ASSESSMENT OF SPREAD AND A RISK ASSESSMENT TOOL FOR WHITEFLY-TRANSMITTED VIRUSES ON VEGETABLES IN GEORGIA

by

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(Under the Direction of Bhabesh Dutta and Robert C. Kemerait, Jr.)

ABSTRACT

Whitefly-transmitted viruses are a major production concern for fall-grown vegetables including snap beans, squash, and tomatoes in the southeastern United States. Currently, no single pest management tactic has proven to provide sufficient control of whitefly-transmitted viruses. To aide in the development of appropriate pest management programs, the spatial distribution and temporal patterns of whitefly transmitted viruses were documented. In squash, symptoms of disease caused by the whitefly-transmitted virus complex (WTVC) were initially randomly distributed but later shifted to an aggregated pattern as the incidence of virus-infected plants increased over time. Mapping of interpolated cluster indices from SADIE analysis revealed an edge effect on the spread of WTVC infection over space and time in squash fields. Similar patterns were observed on snap beans where the incidence of sida golden mosaic virus (SiGMV) was greater along edges in snap bean fields. The presence of SiGMV infected prickly sida in field margins adjacent to snap bean plots was associated with earlier occurrence and higher SiGMV incidences in snap beans. Multiple field trials were conducted to assess different planting dates, mulch types, row cover, planting materials, and types of squash as tactics for managing WTVC in squash. Using data generated from these field trials, a risk assessment index for WTVC in squash was developed through a novel approach. Random forest analysis was used to determine the quantitative importance of different variables as predictors of WTVC. The risk index was validated through survey of commercial squash fields where the calculated risk point values showed a positive linear relationship with the observed WTVC incidences. The risk index developed through this study may be used as a guide to determine suitable integrated pest management programs that would help mitigate high levels of WTVC and yield losses in squash fields.

INDEX WORDS: whitefly-transmitted viruses, spatiotemporal patterns, risk assessment index

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DEDICATION

This work is dedicated to my wife Bencie
and children Ieza Wren and Saphira
who have somehow managed to keep me sane.

To lola Penny, iyaman sin panangisurom en dakami
si mayat ay amag. Iyaman abe ta sin ed mu
ay nasiken kami asam amey ed langit.

To my parents Gloria and Nelson,
thank you for always supporting my dreams.

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To my family and friends, you have contributed in getting me through this quest more than you might recognize.

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INTRODUCTION AND LITERATURE REVIEW

Introduction

Whitefly transmitted viruses have emerged as a global threat to the production of a wide range of crops over the last 20 years (Navas-Castillo et al. 2011). Among the crops affected, whiteflies and whitefly-transmitted viruses are primary concerns in dicotyledonous plants, many of which are economically significant. Some of the greatest losses occur in fiber crops such as cotton (Chavan et al. 2010); vegetable crops such as beans, cucurbits, pepper, tomato (Adkins et al. 2007; Adkins et al. 2009; Guzman et al. 2000; Kao et al. 2000; Kenyon et al. 2014; Polston et al. 1999); and agronomic crops such as soybean (Murgianto and Hidayat 2017). Yield losses due to whiteflies and whitefly-transmitted viruses range from minimal to complete crop failure depending on occurrences of type of viruses, type of crops, age of crops at the time of infection, and the incidence and severity of viral infection (Lapidot et al. 2014).

Management of whiteflies and whitefly-transmitted viruses to reduce losses is challenging. The polyphagous nature, higher reproductive rate, short generation times, and resistance to insecticides aids the whiteflies to survive and dominate in agroecosystems throughout the world (Soumia et al. 2021). While resistance to whitefly-transmitted viruses has been developed in some crops such as tomato (Vidavski et al. 2008) and cassava (Jennings 1994), occurrence of new virus strains or high virus pressure have been reported to overcome resistance (García-Cano et al. 2008; Ohnishi et al. 2016). Control of whiteflies and viruses requires timely use of numerous management tactics, usually with heavy reliance on insecticides, in addition to use of resistant varieties if available.

Whiteflies

Whiteflies belong to the *Aleyrodidae* family (order Hemiptera), which is comprised of more than 1500 species in 126 genera (Martin 2004). Whiteflies have a mealy white wax covering on the wings and bodies of adults thereby giving them a white color and thus the name "whiteflies" (Hodges and Evans 2005). Immature and adult whiteflies have piercing, sucking mouthparts and damage crops by feeding within vascular tissues and sucking plant sap. Of the 126 genera that have been described, only the *Bemisia* and *Trialeurodes* genera are verified plant virus vectors (Mound and Halsey, 1978). Five species within the *Bemisia* and *Trialeurodes* genera are known to transmit plant viruses: *Bemisia afer*, *Bemisia tabaci* species complex (sweetpotato whiteflies), *Parabemisia myricae* Kuwana (bayberry whitefly), *Trialeurodes abutilonea* (banded wing whitefly), and *Trialeurodes vaporariorum* (greenhouse whitefly) (Lapidot et al. 2014; Navas-Castillo et al. 2011). Among the whitefly species, *Bemisia tabaci* species complex has been considered as one of the most damaging pests of the open field and protected cropping systems (De Barro 2011). *Bemisia tabaci* has a global distribution and is a considerable pest of ornamental, vegetable, grain legume, and cotton production (Jones 2003).

