Squash Leaf Curl, a New Disease of Cucurbits in California

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ABSTRACT

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Symptoms of squash leaf curl, a new disease of cucurbits in California, include leaf curl, enations, thickening of veins, stunting, and high plant mortality. The causal agent is transmitted by the whitefly *Bemisia tabaci.*

Banana squash (*Cucurbita maxima*) and other cucurbits in southeastern California were severely damaged in the summer and fall of 1977 and 1978 by a previously unrecognized disease for which we propose the name squash leaf curl (SLC). In the Imperial Valley, the disease caused high mortality and drastically reduced yield.

Virus diseases of cucurbits in the Imperial Valley have been surveyed (7,9), and beet curly top virus has been shown to infect cucurbits in the valley (6). Whitefly-transmitted diseases of cucurbits have been reported in Israel and India (2,11) but not in the United States. However, a whitefly-transmitted disease of cotton, leaf crumple, has been found in Imperial County (3).

We report the results of surveys to determine the distribution and severity of SLC, laboratory studies to distinguish SLC from other cucurbit diseases, and studies to determine possible vectors.

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MATERIALS AND METHODS

Cucurbit plantings in Imperial and Riverside counties were inspected in 1977, 1978, and 1979 to determine the presence and distribution of SLC. Most of the plantings in Imperial County were inspected and sampled throughout the growing season. Insects in the diseased plantings were collected with sweep nets or a D-Vac Suction Sampler (4).

Leaves of diseased plants were triturated in 0.01 M Na-K PO_4 buffer, pH 7.2, and the resultant sap was used to mechanically inoculate plants including *Brassica oleracea* var. *botrytis, Capsicum frutescens* 'Yolo Wonder,' *Chenopodium amaranticolor, C. quinoa, Cucumis sativus* 'National Pickling,' *Datura stramonium, Gomphrena globosa, Hordeum vulgare, Nicotiana tabacum* 'Samsun NN' and 'Turkish,' *N. glutinosa, N. tabacum* × *N. glutinosa, Phaseolus vulgaris* 'Great Northern,' *Triticum aestivum, Vigna unguiculata,* and *Zea mays.*

In addition, several cucurbit hosts were inoculated including pumpkin, acorn and zucchini squash (*Cucurbita pepo*), watermelon (*Citrullus vulgaris*), and cantaloupe (*Cucumis melo*).

Inoculations were made by rubbing Carborundum-dusted leaves with cheesecloth saturated with sap. Healthy sap was used as a control. In addition to mechanical transmission tests, tissue from SLC-infected banana squash was stem-grafted to healthy banana squash seedlings.

Diseased plants were routinely tested for the presence of squash mosaic virus (SMV) and cucumber mosaic virus (CMV), by using antisera produced in rabbits inoculated with partially purified CMV (type strain, ATCC PV 29) or SMV (a common California strain). The procedure was the Ouchterlony double diffusion test (1).

Leaf dip preparations were made by touching freshly cut surfaces of diseased cucurbit leaves to droplets of 2% phosphotungstic acid, pH 6.8, on Formvar-carbon-coated copper grids. These and similar preparations from healthy cucurbit tissue were examined with a Zeiss EM 9 S-2 electron microscope.

Insects were placed on test plants grown under incandescent and fluorescent lights and kept in screen and wood cabinets or in a greenhouse. Cloth and glass covered cages were placed over pots containing three or four plants. Because symptoms in the field were most obvious

