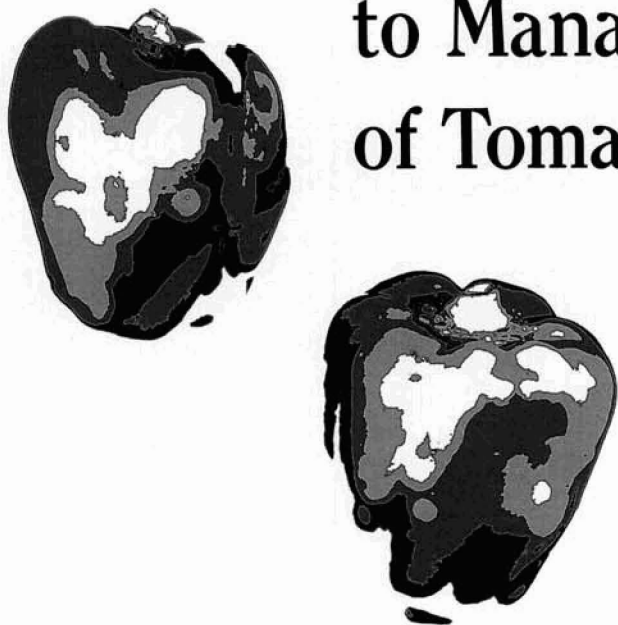


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A Multidisciplinary Approach to Management of Tomato Spotted Wilt Virus in Hawaii



The tomato spotted wilt virus (TSWV) seriously affects production of food and ornamental crops worldwide (2,9). First described in southern Australia (3), the virus is now widespread in temperate and subtropical regions throughout the world. TSWV is unique among plant viruses in that it is covered by a lipoprotein envelope, is the only virus transmitted in a circulative manner by certain species of thrips, is highly unstable in vitro, and has one of the widest host ranges of any plant virus.

The insect vectors of TSWV belong to the family Thripidae (Order: Thysanoptera) (1,2,14,17,20) and include tobacco thrips (*Frankliniella fusca* (Hinds)), western flower thrips (*F. occidentalis* (Pergande)), common blossom thrips (*F. schultzei* (Trybom)), chilli thrips

(*Scirtothrips dorsalis* Hood), *Thrips setosus* Moulton, and onion thrips (*T. tabaci* Lindeman). Virus is acquired only during the larval stages. Larvae can transmit the virus before they pupate, but adults more commonly transmit the virus. Adults can remain infective throughout their life span, but transmission is variable (21).

TSWV has an extensive host range that includes 192 dicotyledonous species in 33 families and eight monocotyledonous species in five families (2,4,6,12). Important crops affected include tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), peanut (*Arachis hypogaea* L.), pineapple (*Ananas comosus* (L.) Merr.), lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* Mill.), and pepper (*Capsicum annuum* L.). Several flowering ornamentals are affected, including chrysanthemum (*Chrysanthemum morifolium* (Ram.) Hemsl.), dahlia (*Dahlia pinnata* Cav.), gloxinia (*Sinningia speciosa* Benth. & Hook.), and touch-me-not (*Impatiens* sp.).

In lettuce, the virus causes brown necrotic spots on leaves, generally on one side of the plant. Systemic infections are characterized by marginal wilting, yellowing, and brown spotting of internal leaves and midribs (Fig. 1). In tomato, symptoms are characterized by an initial chlorosis of leaves and terminal shoots that may develop into bronzing and necrosis (Fig. 2A). Ripe fruit show pale red or yellow areas ranging from irregular mottling or blotches to distinct concentric rings (Fig. 2B). Pepper plants show chlorosis and necrotic spots on leaves, necrosis of terminal shoots, and general stunting (Fig. 3A), and fruit show chlorotic spots, green or red areas surrounded by yellow halos, and concentric rings (Fig. 3B). Affected chrysanthemums show chlorotic and necrotic rings on leaves and stems and stunting and necrosis of terminal shoots (Fig. 4).

Hawaii's Agricultural Ecosystem

Virus diseases limit efficient production of vegetable crops in Hawaii. Several



Fig. 1. Symptoms of tomato spotted wilt virus on lettuce include brown necrotic leaf spots, marginal wilting, yellowing, and stunting.

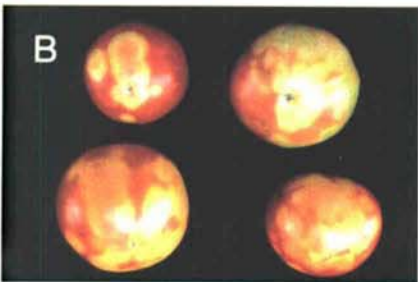


Fig. 2. Symptoms of tomato spotted wilt virus on tomato: (A) Chlorosis, bronzing, and necrosis of terminal shoots. (B) Ripe fruit showing pale red or yellow areas ranging from irregular mottling or blotches to distinct concentric rings.

factors unique to Hawaii are conducive to intensifying virus problems. Hawaiian vegetable farms are small compared with those on the U.S. mainland. They range from 4 to 40 acres, averaging 10 acres, and are located on three major islands. Usually, many farms are clustered in small pockets interspersed among uncultivated pasturelands and/or urban development. Numerous dispersed farms make it difficult to coordinate the implementation of management strategies.

Weeds are prevalent in fallowed fields and in uncultivated areas between farms.