The *B. tabaci* species complex is composed of morphologically indistinguishable species (Boykin et al. 2007). However, they have differences in their biological characteristics (e.g., ability to induce phytotoxic responses, plant virus transmission capabilities, pesticide resistance expression, symbiotic bacteria, and host plants), biochemical attributes (e.g., diagnostic esterase banding pattern), and mitochondrial COI (cytochrome c oxidase subunit 1) DNA sequence divergence (Boykin et al. 2007; Watanabe et al. 2019). A study on the global phylogenetic relationships of *B. tabaci* revealed that the species complex is composed of at least 34 morphologically indistinguishable species based on the mitochondrial cytochrome oxidase one gene (mtCOI) (Boykin et al. 2007). The Middle East–Asia Minor 1 (MEAM1) species (biotypes B and B2) of the *B. tabaci* species complex was the first globally invasive species (Toscano et al. 1998). The MEAM1 species originated in the region that includes Iran, Israel, Jordan, Kuwait, Pakistan, Saudi Arabia, Syria, United Arab Republic, and Yemen. This species has spread to at least 54 countries, including the United States, via trade in ornamentals (Broadbent et al. 1989). The second invasive species of *B. tabaci* was the Mediterranean (MED) species (Q biotype). The MED species originated in countries bordering the Mediterranean Basin (Algeria, Crete, Croatia, Egypt, France, Greece, Israel, Italy, Morocco, Portugal, Spain, Sudan, Syria, Turkey) and has spread to at least 10 countries including the United States (De Barro 2011). The occurrence of *B. tabaci* MED species in agricultural fields is extremely problematic because their populations are prone to develop resistance against commonly used insecticides such as neonicotinoids and insect growth regulators (McKenzie et al. 2012). In the southeastern United States, it has been found from studies conducted in Florida and Georgia that

MEAM1 species are still predominant in vegetable fields (McKenzie et al. 2012; Gautam et al. 2020). Analyses of microsatellite markers exhibited low genetic differentiation among *B. tabaci* MEAM1 populations collected from different host plants and farmscapes in Georgia (Gautam et al. 2020). Further, Gautam et al. (2020) also found that a single panmictic population of *B. tabaci* MEAM1 dominated among hosts sampled across different locations in the state.

Bemisia tabaci was named “sweetpotato whitefly” after its discovery in 1894 on sweet potato plants in the United States (Li et al. 2021). Since the discovery, sweetpotato whiteflies have become widespread throughout the agricultural regions in the southern and western United States. However, sweetpotato whiteflies were not recognized as an economic pest until 1981 when fall grown vegetables, melon crops, and sugar beets were decimated by the whitefly-transmitted lettuce infectious yellows virus (LIYV) in the southwestern United States (Duffus et al. 1986). Viruses transmitted by sweetpotato whiteflies have spread across the southern states where extreme field outbreaks have occurred on melons, squash, tomato, cotton, and other vegetables (Gonzales et al. 1992; Hoelmer et al. 1991; Simmons et al. 2008).

Whiteflies can disperse naturally over short distances. While whiteflies are not efficient in flight, they can cover long distances assisted by wind (Byrne and Bellows 1991). Transportation of infested ornamental plants, grafted crop plants, seedlings, and cut flowers also facilitates the spread of whiteflies over long distances.

The life cycle of *B. tabaci* consists of six stages to include, egg, four nymph stages, and winged adults. The developmental time of *B. tabaci* from egg to adult varies greatly, from 14 to 105 days, depending on the temperature and available host plants

(Wang and Tsai 1996; Xei et al. 2011). The optimal temperature range for *B. tabaci* growth is 20- 33°C. Under suitable conditions, the development time of *B. tabaci* on cotton was reported to be 16- 40 days (Nava-Camberos et al. 2001). Given host availability and optimal conditions, whiteflies can have multiple generations during one cropping season. Moreover, *B. tabaci* females were reported to be capable of laying over 500 eggs throughout their lifetime (Naranjo et al. 2009). The relatively short generation time and high fecundity are key characteristics that allow whiteflies to build populations causing significant damages on economically important crops.

There are three main ways that sweetpotato whiteflies can cause damage to plants. The first is caused by the direct feeding of immature and adult stages. Nymphs and adult whiteflies feed by inserting their proboscises into the leaf, penetrating into the phloem and withdrawing sap (Jones 2003). Heavy infestations of nymphs and adults can cause seedling death, stunting, and reduction in yield (Gangwar and Charu 2018). During feeding, immature whiteflies inject salivary fluids which causes physiological disorders, such as silvering in squash and irregular ripening of tomatoes (McCollum et al. 2004).

The second way that whiteflies cause damage is the excretion of honeydew onto the surfaces of leaves and fruit (Byrne and Bellows, 1991). Honeydew is a sugar-rich sticky liquid excreted by certain insects (Calabuig et al., 2015). The honeydew excreted by whiteflies contain carbohydrates and amino acids which acts as a substrate for the growth of sooty mold fungi which interferes with photosynthesis and reduces the quality of fruit and fiber (Byrne and Bellows 1991).

The third way that sweetpotato whiteflies can cause damage to plants is indirect by acting as vectors of economically important genera of plant viruses, including

Begomovirus (Geminiviridae), Carlavirus (Betaflexiviridae), Crinivirus (Closteroviridae), Ipomovirus (Potyviridae), Polerovirus (Solemoviridae), and *Torradovirus (Secoviridae)* (Fiallo-Olive et al. 2020; Ghosh et al. 2019; Navas-Castillo et al. 2011; Sõmera et al. 2021). Begomoviruses and criniviruses are genera of whitefly-transmitted viruses recognized as important emerging plant virus groups worldwide (Jones 2003; Liu et al. 2013; Tzanetakis et al. 2013).

Pattern of whitefly abundance

Adult whiteflies are usually more concentrated close to the ground and to the source of infestation (Norman et al. 1996). Adults emerge from pupae during the morning and become more active as the temperature increase. Movement of whiteflies is greatest from mid-morning to mid-afternoon. Adult whiteflies can fly short distances within the plant canopy and are carried long distances on air currents (Norman et al. 1996). When choosing a host to settle on, whiteflies prefer younger leaves where they can feed and oviposit (Liu and Stansly 1995). After settling, adults congregate, feed, and mate on the undersurfaces of the leaves of a host plant (Norman et al. 1996).

