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Ecology and management of whitefly-transmitted viruses of vegetable crops in Florida☆

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ABSTRACT

A variety of fresh market vegetables, including watermelon and tomato are economically important crops in Florida. Whitefly-transmitted *Squash vein yellowing virus* (SqVYV) was first identified in squash and watermelon in Florida in 2005 and shown to cause a severe decline of watermelon vines as crops approach harvest. Florida is most economically impacted by SqVYV, although the virus has been detected more recently in Indiana and South Carolina. The origin and evolutionary history of SqVYV, one of the few members of the genus *Ipomovirus* within the family *Potyviridae*, are not known. Sequence diversity of SqVYV isolates collected at different times, from different locations and from different plant species is being analyzed for insights into the origin of the virus. More recently, *Cucurbit leaf crumple virus* (CuLCrV) and *Cucurbit yellow stunting disorder virus* (CYSDV), also whitefly-transmitted, have been detected in watermelon in Florida. *Tomato yellow leaf curl virus* (TYLCV) was first detected in south Florida tomato crops in 1997. Several surveys have been conducted in the region to identify alternative hosts for these four viruses. Cucurbit weeds including Balsam-apple (*Momordica charantia*), creeping cucumber (*Melothria pendula*) and smellmelon (*Cucumis melo* var. *dudaim*) provide reservoirs for SqVYV, CuLCrV and/or CYSDV. Green bean (*Phaseolus vulgaris*) also can be a reservoir for CuLCrV. No wild hosts of TYLCV have been reported in Florida. The effectiveness of insecticides and silver plastic mulch to manage whiteflies and mitigate TYLCV has been demonstrated and is currently being evaluated for SqVYV, CuLCrV and CYSDV. In addition, potential sources of SqVYV resistance have been identified in greenhouse and field screening of watermelon germplasm. Further studies to refine these sources of resistance are underway. Lastly, a comprehensive map of 33,560 hectares (82,928 acres) of vegetable fields in the three counties comprising the majority of the southwest Florida vegetable production area has been developed to identify 'hot spots' and reservoir crops for viruses and whiteflies, and will be useful in evaluation of management strategies to decrease virus incidence in commercial fields.

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1. Introduction

Fresh market vegetables are economically important crops in Florida, with the state ranked first in the U.S. in production of watermelon in 2007, 2008, and 2009 (Anonymous, 2010). Florida is the only U.S. producer of watermelon from December through April (Bertelsen et al., 1994), and the crop had an annual mean value of $138 million to the state between 2007 and 2009 (Anonymous, 2010). Tomato is the most important of Florida's winter fresh market vegetables with an annual mean value of just over $522 million between 2007 and 2009, representing 37% of the total value of Florida's vegetable production (Anonymous, 2010). Florida ranked first annually in the U.S. in production of fresh market tomato between 2007 and 2009 (Anonymous, 2010).

Watermelon, tomato and other vegetable crops are infected by a wide range of viruses, many of which are transmitted by whiteflies. Until the early 2000s, *Papaya ringspot virus* type W (PRSV-W) and other aphid-transmitted potyviruses were the most important group of viruses infecting watermelon and other cucurbit crops in

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Florida and elsewhere in the southeastern U.S. (Webb et al., 2003). However, since 2005 three whitefly-transmitted viruses [*Squash vein yellowing virus* (SqVYV), *Cucurbit leaf crumple virus* (CuLCrV) and *Cucurbit yellow stunting disorder virus* (CYSDV)] have been detected for the first time and become widespread in watermelon in Florida. SqVYV was first identified in 2005, causes a rapid vine decline near harvest and is now widely distributed in southwest and west-central parts of the state (Adkins et al., 2007; Roberts et al., 2004). It has also been detected in Indiana (Egel and Adkins, 2007) and South Carolina (Kousik and Adkins, unpublished data). CuLCrV was first found in north-central Florida in fall 2006 and has spread subsequently to southwest, west-central, and north Florida (Akad et al., 2008). CYSDV was first found in west-central Florida in summer 2007 (Polston et al., 2008) and has since spread to southwest Florida.

Currently widespread and/or economically important tomato viruses in Florida include two that are whitefly-transmitted, *Tomato yellow leaf curl virus* (TYLCV) and *Tomato chlorosis virus* (ToCV), and two that are thrips-transmitted, *Tomato spotted wilt virus* (TSWV) and *Groundnut ringspot virus* (GRSV). TYLCV was first detected in 1997 in south Florida (Polston et al., 1999) and has spread subsequently throughout the peninsula (where it remains the major virus pathogen of tomato), and to other southeastern states including South Carolina and Alabama (Ling et al., 2006; Akad et al., 2007). In contrast, TSWV has been present in north Florida (and much of the southeastern U.S.) since the mid-1980s where it causes economic losses in tomato, peanut and tobacco (Bauske, 1998). ToCV was first identified in 1996 in greenhouse tomato in north Florida (Wisler et al., 1998) and has since been found in field tomato throughout the state although its symptoms are often obscured by those of TYLCV or TSWV. GRSV has recently been detected in south Florida (Webster et al., 2010).