Fig. 3. Symptoms of tomato spotted wilt virus on pepper: (A) Chlorosis and necrotic spots on leaves, necrosis of terminal shoots, and general stunting. (B) Chlorotic spots, green or red areas surrounded by yellow halos, and concentric rings on fruit.



Fig. 4. Symptoms of tomato spotted wilt virus on chrysanthemum: (A and B) Chlorotic and necrotic rings on leaves and stems and stunting and necrosis of terminal shoots. (C) Affected rooted cutting (cv. Polaris) inoculated with *Frankliniella occidentalis* with typical chlorotic ring spots 7-10 days after transmission.

Weed management in cultivated fields has become difficult because of the withdrawal of effective preemergence herbicides by the U.S. Environmental Protection Agency. These factors complicate management of alternate pest reservoirs.

Although several different vegetable crops are planted, lettuce, tomato, and head cabbage account for approximately 60% of all vegetables grown in the state. These crops are generally monocropped in sequential fields on individual farms throughout the year. This practice favors buildup of pests within the crop area. Hawaii's mild annual climate favors the multiplication and dispersion of several pests. The lack of a cold winter period allows pests to survive continuously on cultivated and reservoir hosts.

Vegetable and ornamental crops affected by TSWV are commonly grown in Hawaii from sea level to approximately 1,000 m elevation. Major production areas affected by TSWV epidemics include the Koko Head and Waianae regions (sea level) on the island of Oahu, the Kula region (300-1,000 m elevation) (Fig. 5) on the island of Maui, and the Waimea region (760 m elevation) on the island of Hawaii.

Historical Perspective of TSWV in Hawaii

In Hawaii, a new disease of pineapple called yellow spot, subsequently recognized as caused by TSWV, was observed in 1926 on the island of Oahu by Illingworth (11). Merely a curiosity at first, the disease became increasingly important, causing considerable loss of pineapples on several islands. The onion thrips was identified as the primary vector of the disease agent, and the weed *Emilia sonchifolia* (L.) DC. was identified as an important alternate host of both virus and vector (15). Because of effective weed management, the importance of pineapple yellow spot diminished.

In the 1940s, TSWV became a limiting factor in tomato production on Oahu, with plant losses of 75-100%. These severe losses stimulated a successful breeding program by the University of Hawaii that culminated in a commercial TSWV-resistant tomato cultivar named Pearl Harbor in 1945 (13). Unfortunately, the resistance was overcome by the development or introduction of new TSWV strains.

In 1955, *F. occidentalis* (Fig. 6) was discovered in Hawaii. Subsequently, periodic TSWV epidemics in lettuce, tomatoes, and other vegetable crops began to occur on Oahu. During the late 1960s, TSWV seriously affected tomato production and contributed to the cessation of tomato production in leeward Oahu. Leaf lettuce was also affected by TSWV during this period. Initially, losses in lettuce were tolerated



Fig. 5. Typical lettuce farm located at 640 m elevation in the Kula region on the island of Maui.

because epidemics occurred only during summer months. However, TSWV increasingly forced some growers to stop production and others to produce alternative crops. Today, TSWV frequently causes 50–90% crop losses in lettuce during any season of the year (7).

Similar crop losses paralleling the buildup of TSWV on Oahu have occurred on Maui and Hawaii, where the majority of all vegetables and melons are produced. In the early 1980s on Maui and in the mid-1980s on Hawaii, TSWV started to cause economic losses in lettuce production. Today, major losses on Maui in lettuce, tomato, and pepper have forced several growers from planting TSWV-susceptible crops, particularly during summer and other hot, dry periods. A few lettuce farms on the island of Hawaii have experienced economic losses because of TSWV, but losses have not been as great as on Oahu and Maui.

T. tabaci was the major vector of TSWV on pineapple but does not seem to be important in recent disease epidemics in vegetables and ornamentals. Likewise, *F. schultzei* occurs at low population densities on Maui and Hawaii but is rarely collected on lettuce, tomato, and peppers. Instead, *F. occidentalis* has become the predominant vector. It has a broad host range and has been implicated as the vector in recent epidemics on chrysanthemum, lettuce, pepper, and tomato crops.

In 1984, a group of scientists from the University of Hawaii College of Tropical Agriculture and Human Resources (CTAHR) was convened to review the TSWV problem. A multidisciplinary research project was initiated because of the severe impact of the disease on crop production. Significant strides have been made toward understanding the etiology, vector interrelationships, epidemiology, and management of TSWV in Hawaii.

The Multidisciplinary Program

CTAHR scientists realized that a rapid, easy solution to alleviate losses caused by TSWV was not possible. The complex nature of this disease required that researchers from a number of