In Texas, monitoring of whiteflies showed that the peak of migration occurred in late July and then drastically declined in August (Norman et al. 1996). The rapid increase, peak, and decline in the number of migrating adult whiteflies correlated with the maturation and defoliation of the cotton crop. In Georgia, monitoring of 125 locations revealed that whitefly populations were low from January until late July, followed by a rapid increase in the months of August and September, and then a rapid decline in October (Barman et al. 2019; Candian et al. 2021; Srinivasan et al. 2012). Destruction of crop hosts at harvest, as well as the occurrence of cooler temperatures and fall build-up of natural enemies, contributes to the regional decline of whitefly numbers (Norman et al. 1996).

Sweetpotato whiteflies have a broad host range including crops and weeds. In southern Georgia, various hosts of sweetpotato whiteflies are present year-round allowing sweetpotato whiteflies to be active throughout the year (Sparks et al. 2018). Populations of whiteflies move from winter vegetables (cabbage, collards, kale) to spring vegetables (cucurbits, tomatoes, and beans). From spring vegetables, whiteflies move to agronomic crops (especially cotton) in late spring and summer. Whiteflies then move to fall-grown vegetables (cucurbits, tomatoes, and beans). After the termination of fall vegetables, whiteflies then move to winter vegetables. Prevalence of host plants across the seasons in a year facilitates survival and propagation of whitefly populations and potentially survival of viruses in them.

Whitefly-transmitted viruses

Vector transmission is an essential step in the infection cycle of most plant viruses. Vectors facilitate the spread of the virus from one host to another. Diverse organisms are recognized as vectors of plant-viruses, including fungi, nematodes and insects. Insects are the most common vectors of plant viruses. Many of the insect vectors are plant pests, such as aphids, leafhoppers, thrips, and whiteflies (Catto et al. 2022). There are different types of virus transmission based on retention time of virions inside the vector and mechanism of transmission (Ng and Falk 2006). The types of transmission are nonpersistent transmission or stylet-borne, semi-persistent transmission or foregutborne, persistent and circulative transmission, and persistent and propagative transmission (Ng and Falk 2006).

Whiteflies transmit plant viruses in a persistent or semipersistent manner. Semipersistent transmission of viruses requires minutes to hours for acquisition of virus particles. After acquisition, the virus particles are retained in the foregut of the insect vector for hours to days. In contrast, persistent transmission of viruses requires a longer period for acquisition and the virus particles are retained in the hemolymph for days up to the lifetime of the insect. Persistent transmission of viruses can be further classified as either circulative transmission, where the virus does not replicate in the insect, or propagative transmission, where the virus replicates inside the insect vector (Ng and Falk 2006).

Whiteflies acquire virus particles through their stylet while feeding in the phloem of an infected plant. Depending upon mode of transmission, the virus particles are retained in the insect within minutes or hours. Semipersistently transmitted viruses move from the stylet to the esophagus, and to the foregut where they are retained (Ng and Falk 2006). The transmission of viruses happens during feeding through an ingestion-egestion mechanism (Chen et al. 2011).

Viruses that are transmitted in a persistent manner move from stylet to the esophagus and to the midgut. The virus particles cross the filter chamber and midgut into the hemolymph through receptor-mediated endocytosis. The virions travel through the hemolymph, reach the primary salivary glands, and move through different salivary gland physical barriers before being egested with saliva into the plant phloem during feeding (Whitfield et al. 2015).

Sweetpotato whiteflies (*B. tabaci*) are referred to as "supervectors" for their ability to transmit over 100 plant viruses (Li et al. 2021). Sweetpotato whiteflies can transmit six genera of plant viruses, including *Begomovirus (Geminiviridae), Ipomovirus (Potyviridae), Crinivirus (Closteroviridae), Carlavirus (Betaflexiviridae), Torradovirus (Secoviridae),* and *Polerovirus (Solemoviridae)* (Navas-Castillo et al. 2011; Fiallo-Olive et al. 2020; Sõmera et al. 2021). In the southeastern US, begomoviruses and criniviruses are the two most important genera among the sweetpotato whitefly-transmitted viruses (Gautam 2019).

The genus *Begomovirus* (family *Geminiviridae*) is the largest genus in the entire virosphere, with more than 409 recognized species (Zerbini et al. 2017). Begomoviruses are single-stranded DNA plant viruses and are considered one of the most important groups of emerging plant viruses in tropical and sub-tropical regions (Navas-Castillo et al. 2011). In the southeastern US, some of the economically important begomoviruses include cucurbit leaf crumple virus (CuLCrV) in cucurbits and snap beans, tomato yellow leaf curl virus (TYLCV) in tomatoes, and sida golden mosaic virus (SiGMV) in snap beans (Akad et al. 2008; Pappu et al. 2000; Durham et al. 2007).

It is widely accepted that begomoviruses do not replicate inside their whitefly vector (He et al. 2020). However, studies conducted on TYLCV have shown that this virus could replicate inside whiteflies especially when the whiteflies are stressed by an insecticide application (Pakkianathan et al. 2015). Transovarial and sexual transmission were also reported to occur with TYLCV (Bosco and Accotto 2004; Ghanim and Czosnek 2000). Virus replication in their insect vectors, and transovarial and sexual transmissions could aid in spread and enhance the survival of the virus outside its host plant.

