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IMMUNODIFFUSION TESTS FOR SIX VIRUSES THAT INFECT CUCURBITS IN FLORIDA

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Abstract. **Leaves that exhibited chlorotic flecking were collected from a plant of *Trichosanthes dioica* in Dade County in April, 1986. An extract from the leaves was tested by sodium dodecyl sulfate immunodiffusion tests against antisera to each of 5 viruses previously identified in cucurbits in Florida: papaya ringspot virus type W (PRSV-W), watermelon mosaic virus-2 (WMV-2), zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), and squash mosaic virus (SMV). The extract did not react with any of the antisera. A sample of the leaves from *Trichosanthes* was ground in buffer and rubbed on zucchini squash (*Cucurbita pepo* L.) plants in the greenhouse. The zucchini squash plants developed vein clearing and mild mosaic symptoms in systemically infected leaves. A filamentous virus, designated Trichosanthes virus (TV), was associated with the infection. An antiserum was prepared to TV and serological tests confirmed it was unrelated to PRSV-W, WMV-2, ZYMV, CMV, and SMV. In 1987 and 1988, antisera to each of 6 viruses were used for serodiagnoses of cucurbit samples, which represented different crops, seasons, and locations in Florida. These samples were either collected in disease surveys or were submitted for diagnosis. Of 549 samples, 245 reacted with PRSV-W antiserum, 204 with WMV-2 antiserum, 51 with ZYMV antiserum, 13 with CMV antiserum, none with SMV antiserum, and 22 with TV antiserum. The TV was detected in *Trichosanthes dioica* Roxb. and *Coccinea grandis* (L.) Voigt in Dade County, but not in squash from Dade County, nor in cucurbit samples collected elsewhere in the state. The cucurbit potyviruses, PRSV-W, WMV-2, and ZYMV, continue to be important pathogens in the mosaic disease complex of cucurbits in Florida.**

Viruses that induce mosaics, distortion, and reduced yields have been problems in the production of cucurbits in Florida (1, 2, 3, 5, 6, 12, 14, 15) and in other parts of the world (10) for many years. In recent surveys (3, 12) three potyviruses emerged as important pathogens in Florida: papaya ringspot virus type W (formerly named watermelon mosaic virus-1) (13), watermelon mosaic virus-2, and zucchini yellow mosaic virus. The latter was discovered in Florida for the first time in 1981 (3, 12). At least two other mosaic viruses occur in Florida cucurbits, cucumber mosaic virus (5) and squash mosaic virus (6, 11).

In 1986, a virus distinct from the five viruses mentioned above was isolated from *Trichosanthes dioica* Roxb. in Dade County. During 1987 and 1988, cucurbit samples from various crops and locations in Florida were collected as part of another study on variation of cucurbit potyviruses. Serological assays were performed on these samples and on several samples submitted for diagnosis to determine if they were infected by any of the 6 viruses. This paper presents the results of those assays.

Materials and Methods

The viruses and the antisera used in this study are listed in Table 1. Virus cultures were maintained in 'Small Sugar Pumpkin' (*Cucurita pepo* L.) and were transferred periodically by mechanical transmission. Inocula were prepared by triturating leaf tissue in 0.02 M potassium phosphate, pH 7.5, with carborundum added as abrasive. Gauze pads dipped in the inocula were rubbed on the expanded cotyledonary leaves of cucurbitaceous test plants at a stage when the first true leaf was expanding.

Samples collected in the field usually consisted of leaves from plants that exhibited possible virus-like symptoms, i.e. mosaic, mottle, chlorosis, or distortion. Efforts were made to obtain samples representative of the range of symptoms, but the samples were usually not randomly selected. Sometimes leaves from nonsymptomatic plants also were collected and assayed. Occasionally, symptomatic fruit were tested, especially when they were submitted for diagnosis through the Florida Extension Plant Disease Clinic at Gainesville. The plant samples were bagged, refrigerated and tested within 1-7 days after collection.