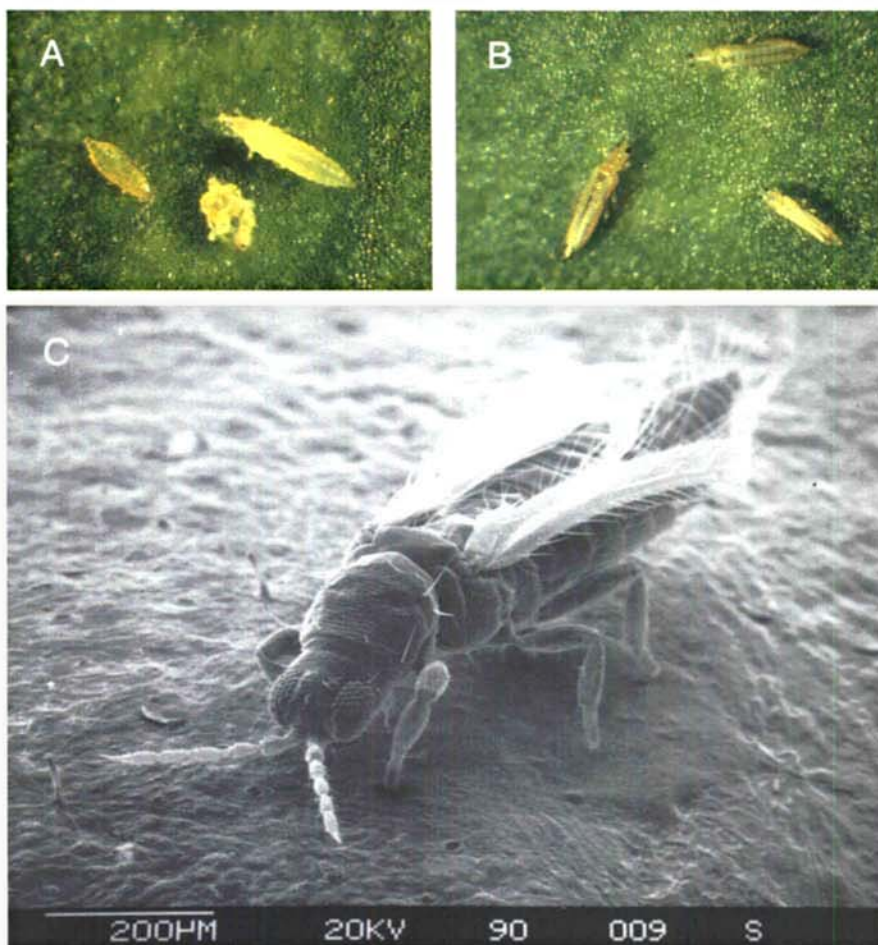


Fig. 6. Thrips vectors of tomato spotted wilt virus: (A, left to right) First instar larva, first instar larval exuvia (molted exoskeleton), and second instar larva of the western flower thrips, *Frankliniella occidentalis*. (B) Adult female (lower left) and adult male (lower right) stages of *F. occidentalis* compared with adult *F. schultzei*, the common blossom thrips. (C) Scanning electron micrograph of *F. occidentalis*. Scale bar = 400 μ m. (Fig. C courtesy Wayne B. Hunter and Diane E. Ullman)

different disciplines participate in development of control methods.

In assembling the TSWV multidisciplinary team, researchers were chosen for their disciplinary expertise and commitment to a goal-oriented program. The team was headed by an extension specialist in entomology and consisted of plant pathologists, a molecular plant virologist, entomologists, a weed scientist, and plant geneticists from the University of Hawaii. In addition, two virologists from the New York State Agricultural Experiment Station (NYSAES), Cornell University, Geneva, were asked to join the team to assist in developing a rapid assay for TSWV and in evaluating lettuce germ plasm.

The team recognized that host resistance offered the best long-term solution but also realized that development of suitable cultivars would require several years of research. Furthermore, available information on TSWV was inadequate to develop short-term disease management programs. A decision was made to set up a series of subprojects in order to develop feasible control solutions in a shorter period of time.

The problem was analyzed and possible solutions were outlined. A comprehensive plan of action was prepared that comprised a list of major and subordinate research objectives. Major objectives of the team were to: 1) develop rapid methods of diagnosis, 2) develop basic knowledge about virus-host, virus-vector, and vector-host associations, 3) develop and evaluate programs for short-term disease management, 4) evaluate lettuce and tomato germ plasm and develop resistant cultivars, and 5) evaluate cross-protection as a method for managing disease losses.

An industry analysis and action plan was presented at a statewide meeting of grower representatives, researchers, and extension workers. The plan was approved by growers and subsequently funded by the Governor's Agricultural Committee.

Plan of Attack and General Chronology of Research

Previous research. A 3-year integrated pest management project on cabbage and lettuce was initiated on the island of



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Dr. Mau is an extension specialist and professor in the Department of Entomology at the University of Hawaii, Honolulu. He presides as research and extension coordinator of the University of Hawaii's multidisciplinary project on the tomato spotted wilt virus and has extension responsibilities for fruit and vegetable crops. His primary research interests include basic and applied investigations on tropical crop pests, particularly vegetables, macadamia, papaya, and guava. He received his B.S., M.S., and Ph.D. degrees from the University of Hawaii.

Dr. German is an associate professor in the Department of Plant Pathology at the University of Hawaii, Honolulu. His

primary research interests involve the molecular biology of plant viruses and the diseases they cause. He received his B.S. degree in zoology from the University of Wisconsin, Madison, his M.S. degree from Michigan State University, East Lansing, and, in 1974, his Ph.D. degree in plant pathology/virology from the University of Wisconsin. He did postdoctoral research in virology until assuming his present position in 1985.

Dr. Hartmann is an associate professor in the Department of Horticulture at the University of Hawaii, Honolulu. He obtained his B.A. degree in botany from Rutgers University, New Brunswick, New Jersey, his M.S. degree in horticulture from Virginia Polytechnic Institute, Blacksburg, and, in 1962, his Ph.D. degree in plant science from the University of California, Los Angeles. His research interests have been in breeding and genetics for disease resistance and tropical adaptation of vegetables, particularly legumes and leafy vegetables.

