Occurrence, Distribution, and Relative Incidence of Five Viruses Infecting Cucurbits in the State of São Paulo, Brazil

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ABSTRACT

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Cucurbits species grown in 38 of 40 agricultural regions in the state of São Paulo, Brazil, were surveyed for the relative incidence of *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-type W* (PRSV-W), *Watermelon mosaic virus-2* (WMV-2), *Zucchini lethal chlorosis virus* (ZLCV), and *Zucchini yellow mosaic virus* (ZYMV) during May 1997 and June 1999. Samples from 621 plants, representing eight cultivated species, six wild species, and one commercial hybrid (*Cucurbita moschata* × *C. maxima*), were analyzed by plate trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA). PRSV-W and ZYMV were the most frequently found viruses, accounting for 49.1 and 24.8%, respectively, of 605 samples tested. ZLCV, CMV, and WMV-2 were detected in 7.8, 6.0, and 4.5% of 612, 497, and 423 samples tested, respectively. Double infection was found in 97 samples, and triple infection was found in 10 samples. Quadruple infection was detected in one *C. pepo* sample. Plants that were symptomatic but negative by PTA-ELISA might be due to abiotic agents, infection by virus for which antiserum was not available, such as *Squash mosaic virus*, or infection with an as yet uncharacterized virus.

Commercial production of zucchini squash (*Cucurbita pepo*), winter squash (*C. maxima* and *C. moschata*), hybrid squash (*C. maxima* × *C. moschata*), cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), chayote (*Sechium edule*) and sponge gourd (*Luffa acutangula*) are produced by several small to medium size growers throughout the state of São Paulo, Brazil. Cucurbit crops occupied approximately 22,130 ha in the state in 1998, with an estimated production of 579,405 t (2). Due to the tropical and subtropical weather predominant in São Paulo, most of the species can be grown throughout the year. In addition, wild Cucurbitaceae species such as bitter melon (*Momordica charantia*), West Indian gherkin (*Cucumis anguria*), calabash gourd (*Lagenaria siceraria*), and others can be found naturally almost everywhere at any time.

Cucurbit virus diseases are a worldwide problem, and in the state of São Paulo they represent one of the most limiting factors for growers. Of about 20 different viruses

reported on the Cucurbitaceae worldwide (35), eight have been reported in Brazil (1,4,5,10,15,24,28,32). Among these, *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-type W* (PRSV-W), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus-2* (WMV-2), *Zucchini yellow mosaic virus* (ZYMV), and *Zucchini lethal chlorosis virus* (ZLCV) have been found in the state of São Paulo (4,5,24,28,32). Although there are no studies regarding the distribution and relative incidence of these viruses in cucurbit crops, past observation in the states of São Paulo (5), Pará (1), Rio Grande do Norte (12), Mato Grosso (unpublished), Minas Gerais (22), Ceará (14), and in the Distrito Federal (7) indicated that PRSV-W was predominant. A recent survey on viruses infecting melon and watermelon crops in the northeast states of Bahia and Pernambuco showed that WMV-2 and PRSV-W were the most prevalent, with incidence of 68.7 and 31.2%, respectively (6). PRSV-W is still regarded as one of the most destructive pathogens infecting cucurbits in several states of Brazil, even though estimates of crop loss are not available.

Despite the importance of cucurbit crops in São Paulo, only limited local information is available about the incidence of viral diseases and their effect on the yield. For this reason, a 2-year survey was carried out to estimate the incidence of CMV,

PRSV-W, WMV-2, ZLCV, and ZYMV on cultivated and some wild species of Cucurbitaceae. These are the only viruses that have been reported affecting cucurbit crops in the state until now.

MATERIALS AND METHODS

Survey. Samples were collected during May 1997 and June 1999 from 38 of 40 agricultural regions of the state of São Paulo (Fig. 1). In each region, cucurbit fields and wild cucurbit species were arbitrarily inspected and selected for virus infection. Surveys were preferably conducted during a mid-developmental stage of the plant. Plants were randomly evaluated based on symptoms thought to be caused by virus infection such as chlorosis, vein clearing, mottle and/or leaf malformation, and fruits that were smaller and malformed and showed abnormal discoloration on their surface. Young leaves from some symptomatic plants were placed in plastic bags and examined at IAC, Campinas, ESALQ/USP, Piracicaba, and FCA/UNESP, Botucatu for serological identification of infecting viruses.

