Reservoir Weed Hosts of Tomato Spotted Wilt Virus

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ABSTRACT

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Surveys were made in Hawaii's major vegetable production areas including Waianae on Oahu, Kula on Maui, and Waimea on Hawaii to determine important reservoir plants of tomato spotted wilt virus (TSWV). More than 9,000 plant samples were collected and analyzed for TSWV by enzyme-linked immunosorbent assay. Forty-four plant species representing 16 families were found infected with TSWV. Twenty-five species are considered important reservoirs of the virus. Twenty-four new TSWV host recordings are identified.

Additional key words: insect vector, thrips

Tomato spotted wilt virus (TSWV) disease affects production of several economically important vegetable and ornamental crops in Hawaii. This disease has been particularly devastating in recent years, when losses of 50–90% have occurred in lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Mill.) production (12).

Development of feasible control procedures has been difficult because TSWV has an extensive plant host range. Best (3) lists 157 dicotyledonous species in 29 families and six monocotyledonous species in five families as hosts. Francki and Hatta (8) list 11 additional host species. Several of these hosts are found along field borders and within crops of Hawaii's major vegetable-growing regions on the islands of Hawaii, Maui, and Oahu.

Six thrips species are known vectors of the virus (1,2,11). Three of these species, *Frankliniella occidentalis* (Pergande), *F. schultzei* Trybom, and *Thrips tabaci* Lindeman, are found in Hawaii (5,12). Yudin (12) found *F. occidentalis* to be associated with several known TSWV reservoir host plant species on Maui.

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This study was initiated to identify plant species that may serve as reservoirs for TSWV in major vegetable production areas of Hawaii.

MATERIALS AND METHODS

Disease surveys were conducted from January through July 1985 in major vegetable-growing regions of the state afflicted with TSWV disease. Surveys included the Lalamilo and Pukapu areas on the island of Hawaii, the Kula area on Maui, and the Waianae area on Oahu.

Random plant samples were selected from known reservoir host plants to estimate the frequency of TSWV infection. A biased sampling was used to determine possible new TSWV reservoir host species. Suspect new plant hosts generally were selected from cropping areas where a high TSWV disease incidence was observed and selected plants showed apparent TSWV symptoms as previously described by Ie (10). TSWV symptoms include a range of chlorotic, necrotic, stunting, and enation symptoms in all parts of the plant.

Major weeds from a few low-elevation (335–430 m) Kula farms were sampled extensively for TSWV. These farms are in an area with a known history of high TSWV disease incidence on lettuce. Plants were selected randomly from abandoned lettuce, cabbage, and corn plantings.

Detection of infected plants was determined by enzyme-linked immunosorbent assay (ELISA). A direct (double-antibody sandwich) ELISA system developed for TSWV was used as previously described (9). The antiserum was produced from a TSWV isolate obtained from an infected lettuce plant on Maui. Preliminary tests indicated that this procedure is more sensitive than the indirect method. Flat-bottom or U-bottom Immulon II microplates (Dynatech) were coated with 200 μl of TSWV immunoglobulin (1-μg/ml concentration) overnight at 4 C. Plates were washed three times with phosphate-buffered saline containing 0.05% Tween 20 between each step. Plant tissues were taken from different parts of each sample and triturated in a mortar and pestle, using about 1 g fresh weight of tissue with 50 ml of ELISA extraction buffer. The remainder of each sample was placed in the refrigerator for subsequent bioassays. Samples were placed in separate wells and incubated overnight at 4 C. Buffer controls consisted of six wells that contained ELISA extraction buffer. A positive control consisted of TSWV-infected lettuce tissue sap diluted 1/50 in ELISA extraction buffer. Alkaline phosphatase-conjugated immunogloblins were cross-absorbed with healthy *Nicotiana benthamiana* Domin tissue and used at 1/2,000 dilution. *p*-Nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) was added, incubated for 1 hr at room temperature, and the reaction stopped by adding of 3 M NaOH. In initial surveys, ELISA reactions were rated visually where strong positive reactions were rated as +++ and weak reactions scored as +. Samples that showed a color intensity judged subjectively to be above the buffer and healthy plant sap controls were recorded as +. Later, reactions were measured spectrophotometrically at 405 nm using an EL307B EIA reader (Bio-Tek Instruments, Inc., Burlington, VT). A sample was considered positive for TSWV if the A_{405} reading was greater than twice the average buffer and/or healthy plant control readings, whichever of the two was higher.