Dr. Yudin is currently working on a postdoctoral fellowship at the University of Hawaii, Honolulu. He received his B.S. and M.S. degrees and, in 1988, his Ph.D. degree in entomology from the University of Hawaii, Honolulu. His research interests have been in the ecology of thrips and tomato spotted wilt virus and in integrated control of insect vectors.

Dr. Gonsalves is a professor in the Department of Plant Pathology at the New York State Agricultural Experiment Station, Cornell University, Geneva. He received his B.S. degree in horticulture and his M.S. degree in plant pathology from the University of Hawaii, Honolulu, and his Ph.D. degree in plant pathology from the University of California, Davis. His research activities include investigations on basic and applied aspects of virus diseases of fruits and vegetables, with emphasis on cross-protection as a control measure.

Dr. Provvidenti is the Liberty Hyde Professor of Plant Pathology in the Department of Plant Pathology at the New York State Agricultural Experiment Station, Cornell University, Geneva. He is a native of Italy, where he received his B.S. and Ph.D. degrees in microbiology. His research has involved the identification and characterization of viral diseases of vegetables, the search for sources of resistance, and the genetics of resistance.

Maui in the fall of 1980. Baseline information on the TSWV disease situation was collected that was useful in formulating a plan of attack.

The project was fortunate in obtaining the assistance of K. Sakimura, an authority in the taxa of thrips. He was invaluable in identifying thrips found within Maui's farmlands. Four of the six known vectors currently occur in Hawaii. *T. tabaci*, *F. occidentalis*, and *F. schultzei* have occurred in Hawaii for more than 30 years. The fourth vector, *S. dorsalis*, was found on the island of Oahu in 1986. Of these, *F. occidentalis* was identified as the predominant vector. This thrips was associated with several weed species within the vegetable farmlands and was abundant within flowers of three leguminous plants commonly found just outside farm borders, along roadsides, and in thickets scattered throughout the vegetable-growing region (26). Several of these reservoir plants were suspected as possible virus sources, and studies were initiated to confirm this suspicion.

TSWV identification and detection. Identification of TSWV reservoirs posed a more difficult problem than identification of insects because a simple, rapid detection method was not available. A test was needed for rapid accumulation of knowledge about TSWV host and vector associations in the vegetable ecosystem. A collaborative arrangement was established with D. Gonsalves, NYSAES, who had prior experience with TSWV and local agricultural conditions. Within 6 months, a high-titer polyclonal antiserum to TSWV was produced. Subsequently, a direct (double-antibody sandwich) enzyme-linked immunosorbent assay (ELISA) was developed as a rapid and sensitive means for detecting TSWV (10). Recently, Cho et al (5) modified this test to detect TSWV in individual thrips.

TSWV vector-host relationships. Alternate hosts were suspected to play an important role in TSWV epidemics, because there were periods during the year when the disease was not present in crop hosts. Several plants that occur on Hawaii's farmlands were known hosts for both the virus and *F. occidentalis* (4,26,27). The importance of these plants as potential reservoir sources was unknown, however.

During 1985-1986, extensive field surveys were initiated to determine actual and potential virus vector reservoirs. The following criteria were used to determine the importance of alternate hosts: 1) Infected plants must occur on farmlands, 2) thrips vectors must utilize the plant for larval development, and 3) the virus must be present in plant parts where vectors feed to allow virus acquisition. Thrips were collected and identified from plants showing symptoms of TSWV infection, and TSWV infection was confirmed by ELISA. Information from these surveys

was used to make a list of important host plant reservoirs (Table 1). In addition, 25 new hosts of the virus (4) were identified. There are now more than 200 species of plants known to be susceptible to TSWV (6).

Chemical control of the vector. In 1984, 26 insecticides were evaluated by a rapid assay method for toxicity to *F. occidentalis*. Nineteen chemicals were registered for lettuce, pepper, and/or tomato. Thrips adults were placed on treated lettuce leaves in forced-air vented acrylic cells and observed for mortality. Although the insecticides did not kill rapidly enough to prevent virus transmission, the data were useful in choosing insecticides for field evaluations on lettuce.

Three separate field experiments were conducted on Maui. Treatments were applied immediately after transplanting and at 3- to 4- or 7-day intervals at 152 or 304 L/ha. A boom sprayer equipped with overhead and drop-nozzles provided thorough coverage of the entire plant. The results summarized below provided us with substantial knowledge about *F. occidentalis* in the lettuce ecosystem:

1. None of the insecticide treatments suppressed TSWV disease occurrence.
2. Lettuce supported large thrips populations. When measured 5 weeks after planting, thrips densities averaged 125-375 thrips per plant (1.1-3.4 million thrips per hectare of crop) in the untreated or ineffective treatment plots.
3. Field application rates of 304 L/ha once or twice a week were required to suppress thrips populations. Acephate, azinphos-methyl, cypermethrin, fluralinate, methomyl, and mevinphos were among the most effective insecticides.
4. Roguing diseased plants at 3- to 4-day intervals did not affect disease occurrence or progression.
5. Thrips continued to emerge from the soil for 2-3 weeks after crop residues were plowed and rototilled. More than a million thrips per hectare of crop

emerged during this period. We concluded that sequential planting schemes were to be avoided during disease epidemics.

6. Cultivation and harvesting activity disrupted and agitated thrips and resulted in their considerable intercrop movement.