Virus identification. The presence of CMV, PRSV-W, WMV-2, ZLCV, and ZYMV was determined using the plate trapped antigen (PTA)-ELISA (18), with some modification. Briefly, test samples diluted 1:20 in 0.05 M carbonate buffer, pH 9.6, were added to wells of ELISA plate and incubated for 1.5 h at 37°C. Polyclonal antiserum diluted 1:1,000 in 0.2 M Tris-HCl buffer, pH 7.2, was incubated in the wells for 1.5 h at 37°C. Goat-anti-rabbit IgG conjugated to alkaline phosphatase (Sigma A-3687), diluted 1:32,000 in Tris-HCl buffer, was added to the plate and incubated as before. Enzyme substrate, *p*-nitrophenyl phosphate (Sigma N-9389), was added in 10% diethanolamine at pH 9.8. Antiserum against CMV was provided by P. S. T. Brioso (UFRRJ, Rio de Janeiro). Antisera against PRSV-W and ZYMV were produced at ESALQ/USP, Piracicaba, and those against WMV-2 and the nucleocapsid protein of ZLCV were kindly provided by A. C. de Ávila (CNPq/EMBRAPA, Brasília, DF). Virus positive and negative controls were included in all tests. PTA-ELISA reactions were recorded by an Σ 960 ELISA reader (Metertech, Taiwan) at A_{405nm} . A sample

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was considered virus positive if the A_{405nm} value was greater than three times that of healthy plant extract.

RESULTS AND DISCUSSION

The results of these surveys are summarized in Table 1 and Figure 1. Symptoms due to virus infection and those caused by abiotic agents (insecticide, herbicide, nutritional deficiency, and insect toxemia) were observed in several fields. Incidence of symptomatic plants varied largely, not only among regions but also among cultivated species of cucurbits (data not shown). Of the five viruses tested, PRSV-W and ZYMV were found in 100 and 82% of the regions, respectively. ZLCV, a new species of *Tospovirus* (25), was detected in 58% of the regions, whereas CMV was found in 35% of the areas surveyed. WMV-2 was detected in only eight agricultural regions (23%).

The incidence of these viruses on different species of Cucurbitaceae surveyed showed that PRSV-W was the most prevalent virus found in almost all species, followed by ZYMV, ZLCV, CMV, and WMV-2 (Table 2). An exception was found for *L. acutangula*, where ZYMV was predominant. This species is usually found as very small isolated plantings throughout the state. Chayote, on the other

hand, is a popular crop grown in almost all agricultural regions, being frequently found near other cultivated cucurbit species. In spite of the large number of chayote plantings visited, only five plants from three agricultural regions (3, Araraquara; 9, Bragança Paulista; and 10, Campinas) were found showing viruslike symptoms and gave positive reactions with PRSV-W and ZLCV antisera. All other 18 samples showing symptoms suspected to be caused by virus infection tested negative on PTA-ELISA. As several chayote plantings were located near cucurbit crops from which collected samples tested positive for at least one virus, it is suggested that the low incidence of symptomatic chayote plants associated with virus infection might be due to field resistance present in this species.

Among the wild species, *Cayaponia tibiricae*, which was previously reported as a natural host for ZYMV (34), was also found infected by PRSV-W and ZLCV. *M. charantia* is another wild species widespread in the state, but despite exhaustive attempts, no plants were found exhibiting viruslike symptoms. Nevertheless, seven symptomless samples of this species were analyzed and were negative for all five viruses tested. Additional studies are under way to determine whether or not *M.*

charantia is resistant to infection with these viruses. One asymptomatic sample of *Sicyos polyacanthus* and one of *Trichosanthes cucurberina* var. *anguina*, which are wild species, also tested negative for all five viruses.