There are several taxonomic affinities between these viruses of watermelon and tomato. For instance, CuLCrV and TYLCV are both members of the genus *Begomovirus*, CYSDV and ToCV are both members of the genus *Crinivirus*, and TSWV and GRSV are both members of the genus *Tospovirus*. There are also several host range and insect vector differences between these viruses. For instance, SqVYV and PRSV-W are only known to infect watermelon (and other cucurbit crops) but not tomato in Florida, whereas TSWV and GRSV both infect tomato (and other solanaceous crops) but are not known to infect watermelon in Florida. Several species of thrips transmit TSWV and GRSV, several species of aphids transmit PRSV-W, and a single species of whitefly (*Bemisia tabaci*, biotype B) transmits SqVYV, CuLCrV, TYLCV and CYSDV. This article will consider the ecology and management of these four whitefly-transmitted viruses, representing three taxonomically distinct types of viruses that infect watermelon or tomato in Florida. Although somewhat disparate topics, ecology and management are really inter-related, for it is exceedingly difficult to manage a pathogen whose ecology is not well understood.

2. Ecology of whitefly-transmitted vegetable viruses in Florida

A central issue for understanding the ecology of these viruses is the determination of reservoir hosts for the viruses and their vectors within the environment. Reservoir hosts can include watermelon or tomato crops (especially if cropping is continuous throughout the year), and other related or non-related crops during the cropping season. Weeds and volunteer watermelon or tomato plants bridging the spring and fall cropping seasons can serve as oversummering hosts in Florida. Crop plants are generally the overwintering hosts in Florida as this is the major cropping season in the state.

Considerable effort has been made to identify non-watermelon hosts for SqVYV, CuLCrV and CYSDV, and cucurbit weeds have been a consistent priority for examination as no non-cucurbit hosts have been identified for SqVYV. Symptomless *Momordica charantia* (Balsam-apple) plants infected with SqVYV have been found within or adjacent to fields with declining watermelon in southwest and west-central Florida since 2005 when the virus was first identified in squash and watermelon (Adkins et al., 2008). Forty-three percent of *M. charantia* plants sampled from major watermelon production areas were infected with SqVYV. Whiteflies were able to acquire and transmit SqVYV from infected *M. charantia*, although virus titre was apparently low in these plants. Subsequently, SqVYV and CuLCrV have been detected in *M. charantia* between spring and fall crops demonstrating that it is an oversummering host for both viruses. *Melothria pendula* (creeping cucumber) was shown to be an experimental host for SqVYV (Adkins et al., 2008). Symptomless *Cucumis melo* var. *dudaim* (smellmelon) plants infected with both SqVYV and CuLCrV have been found adjacent to watermelon fields in southwest Florida since May 2008 (Adkins et al., 2009b). As CYSDV became more widespread in Florida, *C. melo* var. *dudaim* plants with chlorosis of the lower leaves were observed in this same geographic area and shown to be infected with SqVYV, CuLCrV and CYSDV.

Several non-cucurbit hosts have also been identified in areas adjacent to infected watermelon fields. Symptomatic *Phaseolus vulgaris* [fresh market common (green) bean] plants infected with CuLCrV have been found in southwest Florida (Adkins et al., 2009a). Symptomless *Amaranthus* spp. (pigweed) plants infected with CYSDV were identified in southwest Florida in fall 2009 (Webster et al., 2011), following similar reports from California (Wintermantel et al., 2009). Similarities and also differences between Florida and California CYSDV reservoirs are currently being studied and are to be expected because of differences in crops and cropping practices in these geographically and climatically distinct regions of the country.

In contrast to our work with cucurbit virus reservoirs, no wild plant species has been shown in Florida to serve as an oversummering host for TYLCV to infect fall tomato crops. Near-continuous cropping of tomato is suspected to provide a sufficient virus reservoir for infection of the commercial crop (Polston et al., 2009).