The search for resistant germ plasm. *Tomato.* TSWV resistance was first described in *Lycopersicon pimpinellifolium* (Jusl.) Mill. by Samuel et al in 1930 (22). Since then, resistance has been identified in other species, including *L. hirsutum* Humb. & Bonpl. and *L. peruvianum* (L.) Mill. Resistance was reported in tomato in 1941 (23) from a cross between *L. esculentum* and *L. pimpinellifolium*. From this source of resistance, Kikuta and Frazier (13) developed the cultivar Pearl Harbor. Unfortunately, this resistance was strain-specific. Subsequently, Finlay (8) found five genes that controlled resistance to five TSWV strain groups. *L. peruvianum* was shown to be highly resistant to all five strains.

With the objective of developing TSWV-resistant tomatoes for commercial production, workers at Petoseed Co. have made selections from crosses derived from *L. peruvianum*. This program was conducted on a limited scale because of a lack of experience with TSWV. In 1986, a cooperative program between Petoseed Co. and the University of Hawaii was established in order to accelerate the selection and breeding process.

A rapid, efficient, mechanical inoculation procedure was developed to screen large numbers of tomato plants for TSWV resistance (Fig. 7). Inoculum prepared by triturating young, systemically infected tomato leaves in phosphate buffer and adding Carborundum is applied with a pressurized airbrush. With this method, 600-1,000 plants can be routinely inoculated in a day, with an 85-95% infection rate.

Table 1. Important weed hosts of tomato spotted wilt virus and western flower thrips (*Frankliniella occidentalis*) found in the major vegetable-growing regions of Hawaii

Weed host	Reasons for importance		
	Prevalence	Vector density	Perennial
<i>Amaranthus spinosus</i> L.	x	x	...
<i>A. viridus</i> L.	x	x	...
<i>Bidens pilosa</i> L.	x
<i>B. p.</i> var. <i>minor</i> (Bl.) Sherf	x
<i>Chenopodium album</i> L.	x
<i>C. murale</i> L.	x	x	...
<i>Ipomoea congesta</i> R. Br.	x	...	x
<i>Malva parviflora</i> L.	x	x	...
<i>Melilotus officinalis</i> (L.) Lam.	x
<i>Nicandra physalodes</i> (L.) Gaertn.	x
<i>Tropaeolum majus</i> L.	x	...	x
<i>Verbesina encelioides</i> (Cav.) Gray	x	x	...



Fig. 7. Screening for resistance to tomato spotted wilt virus in tomato: (A) Plants in the five- to six-leaf stage are artificially inoculated with an artist's airbrush using high pressure. (B) Local infections occur on inoculated tomato leaves 7-10 days after inoculation.

A TSWV isolate originally from lettuce was selected for artificial inoculations. This isolate produces severe necrosis of the tomato growing shoot and leaves, is the most virulent isolate of approximately 30 evaluated, and is typical of what has been described previously as a tipblight strain (2). At present, several tomato lines highly resistant to TSWV and with reasonable fruit size have been selected. We are optimistic that commercially suitable cultivars will become available within 2-3 years.

Lettuce. Unlike tomato, no sources for resistance have been reported in *Lactuca* species. Therefore, an extensive survey of the lettuce germ plasm collection was initiated to search for genetic resistance to TSWV. Thus far, resistance to certain isolates of TSWV has been found, but resistance appears to be strain-specific.

We screened 609 lines, mostly of *L. sativa* and *L. serriola* L. Several *L. sativa* lines that originated from Holland were found to have a low level of tolerance to TSWV. Compared with a susceptible control, lines showing tolerance had delayed onset of systemic symptoms.

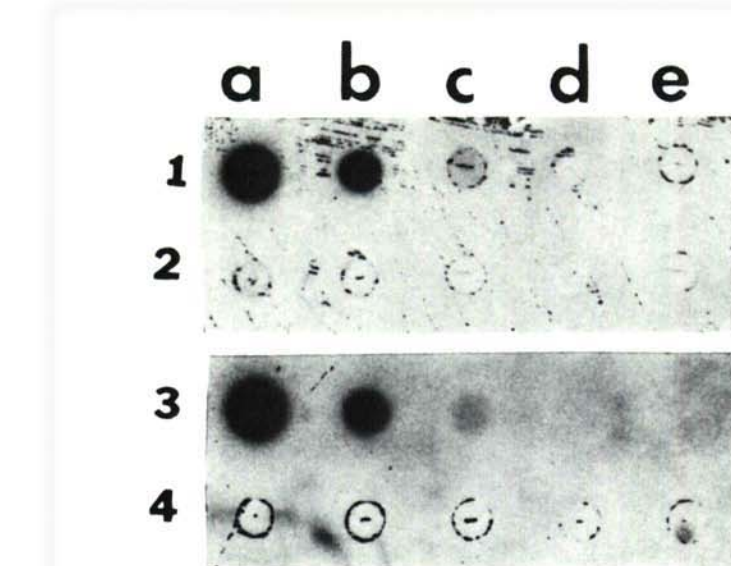


Fig. 8. Detection of tomato spotted wilt virus (TSWV) in infected pepper leaf tissue by dot hybridization using a TSWV cDNA probe. Samples 1-4 were applied to nitrocellulose as serial fivefold dilutions (a-e). Row 1 = TSWV-infected pepper (dilution a equivalent to 5 mg of tissue), row 2 = uninfected pepper, row 3 = TSWV cDNA (dilution a equivalent to 1,000 pg of pBR322 plasmid containing TSWV cDNA probe), and row 4 = no sample. Sensitivity of the assay was equivalent to double-antibody sandwich enzyme-linked immunosorbent assay (T. L. German and J. J. Cho, unpublished).