Two means of establishing the validity of the ELISA were used. In initial tests, several plants collected during field surveys for TSWV host range studies that gave positive ELISA reactions were bioassayed on detached petunia (*Petunia hybrida* Vilm. cv. Purple Plum) leaves to confirm the presence of TSWV. A positive test was based on the development of typical purple local lesions 2–3 days after inoculation when incubated at 24 C. In subsequent tests, a TSWV isolate from lettuce was used in inoculation studies. The homogeneity of this isolate was ensured by five single local lesion transfers in *N. benthamiana*. Several host

plants were inoculated, and the resultant infection was assayed via ELISA and bioassayed on detached petunia leaves and on *N. benthamiana* plants. A positive reaction on *N. benthamiana* plants was based on the development of typical necrotic leaf spots 6–8 days after inoculation.

RESULTS

In initial studies, TSWV was detected by bioassays from several plants collected from several field locations that gave a positive ELISA reading (numbers in parentheses represent number bioassayed positive/total plants tested): *Arctium lappa* (6/6) (authorities for taxa given in Table 1), *Bidens pilosa* (4/5), *Capsella bursa-pastoris* (1/4), *Cordyline terminalis* (3/6), *Galinsoga quadriradiata* (1/1), *Lactuca sativa* (4/4), *Lycopersicon esculentum* (5/5), *Malva parviflora* (5/5), *Nicandra physalodes* (5/5), *Stellaria media* (2/4), *Tropaeolum majus* (1/5), and *Xanthium saccharatum* (3/4).

Table 1. Number of plant species found in the major vegetable-growing regions of Hawaii determined to be infected with tomato spotted wilt virus by ELISA[a]

		Island and location			
		Hawaii		Maui	Oahu
Latin name	**Common name**	**Lalamilo**	**Pukapu**	**Kula**	**Waianae**
Amaranthaceae					
Amaranthus hybridus (L.)*[b]	Green amaranth	···	···	49/791	···
A. spinosus L.*	Spiny amaranth	···	···	1/3	18/21
A. viridus L.*	Slender amaranth	···	···	4/101	4/20
Caryophyllaceae					
Stellaria media (L.) Cyr.	Chickweed	···	9/12	···	···
Chenopodiaceae					
Chenopodium album L.	Lamb's-quarters	1/9	···	···	···
C. ambrosioides L.*	Mexican tea	···	···	3/10	···
C. murale L.*	Nettleleaf goosefoot	4/39	0/4	11/11	0/10
Compositae					
Arctium lappa L.*	Burdock	···	56/73	···	···
Bidens pilosa L.	Spanish needle	···	···	304/1,678	2/2
B. pilosa var. *minor* (Bl.) Sherff*	Spanish needle	···	···	1/12	···
Chrysanthemum coronarium L.*	Garland chrysanthemum	···	···	···	2/5
C. morifolium Ram.	Chrysanthemum	···	···	3/3	9/81
Conyza bonariensis L.	Hairy horseweed	···	···	2/10	···
Emilia sonchifolia (L.) DC.	Flora's paintbrush	···	···	2/3	···
Galinsoga parviflora Cav.*	Galinsoga	0/44	1/18	1/1	···
G. quadriradiata (Raf.) Blake*	Pervian daisy	···	1/1	···	···
Lactuca sativa var. *capitata* L.	Crisphead lettuce	1/3	···	2/7	···
L. sativa var. *crispa* L.	Leaf lettuce	···	···	14/18	6/14
L. sativa var. *longifolia* L.	Cos lettuce	···	···	3/5	···
Sonchus oleraceus L.	Sowthistle	···	···	8/27	2/7
Verbesina enceloides (Cav.) Benth. & Hook*	Golden crownbeard	···	···	5/61	2/14
Xanthium saccharatum Wallr.*	Cocklebur	···	···	10/14	···
Convolvulaceae					
Ipomoea congesta R. Br.*	Blue morning glory	4/4	···	49/180	···
Cruciferae					
Brassica campestris L. subsp. *chinensis**	White stem cabbage	···	···	···	2/6
Capsella bursa-pastoris (L.) Medic.	Shepherd's purse	0/22	7/19	···	···
Coronopus didymus (L.) Sm.*	Swinecress	···	4/8	4/15	···
Labiatae					
Leonotis nepetaefolia R. Br.*	Lion's-ear	···	···	···	3/4
Stachys arvensis L.	Staggerweed	···	7/20	···	···
Leguminosae					
Crotalaria incana L.*	Fuzzy rattlepod	···	···	1/3	···
C. mucronata Desv.*	Smooth rattlepod	···	···	2/12	···
Desmodium unicinatum (Jacq.) DC.*	Spanish clover	···	···	5/6	···
Medicago polymorpha L.*	Bur clover	0/5	···	1/47	···
Melilotus officinalis (L.)*	Sweet yellow clover	3/19	···	285/421	···
Phaseolus vulgaris L.	Snap bean	···	···	3/10	···
Liliaceae					
Cordyline terminalis (L.) Kunth*	···	···	···	10/20	···
Malvaceae					
Malva parviflora L.	Cheese weed	9/82	···	317/1,784	10/17
Plumbaginaceae					
Limonium latifolium (Sm.) Ktze.*	Statice	···	···	3/10	5/7
Portulacaceae					
Portulaca oleracea L.	Purslane	5/29	13/18	0/8	1/10
Solanaceae					
Lycopersicon esculentum Mill.	Tomato	···	1/1	3/3	···
Nicandra physalodes (L.) Gaertn.	Apple of peru	3/25	5/17	0/1	···
Solanum nigrum L.	Black nightshade	···	···	1/1	0/1
Tropaeolaceae					
Tropaeolum majus L.	Nasturtium	···	13/13	1/9	···
Umbelliferae					
Apium graveolens L.	Celery	···	9/11	2/2	···
Verbenaceae					
Verbena litoralis H.B.K.*	Verbena	1/6	···	···	···

