



Occurrence Of Viruses Infecting Watermelon, Other Cucurbits, and Weeds in the Parts of Southern United States

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Abstract

Field surveys were conducted to determine the distribution and frequency of viruses infecting watermelon and other cucurbits in the southern US in 2010 and 2011. Leaf samples were collected from 715 symptomatic plants from 10 states and were tested by dot-immunobinding assays or reverse transcription-polymerase chain reaction for 17 viruses that included *Alfalfa mosaic virus* (AMV), *Bean pod mottle virus* (BPMV), *Cucurbit aphid born yellows virus* (CABYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus-W* (PRSV-W), *Squash leaf curl virus* (SLCuV), *Soybean mosaic virus* (SMV), *Squash mosaic virus* (SqMV), *Squash vein yellowing virus* (SqVYV), *Tobacco ringspot virus* (TRSV), *Watermelon mosaic virus* (WMV), *Watermelon silver mottle virus* (WSMoV), *Zucchini yellow mosaic virus* (ZYMV), and *Zucchini green mottle mosaic virus* (ZGMMV). Thirteen out of 17 viruses were detected in this study. The distribution of detected viruses varied with the highest average frequency for WMV (30.6%), followed by PRSV-W (24.7%), ZYMV (13.9%), TRSV (5.7%), SqMV (3.5%), and MNSV (2.6%). The percent frequency of the remaining viruses was less than 2%. Seven viruses (AMV, BPMV, CMV, SqMV, TRSV, PRSV-W, and ZYMV) were also detected either from nearby agricultural crops or weeds species. Mixed infections were also recorded for some viruses with potyviruses being the most common. There is limited information on frequency and distribution of viruses that occur on watermelon and other cucurbits. These results indicate that potyviruses, particularly PRSV-W, WMV, and ZYMV, are frequently present in infected watermelon and other cucurbits in the southern US.

Introduction

Cucurbits are one of the largest horticultural cash crops in the United States. From 1980 to 2011, cash revenues for cucumbers (*Cucumis sativus*) increased from \$78 million to \$189 million; for cantaloupe (*Cucumis melo*) from \$153 million to \$350 million and for watermelon (*Citrullus lanatus*) from \$149 to \$543 million (11). The increase in revenue for cucumber, cantaloupe, and watermelon was 242%, 129%, and 364%, respectively, from 1980 to 2011. Data for squash (*Cucumis maxima*) and pumpkin (*Cucurbita pepo*) were not available for the same years; however, the value of production for squash and pumpkin in 2011 was \$283 million and \$113 million, respectively. More than 70% of watermelons grown in the US are in the southern states with the highest acreage in Florida (25,900 acres) followed by Texas (24,900 acres) (11). Watermelon is subject to a variety of diseases caused by plant pathogens, particularly fungi and viruses (18).

More than 20 viruses have been associated with cucurbits in the US (5,6,7,8,12,13,15). Plant viruses are a persistent threat to the production of cucurbits throughout the US, although severe outbreaks tend to occur on a sporadic basis. Many viruses are ubiquitous in nature and can result in severe economic losses under favorable conditions (10,18). In some areas of Oklahoma, pumpkins cannot be grown successfully due to virus diseases, particularly those caused by potyviruses (Sue Gray, OSU Extension, Tulsa County, *personal communication*).

Little information is available about the large-scale frequency and distribution of viruses that occur regularly in watermelon and other cucurbits. The objectives of this research were to assess the distribution and frequency of viruses affecting watermelon and to gain information on potentially new or unknown viruses affecting watermelon in the southern US.

Collection of Samples and Detection Protocols

A total of 17 viruses belonging to 12 genera of plant viruses (Table 1) were targeted. Fifteen of the 17 viruses have been reported to be associated with cucurbit crops (1), while two viruses (*Cucumber green mottle mosaic virus*, CGMMV, and *Watermelon silver mottle virus*, WSMoV) had not been reported in the US. Antisera against 15 viruses (Table 1) were purchased from Agdia Inc. (Elkhart, IN) or AC Diagnostics (Fayetteville, AR). Due to non-availability of antiserum against *Cucurbit yellow stunting disorder virus* (CYSDV) and *Squash vein yellowing virus* (SqVYV), virus-specific primers for these viruses were designed (2,17) and used in the reverse transcription polymerase chain reaction (RT-PCR) assays for detection. Positive controls for each virus used in dot-immunobinding assays (DIBA) were obtained commercially from Agdia Inc. or AC Diagnostics while positive controls in RT-PCR assays for CYSDV and SqVYV were provided by Dr. Scott Adkins at the USDA-ARS Horticultural Research Laboratory in Ft. Pierce, FL, and Dr. William Wintermantel at USDA-ARS Agricultural Research Station, Salinas, CA, respectively.