Double virus infection was detected in 97 of all samples that tested positive by PTA-ELISA. Among double infected plants, 55% were infected with PRSV-W and ZYMV, the most frequently detected viruses in the samples. Double infection with PRSV-W + ZLCV and PRSV-W + CMV accounted for 15 and 16%, respectively. A low percentage of doubly infected plants was reported by Luis-Arteaga et al. (16) for mosaic viruses infecting field-grown melon in Spain. Triple infection involving different combinations with all viruses detected in these surveys was found in only 10 samples. Only one sample of *C. pepo* was simultaneously infected with PRSV-W, WMV-2, ZLCV, and ZYMV.

A significant percentage of samples from symptomatic cucurbit plants analyzed did not react with the antisera against any of the five viruses (Table 2). The absence of positive reactions may be due to any other mosaic-causing agents, such as *Squash mosaic virus* (SqMV) or an as yet unidentified virus present in the state. SqMV was once described infecting cu-

Fig. 1. Map of São Paulo State showing the distribution of five viruses found infecting cucurbit species in 38 of 40 agricultural regions surveyed: 1, Andradina; 2, Araçatuba; 3, Araraquara; 4, Assis; 5, Avaré; 6, Barretos; 7, Bauru; 8, Botucatu; 9, Bragança Paulista; 10, Campinas; 11, Catanduva; 12, Dracena; 13, Fernandópolis; 14, Franca; 15, General Salgado; 16, Guaratingueta; 17, Itapetininga; 18, Itapeva; 19, Jaboticabal; 20, Jales; 21, Jaú; 22, Limeira; 23, Lins; 24, Marília; 25, Mogi das Cruzes; 26, Mogi Mirim; 27, Orlândia; 28, Ourinhos; 29, Pindamonhangaba; 30, Piracicaba; 31, Presidente Prudente; 32, Presidente Venceslau; 33, Registro; 34, Ribeirão Preto; 35, São João da Boa Vista; 36, São José do Rio Preto; 37, São Paulo; 38, Sorocaba; 39, Tupã; and 40, Votuporanga.

cumber in São Paulo (4), but antiserum was not available during these surveys. Other factors that might explain negative results on PTA-ELISA are the

low concentration of virus in the sample being tested or abiotic agents causing viruslike symptoms, as reported by Yuki et al. (33).

These data show for the first time the identities of viruses infecting commercially grown cucurbits in São Paulo. PRSV-W, considered the most prevalent and destruc-

Table 1. Occurrence of viruses on cucurbit species with viruslike symptoms collected from 38 agricultural regions in São Paulo State, Brazil, during May 1997 and June 1999

Region	Fields surveyed	PTA-ELISA ^a				
		CMV	PRSV-W	ZLCV	ZYMV	WMV-2
Andradina	4	0/10 ^b	1/10	0/10	3/10	0/10
Araçatuba	9	1/22	18/30	2/30	11/30	0/12
Araraquara	5	0/16	9/16	5/16	7/16	0/16
Assis	3	0/5	5/5	0/5	1/5	0/5
Avaré	3	0/4	3/4	0/4	0/4	0/4
Barretos	1	0/3	1/3	0/3	1/3	0/3
Bauru	5	0/10	10/10	0/10	5/10	3/10
Botucatu	2	0/2	2/2	0/2	0/2	0/2
Bragança Paulista	10	3/44	18/48	6/48	5/48	2/43
Campinas	10	1/32	22/53	4/65	13/53	2/42
Catanduva	7	3/10	6/10	1/10	4/10	2/10
Dracena	2	1/10	7/10	0/10	6/10	NT
Fernandópolis	6	0/11	3/12	0/12	1/12	0/1
General Salgado	1	NT	1/5	NT	4/5	0/5
Guaratingueta	5	0/11	7/11	0/11	1/11	0/11
Itapetininga	6	2/12	6/12	1/12	2/12	1/12
Itapeva	1	0/12	7/12	0/12	0/12	0/12
Jaboticabal	9	0/23	20/29	2/29	10/29	0/23
Jales	7	4/25	8/25	0/25	1/25	NT
Jaú	7	0/10	10/37	1/37	8/37	0/23
Limeira	6	0/10	1/12	3/12	5/12	0/10
Lins	13	3/41	11/41	0/41	6/41	1/35
Marília	3	0/10	9/10	1/10	1/10	0/10
Mogi das Cruzes	5	0/6	5/8	2/8	0/8	1/8
Mogi Mirim	7	0/14	3/14	4/14	2/14	0/14
Orlândia	1	1/8	10/12	2/12	5/12	NT
Ourinhos	9	0/10	8/10	0/10	2/10	0/10
Pindamonhangaba	5	3/15	9/15	1/15	4/15	7/15
Piracicaba	7	0/7	9/20	4/20	2/20	0/20
Presidente Prudente	2	0/2	1/2	0/2	1/2	0/2
Registro	9	6/19	12/27	0/27	0/27	0/19
Ribeirão Preto	10	0/14	8/14	1/14	6/14	0/1
São João da Boa Vista	6	0/7	5/7	2/7	0/7	0/7
São José do Rio Preto	8	0/8	10/12	0/12	6/12	0/10
São Paulo	4	0/10	5/10	1/10	1/10	0/10
Sorocaba	6	0/10	9/10	3/10	0/10	0/7
Tupã	10	1/33	11/33	1/33	23/33	0/7
Votuporanga	5	1/10	2/13	1/13	3/13	0/3
Total	219	30/506	297/614	48/621	150/614	19/432