The presence of SqVYV, CuLCrV and CYSDV in common cucurbit weed hosts raises the question – are viruses moving from weeds to crops or vice versa? SqVYV was first identified in Florida and still causes the largest economic impact there, unlike CuLCrV and CYSDV, which were first found elsewhere in the U.S. before detection in Florida (Brown et al., 2002, 2007; Guzman et al., 2000; Kao et al., 2000; Kuo et al., 2007). As the origin and evolutionary history of SqVYV are not known, sequence diversity of SqVYV isolates collected at different times, from different locations and from different plant species is being analyzed for insights into the origin of the virus. Coat protein gene sequences from watermelon and weed isolates analyzed to date have shown limited diversity with the mean diversity of coat protein gene sequences of $0.55 \pm 0.08\%$ and the maximum percentage divergence of isolates of 2.22%. *Sweet potato mild mottle virus*, the type ipomovirus species, has greater nucleotide diversity reported across the genome of isolates from its centre of origin in East Africa (Tugume et al., 2010), which suggests that the limited diversity of known SqVYV isolates may result from a recent genetic 'bottleneck' event. A likely explanation is that SqVYV was recently introduced to Florida from elsewhere, although the virus has since become established in both cucurbit crops and weeds in the state. Other than the isolated reports from Indiana and South Carolina noted above, SqVYV has not been reported outside of Florida so its origins remain unknown. Phylogenetic reconstructions show close relationships between all isolates and no separation of isolates based on time, host or location. This supports the idea that weed species act as virus reservoirs (e.g. Adkins et al., 2008). Further sequencing and analysis of SqVYV isolates from additional locations and plant species is ongoing, including examination of different and potentially more genetically diverse regions of the genome.

3. Management strategies

A number of strategies have been and are being explored for management of whitefly-transmitted vegetable viruses in Florida. These include management of virus and vector reservoirs, grafting onto resistant rootstocks, application of insecticides combined with the use of silver plastic mulch, host resistance, and improved methods of crop destruction. A decision support system (DSS) is also being developed to help growers select appropriate management techniques based on local virus and insect vector pressure, and weather forecasts.

Control of cucurbit weeds to reduce virus and vector reservoirs is limited in practice by the subtropical year-round climate of Florida and the ubiquitous nature of cucurbit weeds. However, where practical, destruction of cucurbit weeds adjacent to watermelon fields should help to reduce both viruses and vector. Existing, TYLCV-infected tomato fields are often the most important source of this virus and its vector (Polston and Lapidot, 2007). Symptomless infection of pepper can also provide a reservoir of TYLCV for tomato (Morilla et al., 2005; Polston et al., 2006). A tomato- and pepper-free period has been suggested (but not yet tried in Florida) as a means of reducing initial TYLCV inoculum levels (Polston et al., 2009).

Grafting onto resistant rootstocks is effective for managing many soil-borne pathogens such as fungi and nematodes, and various cucurbit vine declines (Cohen et al., 2007; Davis et al., 2008; Thies et al., 2010). In some instances, grafting seedless watermelon on virus-tolerant rootstocks has been reported to help manage two potyviruses, *Zucchini yellow mosaic virus* and PRSV-W (Wang et al., 2002). However, grafting has not proven useful for managing watermelon vine decline caused by SqVYV because watermelon scions grafted to SqVYV-tolerant rootstocks still declined (Kousik et al., 2007).

Insecticides and silver plastic mulch are useful tools to manage whiteflies, watermelon vine decline (caused by SqVYV), and TYLCV in tomato (Kousik et al., 2008; Polston and Lapidot, 2007; Roberts et al., 2007). In studies conducted from 2006 to 2009, significantly fewer SqVYV-symptomatic fruits were observed in watermelon that was treated with insecticides for managing whiteflies (Kousik et al., 2008, 2010a, 2010b). The chemical treatment in these studies consisted of an imidacloprid (Admire; Bayer CropScience, Research Triangle Park, NC) drench at transplanting followed by two sprays of spiromesifen (Oberon; Bayer CropScience) during the season to manage whiteflies. Adult whitefly counts recorded about one month after transplanting were lower in chemically treated plots compared to untreated plots in these experiments (Kousik et al., 2010a). Recent increases in insecticide-resistant whitefly populations in Florida (Schuster et al., 2006) and the emergence of biotype Q whitefly populations resistant to many common insecticides is a matter of great concern and highlight the need for long-term, sustainable management strategies for these viruses and their vectors (Dennehy et al., 2005; Schuster et al., 2007).

Silver plastic mulch has been shown to lower whitefly populations in watermelon (Simmons et al., 2010) and tomato (Kring and Schuster, 1992). Silver plastic mulch was effective in significantly reducing development of watermelon vine decline in 2007 (Kousik et al., 2008) but not in 2009 (Kousik et al., 2010a). Silver plastic mulch is effective in reducing the incidence of TYLCV in tomato fields in Florida (Polston and Lapidot, 2007) and is widely used in north Florida to reduce colonization of tomato by thrips and thereby reduce the incidence of TSWV (e.g. Momol et al., 2004). Some watermelon growers are currently using silver plastic mulch to help manage whiteflies, but because of the additional cost of silver plastic mulch (compared to the more commonly used white or black plastic mulch), growers need to carefully consider the economics of silver plastic mulch use.