Table 1. Viruses that were tested by dot-immunobinding assay (DIBA) using their polyclonal antibodies or reverse transcription-polymerase chain reaction (RT-PCR) using virus specific primers.

Family	Genus	Virus	Acronym	Transmission	Reported in the USA
Bromoviridae	<i>Alfamovirus</i>	<i>Alfalfa mosaic virus</i>	AMV	Aphids	Yes
	<i>Cucumovirus</i>	<i>Cucumber mosaic virus</i>	CMV	Aphids	Yes
Bunyaviridae	<i>Tospovirus</i>	<i>Watermelon silver mottle virus</i>	WSMoV	Thrips	No
Closteroviridae	<i>Crinivirus</i>	<i>Cucurbit yellow stunting disorder virus</i>	CYSDV*	Whitefly	Yes
Geminiviridae	<i>Begomovirus</i>	<i>Squash leaf curl virus</i>	SLCuV	Whitefly	Yes
Luteoviridae	<i>Polerovirus</i>	<i>Cucurbit aphid-borne yellows virus</i>	CABYV	Aphids	Yes
Potyviridae	<i>Potyvirus</i>	<i>Papaya ringspot virus</i>	PRSV-W	Aphids	Yes
		<i>Soybean mosaic virus</i>	SMV	Aphids	Yes
		<i>Watermelon mosaic virus</i>	WMV	Aphids	Yes
		<i>Zucchini yellow mosaic virus</i>	ZYMV	Aphids	Yes
	<i>Ipomovirus</i>	<i>Squash vein yellowing virus</i>	SqVYV*	Whitefly	Yes
Secoviridae	<i>Comovirus</i>	<i>Bean pod mottle virus</i>	BPMV	Beetle	Yes
		<i>Squash mosaic virus</i>	SqMV	Beetle	Yes
	<i>Nepovirus</i>	<i>Tobacco ringspot virus</i>	TRSV	Nematode	Yes
Tombusviridae	<i>Carmovirus</i>	<i>Melon necrotic spot virus</i>	MNSV	Seed/fungus	Yes
Virgaviridae	<i>Tobamovirus</i>	<i>Cucumber green mottle mosaic virus</i>	CGMMV	Seed/fungus	No
		<i>Zucchini green mottle mosaic virus</i>	ZGMMV	Seed/fungus	No

* Tested by RT-PCR using virus specific primers due to non-availability of virus antisera.

In 2010 and 2011, leaf samples were collected from symptomatic watermelon, other cucurbit crops, nearby agricultural crops, and weeds from 10 southern US states (Fig. 1, Table 2). Samples from symptomatic plants in Arkansas, Oklahoma, and Texas were collected, placed in a plastic bag on ice and transported to the laboratory at the University of Tulsa. Samples collected by collaborators in the other seven states were shipped overnight and processed within 24 h of collection.

Table 2. Number of samples collected from cucurbits, other agricultural crops, and weeds in 10 states during the 2010 and 2011 growing seasons.

Hosts	AL	AR	FL	GA	KY	LA	MS	MO	OK	TX	Total
Cucumber	1	0	1	0	0	1	1	0	0	2	6
Cantaloupe	0	3	1	0	0	0	0	0	16	6	26
Watermelon	9	36	1	2	0	1	5	1	142	296	493
Pumpkin	1	16	1	0	1	0	6	0	21	0	46
Squash	2	0	5	0	0	1	5	0	6	1	20
Other crops	0	0	0	0	0	0	0	0	46	4	50
Weeds	0	0	0	0	0	0	0	0	69	5	74
Total	13	55	9	2	1	3	17	1	300	314	715

Other crops included eggplant, pepper, snap bean, soybean, sunflower, and tomato), while weeds contained devil's claw, groundcherry, morningglory, nightshade, Palmer amaranth, and ragweed (see Table 4).



Fig. 1. Map of southern US states where 715 samples were collected from watermelon, other cucurbit crops, and weeds, and were tested against 17 viruses by dot-immunobinding assay (DIBA) or reverse transcription-polymerase chain reaction (RT-PCR).

Approximately 100 mg of tissues from each sample were aliquoted into individual plastic bags and crushed in phosphate-buffered saline (PBS) at room temperature. Sap was extracted and 2 µl were dotted onto a nitrocellulose membrane. Each sample was replicated on 15 separate sheets of membranes in order to test each sample against the antisera of 15 different viruses. Samples that were negative to 15 viruses were used for RNA extraction and tested by RT-PCR against CYSDV and SqVYV. Symptoms on individual leaf samples were photo-recorded. All membranes were tested for a specific virus by DIBA (3).