Single plant selections have been made from two lines, Tinto and Ancora (PI 342517), that showed the best tolerance. These lines have been used in inheritance studies (18) but have been hampered by the difficulties encountered in uniformly infecting individual plants in different experiments.

In further evaluations, 223 lines of wild and cultivated lettuce were screened, including accessions of *L. aculeata* Boiss & Kotschy, *L. altaica* Fisch. & Mey, *L. augustana* All., *L. canadensis* L., *L. capensis* Thunb., *L. dentata* (Thunb.) C. B. Robins, *L. dregeana* DC., *L. indica* L., *L. livida* Boiss & Reut., *L. muralis* (L.) Fresen, *L. perennis* L., *L. quercina* L., *L. saligna* L., *L. serriola*, *L. squarrosa* (Lamb.) Miq., *L. tenerrima* Pourr., *L. tuberosa* Jacq., *L. viminea* (J.) J. & K. Presl., *L. virosa* L., and *L. sativa*. A number of *L. sativa* lines appeared to be resistant. Progeny tests indicated that none of the *L. sativa* lines were homozygous resistant, but the majority of the plants survived up to three or four inoculations.

The performance of wild *Lactuca* species has been disappointing. With some exceptions, plants of wild *Lactuca* species responded with a severe necrotic reaction, followed by premature death. R. Provvidenti found only one species that may offer valuable resistance. Lines of *L. saligna* PI 491208 (Greece) and Acc. 3739 (Israel) yielded a few resistant plants that were confirmed in progeny tests from single plant selections. A few plants of *L. altaica*, *L. capensis*, *L. dregeana*, and *L. quercina* were also found to be

resistant, but further evaluation is needed.

Cross-protection against severe strains of TSWV. A mild nitrous acid-induced mutant of TSWV was selected for cross-protection against severe TSWV strains (M. Wang and D. Gonsalves, unpublished). It produced only mild mottle and necrotic specks on lettuce and did not affect plant size. In further tests, the virus was not evenly distributed among the leaves, and this would probably result in uneven protection of the infected plant. The effects of the strain on other plants are being investigated.

Management Strategies

As a result of knowledge gained during 1984-1985 on crop, virus, virus source plants, and insect vectors, we identified several cultural practices that would help minimize TSWV disease occurrence. Although none was sufficiently effective alone, integrating these practices for significant reduction of disease losses appeared possible.

Pilot program. In early 1986, a TSWV management program was attempted at Waimea, Hawaii. Useful tactics were grouped in relation to crop cycle phases.

Precrop phase. 1) Crop rotation with nonsusceptible crops to reduce buildup of inoculum source. 2) Crop placement to avoid planting TSWV-susceptible crops adjacent to each other. 3) Control of alternate TSWV vector hosts.

Crop phase. 1) Use of virus-free seedlings. 2) Regular applications of insecticides to propagated seedling areas

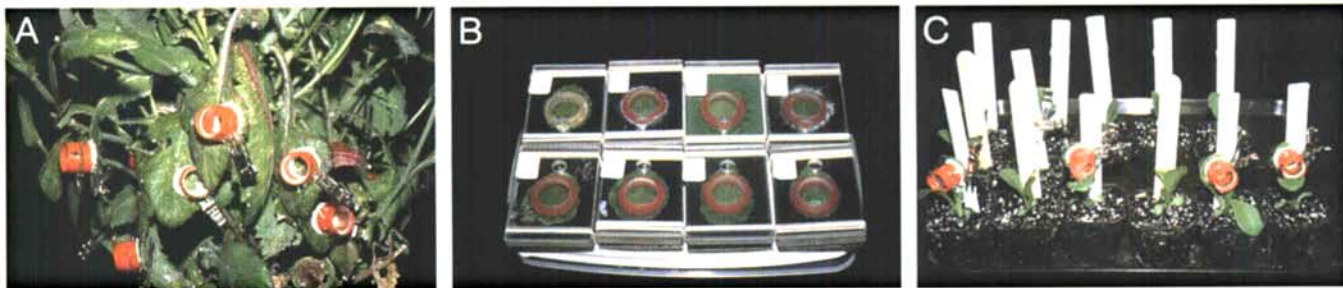


Fig. 9. Cages used in transmission studies to confine thrips on plants: (A) Larval thrips are confined with clip-on cages on an infected *Emilia sonchifolia* plant for 2-3 days to allow acquisition of tomato spotted wilt virus. (B) After acquisition, thrips are held in modified Tashiro cages and allowed to feed on detached cabbage leaves (or other plant food sources) and mature into adults. (C) Late-stage adult thrips are confined singly or in groups with clip-on cages on 1-month-old lettuce seedlings to allow transmission of the virus.

and field plantings (none of the registered insecticides can control or prevent epidemics). 3) Reduced in-field cultivation to avoid movement of thrips from infected sources.

Postharvest phase. 1) Fallow (3-4 weeks) field areas with high disease incidence to allow thrips to emerge from infected crop debris and disperse from the field. 2) Soil fumigation with metham-sodium (Vapam) or 1,3-dichloropropene (Telone) to eliminate thrips associated with crop debris.