^a Plate trapped antigen enzyme-linked immunosorbent assay. *Cucumber mosaic virus* (CMV), *Papaya ringspot virus*-type W (PRSV-W), *Zucchini lethal chlorosis virus* (ZLCV), *Zucchini yellow mosaic virus* (ZYMV), and *Watermelon mosaic virus*-2 (WMV-2).

^b Number of infected plants/number of plants sampled.

Table 2. Occurrence of viruses on cucurbit species with viruslike symptoms collected from fields in São Paulo State, Brazil, during May 1997 and June 1999

Species	PTA-ELISA ^a					Double infection	Triple infection
	CMV	PRSV-W	ZLCV	ZYMV	WMV-2		
<i>Cucurbita moschata</i>	2/182 ^b	116/226	19/217	55/226	4/176	37	3
<i>C. maxima</i>	0/13	8/14	0/14	2/14	0/9	0	0
<i>C. moschata</i> × <i>C. maxima</i>	0/23	11/24	2/24	5/24	0/3	6	0
<i>C. pepo</i>	0/58	54/78	11/88	16/78	9/59	16	5
<i>Cucumis anguria</i>	3/12	11/12	1/12	0/12	0/10	3	0
<i>C. melo</i>	5/26	13/26	0/26	11/26	0/11	4	0
<i>C. sativus</i>	14/109	46/138	12/144	40/138	5/96	19	2
<i>Citrullus lanatus</i>	1/24	22/32	1/32	6/32	1/15	6	0
<i>Luffa acutangula</i>	1/14	3/14	0/14	11/14	0/10	1	0
<i>Sechium edule</i>	0/22	4/23	1/23	0/23	0/21	0	0
<i>Lagenaria siceraria</i>	4/11	8/11	0/11	2/11	0/10	4	0
<i>Cayaponia tibiricae</i>	0/3	1/7	1/7	2/7	0/3	1	0
Total	30/497	297/605	48/612	150/605	19/423	97	10
% infected	6.0	49.1	7.8	24.8	4.5		

^a Plate trapped antigen enzyme-linked immunosorbent assay. *Cucumber mosaic virus* (CMV), *Papaya ringspot virus*-type W (PRSV-W), *Zucchini lethal chlorosis virus* (ZLCV), *Zucchini yellow mosaic virus* (ZYMV), and *Watermelon mosaic virus*-2 (WMV-2).