Common Symptoms Expressed on Watermelon and Other Cucurbits

A range of symptoms caused by commonly prevalent viruses (Figs. 2A-G) were observed on watermelon collected from different states. For example, shoe-stringing of leaves, ringspots, blistering, severe leaf mosaic, mottling, stunting, and leaf cupping were common among the samples collected. Some plants also showed leaf narrowing, yellowing, rolling, interveinal chlorosis, and leaf curling. However, mosaic and mottling symptoms were not commonly observed and were somewhat subtle on virus-infected watermelon leaves in the field making field identification difficult and easily overlooked. Virus diseases typically go unnoticed by growers, unless symptoms are observed on more than 10 to 20% of plants in a field, or fruit set is significantly reduced (Benny Bruton, *personal observation*). In symptomatic virus infections, the only positive role of symptoms in the identification of viruses is that it shows a clear difference between virus infected and healthy plants in the field. However, it is difficult to identify a specific virus on watermelon based on symptoms alone, because of the possibility of asymptomatic or mixed virus infections, different virus strains, and confusion with abiotic disorders, such as nutrient deficiencies. Virus symptoms on large-leafed cucurbit crops, such as squash and pumpkin are more easily recognizable.

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Fig. 2. Field symptoms of commonly prevalent viruses infecting cucurbits: **(A, B)** mosaic, blistering, puckering, and chlorosis caused by *Papaya ringspot virus-W* (PRSV-W); **(C, D)** leaf deformation, mottling, and shoe strings by PRSV-W and *Watermelon mosaic virus* (WMV); **(E)** yellowing and mosaic by *Zucchini yellow mosaic virus* (ZYMV); **(F)** mosaic, mottling, and leaf curling by *Squash leaf curl virus* (SLCuV); **(G)** mild mosaic and chlorosis by *Squash mosaic virus* (SqMV).



A



B



C



D



E



F



G

Incidence of Potyviruses

A total of 715 samples (26 cantaloupe, 6 cucumber, 46 pumpkin, 20 squash, 493 watermelon, 50 other crops, and 74 weeds) (Table 2) were collected from fields in 10 southern states (Fig. 1) during 2010 and 2011 growing seasons. The number of samples collected ranged from 1 to 314 among the states (Table 2). Thirteen of the 17 viruses were detected by DIBA in all 10 states, whereas CGMMV, CYSDV, SqVYV, and ZGMMV were not detected in this study.

Three potyviruses (PRSV-W, WMV, and ZYMV) were frequently detected on watermelon and other cucurbits with WMV being found in 219 samples from 9 of the 10 states (Table 3). Average incidence of WMV was 30.6% and was the most common virus detected in each state with the exceptions of Oklahoma and Florida. The incidence of PRSV-W was always higher than WMV in Florida and Oklahoma, whereas WMV ranked second in incidence in both states. PRSV-W was detected in 177 samples collected from 6 states with an average incidence of 24.7%. However, PRSV-W was also detected at a significant level in samples from Alabama, Arkansas, and Georgia compared to viruses other than WMV. ZYMV was detected in 100 samples from 4 states with an average incidence of 13.9%. Results indicate that aphid-transmitted viruses, particularly the three potyviruses (PRSV-W, WMV, and ZYMV), were the predominant viruses detected in all 10 states.

Table 3. Frequency of known viruses in watermelon, other cucurbits, and weeds or agricultural crops in 10 states during the 2010 and 2011 growing seasons.*

State	Sample (n)	Number of samples positive by DIBA													
		AMV	BPMV	CABYV	CMV	MNSV	PRSV-W	SLCuV	SMV	SqMV	TRSV	WMV	WSMoV	ZYMV	
AL	13	0	0	2	0	0	3	4	0	0	0	6	0	4	
AR	55	0	0	0	1	0	22	0	0	0	2	50	0	9	
FL	9	0	0	0	0	0	8	0	0	0	0	7	0	0	
GA	2	0	0	0	0	0	1	0	0	0	0	2	0	0	
KY	1	0	0	0	0	0	0	0	0	0	0	1	0	0	
LA	3	0	0	0	1	0	0	0	0	0	0	3	0	0	
MS	17	0	0	0	0	0	0	0	0	0	0	6	2	0	
MO	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
OK	300	9	4	0	8	4	110	0	2	14	11	33	0	37	
TX	314	0	6	1	2	15	33	4	0	11	28	111	1	50	
Total	715	9	10	3	12	19	177	8	2	25	41	219	3	100	
% infection		1.3	1.4	0.4	1.7	2.6	24.7	1.1	0.3	3.5	5.7	30.6	0.4	13.9	

