Research Proposal for the National Watermelon Association

Development and evaluation of early monitoring techniques for *Squash vein yellowing virus*, the cause of watermelon vine decline

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Total funds requested from National Watermelon Association: **$11,111**
Duration of project: 1 year

Brief Summary of Proposal:

Watermelon vine decline caused by *Squash vein yellowing virus* (SqVYV) is a new and emerging disease that has caused severe losses to Florida watermelon growers in recent years. First identified in 2005, SqVYV causes a rapid vine decline in watermelon as the crop approaches harvest and is distributed widely in southwest and west-central Florida and has recently been reported from southern Indiana. Although the late stage symptoms of watermelon vine decline are basically diagnostic for the presence of SqVYV, earlier stage symptoms are not as obvious and may be confused with other causes. Hence, we propose to develop a simple and reliable diagnostic test for early monitoring of the virus that causes watermelon vine decline.

A) Early stage, B) middle stage, and C) late stage symptoms of watermelon vine decline.

For the past four years (2007-2010) we have conducted laboratory and greenhouse research at the USDA-ARS U.S. Horticultural Research Laboratory in Fort Pierce, FL to develop and evaluate reliable diagnostic tests for SqVYV. Two SqVYV diagnostic tests,

tissue blots and polymerase chain reaction (PCR), we developed in our laboratories have proven to be functional for identification of the virus. Although useful, both methods unfortunately have significant drawbacks. The tissue blot method allows the processing of many samples simultaneously but is not easily transferrable for use in other laboratories due to special equipment requirements. The PCR method has been transferred to diagnostic laboratories but is not suitable for processing large sample numbers.

After several years of difficulty, we have recently produced a rabbit antibody that preliminary tests show to be specific for (able to uniquely detect) SqVYV. With this new SqVYV antibody in hand, and our previous success in developing simple, rapid, robust, and transferrable ELISA (enzyme linked immunosorbent assay) diagnostic tests for other viruses, we will develop, optimize and evaluate a simple ELISA diagnostic test for watermelon vine decline and confirm its effectiveness using field samples. All experiments will be conducted at the U.S. Horticultural Research Laboratory in Fort Pierce, FL.

Significance of Proposed Project to Watermelon Growers:

Squash vein yellowing virus (SqVYV) was identified in 2005 as the cause of a rapid vine decline in watermelon, and fruit rind necrosis and discoloration, as the crop approaches harvest (Adkins et al., 2007b; Roberts et al., 2004; 2005). SqVYV is transmitted by whiteflies (*Bemisia tabaci*, biotype B). The virus is now distributed widely in southwest and west-central Florida, and has recently been reported from southern Indiana (Adkins et al., 2007b; Egel and Adkins, 2007). Crop losses in the 2003-2004 seasons due to SqVYV ranged from 50% to 100% with monetary losses to Florida watermelon growers estimated at 60-70 million U.S. dollars in 2004 alone (Huber, 2006).

Accurate identification is the first step in management of any pathogen including SqVYV. A rapid, reliable yet simple virus detection method is required to analyze sufficient numbers of plant samples to identify a given virus with certainty. ELISA diagnostic tests are routinely used for this purpose with other viruses due to their specificity and simplicity. However, we have only recently been able to produce an SqVYV antibody that appears to be suitable for development of an ELISA diagnostic test for this virus.

Although late stage symptoms of watermelon vine decline are often diagnostic in and of themselves, the availability of an SqVYV ELISA diagnostic test would potentially give watermelon growers an early monitoring technique for this virus facilitating its detection before symptoms of watermelon vine decline are obvious. This simple test will be ideally suited for analysis of large numbers of samples and should permit quantification of relative amounts virus present. Perhaps SqVYV could even be detected at or shortly after the first appearance of whiteflies in a field, or in weeds before transplanting, giving growers additional time to implement management strategies and alter cropping practices.

