. 3B, lane 4), TMV (Fig. 3B, lane 3), and CMV (Fig. 3B, lane 5). Extractions from the symptomatic tissue contained some SPWF eggs, but extreme care was taken to ensure that the sample did not contain nymphs. The detectable amount of dsRNA in extracts from the nymph-infested leaves began in approximately 4 days and increased through day 10. During this time, the nymphs increased in size, but the infested leaves remained without symptoms of SSL. Nymphs were not present on cotyledons except when no other host tissue was available. No dsRNAs were observed from cotyledons of infested plants. New growth of SSL-affected plants which was disinfested and kept SPWF-free was normal in appearance and did not contain detectable levels of dsRNAs. Similarly, new growth and roots from the SSL-regenerated plants was healthy and did not contain dsRNAs. A dsRNA pattern identical with that from nymph-infested tissue was detected in extracts from both the nymph and the adult SPWF (Fig. 3B, lanes 1 and 2). The amount of the dsRNAs was greater from nymphs than from adults.

DISCUSSION

The results presented in this study indicated that the SSL symptoms were induced by SPWF nymphal feeding activity. Silverleaf symptoms developed on new foliar growth after 3 days of feeding by as few as three nymphs per plant. All test plants infested with nymphs developed SSL symptoms, and symptom severity increased with time as long as nymphs were present. Chlorotic spots developed where larger nymphs were feeding, but this tissue did not develop SSL symptoms. These data suggested that the factor responsible for inducing SSL was potent and was translocated to the growing point where new foliage was symptomatic. Adult SPWFs were not able to induce SSL symptoms even with 15 days of continuous feeding. When nymphs were removed from SSL-affected plants, the disorder did not persist and the plant "recovered," with new growth appearing healthy. This suggested that a factor produced by the whitefly nymph was responsible for the induction of SSL symptoms. We have

TABLE 3. Effect of chemical control of the sweetpotato whitefly (SPWF) (*Bemisia tabaci*) on squash silverleaf (SSL) expression in squash (*Cucurbita pepo* 'Senator')

Treatment	Bifenthrin (g a.i./L)	Acephate (g a.i./L)	Applications[a] (no.)	SPWF(SD) (Av. no.) Adults	SPWF(SD) (Av. no.) Nymphs	SSL rating[b]
A	2.41	0.79	4	1.1(1.1)	3.9(5.7)[c]	0
B	4.78	0.79	2	1.7(1.4)	0.6(1.3)[c]	0
Control	...	...	...	10.0(4.1)	93.5(40.8)	4.9

[a] Chemicals were applied at 7-day intervals. Final whitefly counts and SSL ratings were taken on day 30. There were 10 plants per treatment.

[b] Silverleaf symptoms rated as follows: 0, asymptomatic; 1, secondary veins pale on a sector or entire young leaf; 2, veins pale and appearing "netted"; 3, primary veins silver; 4, silvering extends between the veins and the leaf appears "etched"; 5, leaf entirely silver on the upper surface.

[c] Early instars.

recently reported a similar recovery of SPWF-infested crossandra with distorted foliar growth by effective insecticidal control (11).

Extracts from SSL-affected tissue did not react with any of the viral antisera tested. Silverleaf was not graft or mechanically perpetuated, nor was it perpetuated in rooted propagations made from SSL-affected tissue. These data virtually eliminate the possibility that a whitefly-transmitted plant pathogenic agent is involved in SSL etiology.

Studies of dsRNAs from plants suspected of having a viral etiology have been based on the observation that high molecular weight dsRNAs can be detected in plants infected with RNA plant viruses (13,25). Double-stranded RNA patterns have been used to support the hypothesis of a viral etiology in the absence of other data to describe a latent or cryptic virus in avocado (14), a latent viruslike agent in the cassava clone Secundina (8), carnation cryptic virus (16), and lettuce big vein (18). Bharathan et al (2) have reported that dsRNA bands of approximately 4.2 and 4.6 kb were associated with Dixie squash with symptoms of SSL in Homestead, FL, and suggested that SSL had a viral etiology. In our study, similar dsRNAs were consistently detected from SSL-affected plants only when the extracted tissue was contaminated with SPWF nymphs; whereas plant tissue not infested with whiteflies did not contain dsRNAs even though the tissue extracted had SSL symptoms. These dsRNAs had an identical "doublet" pattern as those in the Homestead study, but our size estimates of 4.0 and 4.4 $\times$ 10^6 daltons were larger than those reported by Bharathan et al (2). Since we were unable to associate the dsRNAs with SSL-symptomatic tissue, we concluded that the dsRNAs were not directly related to the induction of SSL in our tests. Double-stranded RNAs detected from both nymph and adult SPWFs were identical to those detected in extracts from SPWF-contaminated leaves. This suggested that the dsRNAs observed were of insect rather than plant origin. In addition, we have found the same dsRNAs in SPWF-infested tomatoes, beans, tobacco, and poinsettias (Yokomi, *unpublished data*). These colony hosts, while infested with whitefly nymphs, were otherwise normal in appearance. Whiteflyfree control plants did not contain dsRNA.

