Occurrence and Distribution of Cucurbit Viruses in the Hawaiian Islands

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ABSTRACT

Ullman, D. E., Cho, J. J., and German, T. L. 1991. Occurrence and distribution of cucurbit viruses in the Hawaiian Islands. Plant Dis. 75:367-370.

Zucchini yellow mosaic virus (ZYMV), an isolate of papaya ringspot virus infecting watermelon (PRSV-W), and cucumber mosaic virus (CMV) were found infecting commercially grown cucurbits on the Hawaiian islands of Oahu, Maui, and Molokai during 1988 and 1989. Although previously reported in the Hawaiian Islands, watermelon mosaic virus 2 (WMV-2) was not found. Composition of virus epidemics varied widely among the three islands, even though all three viruses, alternate weed hosts, and aphid vectors are present throughout the state. Twenty-eight weed species were sampled. CMV, PRSV-W, and ZYMV infections were found in three species of the family Cucurbitaceae: *Mormordica charantia* (weedy form of bittermelon), *Cucumis dipsaceus* (wild cucumber), and *Lagenaria siceraria* (bottle gourd). Although many of the weed species tested did not appear to serve as alternative virus hosts, aphid surveys demonstrated that many of the same weed species are alternate hosts for at least five important aphid vector species, including *Aphis gossypii* and *Myzus persicae*. This information has implications for control of CMV, PRSV-W, and ZYMV in commercially grown cucurbits.

Commercial production of cucurbits, specifically, cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and summer squash (*Cucurbita pepo* L.), represents an important agricultural industry in the Hawaiian Islands, grossing nearly $3 million per year. Cucurbits are grown predominantly on the islands of Oahu, Maui, and Molokai, with watermelon accounting for 62% of the approximately 14 million pounds harvested each year and cucumbers and squash accounting for 30 and 8%, respectively. In the University of Hawaii's 1987 *Industry Analysis for Solanaceous and Cucurbit Crops* (K. Takeda, *unpublished*), cucurbit growers cite aphid-transmitted viruses as one of their most important problems, some reporting crop losses of 100% in squash and cucumber and of 10–100% in watermelon.

Only three viruses infecting commercially grown cucurbits—an isolate of papaya ringspot virus infecting watermelon (PRSV-W), watermelon mosaic virus 2 (WMV-2), and cucumber mosaic virus (CMV)—have been reported previously in the state of Hawaii (12). During 1987, virus epidemics in cucurbits, particularly in zucchini squash on the island of Maui, were severe and symptoms not typical of the previously reported viruses were observed. Zucchini plants showed severe stunting, prominent yellowing, mosaic, and foliar distortion. Most of the zucchini on infected plants were reduced in size, severely distorted, and covered with numerous round knobs. Similarity of these symptoms to those ascribed to zucchini yellow mosaic virus (ZYMV) (11) led us to hypothesize that ZYMV was present in the Hawaiian Islands.

ZYMV was first reported in Italy and France in 1981 as a destructive pathogen of cucurbits (7,8). Since that time, ZYMV has caused severe epidemics in cucurbit crops in Germany, Israel, Morocco, Spain, and many locations in

Journal Series No. 3480 of the Hawaii Institute of Tropical Agriculture and Human Resources.

Accepted for publication 26 September 1990 (submitted for electronic processing).

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the continental United States (5,10,11). Because of its aggressiveness and virulence, ZYMV is considered a serious threat to cucurbit production worldwide (5).

This investigation was undertaken to assess the current composition of virus epidemics in Hawaiian cucurbit crops. More specifically, our objectives were: 1) to determine the possible presence of ZYMV as a newly introduced virus, 2) to determine the incidence and relative importance of viruses causing disease epidemics and yield losses in cucurbit crops, 3) to establish the incidence of the viruses alone and in mixed infections, and 4) to determine if common weed species serve as alternate plant hosts for viruses infecting commercial cucurbits, as well as for their aphid vector species. This information is a critical first step toward formulation of improved strategies of control aimed at reducing economic losses due to virus diseases in cucurbits.