[a] Number ELISA-positive/total tested.
[b] * = New host recording.

TSWV was not detected by bioassay in *Coronopus didymus* (0/5), *Limonium latifolium* (0/2), and *Portulaca oleracea* (0/4), which were ELISA-positive.

In our inoculation studies, TSWV could be detected by ELISA and confirmed via bioassays in all instances from the following hosts: *Datura stramonium* (4/4), *Leonotis nepetaefolia* (3/3), *N. benthamiana* (10/10), *N. tabacum* L. cv. Blue Pryor (2/2), *L. esculentum* (2/2), and *Sonchus oleraceus* (4/4).

About 9,000 plant samples were processed in surveys of major vegetable regions of the state to determine important and new reservoir hosts for TSWV. In general, plants that gave a positive ELISA reaction also showed disease symptoms characteristic for TSWV infections.

Forty-four plant species representing 16 families were found infected with TSWV (Table 1). Twenty-four of these are new host recordings.

Most of the weed species sampled from low-elevation Kula farms were found to have a high incidence of TSWV infection. For example, TSWV incidence for *B. pilosa* was 55.0% (numbers in parentheses represent number of plants infected/total tested) (424/771), *Chenopodium murale* was 100% (11/11), *M. parviflora* was 33.1% (380/1147), *Melilotus officinalis* was 75.1% (383/510), *S. oleraceus* was 53.3% (16/30), and *Verbesina enceloides* was 23.5% (36/153). On the other hand, *Amaranthus hybridus* and *A. viridus* sampled from these farms showed a low incidence of infection, 12.0% (80/667) and 0.8% (2/249), respectively.

T. majus found in Pukapu and *Ipomoea congesta* found in Lalamilo and Kula regions are widely distributed, growing along roadsides and within farmland borders. Several of these plants were found infected with TSWV.

Other than lettuce and tomatoes, 23 plant species (Table 2) are potentially important TSWV reservoirs. These are considered important reservoirs because 1) they occur in abundant numbers within vegetable farmlands, 2) they are associated with vector thrips species, and 3) many were found to be naturally infected with TSWV. Two plant species, *I. congesta* and *T. majus*, are perennial in growth habit. The other plants are herbaceous annuals. Eighteen of these hosts commonly are found on the islands of Hawaii and Maui. Only 11 are common to the Oahu growing region.

TSWV was not detected in 12 weeds common to the vegetable-growing areas (numbers in parentheses represent total number of plants tested): *Cassia occidentalis* L. (5), *Diodia teres* Walt.(5), *Euphorbia geniculata* Ortega (2), *Foeniculum vulgare* Hill (22), *Indigofera suffruticosa* Mill. (7), *Lantana camara* L. (4), *Leucaena glauca* (L.) (50), *Malvastrum coromandelianum* (L.) Garcke (40), *Momordica charantia* L. (5), *Ricinus communis* L. (24), *Vicia angustifolia* L. (15), and *Waltheria americana* L. (5).