Another genus of whitefly-transmitted viruses with worldwide importance is the *Crinivirus*. The transmission of *Crinivirus* is species-specific and performed exclusively by whiteflies belonging to the genera *Trialeurodes* and *Bemisia* in a semi-persistent manner. There has been a steady increase in the number of new species identified over the past 20 years, including the cucurbit yellow stunting disorder virus (CYSDV) and cucurbit chlorotic yellows virus (CCYV) (Kavalappara et al. 2021; Tzanetakis et al. 2013). Several criniviruses cause diseases as single infections whereas others remain asymptomatic and only cause disease when found in mixed infections with other viruses resulting in increased disease severity (Karyeija et al. 2000). An example of severe symptoms resulting from mixed infection between two whitefly-transmitted viruses is that of CuLCrV and CYSDV. Such commonly occurs in squash plants resulting in severe stunting and leaf crumpling (Gautam et al. 2020).

Begomoviruses and criniviruses occur in Georgia and pose concern in the fall vegetable cropping season (Gautam et al. 2020; Kavalappara et al. 2021). For example, in the 2017 fall growing season an estimated 35% reduction in squash crop value amounting to $38 million occurred due to dry conditions and an abundance of whitefly-transmitted viruses (Little et al. 2019).

The whitefly-transmitted viruses reported present in Georgia include viruses that infect economically important vegetables. In cucurbit crops the whitefly-transmitted viruses are CuLCrV, CCYV, CYSDV, and squash vein yellowing virus (SqVYV) (Adkins et al. 2011; Gadhave et al. 2018; Kavalappara et al. 2021). Whitefly-transmitted viruses have been reported in other crops such as TYLCV in tomato and CuLCrV and SiGMV in snap bean (Larsen 2010; Momol et al. 1999). Whitefly-transmitted viruses usually occur as mixed infection causing more severe symptoms in their host as compared to when they occur as a single infection (Gautam et al. 2020, Kavalappara et al. 2021). As for any insect-transmitted viruses impacting cultivated crops, management of the insect vector is critical for delaying the onset of disease and delaying the spread of disease in the field (Castle et al. 2009).

Management of sweetpotato whiteflies and whitefly-transmitted viruses

At present, management of whiteflies in commercial fields in southern Georgia relies heavily on application of insecticides (Li et al. 2021; Perring et al. 2018). The insecticide groups that are widely used for the management of whiteflies include neonicotinoids, insect growth regulators (pyriproxyfen and buprofezen), ketoenols (spiromesifen and spirotetramat), and diamides (anthranilic diamides, cyantraniliprole, and chlorantraniliprole) (Li et al. 2021). However, the polyphagous nature, higher reproductive rate, short generation times, and resistance to insecticides allow whiteflies to survive and dominate the agroecosystem making it challenging to manage them (Soumia et al. 2021). Additionally, adult and immature whiteflies frequently escape contact with foliar insecticide sprays because they infest the underside of leaves (McAuslane and Smith 2018). Occurrence of high populations of whiteflies facilitate the spread and prevalence of whitefly-transmitted viruses in the environment.

Previous studies have reported the use of UV-reflective mulch for management of insect-transmitted viral diseases (Frank and Liburd 2005; Nyoike and Liburd 2010;

Simmons et al. 2010; Riley and Srinivasan 2019); however, adoption of this practice has been slow. Black plastic mulch is used for spring crops to retain heat in the soil to promote crop growth. The growers reuse the plastic mulch for their fall crop by painting them white. Hence, the use of UV-reflective mulch may not be feasible for most growers.

Resistance to the whitefly-transmitted virus TYLCV has been developed in tomato, but is not yet available in other economically important crops. In yellow squash and zucchini, varieties with resistance to whitefly-transmitted viruses are currently not available (Candian et al. 2021; Coolong 2017). It should be noted that although both are susceptible to whitefly transmitted viruses, zucchini plants are still able to produce marketable fruits when infected. Yellow squash infected with whitefly-transmitted viruses, CuLCrV and/or CYSDV, will produce fruits with green streaking rendering them non-marketable (Webb et al. 2013). In years with high whitefly and virus pressure, complete losses of squash plantings can occur in susceptible varieties (Coolong 2017).

The use of physical barriers has been recommended for the management of whiteflies (LaTora et al. 2022; Norman et al. 1996). Under field conditions, the types of barriers that can reduce the potential for whitefly infestation include the use of oil-coated yellow mulch that acts as a trap for whiteflies and use of floating row covers. Floating covers are generally made of insect-proof mesh that are placed over newly planted crops and removed at flowering to allow pollination.

Adjusting planting dates to avoid the heaviest insect migration periods or crop overlap is another tactic that was recommended by Norman et al. (1996) to avoid or reduce whitefly infestations. Because whiteflies reproduce more rapidly under hot and dry conditions, early planting of spring and summer crops would allow the crops to mature before conditions are favorable for rapid increase of whitefly populations. Crops that are highly susceptible to whiteflies such as cucurbits, crucifers, and tomatoes are not recommended to be planted when high populations of whiteflies are expected. However, while whitefly populations reach their peak in mid-September to early October in southern Georgia, cucurbits and tomatoes are planted during this period to meet market demand. Hence, for growers who cannot adjust their planting dates, alternative strategies are needed to protect profitability.

The earliest and most severe whitefly infestations usually occur in fields located near crops with prior or current whitefly infestations (Norman et al. 1996). Hence, the location of a field can also affect the potential for whitefly infestation. Continuous planting of crops that are susceptible to whiteflies and whitefly-transmitted viruses in the same field is not recommended as it provides a constant source of whiteflies and virus inoculum.

Management of whitefly-transmitted viruses through application of insecticides to manage the whiteflies works best when whitefly populations are low (Norman et al. 1996). However, high whitefly populations and increased tolerance to insecticides make insecticides applications an insufficient management tool. Moreover, sweetpotato whiteflies are difficult to manage as adults and immatures frequently escape contact with foliar insecticide sprays because they infest the underside of leaves. Also, development of resistance to commonly used insecticides is well documented (McAuslane and Smith 2018). Thus, management of whitefly-transmitted viruses cannot rely solely on application of insecticides. Because no single pest management tactic has been shown to be consistently effective in managing sweetpotato whiteflies and whitefly-transmitted viruses, there is a need to develop alternative strategies that growers can use to protec their crop.