Double-stranded RNAs have been found in other insect vectors of plant viruses, such as the aphid *Rhopalosiphum padi* (L.) infected with the *Rhopalosiphum padi* virus (RhPV) (9) and the leafhopper *Cicadulina bimaculata* (Evans) infected with leafhopper A viruslike particle (LAV) and leafhopper C viruslike particle (LCV) (3,26). LAV and LCV were found to be associated with maize wallaby ear disease (MWED) (3). Maize plants developed MWED symptoms only when the plant was colonized by leafhoppers infected with viruslike particles. When these leafhoppers were removed from MWED-affected plants, new foliar growth was normal. The virus replicated in the leafhopper but not in the plant (3,22). Ofori and Francki (21), however, reported that one of the viruses, LAV, may not be involved in MWED etiology. Boccardo et al (3) and Ofori and Francki (21) concluded that MWED was induced by a toxin produced by the leafhopper. The RhPV also replicated in the insect rather than in the plant (9). The dsRNAs detected in *B. tabaci* in our study suggest that a RNA virus is replicating in the insect and a mechanism similar to that in MWED may be involved in SSL symptom induction. Experiments are underway in our laboratory to compare Florida SPWFs with a *B. tabaci* colony from California which does not induce SSL (L. S. Osborne and K. A. Hoelmer; and D. J. Schuster, *unpublished data*) to further determine the role of the whitefly dsRNAs and a putative insect virus with SSL etiology.

Our data suggested that SSL symptoms resulted from SPWF feeding activity. A translocatable toxicogenic product or a plant material is proposed as the factor responsible for symptom induction. This mechanism has been proposed for virulent biotypes of the greenbug, *Schizaphis graminum* (Rondani) (5,28). Campbell and Dreyer (5) speculated that a translocatable oligomeric pectic fragment released by the activity of the aphid's salivary enzymes on host-plant cell wall may be responsible for some of the toxicogenic effects of *S. graminum*.

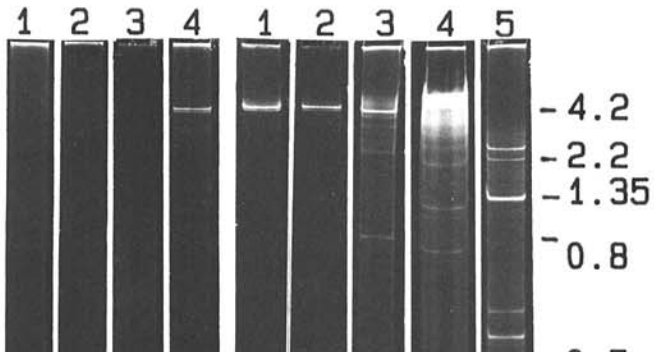

Fig. 3. Ethidium bromide-stained 6% polyacrylamide gels of dsRNAs. **A,** Lane 1, newer foliage from healthy pumpkin (*Cucurbita pepo* 'Small Sugar'); lane 2, older foliage from healthy pumpkin; lane 3, newer pumpkin foliage with squash silverleaf (SSL) symptoms which was free of *Bemisia tabaci*; lane 4, older pumpkin foliage contaminated by *B. tabaci* nymphs. **B,** Lane 1, *B. tabaci* adults; lane 2, *B. tabaci* nymphs; lane 3, tobacco mosaic virus (V-363) in tobacco (*Nicotiana tabacum*); lane 4, citrus tristeza virus (field isolate) in Temple orange (*Citrus sinensis* Osbeck hybrid); lane 5, cucumber mosaic virus (FC 2147) in pumpkin. Electrophoresis was conducted at 100 V per gel for 4 hr. Molecular weight markers are expressed in 10^6 daltons.

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