^b Number of plants positive/number of plants sampled.

tive virus in several parts of the country, including São Paulo, was confirmed to be the most frequent virus detected. ZYMV was not detected in the state until early 1990. It was first reported in São Paulo in 1992, infecting a few watermelon plants in the agricultural region of Votuporanga, no. 40 (32). Since then, this virus has quickly spread and become the second in incidence, being found in 82% of the regions surveyed throughout the state. It has also been reported infecting cucumber in the state of Santa Catarina (3), melon in the state of Ceará (13), and *L. acutangula* in the state of Bahia (21). More recently, it was detected in watermelon samples from the state of Pará (L. S. Poltronieri, unpublished), suggesting that ZYMV is already established throughout the country. ZYMV is considered aggressive and highly virulent. Due to its aphid transmission properties, the virus may have important epidemiological advantages. When inoculations were done from mixed infections with PRSV-W or WMV-2, more often ZYMV was transmitted (11). If this happens from plants with double infection, it could provide an advantage to ZYMV and contribute to its rapid spread.

The occurrence of a tospovirus infecting cucurbit species in Brazil was first noticed by Costa et al. (5) on *C. sativus* in São Paulo, but for several years it was not considered a major problem in Cucurbitaceae. Since the early 1990s, however, an increased incidence of ZLCV infection on cucurbits has occurred. ZLCV is transmitted by *Frankliniella zucchini* (19), but other species of thrips may also be vectors of this virus. The susceptibility of several cucurbit species to ZLCV, as shown in this survey, and the presence of several species of thrips may provide conditions for further increase in the incidence of this tospovirus on Cucurbitaceae. On the other hand, the low incidence of WMV-2, which was first reported in São Paulo in 1985 in the Campinas agricultural region, no. 10 (28), and which is the most prevalent virus in the northeast states of Bahia and Pernambuco (6), is puzzling. Even though WMV-2 is transmitted in a nonpersistent way by several species of aphids (23), its dissemination throughout São Paulo appears to be restricted for some unknown reason. Studies on aphid transmission and alternative hosts of WMV-2 could help us understand its lower incidence in São Paulo.

Although PRSV-W is the most prevalent virus affecting cucurbits in the state of São Paulo, its relatively high incidence cannot be considered a stable pattern, as has been shown for cucurbit viruses in the United States. Studies in California conducted from the 1950s through 1981 indicated that WMV-2 and CMV were the most prevalent viruses (9,17,20). In 1982, ZYMV was identified for the first time in that state (20). Surveys of melon fields from the

Central Valley of California during 1988 and 1989 showed that WMV-2 was the most prevalent virus, followed by CMV and ZYMV (8). In the southernmost region of California, WMV-2 and ZYMV were the predominant viruses (23). Recent studies showed that WMV (possibly WMV-2), CMV, and *Cucurbit aphid-borne yellow luteovirus* were the three viruses detected in Cucurbitaceae in the San Joaquin and Sacramento valleys of California (31).

The same has also been observed for cucurbit viruses in some Hawaiian Islands. Shanmugasundaram et al. (29) reported that in the 1960s, PRSV-W, WMV-2, and CMV were the most common viruses. In 1991, ZYMV and PRSV-W were the most prevalent in cucurbit crops, whereas CMV was seldom found and WMV-2 was no longer detected (30).

Surveys of melon during 1995 and 1996 showed that CMV and WMV-2 were the most frequently found viruses in Spain, whereas PRSV-W and ZYMV were detected at fewer sites in lower incidences. PRSV-W accounted for only 9% of total virus-infected plants in 1995 and was not detected in 1996 (16).

The presence of cultivated and wild cucurbit species in São Paulo throughout the year, plus favorable weather for vector populations, make virus diseases one of the most important problems for growers. Therefore, a better understanding of virus and vector reservoirs, vector population behavior, and vector transmission efficiency are necessary for the development of effective control measurements. The most important strategy for control of virus diseases on cucurbits in São Paulo would be the elimination of the common practice of starting new crops near old infected plantings. Old crops should be destroyed before starting new plantings. Control of wild species of Cucurbitaceae in the vicinity of cucurbit crops would reduce virus sources. Control of weeds that harbor vectors is also recommended. Use of resistant or tolerant cultivars and/or mild strain protection should be practiced whenever available. Mild strain protection has been successfully applied for control of PRSV-W on zucchini squash in São Paulo since 1997 (26). Use of tolerant *C. moschata* cv. Menina Brasileira in combination with mild strains of PRSV-W has also provided better disease control, with approximately 30% yield increase (27).

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