Current vegetable production systems and whitefly management practices in southern Georgia

Sweetpotato whiteflies can feed and reproduce on a wide range of host plants, which allows them to persist from one growing season to another. In southern Georgia, many of the vegetables grown across seasons are hosts for sweetpotato whiteflies. Cold season crops grown from January to April and August to December, such as broccoli, cabbage, and kale, serve as overwintering hosts for whiteflies. Frost events during the winter can kill adult whiteflies, but the impact on eggs and immatures whiteflies are minimal (Sparks et al. 2018). Hence, new generations of whiteflies still emerge from winter hosts.

By the spring, cucurbit crops, eggplant, snap bean, and tomato are grown serving as primary hosts of whiteflies. From spring crops, whiteflies move to crops planted in late spring and early summer, including collards, cotton, soybeans, and sweet potatoes (Sparks et al. 2018). Availability of host crops during warmer months is key in building high levels of whitefly populations that later impact crops grown in the fall. Female sweetpotato whiteflies can lay more than 300 eggs in their lifetime depending on temperature. For example, at 20 °C a *B. tabaci* female produced 324 eggs while at 37 °C a female produced only 12 eggs (Li et al. 2021). Whitefly populations established through the summer season move to fall crops such as squash, tomatoes, and snap beans. Whitefly populations typically reach their peak during the fall (Barman et al. 2019;

Candian et al. 2021; Srinivasan et al. 2012) and can cause overwhelming damage to susceptible crops. Occurrence of high whitefly populations and whitefly-transmitted viruses on susceptible crops including squash, tomato, and snap beans can result in substantial economic losses.

The diverse cropping systems in Georgia provide a continuous supply of host crops allowing whiteflies to persist all year in commercial fields. It provides an opportunity for whiteflies and whitefly transmitted viruses to reach high levels and overwhelm the current pest management tactics used by growers. In order to preserve the quality and quantity of yield in a whitefly susceptible crop, appropriate pest management programs need to be developed. The movement of whiteflies from one crop to another should be minimized though application of effective management tactics during the crop growing season and timely crop termination after harvest. Cross-commodity cooperation between growers of vegetables and agronomic crops with the objective to minimize whitefly populations would be a key to an areawide management of whiteflies that would benefit Georgia growers.

Risk assessment index

Prevention is critical in managing insect pests and pathogens with low economic threshold levels or those which cannot be managed through application of curative tactics. Preventive pest management tactics include production practices that are implemented prior to or immediately after planting. Examples of pest management tactics that are implemented prior to or during planting include use of UV-reflective mulch, selection of resistant varieties, planting away from potential sources of inoculum, and planting during periods when plants are less exposed to diseases. However, use of such pest management tactics can be expensive and may be unnecessary in fields where the pest or diseases are less likely to occur. Estimation of risk to disease would help growers in making better informed decisions with regards to application of preventive tactics.

A risk assessment index is a decision tool that allows the user to get an estimate of risk to disease associated with one or more underlying factors. Risk is defined as "the concept of possible, but uncertain, harm which includes elements of the intensity or size of potential negative effects and/or the probability that such effects will be suffered" (McRoberts et al. 2011). Factors that were found to influence disease intensity can be used as predictors of risk. For example, grape powdery mildew requires three consecutive days of rain for disease to develop (Flaherty et al. 1992), thus a forecasted occurrence of rain lasting three or more days would translate to increased risk of infection to occur.

The occurrence of whitefly-transmitted viruses impacting vegetable production was not the first insect- transmitted pathogen to become a major concern in crop production in the southeastern U.S. The establishment of the thrips (*Frankliniella spp*.)-transmitted tomato spotted wilt orthotospovirus (TSWV) has occurred throughout the southeastern peanut growing region since the late 1980s and has caused significant yield losses in peanuts (Culbreath and Srinivasan 2011; Hagan and Week 1998). In 1997, yield losses due to TSWV in peanut were estimated to be 12% amounting to $40 million in Georgia alone (Bertrand 1998). No single disease management tool provided adequate and consistent management of TSWV in peanuts. However, integration of multiple pest management strategies resulted in effective management and reduction of losses due to TSWV (Culbreath and Srinivasan 2011). The development and use of the spotted wilt risk index (Brown et al. 2005), now known as PEANUT Rx, with the addition of a risk assessment for fungal diseases (Kemerait et al. 2018) has enhanced the adoption of genetic, chemical and cultural practices for management of TSWV in peanuts. The PEANUT Rx considers various peanut production practices and pest and disease management inputs as risk-mitigation factors influencing TSWV risk (Brown et al. 2005; Chappell et al. 2020; Culbreath and Srinivasan 2011). The PEANUT Rx index values are based on results from numerous field studies that estimate the average reduction in TSWV incidence associated with each risk mitigation practice (e.g. peanut variety, tillage, planting date, plant density and row spacing, herbicide and insecticide use) (Brown et al. 2005; Hurt et al. 2006; Marois and Wright 2003). For any given suite of risk mitigation practices that a grower plans to implement, the sum of indices associated with each practice is used to obtain an overall estimate of risk. PEANUT Rx provides a method by which growers can assess the relative risk to TSWV in a particular peanut field based on the overall production practices employed. Assessment of risk also allowed growers to identify and adjust the combination of TSWV-suppressive factors that best apply to their situation (Brown et al. 2005). The wide adoption of PEANUT Rx by peanut growers contributes to the area-wide management of TSWV in the southeastern US.

