

Project Title: Adopting New IPM Methods for Cucurbit Virus Diseases

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Summary

The virus diseases of pumpkin which have been reported in the mid-Atlantic region of the U.S. are *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* type W (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Visual differentiation of these diseases is difficult for growers because symptoms often overlap. As virus resistance becomes available in pumpkin cultivars (varieties), growers must decide which cultivars to plant based on the prevalence of each virus disease in their region. The objectives of the project were to determine the most widely prevalent virus diseases. A field survey was conducted in 2008 and 2009 in Maryland, Delaware, Pennsylvania (2008) and Virginia (2009). In 2008, anomalous ELISA results made it difficult to determine the most prevalent virus. In 2009, the most prevalent virus was WMV, with 25 fields testing positive or with an elevated WMV ELISA reaction. Four samples tested positive for PRSV and one for ZYMV. Six weeds, which were sampled, tested positive for potyvirus in 2008 and 2009. However, no weed sample, in either year, tested positive for CMV, WMV, PRSV-W, or ZYMV specific antibodies. These results suggest that cultivars with WMV and PRSV resistance would be most beneficial for growers in Maryland and Delaware.

Problem, Background, and Justification

Pumpkin production in the mid-Atlantic comprises farms that vary from one to over 100 acres, and half of these operations have five or fewer acres of pumpkins. These acres are very high value with a profit of up to \$2000 per acre. As a result, most growers are concerned about consistent production including minimizing losses due to virus diseases.

Although there are approximately thirty virus diseases of cucurbits reported worldwide, the common pumpkin (*Cucurbita pepo* L) virus diseases previously reported in the mid-Atlantic were *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* type W (PRSV-W, which was previously called *Watermelon mosaic virus* 1 or WMV-1), *Watermelon mosaic virus* (WMV, which was formerly called WMV-2), and *Zucchini yellow mosaic virus* (ZYMV).

These virus diseases of cucurbit are spread by aphids in a non-persistent manner (i.e., viral particles (virions) are acquired by the aphid on its stylet and are retained in association with the stylet for short periods of time (usually hours). Because these cucurbit virus diseases are non-persistent in their aphid vectors, there are limited options for virus management. Insecticide

applications have not been effective. Reflective mulches have been used on squash, but not pumpkins, which are grown primarily on bare ground. Likewise, frequent applications of mineral oil are expensive, and deemed not reliable enough to justify the expense. Cross protection in cucurbits with attenuated virus has been evaluated experimentally, but is not used commercially.

The recommendation for managing cucurbit virus diseases has been to plant pumpkins as far away as possible from other cucurbit crops to minimize the spread of infected aphids into a pumpkin planting. Recently, virus resistance has become available in some cucurbits, most notably squash, and virus host resistance in pumpkin now available in a few cultivars. It is expected that in the future many, if not most, pumpkin cultivars that will be commercially available will have virus resistance. Growers are anxious to use resistance and minimize pumpkin yield and quality losses due to virus infection. Information on how to manage virus diseases remains one of the most frequent requests from pumpkin growers on pest management.

Identification of pumpkin virus diseases by farmers and their advisors is difficult because the diseases cannot be identified reliably by their symptoms. CMV, PRSV-W, WMV and ZYMV may exhibit different symptoms at times, and at other times have overlapping symptoms. Mixed infections also may yield unusual symptoms.

Previous surveys throughout the eastern U.S. indicated that the most prevalent cucurbit viruses vary both temporally and geographically. The most recent survey in the mid-Atlantic region, conducted in 2005 in New Jersey, indicated that WMV was most widespread followed by ZYMV and then PRSV.

Objectives

A survey was conducted in the mid-Atlantic region of the U.S. in 2008 and 2009. The objective of the survey was to determine the prevalence of virus diseases on pumpkins in the mid-Atlantic region of the U.S. to assist pumpkin growers in selection of cultivars with virus resistance for the region. A second objective was to obtain preliminary information on weeds as potential alternate hosts of pumpkin virus diseases.

Approach and Procedures

Pumpkin foliage tissue samples from commercial and experiment station fields in Maryland, Delaware, and Pennsylvania (2008 only) and Virginia (2009 only) were sampled for the presence of CMV, PRSV-W, WMV, and ZYMV. Fields (twenty-seven in 2008 and thirty-one in 2009) were located in areas that represented a cross-section of Maryland and Delaware pumpkin production. In addition, eight fields in Pennsylvania and three fields in Virginia were sampled in 2008 and 2009, respectively. Leaves from ten plants in each field were collected. When possible, plants with virus disease symptoms such as mosaic, distortion, stunting or mottling were collected. If no symptoms were present, plants were sampled in a random pattern across the field. Leaves from plants in each field were combined for ELISA testing. Weed species also were collected in selected fields. Leaves of individual weed species were combined, however each species was tested separately. Samples were transported to the University of Maryland Plant Diagnostic Lab and tested for CMV, PRSV-W, WMV, ZYMV and Potyvirus Group (PVG)

using Enzyme Linked Immunosorbent Assay (ELISA) test kits from Agdia, Inc. (Elkart, IN) in 2008. In 2009, samples were sent directly to Agdia, Inc. for ELISA testing.