For serological tests, the tissue samples were triturated in water (1 ml per g of tissue), 1 ml of 3% sodium dodecyl sulfate (SDS) was added, and the samples were expressed through cheesecloth. They were either tested on that day or frozen for a few days prior to assay. As previously described, immunodiffusion tests were conducted in Petri dishes in a medium consisting of 0.5% SDS, 1.0% sodium azide, and 0.8% Noble agar (11, 12, 14). Antigens were added to the 6 peripheral wells and undiluted antiserum was added to the central well of each 7-well pattern (Fig. 1). Each sample was tested at least once against each of the 6 antisera (Table 1). The plates were incubated at 25C and observed once or twice a day for up to 3 days. In all cases the test samples were placed adjacent to antigen samples of the homologous known antigens so that the extent of their relationship, if any, could be observed.

Throughout this study, representative samples were inoculated by mechanical means to pumpkin for confirmatory serological testing, for preservation of the viruses involved, and for further characterization.

Results and Discussion

Partial characterization of a virus isolated from Trichosanthes. In April 1986, leaves that exhibited chlorotic flecking were collected from a plant of *Trichosanthes dioica* grown commercially in Dade County. Extracts were rubbed on zucchini squash, which subsequently developed systemic veinal chlorosis and a mild mosaic. Extracts from plants infected with subcultures of this sample, which was designated Trichosanthes virus (TV), did not react serologically with PRSV-W, WMV-2, ZYMV, CMV, or SMV, but they did react strongly with homologous anti-

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Table 1. List of antisera used in assays for six viruses in naturally infected cucurbits.

Name of virus	Abbreviation	Source of virus	Antiserum prepared to	Antiserum number	Source of antiserum
Papaya ringspot virus type W (formerly watermelon mosaic virus-1	PRSV-W	Purcifull & Hiebert (14)	virus coat protein	1125	Baker & Purcifull (7)
Watermelon mosaic virus-2	WMV-2	Purcifull (unpublished)	virus coat protein virus	1117 1134	Purcifull & Hiebert (unpublished) Purcifull & Hiebert (unpublished)
Zucchini yellow mosaic virus	ZYMV	Purcifull et al. (12)	virus coat protein	1133	Purcifull, Zhao, & Hiebert (unpublished)
Cucumber mosaic virus	CMV	Kuwite & Purcifull (9)	detergent treated virus	965	Kuwite & Purcifull (9)
Squash mosaic virus	SMV	American type culture PV-36	detergent treated virus	876	Purcifull et al. (11)
Trichosanthes virus	TV	Purcifull & Simone, unpublished	detergent treated virus	1129	Purcifull, Hiebert, & Simone (unpublished)

serum in immunodiffusion (Fig. 1). These results indicated that the TV was a distinct virus and that the antiserum prepared to detergent-treated virus (Table 1) could be used for assaying field samples. The TV was transmitted mechanically to cantaloupe (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita pepo* L.), and watermelon [*Citrullus lanatus* (Thunb.) Matsumi & Nakai]. Serological assays of leaves from these plants indicated that cantaloupe, cucumber, squash, and watermelon were susceptible to TV. Accordingly, samples collected in disease surveys also were tested against antiserum to TV.

Specificity of the serological tests. In most cases, the immunodiffusion tests gave specific results and each of the viruses was readily distinguished by the reactions that were detected (Fig. 1). In the majority of cases, closely related viruses gave reactions of identity (no spur formation). However, some isolates related to PRSV-W (Baker and Purcifull, unpublished) showed reactions of partial identity (spur formation) even though they showed strong cross-reactivity. Likewise, there was evidence for antigenic variation in some of the isolates that reacted with CMV antiserum. For example, the precipitin line formed by isolate 2147 spurred slightly over the precipitin line formed by isolate 2148 (Purcifull and Jacono, unpublished data).