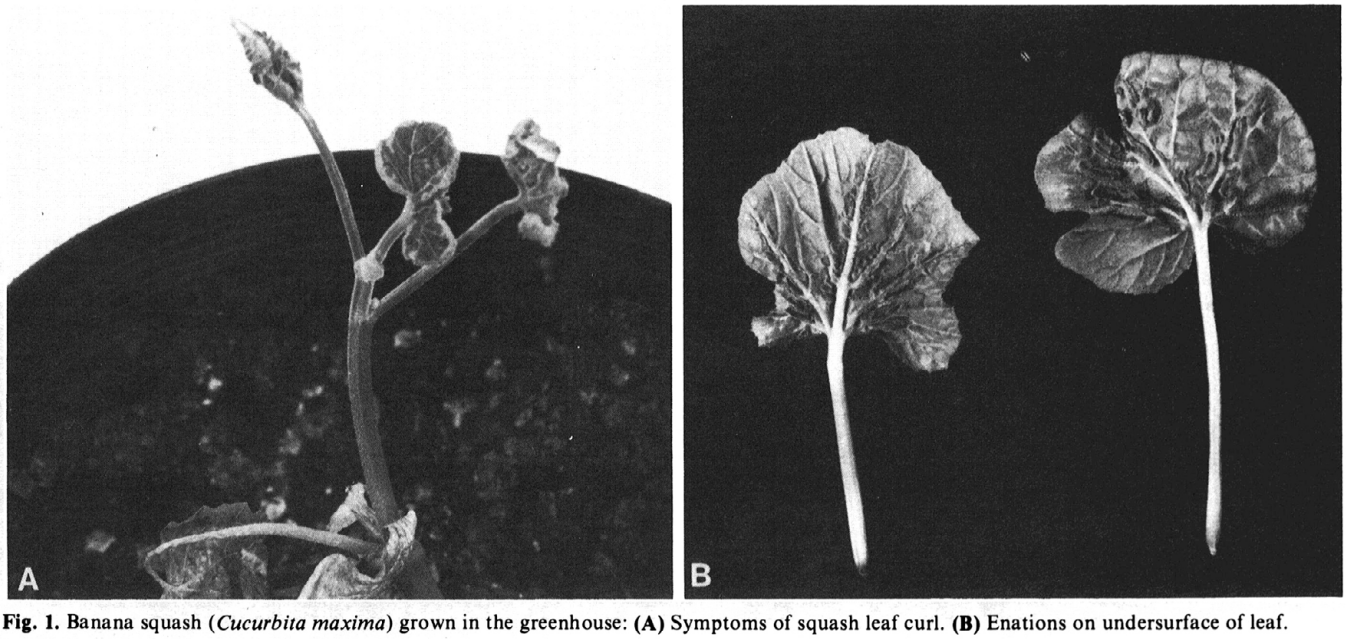

Fig. 1. Banana squash (*Cucurbita maxima*) grown in the greenhouse: **(A)** Symptoms of squash leaf curl. **(B)** Enations on undersurface of leaf.

Table 1. Transmission of the squash leaf curl agent by *Bemisia tabaci* to banana squash, *Cucurbita maxima* 'Pink Jumbo'

Source of vector	Plants (no.)	Positive[a] (%)	Leaf curl (%)	Enations (%)	Mortality (%)
Isolated squash planting[b]	47	87	85	0	59
Squash adjacent to cotton[b]	89	91	82	19	41
Isolated cotton planting[b]	44	91	77	29	36
Inoculated plants[c]	28	100	60	28	82
Checks[d]	178	0	0	0	14

[a] One or more symptoms of squash leaf curl.
[b] 100 insects used per plant.
[c] Plants with SLC symptoms from the first three sources; 1–20 insects were used per plant.
[d] Plants not exposed to insects.

in July and August (32.2 C mean), temperatures between 26.6 and 32.2 C were used in the laboratory as much as possible. Soil was sterilized by a sodium hypochlorite solution. Plants were checked for symptoms at weekly intervals.

Insects for transmission studies were collected mostly from diseased squash fields and included the striped flea beetle (*Systena* spp.), the fruit bud beetle (*Conotelus mexicanus*), the sweet potato whitefly (*Bemisia tabaci*), and the whiteflies *Trialeurodes abutiloneus* and *Aleurocybotus occiduus*. A few insects were raised on diseased squash or held on them for 4 hr. Beet leafhoppers (*Circulifer tenellus*) and cucumber beetles (*Diabrotica* sp.) were obtained a distance from squash fields. Insects were collected with a sweep net or by a D-Vac Suction Sampler.