Two tactics seemed valuable for reducing crop losses. Crop placement was used successfully by growers on several occasions to reduce losses caused by TSWV from 60% to less than 10%. In one locality, a grower was able to utilize a field that was isolated from the disease epidemic. Although the field was no more than 200 m from one where disease incidence was over 60%, he was able to grow several complete crops with less than 10% incidence of TSWV. This field had no TSWV-infected plants in the vicinity before planting and was isolated from infectious thrips by the grower's home and several rows of trees. The grower also successfully utilized the precrop tactic in another locality by planting crops after nearby growers had harvested and plowed the crop residues and the thrips numbers had decreased. Although plants in the area became infected, the field was isolated from infectious thrips by pastures, less susceptible TSWV hosts, and homes. At least two other growers have successfully reduced losses caused by TSWV from 60-65% to 10% by reducing cultivation.

Our conclusions. From the Waimea experience we drew the following conclusions:

1) Management is not totally effective if virus and vector occurrence is high throughout the area. During these periods, it makes little sense to continue planting susceptible crops. Instead, growers should conserve their capital.

2) Because of substantial movement of thrips between crops within and between farms, areawide cooperation of growers is essential. Growers must control

alternate hosts of the virus and thrips, use virus-free seedlings, avoid sequential planting, and plow harvested or abandoned crops immediately.

3) Crop placement helps reduce the incidence of TSWV. The most successful practice at Waimea was to plant fields with low incidence of TSWV. Another successful practice was to separate lettuce blocks with blocks of nonsusceptible crops such as broccoli and cauliflower.

Current Research

Molecular biology. Recombinant DNA techniques are being used to determine how the tripartite genome of TSWV is structured and organized. The information obtained will contribute to understanding the molecular basis of viral infection and also be useful in developing improved diagnostic capabilities and in genetically engineering disease-resistant plants.

Because of difficulties in obtaining purified virus free from contaminating host materials, a screening procedure for differentiating complementary DNAs (cDNAs) made to the virus rather than to host material had to be developed. In the procedure, cDNAs obtained from partially purified viral RNAs were cloned in the bacterial plasmid pBR322, then screened against cDNA prepared from partially purified virus and compared with uninfected "mock"-inoculated plant preparations to select clones unique to plants infected with TSWV. Virus-specific clones could then be identified from dot and northern blot hybridization analyses (D. J. Rice and T. L. German, unpublished). From the library of clones, 20 are being used to derive sequence information, do northern blot analysis of infected tissues, and determine the organization and structure of the viral genome.

By dot blot analysis, several cloned cDNAs have been identified that can detect four biological strains of TSWV in high dilutions of infected plant material from nine different plant species (T. L. German and D. J. Rice, unpublished). The probes have been used to detect

TSWV infection of a previous unreported host, *Anthurium andraeanum* Lind. Moreover, the probes have proved to be especially valuable for detection of low titers of TSWV in hosts (e.g., pepper, Fig. 8) that give high background readings with ELISA but not with the dot blot test. These probes will be a useful addition to techniques available for screening for the presence of virus.

In addition, cDNAs should be useful for evaluating the taxonomic position of TSWV. TSWV is the only membrane-bound plant virus containing RNA reported to have positive polarity. The protein composition and the noninfectious nature of its RNA components have led to speculation that TSWV is closely related to the Bunyaviridae that infect both arthropods and vertebrates (16,17,24). This question can be addressed by determining the polarity of the viral RNA with strand-specific probes and investigating the transcriptional and translational mechanisms. TSWV is the only virus transmitted by thrips. We are interested in examining this unique relationship and determining whether the virus replicates in the vector by searching for negative strand RNA.

Monoclonal antibodies (MCAs). The existence of strains of varying degrees of virulence has been a problem in developing resistant cultivars of tomato and lettuce. Five or six strains of TSWV have been reported based on differential host-plant responses (2). We have characterized two additional strains in our isolate collection. MCAs could be a sensitive and rapid means of facilitating genetic breeding programs and also could be useful in epidemiological studies that trace and identify origins of TSWV infections.

MCAs have been produced against a mixture of several TSWV isolates. MCAs were found to differentiate between two TSWV strain groups that can be separated biologically on the basis of symptom expression on certain host plants. We feel further efforts to isolate additional MCAs are justified.