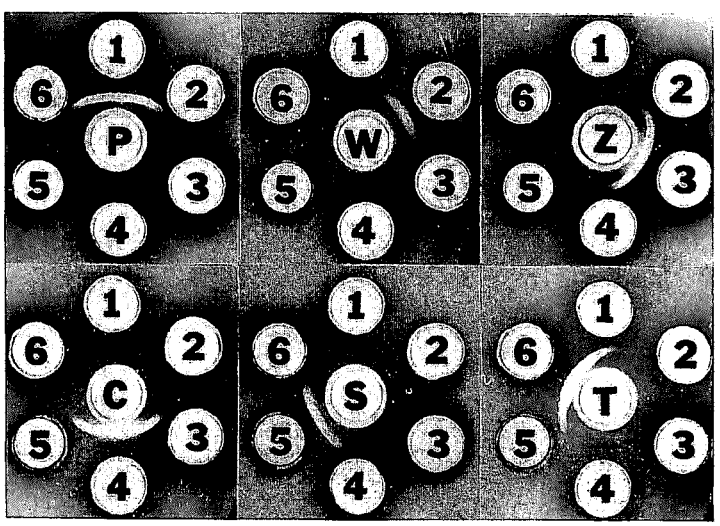

Fig. 1. Immunodiffusion test for serological detection of six viruses that infect cucurbits. The following undiluted antisera prepared to sodium dodecyl sulfate-treated immunogens (Table 1) were added to the central wells: P = papaya ringspot virus type W; W = watermelon mosaic virus-2; Z = zucchini yellow mosaic virus; C = cucumber mosaic virus; S = squash mosaic virus; and T = Trichosanthes virus. Sodium dodecyl sulfate treated leaf extracts from pumpkin plants infected with the following viruses were added to the outer wells: 1 = papaya ringspot virus type W; 2 = watermelon mosaic virus-2; 3 = zucchini yellow mosaic virus; 4 = cucumber mosaic virus; 5 = squash mosaic virus; and 6 = *Trichosanthes* virus.

As noted previously (3, 12), antigenic cross-reactivity of ZYMV with WMV-2 was sometimes observed, particularly with the ZYMV antiserum used in this study. ZYMV and WMV-2, however, could usually be distinguished readily by differences in the intensity of precipitin bands (homologous reactions were much stronger) and by the formation of strong spurs (e.g., where the ZYMV precipitin band spurred over the WMV-2 precipitin band when tested against ZYMV antiserum).

General Comments About Results of Serological Assays for Viruses in Cucurbit Samples. During the two year period of 1987-88, 549 cucurbit samples were collected in Florida and assayed by immunodiffusion tests for the presence of the 6 viruses. Samples from different cucurbit crops, different locations in the state, and different seasons were assayed (Tables 2, 3, 4). Overall, the percentages of samples that assayed positively were as follows (Table 2): PRSV-W (44.6%), WMV-2 (37.1%), ZYMV (9.2%), CMV (2.3%), SMV (0%), and TV (4.0%). The occurrences of the viruses varied in some instances depending on the crop (Table 2), on location in the state (Tables 3 and 4), and on the time of year that the samples were collected (Table 4). Samples that reacted with 2 different antisera were common and were determined to be the result of plants that were doubly infected. These samples included those that reacted with antisera to PRSV-W and WMV-2, WMV-2 and ZYMV, PRSV-W and ZYMV, WMV-2 and CMV, and PRSV-W and TV. Triply infected plants also were detected, including those that reacted with antisera to PRSV-W, ZYMV, and WMV-2, or one that reacted with antisera to PRSV-W, ZYMV, and CMV. All samples were tabulated individually for their reactivity to each virus antiserum.

The number of samples that did not react with any of the 6 antisera constituted 17.3% of the total (Table 2). These samples either were not infected with any of the 6

Table 2. Results of serological assays for six viruses in various cucurbit crops in Florida, 1987-1988.