DISCUSSION

The ELISA technique was used to identify TSWV reservoir hosts in major vegetable-growing areas of Hawaii. Inoculation studies of several plant species with a known TSWV strain and confirmation of the presence of TSWV by both ELISA and bioassays validate the ELISA technique as a reliable, sensitive, and rapid method for detecting TSWV. Negative bioassay results from ELISA-positive plants during farm surveys may be due to the lability of the virus (2) or the sensitivity of the bioassay hosts used. This method helped identify TSWV reservoir hosts found in the major growing areas that may be important sources for disease outbreaks in commercial vegetable crops in Hawaii. Several of these plants are weeds that occur within and outside farmlands.

Weeds are important as virus and insect vector reservoirs for several important crop diseases (7). Bos (4) noted that susceptible weeds assist in virus survival and play an essential role in virus spread. Association between vector and virus source plants and subsequent movement to other susceptible plants are essential for transmission of plant virus diseases by insects (6). *Emilia sonchifolia* has been shown to be a major reservoir for TSWV and its insect vector, *T. tabaci*, in the epidemiology of TSWV disease in pineapples (5). Although infected *E. sonchifolia* plants have been associated with one vegetable farm on Maui, it is not considered an important reservoir within vegetable farmlands because it is not commonly found in those areas. *Ipomoea congesta* and *T. majus* are present thoughout the year on the islands of Hawaii and Maui. On Oahu, *A. spinosus* is commonly found. A high percentage of those plants found within farmland areas harbored the virus and were associated with abundant vector thrips species. It is suspected that these three plant species serve as major sources for the perpetuation of TSWV.

An understanding of TSWV vector relationships with specific plant species is important in determining important reservoir hosts. Bailey (2) demonstrated that *F. occidentalis* only acquired TSWV during larval stages of development and not as an adult. This requires that a potential reservoir host not only be susceptible to TSWV but also serve as a food source for development of immature thrips vectors that can aquire the virus. Yudin (12) found abundant numbers of larval and adult *F. occidentalis* associated with 16 of the 25 hosts identified as important in our study. A few plant species harbored low insect numbers, and their impact in disease epidemics needs to be elucidated. Further investigations on the association of insect vectors with other TSWV reservoir plants are needed.

This study is one in a series of steps to understand the epidemiology of TSWV disease in Hawaii. A thorough understanding of interrelationships between the crops, virus, insect vectors, and reservoir hosts is necessary for development of feasible management procedures. Further studies have been initiated in this regard.

Table 2. Important tomato spotted wilt virus hosts found in the major vegetable-growing regions of Hawaii

Plant species	Common name	Where abundant[a]
Amaranthus hybridus	Green amaranth	H,M
A. spinosus	Spiny amaranth	H,M,O
A. viridus	Slender amaranth	H,M,O
Apium graveolens	Celery	H
Arctium lappa	Burdock	H
Bidens pilosa	Spanish needle	H,M,O
B. pilosa var. *minor*	Spanish needle	H,M,O
Capsella bursa-pastoris	Shepherd's purse	H
Chenopodium album	Lamb's-quarters	H,M
C. murale	Nettleleaf goosefoot	H,M,O
Coronopus didymus	Swinecress	H,M
Datura stramomium	Jimsonweed	M
Ipomoea congesta	Blue morning glory	H,M
Lactuca sativa	Lettuce	H,M,O
Leonotis nepetaefolia	Lion's-ear	O
Lycopersicon esculentum	Tomato	H,M,O
Malva parviflora	Cheese weed	H,M,O
Melilotus officinalis	Sweet yellow clover	H,M
Nicandra physalodes	Apple of peru	H,M
Portulaca oleracea	Purslane	H,M,O
Sonchus oleraceus	Sowthistle	H,M,O
Stellaria media	Chickweed	H
Tropaeolum majus	Nasturium	H,M
Verbesina enceloides	Golden crownbeard	M,O
Xanthium saccharatum	Cocklebur	M

[a] Abbreviations for islands, where: H = Hawaii, M = Maui, and O = Oahu.

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