* *Alfalfa mosaic virus* (AMV), *Bean pod mottle virus* (BPMV), *Cucurbit aphid born yellows virus* (CABYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus-W* (PRSV-W), *Squash leaf curl virus* (SLCuV), *Soybean mosaic virus* (SMV), *Squash mosaic virus* (SqMV), *Squash vein yellowing virus* (SqVYV), *Tobacco ringspot virus* (TRSV), *Watermelon mosaic virus* (WMV), *Watermelon silver mottle virus* (WSMoV), *Zucchini yellow mosaic virus* (ZYMV), and *Zucchini green mottle mosaic virus* (ZGMMV). None of the samples was positive for CGMMV, SqVYV, CYSDV, and ZGMMV. Samples include 6 cucumber, 26 cantaloupe, 46 pumpkin, 20 squash, 493 watermelon, 50 from six other crops (eggplant, pepper, snap bean, soybean, sunflower, and tomato), 74 from six weeds (devil's claw, groundcherry, morningglory, nightshade, Palmer amaranth, and ragweed) and were tested by dot-immunobinding assays (DIBA).

Seven of the tested viruses (Table 4) were detected in other nearby agricultural crops and weed species. Two of the six crops contained at least three viruses. For example, 20% pepper (*Capsicum annuum*) plants collected in Oklahoma were infected with AMV (20%), and 5.7% with each of the BPMV and CMV. Snap bean (*Phaseolus vulgaris*) plants collected from Texas contained CMV, SqMV, and ZYMV. None of the 17 tested viruses was detected in eggplant

(*Solanum melongena*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), and tomato (*Solanum lycopersicum*). It is possible that viruses other than those tested in this study infected these crops. Among the six weeds species, groundcherry (*Physalis angulata*), Palmer amaranth (*Amaranthus palmeri*), and ragweed (*Ambrosia artemisifolia*) harbored AMV, PRSV-W, or TRSV (Table 4), while no virus was detected from morningglory (*Ipomoea hederacea*), nightshade (*Solanum nigrum*) or devil's claw (*Proboscidea louisianica*). Overall, the number of weed species sampled was low (Table 4), which may have resulted from only symptomatic plants being collected for the survey.

Table 4. Frequency of viruses in agricultural crops and weeds in two states during the 2010 and 2011 growing seasons.*

State	Host species name		Samples collected	Number of samples positive by DIBA						
	Common	Scientific		AMV	BPMV	CMV	SqMV	TRSV	PRSV-W	ZYMV
OK	Eggplant	<i>Solanum melongena</i>	3	0	0	0	0	0	0	0
	Pepper	<i>Capsicum annuum</i>	35	7	2	2	0	0	0	0
	Soybean	<i>Glycine max</i>	2	0	0	0	0	0	0	0
	Tomato	<i>Solanum lycopersicum</i>	6	0	0	0	0	0	0	0
	Devil's claw	<i>Proboscidea louisianica</i>	3	0	0	0	0	0	0	0
	Groundcherry	<i>Physalis angulata</i>	25	0	0	0	0	0	1	0
	Morningglory	<i>Ipomoea hederacea</i>	16	0	0	0	0	0	0	0
	Nightshade	<i>Solanum nigrum</i>	2	0	0	0	0	0	0	0
	Palmer amaranth	<i>Amaranthus palmeri</i>	23	1	0	0	0	0	0	0
TX	Snap bean	<i>Phaseolus vulgaris</i>	3	0	0	1	1	0	0	1
	Sunflower	<i>Helianthus annuus</i>	1	0	0	0	0	0	0	0
	Nightshade (s)	<i>Solanum nigrum</i>	1	0	0	0	0	0	0	0
	Palmer amaranth	<i>Amaranthus palmeri</i>	2	0	0	0	0	0	0	0
	Ragweed	<i>Ambrosia artemisifolia</i>	2	0	0	0	0	1	0	0
Total			124	8	2	3	1	1	1	1

* No weeds samples were received from other states. The remaining viruses (CABYV, CGMMV, CYSDV, MNSV, SLCV, SMV, SqYVY, WMV, WSMoV, and ZGMMV) were not detected.