MATERIALS AND METHODS

Identification of virus isolates with serology. Leaves and fruit of cucurbit crop plants (zucchini, cucumber, and watermelon) were collected randomly from farmers' fields with known virus epidemics on the islands of Oahu, Maui, and Molokai during 1988 and 1989. In addition, 194 samples representing 28 weed species were collected on these three islands. When they could be found, weed species showing possible virus symptoms were collected; otherwise, weeds were sampled randomly in the vicinity of infected commercial cucurbit crops. All specimens were tested for the presence of ZYMV, PRSV-W, WMV-2, and CMV using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method (1,2). Antisera for CMV and ZYMV (Florida isolate, ZYMV-FL) were kindly provided by D. Gonsalves of Cornell University; for WMV-2, by H. A. Scott of the University of Arkansas; and for PRSV-W, by J. W. Moyer of North Carolina State University.

U-bottom Immulon II micro-ELISA plates (Dynatech, Alexandria, VA) were coated separately with 200 μl of each immunoglobulin (1 μg/ml). Alkaline phosphatase-conjugated immunoglobulins were cross-absorbed with healthy *C. pepo* leaf tissue extract (1:20, w/v) for 15 min at room temperature (25–27 C) and used at 1/6,000 dilution for ZYMV-FL, 1/2,000 dilution for WMV-2, and 1/1,000 dilution for CMV and PRSV-W. Negative controls consisted of six wells containing ELISA extraction buffer (2) and three wells containing healthy *C. pepo* leaf tissue extracts. Positive controls consisted of single wells containing *C. pepo* leaf tissue extracts from plants infected separately with each of the four viruses tested.

ELISA reactions were measured spectrophotometrically at 405 nm using an EL307B EIA reader (Bio-Tek Instruments, Winooski, VT). A sample was considered virus-positive if the A_{405nm} values were greater than twice the mean healthy plant sap controls and/or average buffer, whichever was higher. Positive readings were confirmed by visual observations.

Aphid colonization of weed species. All samples of weed species were examined for the presence of apterous (nonwinged) aphids. The presence of apterae indicates colonization by an aphid species, but we were unable to key apterae to species. Therefore, samples with apterae were placed in plastic bags and held until alate (winged) aphids were produced. These alate aphids were then collected, placed in alcohol, and identified using techniques of Irwin (4) adapted for Hawaiian species (D. E. Ullman, J. J. Cho, and R. Ebesu, *unpublished*).

Virus isolates and diagnostic species. Virus isolates used as positive controls in ELISA were obtained from R. Provvidenti of Cornell University and H. A. Scott of the University of Arkansas. To ensure and maintain the purity of each virus isolate, all isolates were sap-inoculated to the six diagnostic plant species listed in Table 1. Each isolate was then maintained separately by periodic subculturing with sap inoculation to different plant hosts. PRSV-W and ZYMV-FL were maintained on the zucchini cultivar Ambassador, CMV on *Luffa acutangula* (L.) Roxb., and WMV-2 on the bean (*Phaseolus vulgaris* L.) cultivar Black Turtle 2. To ensure the accuracy of ELISA results, subsamples of field-collected virus isolates were also sap-inoculated to the aforementioned diagnostic plant species. Sap inoculations were done by triturating individual samples with 0.01 M potassium phosphate buffer containing 0.01 M sodium sulfite (pH 7.0) and rubbing the extracts on Carborundum-dusted (360 mesh) leaves.

Electron microscopy. Identities of virus isolates were further confirmed by viewing virus particles with transmission electron microscopy. Expressed sap from infected leaves was stained with 2% phosphotungstic acid (pH 6.8) and examined with a Hitachi H-8 transmission electron microscope.

RESULTS

Virus distribution in cucurbit crops and weed species. Our results show that the composition of virus epidemics in crops varied widely among the three islands (Table 2). On Oahu, PRSV-W and ZYMV were present in zucchini, watermelon, and cucumber, whereas CMV was found only in cucumber. PRSV-W predominated in Oahu epidemics, with 60% of the samples ELISA-positive for this virus alone. Single infections of ZYMV were detected in only 3% of the plants tested, and CMV was found in less than 1% of the cucumber plants tested. Mixed infections of PRSV-W and ZYMV were found in 17% of the plants tested. In contrast, on Maui ZYMV predominated, representing 67% of the single infections detected. Single infections of PRSV-W were found in just 14% of the plants tested. Again, a relatively small number of mixed infections of ZYMV and PRSV-W was noted (14% of the plants tested). On Molokai, ZYMV was present in 97% of the positive plant samples and PRSV-W, in only 3%. CMV and mixed infections of ZYMV and PRSV-W were not detected.