Potential sources of SqVYV resistance have been identified in wild watermelon germplasm (Kousik et al., 2009) including a wild citron (*Citrullus lanatus* var. *citroides*) collected in Florida (C.G. Webster, C.S. Kousik, S.E. Webb, W.W. Turechek and S. Adkins, unpublished). Disease progress was significantly slower on selected watermelon plant introductions (PIs) compared to susceptible watermelon cultivars. However, none of the germplasm evaluated was completely immune to SqVYV. PI 500354 (*C. lanatus* var. *citroides*), PI 386024 (*C. colocynthis*), and PI 459074 and PI 392291 (*C. lanatus* var. *lanatus*) also had significantly lower incidences of watermelon vine decline in field studies compared with highly susceptible commercial watermelon cultivars. Variability in the resistance reaction to SqVYV within these and other accessions was observed and therefore single plant selections are underway to develop resistant lines (Kousik et al., 2009). The identification of potential sources of resistance to SqVYV suggests that watermelon germplasm with moderate resistance can be developed by careful screening and selection of individual resistant plants within these PIs for use in breeding programmes. Such SqVYV-resistant material may also be useful as pollenizers for management of watermelon vine decline in seedless watermelon production, and is currently being evaluated for this purpose. TYLCV can also be managed by using resistant tomato cultivars, but these are not universally available or acceptable (Polston and Lapidot, 2007).

After crops are harvested, it is common practice to destroy or ‘burn down’ the fields with a fast-killing herbicide to eliminate them as a source of viruses or vectors for adjacent fields still in production. Insecticide and/or oil is often added to the herbicide treatment to kill whiteflies and prevent them from leaving the treated crops as the plants collapse, and infecting the remaining production. Although this practice appears justifiable, it is not known whether the addition of an insecticide and/or oil is fully effective and, consequently, worth the additional expense. We are currently examining crop destruction herbicide treatments with and without insecticide and/or oil in tomato and watermelon. Preliminary experiments suggest little effect of treatment on the number of whiteflies leaving a treated area.

Recent efforts have focused on development of an online scouting and DSS for the whitefly-transmitted viruses important in Florida vegetable production. Ideally, such a system would provide real-time information on the status of viruses and their vectors, and help identify ‘hot spots’ for both viruses and vectors. The current practice for scouting Florida vegetables for diseases and insects is use of pen and paper on a clipboard. Hardcopies are then collated, summarized and distributed to growers. Recently, we developed a comprehensive map of 33,560 hectares (82,928 acres) of vegetable fields in the three counties (Collier, Hendry and Lee) comprising the majority of the southwest Florida vegetable production area. Historical TYLCV incidence and whitefly counts were entered into the system for analysis and display and are being used as ‘proof of concept’ for the system. With this data from the recent growing seasons, relationships between whitefly numbers and TYLCV incidence are being examined, and ‘hot spots’ for whiteflies and TYLCV are being identified. Ultimately, this should lead to an all-electronic format where scouts directly enter insect counts and disease severity using a handheld wireless device that is GPS capable. This information would then be immediately available to a grower via computer or smart phone. This system is already being expanded to include

SqVYV, CuLCrV and CYSDV. It is also easily extendable to include newly detected viruses and insects, like the recently detected and thrips-vectored GRSV. A web-based scouting and DSS for tracking and managing viruses and vectors across commodities would lead ideally to more cost-effective, environmentally sound choices, and present interesting research opportunities.

4. Conclusions

Although SqVYV, CuLCrV, TYLCV and CYSDV differ taxonomically, they are all whitefly-transmitted viruses that have become increasingly problematic for watermelon and tomato producers in Florida following their introduction into the state. Until 2005 no whitefly-transmitted viruses of cucurbits were known in Florida; now there are three. Reservoir hosts are important sources of both the viruses and their vector, although the relative importance of weeds and crop plants differs depending on the specific virus considered. Complete elimination of reservoirs is virtually impossible but intensive efforts to remove localized areas of reservoir hosts may be useful to reduce whitefly sources and disease pressure on a smaller scale. Remaining whiteflies can be managed with a combination of insecticides and silver plastic mulch, but insecticides must be used judiciously to decrease development of resistance by the whitefly. In the long-term, use of germplasm that offers resistance to the viruses is likely to be the most sustainable practice. The relative merits of various methods of crop destruction following harvest are still being evaluated. Integrated management involving different measures is required as no single technique is likely to be successful as previously noted by Jones (2001, 2004). The web-based scouting and DSS currently in trial should provide an effective means of integration.

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