Incidence of Other Viruses

TRSV was detected in 41 samples collected from Arkansas, Oklahoma, and Florida with an average incidence of 5.7%. Similarly, SqMV was detected in 25 samples mainly from Oklahoma and Texas with an average incidence of 3.5%. MNSV was only detected in 19 samples from Oklahoma and Texas with an average incidence of 2.6%. CMV was detected in 12 samples from Arkansas, Louisiana, Oklahoma, and Texas with an average incidence of 1.7%. The remaining viruses were detected in 10 or less samples collected from one or two states with an average infection of less than 1.5%. According to the available literature, some viruses identified in this work have never been reported in their respective states. For example, watermelon and squash samples from Alabama were positive by DIBA against the antisera of CABYV and SLCV (Table 3). Similarly, pumpkin samples from Mississippi and watermelon samples from Texas tested positively with WSMoV. In Oklahoma and Texas, MNSV has not been reported prior to this work. However, further tests such as biological characterization and nucleic acid-based detection methods are needed to confirm the identity of the above mentioned viruses in these states. Viruses are ubiquitous and it is not surprising to observe the presence of previously unreported viruses. No systematic study has been conducted for a number of viruses which occur at a subliminal level of less than 1%.

Mixed Infection of Viruses

Mixed infections of two or more viruses were observed among the cucurbit crops, particularly watermelon in five different states (Table 5). This was often observed with the potyviruses (PRSV-W, WMV, and ZYMV). The highest average mixed infection of at least two viruses was recorded for PRSV-W and WMV (15.3%) followed by WMV and ZYMV (3.5%) and PRSV-W and ZYMV (1.9%) (Table 5). The average mixed infection of three viruses was 2.9% for PRSV-W, WMV, and ZYMV, followed by PRSV-W, ZYMV, and TRSV (1.3%). In less than 1% of the samples, a combination of four viruses (PRSV-W, WMV, ZYMV, and SqMV) was recorded. Mixed infection of PRSV-W and WMV was also reported in squash in Alabama (9).

Table 5. Frequency of mixed infection of viruses infecting watermelon and other cucurbit crops in southern United States during 2010 and 2011 growing seasons.*

Virus combination	AR	FL	MS	OK	TX
	Frequency (%)				
WMV+PRSV-W	27.2	77.7	0	5.3	14.9
WMV+ZYMV	12.7	0	0	0	9.2
PRSV-W+ZYMV	7.2	0	0	2.6	5.0
WMV+PRSV-W+ZYMV	5.4	0	0	0	2.8
TRSV+MNSV	0	0	0	0.6	0.6
TRSV+SqMV	0	0	0	1.0	0.3
Potyvirus*+SLCV	0	0	23.5	0	2.2
Potyvirus*+SqMV	1.8	0	0	5.6	3.8
Potyvirus*+TRSV	1.8	0	0	3.3	4.8
Potyvirus*+MNSV	1.8	0	0	3.0	4.1

* Potyviruses included PRSV-W, WMV-2, or ZYMV.

Conclusions and Recommendations

This work illustrates the distribution and frequency of known and emerging viruses in watermelon, other cucurbits, and weeds found in watermelon fields in 10 southern states. The high incidence of PRSV-W was consistent with a recent report from watermelon in Oklahoma (4). Interestingly, the frequency and distribution of WMV was higher than PRSV-W in other southern states.

Despite low sampling frequencies, some of the viruses identified in this survey were found to be associated with various weed species (Table 4). It is important to efficiently managed weeds before or during the cropping season, as weeds not only compete for resources with the main crop, but may also serve as a source of initial inoculum and harbor insect vectors. All six weed species (Table 4) are commonly found in cucurbit fields in southern US and should be managed accordingly. Future work will be focused to thoroughly sample both symptomatic and non-symptomatic weeds species throughout the growing season and determine the impact of disease incidence of a particular virus in cucurbit crops.

More than 50% of viruses that infect cucurbits are transmitted by aphids and whiteflies and are difficult to control (Table 1). Early infection can significantly reduce yield of cucurbits. Damage can be minimized through the use of integrated pest management strategies. Aphid and whitefly populations can be reduced through the use of insecticides combined with cultural practices, such as border crops and reflective mulches. Use of virus-resistant cultivars has been shown to be a highly effective way to combat virus disease of cucurbits (14).

New viruses keep emerging and timely and accurate identification is essential for control. For example, a new disease of watermelon, which caused severe yield losses, was observed in Florida in 2002. The causal agent was identified as a new virus, SqVYV, that was originally detected in Florida in the spring of 2003 in squash and later detected in watermelon (16). The virus is now

known to cause watermelon vine decline (WVD) in Florida (2). It is important to identify the most common and damaging viruses in a locality to allow development of a comprehensive management strategy that growers can utilize to reduce losses from these economically important diseases.

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