The data in Table 2, based on random samples, suggest that commercial zucchini fields on Maui reached 95% infection, while less than 20% of nearby cucumber fields were infected. Epidemics appeared to be less severe on Molokai, with rates of infection less than 65 and 40% in commercial zucchini and watermelon fields, respectively. On Oahu, where epidemics were dominated by PRSV-W, more than 70% of the commercial cucumber was infected. As on Maui, zucchini was severely infected (95%). Watermelon on Oahu was not sampled.

CMV, PRSV-W, and ZYMV infections were found in three species of the family Cucurbitaceae: *Mormordica charantia* L. (weedy form of bittermelon), *Cucumis dipsaceus* Ehrenb. ex Spach (wild cucumber), and *Lagenaria siceraria* (Molina) Standl. (bottle gourd). Among the 42 samples tested from these three species, 69% tested positive for one

Table 1. Reactions of six diagnostic species to four viruses infecting cucurbits

	Reactions of viruses[a]			
Species	CMV	PRSV-W	WMV-2	ZYMV
Chenopodium quinoa	L	L	L	L
Cucumis melo cv. Bush Star	SM	SM	SM	SM
C. metuliferus PI 292190	SM	NR	SM	L,SM,D
Luffa acutangula	SM	SM	NR	L,SM
Nicotiana benthamiana	SM	NR	NR	NR
Phaseolus vulgaris cv. Black Turtle 2	L	NR	SM	L

[a]CMV = cucumber mosaic virus, PRSV-W = an isolate of papaya ringspot virus infecting watermelon, WMV-2 = watermelon mosaic virus 2, ZYMV = zucchini yellow mosaic virus. L = local infection, SM = systemic mottle, D = leaf distortion, NR = no reaction.

or more of the viruses found in crop plants, i.e., CMV, PRSV-W, and ZYMV. CMV was found in bittermelon on Oahu and Maui, PRSV-W and ZYMV were found in bittermelon and bottle gourd on Oahu and Maui, while only ZYMV was detected in wild cucumber on Maui. The cucurbit viruses were not detected in 25 other weed species common to the vegetable-growing areas sampled: *Abutilon grandifolium* (Willd.) Sweet, one plant tested; *Amaranthus hybridus* L., 15 plants; *A. spinosus* L. 15 plants; *A. viridis* L., one plant; *Bidens pilosa* L., eight plants; *Chenopodium album* L., six plants; *C. carinatum* R. Br., one plant; *Coronopus didymus* (L.) Sm., two plants; *Datura stramonium* L., one plant; *Digitaria violascens* Link, one plant; *Foeniculum vulgare* Mill., four plants; *Galinsoga parviflora* Cav., four plants; *Ipomoea congesta* R. Br., three plants; *Malva parviflora* L., 18 plants; *Melodinus officianalis* L., three plants; *Nicandra physalodes* (L.) Gaertn., five plants; *Panicum glaucum* L., one plant; *Passiflora* sp., one plant; *Portulaca oleracea* L., two plants; *Ricinus communis* L., three plants; *Saccharum officinarum* L., one plant; *Solanum nigrum* L., six plants;, *Sonchus oleraceus* L., 26 plants; *Verbecena enceloides* Cav., one plant; and *Xanthium saccharatum* Wallr., two plants.

Aphid colonization of weed species. Although many of the weed species sampled did not appear to serve as alternative virus hosts, aphid surveys demonstrated that some, i.e., *Amaranthus hybridus, A. spinosus, B. pilosa, G. parviflora, Panicum glaucum, M. parviflora, Portulaca oleracea*, and *N. physalodes*, serve as alternate hosts for at least six aphid vector species: *Aphis gossypii, Brachycaudus helichrysi, Hyperomyzus lactucae, Macrosiphum euphorbiae, Myzus persicae*, and *Rhopalosiphum maidis*.

Serological parameters. Table 3 shows typical absorbance (A_{405nm}) values from zucchini samples tested for PRSV-W, WMV-2, ZYMV, and CMV. Mean absorbance values for healthy plant tissue or plant tissue negative for a particular virus were consistently 0.15 or less. Mean absorbance values for PRSV-W, ZYMV, and PRSV-W and ZYMV mixed infections were consistently more than seven times higher than those for the healthy control. The antisera used were very specific for each of the four viruses, as indicated by mean absorbance readings similar to those for the healthy controls. Typical virus particles were visualized in infected controls and absent in healthy controls when viewed with the electron microscope.

