Report 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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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, earlier stage symptoms are not as obvious and may be confused with other causes. We have developed a simple and reliable ELISA (enzyme linked immunosorbent assay) diagnostic test for early monitoring of SqVYV. This easily transferrable ELISA assay complements two previously developed diagnostic assays [tissue blots and polymerase chain reaction (PCR)] that have significant drawbacks limiting widespread implementation.

Project Outcomes:

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 is suitable for development of an ELISA diagnostic test for this virus.

During the current funding cycle, we developed and optimized a rapid and robust ELISA diagnostic test for SqVYV using this antibody. The new ELISA diagnostic test was capable of sensitively detecting SqVYV in watermelons and several related cucurbits, while showing no significant reaction with healthy (uninfected) watermelon and squash plants.

A panel of additional viruses commonly infecting watermelons and other cucurbits in Florida, including recently introduced whitefly-transmitted *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucurbit leaf crumple virus* (CuLCrV), and long-present aphid-transmitted *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV), did not react in the SqVYV ELISA diagnostic test. In contrast, a variety of SqVYV isolates collected from watermelons and cucurbit weeds were detected by the ELISA diagnostic test highlighting its ability to detect multiple isolates of

the virus. 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.

We have tested field samples of watermelon and squash using the SqVYV ELISA diagnostic test for growers and scientists (including Dr. Pam Roberts and Dr. Susan Webb at University of Florida) to further demonstrate the usefulness of this technique. We have also transferred the method to Dr. Webb and Dr. Carlye Baker (Florida Department of Agriculture and Consumer Services) for testing in their diagnostic programs.

Conclusions and Continuing Research Needs:

The SqVYV ELISA diagnostic test we developed is able to accurately detect SqVYV in infected watermelon plants, even if they are also infected with closely related viruses. The simplicity of this new diagnostic test makes it easily transferrable to diagnostic and research laboratories, and adaptable for large scale testing.

We will continue to improve the SqVYV ELISA diagnostic test and are currently using it to determine how soon after infection of watermelon that SqVYV can be detected. The SqVYV ELISA diagnostic test is also being compared with our previously developed tissue blot and PCR diagnostic tests.

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.