RESULTS

SLC was found in the California desert agricultural area of Imperial and Riverside counties north to Coachella and Blythe and the adjacent areas of Mexicali, Mexico, and Posten, AZ. In 1977 and 1978, most summer and fall plantings of squash were damaged to some extent by SLC. In 1979, only a portion of one field was diseased. Banana squash, Hubbard squash, and pumpkins were most severely damaged, probably because they have a long growing season and were planted in July. In 1977 and 1978, 48 and 56 ha, respectively, were plowed up or almost completely destroyed. Early plantings of other squash such as yellow crookneck, scallop, and zucchini were severely damaged, but plants that were not killed usually produced a crop when the weather cooled.

In the field, SLC caused severe stunting of new growth and, frequently, death of plants. New shoots were stunted and bent upward. The margins of the leaves curled up and the veins were thickened (Fig. 1A). The interveinal tissue usually became chlorotic or mottled with associated vein clearing or green vein banding. Occasionally, blossoms failed to develop or set fruit, or fruit was small and distorted.

Enations frequently formed on the underside of the leaves (Fig. 1B). They were of various sizes, often oval, leafy cuplike outgrowths, or raised areas. Enations were produced on banana squash, sugar pumpkin, Hubbard squash, Japanese pumpkin, and Mediterranean squash.

Mortality was highest if plants were infected before the four-leaf stage. Mortality increased at temperatures above 32.2 C, and no distinct symptoms developed when temperatures were lower than 21 C. Enations and other symptoms developed best between 26.6 and 32.2 C under artificial light.

Symptoms on watermelon, cucumber, cantaloupe, casaba, and crenshaw melon were mild chlorosis and slight stunting, with some vein clearing.

No virus or viruslike particles were observed in leaf dip preparations made from diseased cucurbit leaves, with the exception of 30-nm isometric particles in plants shown to be infected with SMV by serology, and rod-shaped particles (750 nm) in one sample presumably infected with watermelon mosaic virus.

Serologic tests showed SMV in 5–10% of the field plants. CMV was confirmed in only one plant. Attempts to mechanically transmit SLC were unsuccessful, but SLC developed in 75% of squash plants successfully grafted with infected tissue.

Plants inoculated with field-collected cucumber beetles failed to develop SLC symptoms.

Of the insects tested, only *Bemisia tabaci* proved to be a vector of the SLC agent, and additional tests were made with this whitefly (Table 1). Whiteflies collected from isolated cotton plantings were as effective in transmitting the SLC agent as those collected from squash, suggesting that cotton may be a host of the SLC agent.

DISCUSSION

SLC appears to be similar to but distinct from cotton leaf curl and similar diseases (5,8,10). Symptoms of cotton leaf curl in the Sudan, Africa (11), include similar enations and upward leaf curl on the Sakel variety of cotton. Squash has not been reported as susceptible to cotton leaf curl. Enations and typical upward leaf curl symptoms were not observed on cotton during this study.

Cotton leaf crumple appears to be distinct from SLC. It has occurred erratically in the Imperial Valley, apparently after the growing of perennial or stub cotton. It was common until 1961 when a cotton plow-up regulation was enacted. An outbreak of cotton leaf crumple in 1967 was associated with failure to plow up cotton in adjacent Baja, CA. The disease has not been observed in California since 1967.

SLC differs from the bottle gourd virus disease of Israel by the lack of vein yellowing and mechanical transmission (2). It differs from pumpkin yellow vein of India by the lack of vein banding (11).

SLC can be distinguished from most cucurbit virus diseases by the upward leaf curl and enations. The leaf curl agent was not mechanically transmissible, and no virus particles were observed by electron microscopy. Squash plants with beet curly top may have leaves curled upward but no leafy enations. Beet curly top can be identified by transmission tests with the beet leafhopper to sugar beet on which the leaves curl up and spinelike outgrowths or enations are produced on the underside of the leaves.

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