Crop[y]	Number of samples assayed	Number of samples positive for[z]						
		PRSV-W	WMV-2	ZYMV	CMV	SMV	TV	N
Squash	305	177	89	27	6	0	0	39
Watermelon	130	34	89	15	0	0	0	17
Cucumber	39	19	2	7	3	0	0	16
Cantaloupe	36	4	23	2	3	0	0	8
Coccinea	15	11	0	0	0	0	14	1
Trichosanthes	11	0	0	0	0	0	8	3
Miscellaneous	13	0	1	0	1	0	0	11
Totals	549	245	204	51	13	0	22	95

[z]Data indicate the number of samples that were identified based on their reactivity to papaya ringspot virus type W (PRSV), watermelon mosaic virus-2 (WMV-2), zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), squash mosaic virus (SMV), and the Trichosanthes virus (TV). N = the number of samples that did not react with any of the six antisera.
[y]Squash primarily consisted of yellow summer squash or zucchini but includes all samples of the genus *Cucurbita*. The miscellaneous category includes unidentified *Cucumis* samples, (one reacted with WMV-2 antiserum and one with cucumber mosaic virus antiserum), and 4 samples each of *Luffa* and *Momordica* (all of which failed to react with any of the antisera).

viruses or the virus titer was not high enough to be detected in this test system. Most of these samples either had no characteristic virus-induced mosaics or distortion symptoms, or had symptoms that may have been due to other causes. There is a possibility that some samples were infected with viruses not detectable with the antisera used.

Comments about the Occurrence and Distribution of Specific Viruses. PRSV-W is one of the most prevalent viruses in the state (Tables 3 and 4). Although it occurs in South Florida in the spring and fall (Table 4), its occurrence in north and central Florida seems primarily to be in the fall crops. PRSV-W was detected in all the major cucurbit crops and in *Coccinea grandis*, but not in *Trichosanthes dioica* (Table 2). The pattern of occurrence of PRSV-W in Florida was similar to that reported previously by Adlerz and others (1, 2, 3).

WMV-2 is an important pathogen of squash, watermelon, and cantaloupe, and it also was detected in cucumber (Table 2). WMV-2 was detected in both north and central Florida in the spring and fall (Table 4). It was not detected at all in the south Florida counties of Collier, Dade, and Palm Beach. Adlerz and others obtained similar results with WMV-2 (1, 3). Interestingly, Webb and Scott (15) noted that only WMV-2 was detected in Collier County in 1962-63, but only WMV-1 was detected there in 1964-65.

In 1981, ZYMV was found for the first time in Florida (3, 12). Because it was a new virus, its severe effects on several cucurbits were the cause for concern. In the 1987-1988 seasons, ZYMV was detected in various parts of the state, but most of the ZYMV-infected cucurbits were detected in central Florida (Tables 3 and 4). Although ZYMV was detected in fewer samples than either PRSV-W or WMV-2, it is still regarded as an important pathogen in cucurbit production in Florida because of its severity, its occurrence in 9.2% of the cucurbits tested, and its distribution. The sources of inoculum of ZYMV in the state are not well known, although natural infection of *Melothria pendula* in central Florida has been reported (3).

The TV was detected only in *Trichosanthes dioica* and

Table 3. Results of serological assays for six viruses in cucurbits in Florida in 1987-1988, tabulated by county.

County	Region[y] of state	Number of samples assayed	Assay Results Number of samples positive for[z]:					
			PRSV-W	WMV-2	ZYMV	CMV	SMV	TV
Alachua	NF	79	27	30	1	4	0	0
Citrus	CF	8	0	8	0	0	0	0
Collier	SF	32	28	0	2	0	0	0
Dade	SF	104	73	0	1	0	0	22
Dixie	NF	8	0	8	0	0	0	0
Duval	NF	3	0	0	0	2	0	0
Gadsden	NF	13	2	9	0	0	0	0
Gilchrist	NF	8	1	8	0	0	0	0
Hillsborough	CF	5	4	0	5	1	0	0
Jackson	NF	38	3	17	0	0	0	0
Jefferson	NF	33	15	7	0	0	0	0
Lafayette	NF	1	0	1	0	0	0	0
Lake	CF	43	35	18	14	0	0	0
Levy	CF	18	1	17	0	0	0	0
Marion	CF	43	8	38	6	0	0	0
Palm Beach	SF	50	42	0	3	0	0	0
Pasco	CF	29	3	22	12	4	0	0
St. Lucie	CF	2	2	0	0	1	0	0
Sumter	CF	16	0	16	1	1	0	0
Suwannee	NF	7	1	3	2	0	0	0
Union	NF	9	0	2	4	0	0	0