The concept of using a risk index as an aid in the successful management of the insect-transmitted virus, similar to the use of PEANUT Rx for TSWV in peanuts, may also be implemented for the management of whitefly-transmitted viruses. While these are different pathosystems and insect-vectors and hosts differ, there are similarities between the thrips-transmitted TSWV and whitefly-transmitted viruses. The pathosystem of whitefly-transmitted viruses and TSWV both requires the presence of insect vectors for the spread of disease (Navas-Castillo et al. 2011; Ullman et al. 1997). These pathosystems are also similar in terms of the seasonality of the vectors. In Georgia, thrips populations gradually increase until they reach their highest peak in the month of May, after which thrips populations drop drastically (Wells et al. 2002). Similarly, whitefly populations in southern Georgia gradually increase and reach their peak around mid-September, and then drop drastically (Barman et al. 2019; Candian et al. 2021). With these similarities between the whitefly-transmitted viruses and TSWV pathosystems, the use of an integrated pest management strategy with the aid of a risk index may be an effective means for the management of whitefly-transmitted viruses.

Project objectives

This study was developed to gain a better understanding of the epidemiology of whitefly-transmitted viruses, to identify factors that influence the intensity of whitefly-transmitted virus complex, and to develop a risk assessment index for the management of whitefly-transmitted viruses. The specific objectives are: 1) to characterize the spatial distribution and temporal pattern of whiteflies and whitefly-transmitted viruses in commercial and experimental squash and tomato fields; 2) to assess prickly sida as a natural reservoir of SiGMV in commercial snap beans fields in southern Georgia; and 3) to develop a risk assessment index for the management of WTVC in fall-grown squash in southern Georgia.

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CHAPTER 2

CHARACTERIZATION OF THE SPATIAL DISTRIBUTION OF THE WHITEFLY-TRANSMITTED VIRUS COMPLEX IN YELLOW SQUASH FIELDS IN SOUTHERN GEORGIA, USA[1]

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[1] Codod, C.B., Severns, P.M., Sparks, A.N., Srinivasan, R., Kemerait, R.C., Jr. and Dutta, B. (2022). Characterization of the spatial distribution of the whitefly-transmitted virus complex in yellow squash fields in Southern Georgia, USA. *Front. Agron*. 4:930388. doi: 10.3389/fagro.2022.930388

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Abstract

A two-year study was conducted in 2019 and 2020 to characterize the spatial distribution of whitefly-transmitted virus complex (WTVC) in experimental and commercial yellow squash (*Cucurbita pepo*) fields in southern Georgia, USA. Field trials planted in Tifton, Georgia, USA were comprised of 30 rows of squash (variety: Gentry) divided into 300 quadrats. Six commercial fields in Tift, Worth, and Colquitt Counties in Georgia, USA were surveyed. In each field, 10 rows of plants per field were partitioned into 70 quadrats so that their approximate location could be mapped and disease incidence tracked in space over time. Plants in each quadrat were visually assessed for WTVC symptoms at seven-day intervals (experimental fields) or at fourteen-day intervals (commercial fields). The spatial distribution of WTVC in a one-dimensional space was determined through ordinary runs analysis and in two-dimensional space through spatial autocorrelation analysis and spatial analysis by distance indices (SADIE). Ordinary runs analysis down and between rows suggested that disease was more likely to be transmitted from infected plants within rows than across rows. WTVC incidence in space was positively correlated with adult whiteflies on squash leaves taken one or two weeks prior to the assessment of virus incidence coinciding with latent period of Geminiviruses. SADIE generated disease severity maps indicated a shift from sparse and randomly distributed disease early in the infection cycle to denser, more aggregated patterns later in time in both experimental and commercial fields. Contour plots of interpolated indices from SADIE analysis suggested an edge effect on the spatial distribution of WTVC in experimental fields as well as in two of the commercial fields. An understanding of the

shift from random to aggregated distribution and the edge effect on WTVC incidence are considerations for refining current management strategies.

Introduction

Characterization of the spatial and temporal patterns of plant disease development is needed to fully understand the disease dynamics, develop more accurate sampling plans, better assess crop loss in relation to disease intensity, and design and analyze experiments more efficiently (Xu and Madden 2004; Turecheck and Madden 1999). Information on the spatial and temporal patterns of pests could also be used in the prediction of risk for a given crop at a given time (Reich et al. 2013). Risk predictions allow growers to plan and implement appropriate management strategies to reduce their risk of pests and diseases and thereby reducing the impact of disease to yield.

Whitefly-transmitted viruses have emerged as a global threat to the production of a wide range of crops over the last 20 years (Navas-Castillo et al. 2011). Of the crops affected, whiteflies and whitefly-transmitted viruses are primary concerns in dicotyledonous plants, many of which are economically important. Some of the greatest losses occur in vegetable crops such as cucurbits, tomato, pepper, beans (Polston et al. 1999; Guzman et al. 2000; Adkins et al. 2009). Yield losses due to whiteflies and whitefly-transmitted viruses range from minimal to complete crop failure depending on what virus occur in the field, what crop, age of crops at the time of infection, and the incidence and severity of virus infection (Lapidot et al. 2014).