Outcomes

In 2008, a total of 92 pumpkin or weed samples were ELISA tested (Appendix 1). ZYMV was detected in five pumpkin tissue samples. One sample from Montgomery County, MD was positive for PRSV. Only two pumpkin tissue samples tested positive for WMV. However, twenty-eight symptomatic plants reacted positive to the potyvirus group (PVG) ELISA tests but did not test positive to PRSV, WMV, or ZYMV specific antibodies. During this same year, a virus survey conducted in thirteen other states reported similar anomalous results (Vincelli and Seebold, 2009, Plant Health Progress, doi 10.1094/PHP-2009-0313-01-BR). Subsequent analysis on virus infected pumpkin tissue collected in Kentucky indicated that there was a strain of WMV present in Kentucky that did not react to the Agdia, Inc. ELISA test for WMV, but was positive to PVG ELISA. It is likely that a number of the samples collected in Maryland, Delaware and Pennsylvania in 2008 also were infected with a strain of WMV that did not react positively to the Agdia, Inc. ELISA test for WMV.

Following the anomalous results of 2008, Agdia, Inc. developed a new WMV ELISA test that was capable of detecting the previously undetectable strain of WMV. In 2009, 40 pumpkin or weed samples were ELISA tested (Appendix 2). There were only two pumpkin tissue samples that tested positive to PVG but not to PRSV, WMV, or ZYMV specific antibodies. Twenty-one samples tested positive, or had an elevated reaction, to both PVG and WMV; one tested positive to PVG, and had an elevated reaction to WMV and ZYMV; one tested positive to PVG and PRSV; and three tested positive for PVG, WMV, and PRSV. The most prevalent pumpkin virus in the mid-Atlantic region in 2009 was WMV, followed by PRSV and ZYMV. Four pumpkin samples had mixed infections in 2009. The most beneficial virus resistance in pumpkin in 2009 would have been WMV resistance. Magician, which is the only virus resistant pumpkin in the Commercial Vegetable Production Recommendations, has resistance only to ZYMV. We did not detect CMV in any pumpkin tissue sample in either year.

In 2008 and 2009, no weed tissue samples tested positive to CMV, WMV, PRSV, or ZYMV specific antibodies. However, in 2008 when 58 weed samples were tested, five were positive to potyvirus group PVG (Appendix 1). Because of the anomalous results in 2008, where most pumpkin samples as well as weed samples tested positive to PVG ELISA but not to PRSV, WMV or ZYMV, the contribution of weeds as alternate hosts is unknown. In 2009, one of six weeds which were sampled tested positive for PVG, but not WMV, PRSV or ZYMV specific antibodies (Appendix 2). Additional weed sampling will be necessary to determine if weeds in the mid-Atlantic region serve as reservoirs for WMV, PRSV or ZYMV.

Impacts

The information on virus prevalence from our project has been used to advise growers and extension personnel on management of cucurbit disease. We presented several talks on virus management in pumpkins (Pumpkin twilight meetings in Queenstown and Keedysville, MD; Caroline Vine crops; Central Maryland Vegetable Growers, Upperco; Southern Maryland

Vegetable and Fruit Growers, Clements.) In-service training for extension educators was conducted at the mid-Atlantic Vegetable Workers Conference in Newark, DE. Web-based outreach also has been developed for the Maryland vegetables website (<http://mdvegetables.umd.edu>).

The information that resulted from our survey has been timely. When the project began, there were no multiple virus resistant pumpkin cultivars that were commercially available. Magician, which was available, was resistant only to ZYMV, and often had severe virus symptoms in our region. Recently, Hollar Seeds and Seigers have introduced 'Corvette' which has tolerance to WMV and ZYMV. Outstanding Seed Company, LLC has introduced 'Triumph', which has intermediate resistance to WMV, PRSV and ZYMV. 'Orange Bulldog', which was developed at the University of Georgia, is also available and has virus tolerance (virus not specified). These cultivars are not yet recommended due to lack of field testing. Our survey results indicate that a cultivar with WMV and ZYMV tolerance, such as Corvette or Triumph, would perform well in our region.

