

## Report for the National Watermelon Association

### Development and evaluation of quantitative early monitoring techniques for *Squash vein yellowing virus*, the cause of watermelon vine decline – towards detection of two viruses with one test

**Scott Adkins, Craig G. Webster**  
USDA, ARS  
U.S. Horticultural Research Laboratory  
2001 South Rock Road  
Fort Pierce, FL 34945  
Phone: 772-462-5885  
Email: [scott.adkins@ars.usda.gov](mailto:scott.adkins@ars.usda.gov)

**C.S. (Shaker) Kousik**  
USDA, ARS  
U.S. Vegetable Laboratory  
2700 Savannah Highway  
Charleston, SC 29414

#### **Brief Summary of Research:**

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. Although the late stage symptoms of watermelon vine decline are basically diagnostic for the presence of SqVYV, early symptoms are not as obvious and may be confused with other causes including the recently introduced whitefly-transmitted *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucurbit leaf crumple virus* (CuLCrV). We have developed a quantitative early monitoring test for SqVYV that is able to detect the virus up to eight days before the earliest symptoms of vine decline appear. We have also developed a similar test for CYSDV for future combination into a single test capable of detecting both viruses.

#### **Project Outcomes:**

Accurate identification is the first step in management of any pathogen including SqVYV. Multiple, independent detection methods are required to identify a given virus with certainty. During the previous NWA funding cycle, we developed a rapid and reliable ELISA (enzyme linked immunosorbent assay) diagnostic test for SqVYV complementing two previously developed diagnostic assays [tissue blots and conventional polymerase chain reaction (PCR)] that have significant drawbacks limiting widespread implementation. The ELISA diagnostic test is capable of sensitively detecting SqVYV in watermelons and several related cucurbits, while showing no significant reaction with healthy (non-infected) watermelon and squash plants. The SqVYV ELISA diagnostic test has been demonstrated for growers, and transferred to scientists at University of Florida and Florida Department of Agriculture and Consumer Services for use in their diagnostic programs. Given the similarity of SqVYV isolates collected across Florida during the past six years, we expect this ELISA diagnostic test will detect all SqVYV isolates currently known, if the virus is present in sufficient concentration in the sample. However, there remains a need for a more sensitive (able to detect smaller amounts of SqVYV) and quantitative nucleic acid-based diagnostic test for SqVYV as a second, independent means of early monitoring.

During the current funding cycle, we developed and optimized a robust real-time PCR diagnostic test for SqVYV to address this need. The nuclear inclusion b gene of SqVYV was specifically targeted because sequence comparison suggested this region was unique among cucurbit viruses. Validation with greenhouse and field samples

demonstrated that the new real-time PCR diagnostic test was capable of sensitively detecting SqVYV in watermelons and several related cucurbits, while showing no significant reaction with healthy (non-infected) watermelon and squash plants.

A selection of additional viruses commonly infecting watermelons and other cucurbits in Florida, including recently introduced whitefly-transmitted CYSDV and CuLCrV, and long-present aphid-transmitted *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon leaf mottle virus* (WLMV), did not react in the SqVYV real-time PCR diagnostic test. In contrast, a variety of SqVYV isolates collected from watermelons and cucurbit weeds were detected by the real-time PCR diagnostic test highlighting its ability to detect multiple isolates of the virus. We expect this real-time PCR diagnostic test will detect all SqVYV isolates currently known given the similarity of SqVYV isolates collected across Florida during the past six years. Preliminary attempts to detect SqVYV in whiteflies were not successful.

The sensitivity of the newly developed real-time PCR diagnostic test was compared with our previously developed SqVYV diagnostic tests in two ways:

- 1) Our real-time PCR and conventional PCR diagnostic tests were used to evaluate 58 field samples for SqVYV infection. Both techniques correctly detected SqVYV in the same 24 samples. However, the real-time PCR diagnostic test also detected SqVYV in 6 additional samples demonstrating the increased sensitivity of real-time PCR.
- 2) We inoculated watermelon and squash plants with SqVYV in the greenhouse and monitored them daily for symptoms. We also collected samples daily for testing by the newly developed real-time PCR and previously developed conventional PCR and ELISA diagnostic tests. The real-time PCR diagnostic test detected SqVYV eight days before symptoms appeared in watermelon highlighting the promise of its use for early monitoring because the virus was detected one day earlier than by conventional PCR and two days earlier than by ELISA. In contrast, all three diagnostic tests detected SqVYV one day before symptoms appeared in squash.

Towards the goal of detecting two viruses with a single test, we also developed and optimized a robust real-time PCR diagnostic test for CYSDV that specifically targets the coat protein gene. SqVYV, CuLCrV and PRSV did not react in the CYSDV real-time PCR diagnostic test. In contrast, CYSDV was detected by the real-time PCR diagnostic test in a selection of field samples previously determined to be infected with CYSDV by conventional PCR.

### **Conclusions and Continuing Research Needs:**

The real-time PCR diagnostic tests we developed are able to accurately detect SqVYV or CYSDV in infected watermelon plants, even if they are also infected with closely related viruses. The sensitivity of the SqVYV real-time PCR diagnostic test allows it to reliably detect even small amounts of virus, making it possible to detect SqVYV in watermelon plants eight days before the earliest symptoms of vine decline appear.

We will continue to improve the SqVYV and CYSDV real-time diagnostic tests. We are currently working to combine them into a single test capable of detecting both viruses, and to optimize them for virus detection in whiteflies.

In future funding cycles, we would like to develop a single test for SqVYV, CYSDV and CuLCrV so that these three whitefly-transmitted watermelon viruses could all be detected at once.