Among the whiteflies, the sweetpotato whitefly (*Bemisia tabaci*) is considered one of the most damaging pests of open field and protected cropping systems throughout major crop growing regions of the world (De Barro 2011). There are three main ways that sweetpotato whiteflies can cause damage. The first is caused by the direct feeding of both immature and adult stages on plants. Nymphs and adult whiteflies feed by inserting their proboscises into the leaf, penetrating the phloem and withdrawing sap (Jones 2003). Heavy infestations of nymphs and adults could cause seedling death, stunting, or reduction in yield of older plants (Gangwar and Charu 2018). During feeding, immature whiteflies can inject salivary fluids which causes physiological disorders, such as silvering of foliage in squash and irregular ripening of tomato fruits (Maynard and Cantliffe 1990; McCollum et al. 2004). The second way that whiteflies causes damage is the excretion of honeydew onto the surfaces of leaves and fruit. The honeydew acts as a substrate for the growth of sooty mold fungi which interferes with photosynthesis and reduces the quality of fruit and fiber (Byrne and Bellows 1991). The third is indirect damage by acting as vectors of economically important genera of plant viruses, including *Begomovirus (Geminiviridae), Ipomovirus (Potyviridae), Crinivirus (Closteroviridae), Carlavirus (Betaflexiviridae), Torradovirus (Secoviridae)*, and *Polerovirus (Solemoviridae)* (Navas-Castillo et al. 2011; Fiallo-Olive et al. 2020). Begomoviruses and criniviruses are genera of whitefly-transmitted viruses recognized as important emerging plant virus groups worldwide (Liu et al. 2013; Jones 2003; Tzanetakis et al. 2013).

In the southeastern US, the whitefly-transmitted cucurbit leaf crumple virus (CuLCrV) and cucurbit yellow stunting disorder virus (CYSDV) have impacted squash production during the fall growing season (Kavalappara et al. 2021; Nyoike et al. 2008). In 2017, an estimated 35% reduction in squash crop value amounting to $38 million occurred due to simultaneously dry conditions and an abundance of whitefly-transmitted viruses during the fall growing season (Little et al. 2019). The CuLCrV, CYSDV, and the recently reported cucurbit chlorotic yellows virus (CCYV) more often occur as mixed infection complex, as opposed to single infection (Kavalappara et al. 2021). Compared with symptoms caused by either CuLCrV or CYSDV in a single infection, more severe symptoms, characterized by a combination of stunting, leaf crumpling, and interveinal chlorosis, were observed among plants that were mixed infected with both CuLCrV and CYSDV in squash (Gautam et al. 2020). The mixed infection of two or more viruses transmitted by whiteflies in squash was collectively referred to as whitefly-transmitted virus complex (WTVC) in this paper.

Management of whiteflies and whitefly-transmitted viruses is challenging. The broad range of acceptable host plants (plants belonging to *Cucurbitaceae, Euphorbiaceae, Fabaceae, Solanaceae, Malvaceae*, *Graminae,* and *Musaceae*), high reproductive rate, short generation time, and insecticide resistance allow whiteflies to colonize and quickly dominate an invaded agroecosystem (Hidayat et al. 2018; Soumia et al. 2021). The current management strategy for whiteflies and their associated viruses in agricultural systems employs the timely use of numerous management tactics, usually with heavy reliance on insecticides, complemented by resistant plant cultivars if available (Lapidot et al. 2014). While resistance to whitefly-transmitted viruses has been developed for tomatoes (Vidavski et al. 2008) and cassava (Jennings 1994), there are no available squash (*C. pepo*) varieties with whitefly-transmitted virus resistance. Thus, optimizing the use of available pest management tactics is critical in reducing the impact of whitefly-transmitted viruses. An important first step in developing a refined disease management strategy is to identify the spatial and temporal patterns of whitefly and disease occurrence in squash fields. If there are consistent spatio-temporal patterns of whitefly and disease occurrence and spread, this information can be used to develop tactics to reduce the numbers of vectors, slow the rate of disease progression, and suppress increases in disease severity over time. We conducted a study to characterize and track the changes in the spatial and temporal distribution of whitefly abundance and WTVC in experimental as well as commercial squash fields in southern Georgia to gain a better understanding of the patterns of spread of whitefly-transmitted viruses in squash fields over time. Characterization of the spatiotemporal distribution could help to determine appropriate tactics that could be implemented to slow or suppress WTVC spread.

Materials and methods

Experimental fields

There were two types of fields in our study, one designed specifically for experimentation (this subsection) and commercial squash production fields. Experimental field trials were established at the University of Georgia's Black Shank Farm in Tifton, Georgia, USA during the 2019 and 2020 fall growing seasons. Field trials occurred in the same location in both years and consisted of 15 raised beds (0.11 ha for each experimental field). Two rows of squash (cv. Gentry) were planted (3-Sept-2019 and 3-Sept-2020) on bare soil in each bed. Each row was partitioned into ten 3.05m long quadrats resulting in a total of 300 quadrats (Fig. 2.1), with each quadrat having an average of 9 squash plants. To create an alley between each quadrat, plants in 0.9m sections were cleared after each 3.1 m long quadrat. Each quadrat in the field was assigned an X and Y coordinate value in a Cartesian X-Y plane based on the specific location of the quadrat in the field, enabling us to track changes in whitefly abundance and disease levels over time. The corresponding distance (meters) of each quadrat to the edge of the field was used on the y-axis. The x-axis contains the distance (m) of each row of plants from the left side of the sampling plot. Fertilization and management of fungal disease as well as pest insects other than whiteflies were implemented following recommendations from vegetable extension specialists for southeastern US (Arancibia et al., 2018). No insecticides known to be antagonistic to whiteflies were applied.