Identification of virus isolates with diagnostic plants. Sap inoculations of diagnostic plant species with virus isolates used as positive controls induced the plant responses summarized in Table 1. The plant responses we observed are representative of those reported for similar diagnostic plant ranges used for CMV, PRSV-W, WMV-2, and ZYMV (11). These results and visualization of typical virus particles by electron microscopy verify purity of the virus isolates used as positive controls in ELISA, as well as representative ELISA-positive field samples.

DISCUSSION

Our results show that the composition of nonpersistent virus epidemics in commercially grown cucurbits in Hawaii has changed dramatically in the 20 yr since the last survey, when PRSV-W, WMV-2, and CMV were all widespread (12). Our survey suggests that WMV-2 is no longer present in our cucurbit crops and that CMV is seldom found. ZYMV, reported here for the first time in the Hawaiian Islands, is widespread, causing more than 60% of the infections seen in cucurbits on the islands of Maui and Molokai. ZYMV is far less common on the island of Oahu, where PRSV-W now predominates. The possible factors influencing this shift in virus incidence are many and varied, including variation in alternate host plant populations, presence and efficiency of aphid vectors, and virus interactions in infected plants (3). Exactly why WMV-2 is currently absent and why the islands vary in epidemic composition is somewhat of a dilemma. Certainly, the aphid vectors for all four of these cucurbit viruses are abundant, landing in cucurbit crops and colonizing many common weed species (D. E. Ullman, J. J. Cho, and R. Ebesu, *unpublished*). Weed species acting as alternate hosts to the cucurbit viruses are also present in all surveyed locations. Indeed, the continuous cropping system common to all the Hawaiian islands and year-round presence of alternate plant hosts for viruses and aphid vectors provide conditions that should favor epidemics of all the cucurbit viruses. The shift in epidemic composition that we report herein is likely linked to as yet unknown complex interactions occurring within the virus/plant/aphid triad.

Similar shifts in cucurbit virus incidences have been reported in California, where PRSV-W and CMV were absent from large surveys in 1981 after having been reported in several earlier surveys (9). ZYMV is aggressive and highly virulent and because of its aphid transmission properties may have important epidemiological advantages (3,6). When inoculations are done from mixed infections of ZYMV and PRSV-W or WMV-2, ZYMV is more often transmitted (3). In addition, helper component of other cucurbit viruses has been shown to support more efficient transmission of ZYMV than the homologous virus (6). In light of these factors, one may speculate that ZYMV

Table 2. Distribution of cucurbit viruses infecting zucchini, watermelon, and cucumber on three Hawaiian islands during 1988–1989 as identified by double antibody sandwich enzyme-linked immunosorbent assay

Island	Collection date	Crop	No. of samples tested	No. of negative samples	No. of samples infected with viruses[a] CMV	PRSV-W	WMV-2	ZYMV	WMV-2 + ZYMV
Maui	7 Dec.1988	Zucchini	39	1	0	9	0	19	10
	14 Dec. 1988	Zucchini	30	0	0	1	0	28	1
	21 Dec. 1988	Cucumber	30	6	0	0	0	22	2
	29 Dec. 1988	Zucchini	35	4	0	4	0	21	6
Molokai	13 Sept. 1988	Watermelon	12[b]	4	0	0	0	8	0
	5 Jan. 1989	Zucchini	35	12	0	0	0	23	0
	12 May 1989	Watermelon	22	13	0	2	0	7	0
Oahu	9 Dec. 1988	Zucchini	43	2	0	33	0	0	8
	9 Dec. 1988	Watermelon	10	9	0	0	0	0	1
	11 Dec. 1988	Cucumber	6	1	0	2	0	2	1
	11 May 1989	Watermelon	5[b]	0	0	5	0	0	0
	11 May 1989	Cucumber	23	7	0	8	0	6	2
	23 Nov. 1989	Cucumber	6	1	0	2	0	2	1
Total			296	60	0	66	0	138	32

[a]CMV = cucumber mosaic virus, PRSV-W = an isolate of papaya ringspot virus infecting watermelon, WMV-2 = watermelon mosaic virus 2, ZYMV = zucchini yellow mosaic virus.
[b]Symptomatic tissue rather than random samples.