Safeguarding human health and the environment:

Because pumpkins are grown for ornamental use, frequently in "pick-your-own" enterprises, and because pumpkins receive multiple insecticide sprays, the potential pesticide exposure of the public is greater for pumpkin than for many other crops. This study, coupled with the production of pumpkin cultivars that have virus resistance should result in the reduction in insecticide applications.

Economic benefits:

A pumpkin budget produced by Pennsylvania State University <http://agalternatives.aers.psu.edu/Publications/pumpkin.pdf> indicates that a reduction of one application of Admire, which is commonly used for aphid management, would reduce production costs by about \$108 per acre. Additional economic benefits would be experienced by growers that do not successfully manage aphids. The yield loss due to viruses, when aphid control is not achieved, would be mitigated by the use of pumpkins with virus resistance.

Implementation of IPM:

In addition to the outreach already conducted as part of this grant, additional follow up to educate growers about the availability of pumpkins with virus resistance needs to be accomplished over the next few years. One critical component for grower IPM implementation will be field demonstration of new cultivars in the mid-Atlantic region.

Appendix 1. Reaction of pumpkin and weed tissue samples collected in 2008 to Agdia, Inc. ELISA tests.

Host Plant	Region, State County	Positive Results				
		None	PVG	PVG + ZYMV	PVG + WMV + ZYMV	PVG + PRSV
	Eastern Shore, MD					
Pumpkin	Wicomico		4			
Weed	Wicomico	6				
Pumpkin	Cecil		1			
Weed	Cecil	4				
Pumpkin	Queen Anne's		1			
Pumpkin	Caroline		1			
	Southern MD					
Pumpkin	St. Mary's		2			
Pumpkin	Charles			1		
Pumpkin	Prince George's		2			
Weed	Prince George's	3				
	Central MD					
Pumpkin	Howard				1	
Weed	Howard	2				
Pumpkin	Carroll		1			
Weed	Carroll	1				
Pumpkin	Montgomery		1			1
Weed	Montgomery	6	1 ^y			
	Western MD					
Pumpkin	Allegany		1			
Pumpkin	Garrett			1		
Weed	Garrett	1				
Pumpkin	Washington		3		1	
Weed	Washington	7				
Pumpkin	Frederick		1			
Weed	Frederick	1				
	Delaware					
Pumpkin	Sussex		1	1		
Weed	Sussex	14	1 ^x			
Pumpkin	New Castle		2			
Weed	New Castle	8				
	Pennsylvania					
Weed	Lancaster		3 ^w			
Pumpkin	Lancaster	6	2			
Total	92 samples	59	28	3	2	1

^zWeeds tested included *Solanum* spp. *Amaranthus* spp. *Ipomoea* spp., common lambsquarter (*Chenopodium album* L.), curly dock (*Rumex crispus* L.), jimsonweed (*Datura stramonium* L.), prickly sida (*Sida spinosa* L.), spurred anoda (*Anoda cristata* L.), velvetleaf (*Abutilon theophrasti* Medicus), common pokeweed (*Phytolacca americana* L.), burcucumber (*Sicyos angulatus* L.) and ground cherry (*Physalis* spp.).

^ySpurred anoda

^xCommon pokeweed

^wCommon pokeweed, burcucumber, and groundcherry

Appendix 2. Reaction of pumpkin and weed tissue samples collected in 2009 to Agdia, Inc. ELISA tests.

Host Plant	Region, State County	Positive Results					
		None	PVG	PVG + WMV	PVG + PRSV	PVG + WMV + PRSV	PVG + WMV + ZYMV
	Eastern Shore, MD						
Pumpkin	Queen Anne's		1	1(4 ^y)			
Pumpkin	Caroline			2			
Pumpkin	Dorchester	6	1	1		2	
Weed	Dorchester	1					
	Central MD						
Pumpkin	Howard			1			1 ^x
	Southern MD						
Pumpkin	Charles			1			
Pumpkin	Calvert			2			
	Western MD						
Pumpkin	Frederick			2			
	Virginia						
Pumpkin	Westmoreland			3			
	Delaware						
Pumpkin	Sussex			2	1	1	
Weed	Sussex	4	1 ^w				
Pumpkin	Kent			1			
Pumpkin	New Castle			1			
Total	40 samples	11	3	21	1	3	1

^zWeeds collected in 2009 were horsenettle (*Solanum carolinense* L.), spurred anoda (*Anoda cristata* L.) and morningglory (*Ipomoea* spp.)

^yWMV was elevated in four samples

^xElevated WMV and ZYMV

^wSpurred anoda