[z]Number of samples that were identified based on their reaction with the designated antisera. See footnotes of Table 1 for explanation of abbreviations.
[y]Region of state: NF = Northern Florida; CF = Central Florida; SF = Southern Florida.

Table 4. Results of serological assays for six viruses in cucurbits tabulated by region and time of year.[z]

Region/time of year[x]	Number of samples assayed	Number of samples positive for[y]: PRSV-W	WMV-2	ZYMV	CMV	SMV	TV
NF/Jan.-July	97	3	52	0	6	0	0
NF/Aug.-Nov.	102	46	33	7	0	0	0
CF/Jan.-July	111	4	101	13	5	0	0
CF/Aug.-Nov.	53	49	18	25	2	0	0
SF/Jan.-July	112	83	0	4	0	0	19
SF/Aug.-Nov.	74	60	0	2	0	0	3

[z]Combined results of assays conducted in 1987 and 1988.
[y]Number of samples that were identified based on their reactivity with the designated antisera. See footnote of Table 1 for explanation of abbreviations.
[x]Region of state where samples were collected: NF = Northern Florida; CF = Central Florida; and SF = Southern Florida (see Table 3 for counties included in each region). Time of year indicates that samples were collected from January to July or from August to November.

Coccinea grandis (Table 2) in Dade Co. (Table 3), the only location where these 2 species were collected for assay. Although squash is susceptible to the TV, this virus was not detected in any of the more than 70 squash samples collected in Dade Co. The pathological effects of TV on *Coccinea* and *Trichosanthes* are unknown at this time.

Preliminary tests indicate that TV is filamentous and that it may be serologically related to at least one potexvirus (Purcifull, unpublished data). However, its relationships to other potexviruses that infect cucurbits have not yet been studied in detail.

Cucumber mosaic virus has occurred in cucurbitaceous crops in Florida for many years (5), and it has been detected in commelinaceous weeds in northern, central and southern Florida (2, 8). In our study, CMV was detected only in a small number of the cucurbit samples collected in north and central Florida (Table 4). It was not detected in any of the 186 cucurbit samples collected in south Florida.

Squash mosaic virus was not detected in this study, although it has been reported previously in cantaloupe in Florida (6, 11). The number of cantaloupe samples assayed during the 1987-1988 seasons, however, was not large (36 samples). Anderson reported more than 30 years ago that SMV (then called muskmelon mosaic virus) was uncommon in central Florida. Two cantaloupe isolates previously collected in Florida reacted serologically (11) with the same SMV antiserum used in the present study.

Mosaic virus diseases continue to constitute significant problems in the production of cucurbits in Florida. This is particularly true of the diseases caused by the aphid-borne potyviruses, PRSV-W, WMV-2 and ZYMV. The *Trichosanthes* virus represents a pathogen that apparently has not previously been described in Florida (4). There are no indications at this time that it occurs to any significant extent, if at all, in major cucurbit crops in Florida. However, this situation might change in the future. Because of the large number and diverse types of viruses that infect cucurbits worldwide (10), it seems likely that more viruses will be found in Florida as more surveys are conducted, antisera to additional viruses are used, and more diverse detection techniques are used. More extensive application of serological and other indexing techniques could also help to obtain additional informationabout the epidemiology of the viruses affecting cucurbits in this state.

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