Virus-vector relationship. Although thrips have been recognized as vectors of

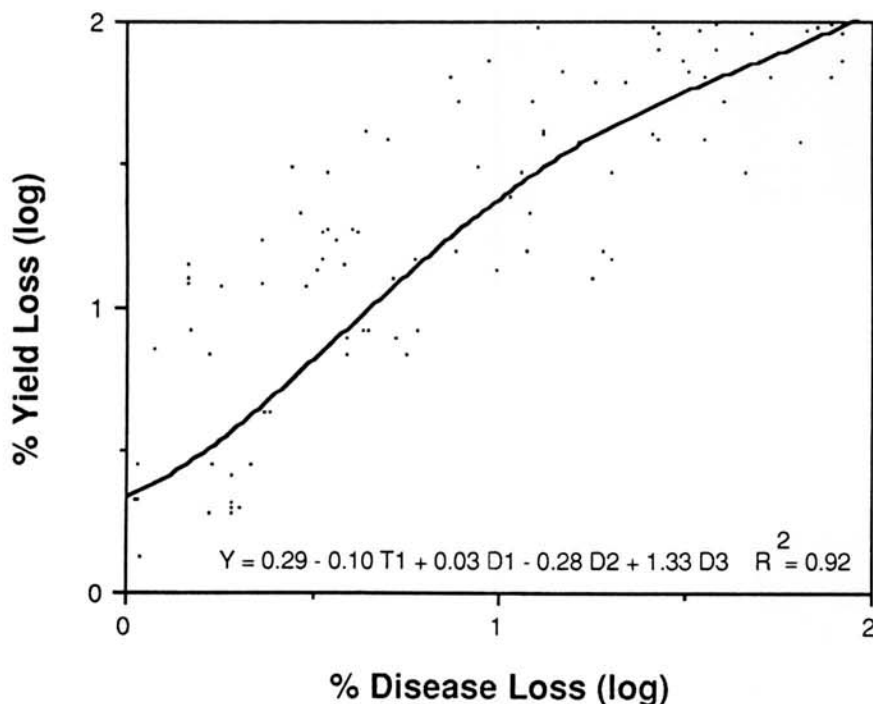


Fig. 10. Predictive model of lettuce yield loss in relation to tomato spotted wilt virus disease incidence. The model is based on assessment of thrips populations 1 week (T1) and disease incidence 1 week (D1), 2 weeks (D2), and 3 weeks (D3) after crop transplanting. This model explained 92% of the total variation in yield loss due to the disease.

TSWV for more than 50 years (20), the virus-vector interaction is not fully understood. Sakimura (21) discussed much of what was known of the virus-vector relationships and suggested that TSWV was similar to aphid-borne persistent viruses.

Research is under way to clarify the mechanisms underlying vector specificity, virus persistence, and replication in thrips tissues (Fig. 9). Using ELISA to assay individual thrips, we obtained indirect evidence of virus multiplication within the vector. Transstadial passage of the virus after acquisition was investigated after high postacquisition titers in larval thrips. Virus titers decreased during the pupal stage and in recently emerged adults but increased in 7- to 10-day-old adults. Further comparative tests with cDNA probes and tests for transovarial passage of the virus are being conducted to obtain direct evidence of TSWV multiplication in the vector.

ELISA of thrips has been useful in studying other aspects of the virus-vector association. Best (2) and Paliwal (19) noted that transmissibility by thrips can be substantially reduced by long-term passage of the virus by mechanical inoculation. We have found that although transmissibility by thrips is reduced, TSWV is acquired by the larvae, transstadially passed, and readily detected by ELISA in adult thrips. Obviously, continuous passage by mechanical inoculation does not affect

acquisition but in some way affects the thrips ability to transmit the virus.

Disease forecasting. A forecasting model would be an important tool for management of TSWV because effective control measures are unavailable. Yudin (25) generated such a model for early prediction of TSWV disease incidence in lettuce at crop maturity. Early disease incidence and thrips populations were found to be good predictors of disease losses (Fig. 10). By combining disease forecasting with economic data, yield loss and expected profits can be estimated.

A computer program has been developed from the economic and prediction model. This program allows growers to scout their farms to determine disease incidence and thrips numbers, enter these data, and obtain information to make economic decisions about their crops. We believe this program is flexible and can be adapted to predict the incidence of TSWV in other crops.

ELISA is being developed for detection of vector adults to assist in predicting epidemics. We initially found that ELISA detected TSWV in viruliferous adult vectors as well as nonviruliferous adults that had just fed on infected hosts. Experiments with two vectors, *F. occidentalis* and *F. schultzei*, showed that viruliferous *F. occidentalis* can be identified by allowing adults to feed on healthy plants for a minimum of 2 days before ELISA. Some thrips, however,

tested positive for the virus even after 8 days of feeding on healthy plants.

An Overview

TSWV has become an increasingly important factor contributing to economic losses in many agronomic and ornamental crops throughout North America. Why the disease has become more prominent is not clear, but it has been suggested that the establishment of western flower thrips populations east of the Rocky Mountains, development of insecticide resistance by these thrips, and movement of infected plant materials have contributed to the problem.

The disease has become a major threat to peanut, pepper, tobacco, and tomato, especially in the southern United States. Initially confined to Louisiana, Mississippi, Alabama, and Arkansas, the disease has spread to Oklahoma, the Florida panhandle, Tennessee, Kentucky, and Georgia. Now that this disease is well established in the field, it would be prudent to begin studies of its importance in some of the major crops known to be TSWV-susceptible, including soybean, dry bean, potato, and other vegetable crops.

Outbreaks of TSWV have occurred in greenhouse culture and affected many ornamental crops. The disease has been particularly devastating in California on chrysanthemums and has caused severe losses in other flowering ornamentals in North Carolina, Ohio, New York, and Ontario, Canada. Because of the potential threat to the ornamentals industry, the American Floral Endowment sponsored a symposium hosted by Yoder Brothers, Inc., on TSWV and the western flower thrips in June 1987. The symposium was successful in providing a multidisciplinary meeting for research, extension, and industry to discuss the problem and develop research priorities for the ornamentals industry. In March 1989, the TSWV symposium was expanded to include discussions on all crops affected by the disease. The symposium was held in Honolulu and hosted by the University of Hawaii.

The multidisciplinary approach has resulted in relatively rapid progress on a difficult research problem. Progress made on a broad range of subordinate projects validates the approach taken. The same approach—analysis of the problem, identification of major and subordinate objectives, identification and selection of a multidisciplinary group, semiannual reporting of progress—could be used selectively to investigate other viral diseases vectored by insects.

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