Table 3. Mean absorbance values of zucchini samples tested by double antibody sandwich enzyme-linked immunosorbent assay for four viruses infecting cucurbits

Antisera[a]	Absorbance at 405 nm: Healthy ($n=3$)	PRSV-W ($n=4$)	ZYMV ($n=59$)	WMV-2 + ZYMV ($n=6$)
CMV	0.11	0.11	0.11	0.11
PRSV-W	0.11	2.26	0.14	0.77
WMV-2	0.10	0.11	0.12	0.13
ZYMV	0.11	0.14	0.92	1.33

[a]CMV = cucumber mosaic virus, PRSV-W= an isolate of papaya ringspot virus infecting watermelon, WMV-2 = watermelon mosaic virus 2, ZYMV = zucchini yellow mosaic virus.

predominates on Maui and Molokai because it may be competitively excluding the other cucurbit viruses. The relatively low incidence of ZYMV on Oahu suggests that the virus was very recently introduced to the island. If this is the case, our continuing surveys should indicate an increase in ZYMV incidence and a decrease in PRSV-W incidence.

The dynamics of mosaic virus epidemics in Hawaiian cucurbit crops make it clear that a sound basis in understanding virus interactions, virus reservoirs, and aphid transmission efficiency under tropical conditions will be critical to development of effective control strategies. Clearly, control of bittermelon, bottle gourd, and wild cucumber in the vicinity of cucurbit crops is important in reducing virus inoculum. In addition, control of other weed species that support aphid vector populations should help to reduce aphid presence in commercial production areas. Our data also demonstrate that under tropical conditions with continuous cropping systems, cucurbit crops themselves represent a significant source of virus inoculum and an important aphid vector species, the melon aphid. A large amount of virus inoculum would be eliminated if growers quickly removed plants from infected fields after harvest. Unlike in more temperate climates, crop-free periods are seldom feasible in the tropics, yet it is still possible to isolate cucurbit plantings spatially and temporally by alternating planting with nonsusceptible crops. Our future work will focus on the interaction of ZYMV with other cucurbit viruses and how these interactions influence aphid transmissibility, as well as the composition and severity of virus epidemics in Hawaiian cucurbits.

ACKNOWLEDGMENTS

We thank Diana Custer, Lance Kanemoto, and Aubrey Moore, for technical assistance, and Lee Yudin, Randy Hamasaki, Alton Arakaki, and Glenn Teves, for sample collection. Investigations were supported in part by the State of Hawaii Governor's Agricultural Coordinating Committee (contract 87-9).

LITERATURE CITED

1. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
2. Cho, J. J., Mau, R. F. L., Gonsalves, D., and Mitchell, W. C. 1986. Reservoir weed hosts of tomato spotted wilt virus. Plant Dis. 70:1014-1017.
3. Davis, R. F., and Mizuki, M. K. 1987. Detection of cucurbit viruses in New Jersey. Plant Dis. 71:40-44.
4. Irwin, M. E. 1980. Sampling aphids in soybean fields. Pages 239-259 in: Sampling Methods in Soybean Entomology. M. Kogan and D. C. Herzog, eds. Springer-Verlag, New York.
5. Lecoq, H., Lisa, V., and Dellavalle, G. 1983. Serological identity of muskmelon yellow stunt and zucchini yellow mosaic viruses. Plant Dis. 67:824-825.
6. Lecoq, H., and Pitrat, M. 1985. Specificity of the helper-component-mediated aphid transmission of three potyviruses infecting muskmelon. Phytopathology 75:890-893.
7. Lecoq, H., Pitrat, M., and Clement, M. 1981. Identification et caracterisation d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. Agronomie 1:827-834.
8. Lisa, V., Boccardo, G., D'Agostino, G., Dellavalle, G., and d'Aquilio, M. 1981. Characterization of a potyvirus that causes zucchini yellow mosaic. Phytopathology 71:667-672.
9. Nameth, S. T., Dodds, J. A., Paulus, A. O., and Laemmlen, F. F. 1986. Cucurbit viruses of California: An ever-changing problem. Plant Dis. 70:8-12.
10. Provvidenti, R., Gonsalves, D., and Humaydan, H. S. 1983. The occurrence of zucchini yellow mosaic virus in the United States. Cucurb. Genet. Coop. 6:99.
11. Provvidenti, R., Gonsalves, D., and Humaydan, H. S. 1984. Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida, and California. Plant Dis. 68:443-446.
12. Shanmugasundaram, S., Ishii, M., Gilbert, J. C., and Nagai, H. 1969. Cucurbit virus studies in Hawaii. Plant Dis. Rep. 53:70-74.