Preliminary Results and Potential for Success:

After several years of difficulty, our recent production of a rabbit antibody that appears to be specific for the detection of SqVYV puts development of a simple, reliable, robust and transferrable ELISA diagnostic test within reach. ELISA tests are already conducted in plant disease diagnostic laboratories worldwide and require no high dollar specialized equipment. An SqVYV ELISA diagnostic test would be faster than the other two types of diagnostic tests we have developed and are currently using for detection of SqVYV in watermelon and other cucurbit crop and weed hosts (Adkins et al., 2008; 2009).

We have previous experience in successfully developing ELISA diagnostic tests for other new viruses we have discovered including *Hibiscus latent Fort Pierce virus*, *Angel-*

onia flower break virus and Tropical soda apple mosaic virus (Adkins et al., 2006; 2007a; Kamenova and Adkins, 2003). Practical implementation of the ELISA diagnostic tests we have developed for these three viruses has seen their transfer to far-flung diagnostic laboratories to be put into use for routine identification of these viruses for growers.

Our recent generation of an SqVYV antibody coupled with our previous successes in using similar antibodies to develop useful ELISA diagnostic tests for other viruses gives us hope and incentive to develop and optimize an ELISA diagnostic test for SqVYV. Hence, we are requesting funds in 2010 to develop, optimize and critically evaluate a simple ELISA diagnostic test as an early monitoring tool for watermelon vine decline.

Outline of Specific Research to be Conducted:

Sufficient laboratory and greenhouse space is available at the U.S. Horticultural Research Laboratory in Fort Pierce, FL. All supply charges will be cost-shared by Adkins and Kousik from other funding as noted in "2010 Budget Contributions" below. Preliminary experiments have shown our latest SqVYV antibody to be specific for (detect only) SqVYV. However, to get this antibody out of our laboratory and into practical use by diagnosticians to help watermelon growers, it needs to be incorporated into a simple, rapid and robust ELISA diagnostic test. We will draw on our experience and initially explore several different methods to purify the antibody. Once purified, the antibody will be tested in several different types of ELISA diagnostic tests with several different buffer systems to fully optimize the test for watermelon tissue. The optimized ELISA diagnostic test will then be evaluated for its ability to detect SqVYV in other cucurbit crops and weeds, and re-optimized for this purpose if necessary. Finally, the ELISA diagnostic test will be transferred to other watermelon researchers for independent verification in their laboratories. We will also test the ability of the newly developed ELISA diagnostic test to detect SqVYV in the whitefly vector and quantify relative amounts of virus, and determine how soon after infection of watermelon that SqVYV can be detected. This will help determine the suitability of the ELISA diagnostic test to function as an early detection method for SqVYV and forecaster for watermelon vine decline. The sensitivity of the ELISA diagnostic test will be compared to our two existing but more complicated and time-consuming SqVYV diagnostic methods.

Publication of Results:

Diagnostic methods developed in these trials will be published in the NWA's online magazine "Vineline" and other appropriate grower and scientific publications, and also transferred to regional diagnostic laboratories and crop consultants that serve growers.

2010 Budget Contributions:

Funding Requested From National Watermelon Association:	
20% time of Research Associate Webster	**$11,111**
Total	**$11,111**
Cost-Share From USDA-ARS:	
10% time of Scientists Adkins & Kousik	$10,000
15% time of Technicians	$7,500
Supplies	$3,000
Total	$20,500
Overall Project Total	$31,611

Budget Justification:

The National Watermelon Association funding will be used to support Research Associate Webster (already in place) for 20% of his time to explore alternative methods to purify the SqVYV antibody, test different ELISA diagnostic test formats and buffer systems, optimize an ELISA diagnostic test for SqVYV detection in watermelon, other cucurbits and whiteflies, and compare the sensitivity of this new ELISA diagnostic test to our two existing but more complicated and time-consuming SqVYV diagnostic tests.

Scientists Adkins and Kousik will each devote 5% of their time to direct the project, coordinate transfer of reagents and protocols to other laboratories for independent verification of methods and to get the ELISA diagnostic into routine use to help watermelon growers, analyze data, and prepare and present reports. Technicians of Adkins will devote 15% of their time to sample collection (from greenhouse and field samples) and preparation for diagnostic testing with newly developed methods.

Literature Cited:

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