Plants within each quadrat were visually assessed for WTVC symptoms every seven days beginning at eight days after planting (DAP) in 2019 and at 11 DAP in 2020. Individual plants were scored as “1” if it was showing symptoms of WTVC and “0” if it was not showing WTVC symptoms. WTVC infected plants show crumpling of leaves, stunting, chlorosis, and green streaking on fruits. Yellow sticky cards were used as traps, 7.6cm wide and 12.7cm long, (BASF Corporation, Research Triangle Park, NC) and installed between two rows of squash in the 2nd, 5th, and 8th quadrat to represent dispersing whiteflies. A total of 45 traps were installed within the experimental field and each trap was fastened to a 30cm metal wire to raise the traps at approximately 20cm from the ground. The traps were installed immediately after planting and replaced at a seven-day interval and the number of whiteflies were counted on each sticky card. To account for the abundance of whiteflies on plants, we used the leaf-flip method (Turechek et al., 2014) on three arbitrarily selected leaves per two adjacent quadrats. Leaf-flip counts of whiteflies were taken from a total of 450 leaves at each seven-day interval. The number of whiteflies counted through the leaf-flip method is hereafter referred to as leaf-flip counts. During instances when a leaf had more than 100 adult whiteflies, the number of whiteflies was counted in half of the leaf and then multiplied by two to get an estimated number for the whole leaf. Rating for WTVC and whitefly counts were performed until the plants collapsed from whitefly feeding and WTVC infection. In 2019, four WTVC ratings and leaf-flip counts were taken starting at 8days after planting (DAP) to 29 DAP. Four WTVC ratings, leaf-flip counts, and trap counts were taken in 2020 starting 11 to 32 DAP.

Commercial fields

Six commercial squash fields located in Tift, Worth, and Colquitt counties in Georgia, USA were surveyed (Table 2.1). In each field, 10 rows of plants per field were partitioned into 1.83m x 9.14m quadrats resulting in a total of 70 quadrats with an average of 26 plants per quadrat (Fig. 2.2). Orange marking flags with 0.8m long wire stakes (Gempler's, Janesville, WI) were used to mark the partition of rows into quadrats. Between each row of plants, four rows of plants were skipped, generating 7.3m of space between each row of plants that were included in the study for a total study area of 0.5 ha per field. Similar to the experimental field, each quadrat in the field was assigned X and Y coordinate values based on the specific location of the quadrat in the field. Plants in each quadrat were visually assessed for WTVC symptoms at a 14-day interval. Within each row of squash, whitefly traps were installed in the 2nd, 4th, and 6th quadrats. A total of 30 traps per field were installed during the first rating date and replaced at a 14-day interval. Damaged traps were discarded. The abundance of whiteflies on plants was assessed using the leaf-flip method on four arbitrarily selected plants (one leaf/plant) in the vicinity (within a meter) of each sticky trap. Leaf-flip counts of whiteflies were taken from a total of 120 leaves at each 14-day interval. Rating for WTVC and trapping of whiteflies were performed until the crop was terminated by the growers. Two to four ratings were taken in commercial squash fields depending on the age of the crop during the first visit and when the crop was terminated.

Preliminary data processing

The percent incidence of WTVC was calculated per quadrat for both the experimental and commercial squash fields. The proportion of WTVC symptomatic plants over the total number of plants was multiplied by 100 to get the percent incidence. The mean number of plants per quadrat was 9 and 26 plants for the experimental and commercial fields, respectively. The higher number of plants per quadrat in the commercial fields was due to larger size of quadrats to account for the larger area under study.

The number of adult whiteflies caught on traps was counted under the stereomicroscope or with the aid of a magnifying glass. The traps used in this study had vertical and horizontal gridlines forming $2cm^2$ cells in the trap. Adult whiteflies were counted in four cells on each side of the trap. The mean number of whiteflies per $2cm^2$ cell was calculated per trap and used for subsequent analyses.

Detection of CuLCrV and CYSDV mixed infection

Samples from plants showing virus infection symptoms were collected to confirm CuLCrV and CYSDV infection through genotyping. DNA and RNA were extracted, and

PCR and RT-PCR were conducted using virus-specific primer sets (CuLCrV: 3FAC3= 5′-TTTATATCATGATTTTCGAGTACA-3′ & 5RAC1= 5′-AAAATGAAAGCCTAAGAGAGTGGA-3′ and CYSDV: CYSCPf = 5′-ATGGCGAGTTCGAGTGAGAATAA-3′ & CYSCPr= 5′-ATTACCACAGCCACCTGGTGCTA-3′) and established protocols (see Gautam et al. 2020 for a detailed description). A total of 149 samples showing WTVC symptoms from experimental (49 samples) and commercial squash fields (100 samples) were tested through PCR for presence or absence of CuLCrV. Among the samples that tested positive for CuLCrV, 56 samples were tested for mixed infection with CYSDV. Of the 56 samples tested for CYSDV, 20 samples were collected from experimental fields and 36 were from commercial fields.

Analysis

Temporal relationships between whitefly abundance and WTVC disease incidence

To understand the relationship between whitefly abundance and the incidence of WTVC, we performed a series of correlation analyses between combinations of concurrent and delayed time periods featuring within experimental field mapped quadrat whitefly counts (from leaf flips and traps) and WTVC incidence in 2019 and 2020. Two separate correlation analyses were performed for whitefly counts from traps and WTVC incidence, and for leaf-flip counts and WTVC incidence. Correlation analysis for whitefly trap counts and disease incidence was conducted using WTVC incidences in the 2nd, 5th, and 8th quadrats where traps were placed. Whiteflies trap counts represent abundance of adult whiteflies that are dispersing while leaf-flip counts represent adult whiteflies that are actively feeding and potentially inoculating WTVC on squash plants. Because the count and incidence data were non-normally distributed, we used a Spearman’s rank correlation for comparing these datasets within and between the different time periods. Correlation analysis was performed using the ‘rcorr’ function in the ‘Hmisc’ package (v4.6.0; Harrell 2019), in the R software (R Core Team 2020).

WTVC disease progression within and across rows

The spatial distribution of disease can be characterized on a one-dimensional scale (such as within rows of plants) or a two-dimensional scale. For characterization of the one-dimensional spatial pattern of a population, ordinary runs analysis was used. Ordinary runs analysis provides information on the pattern of disease spread within rows of plants in a field (Madden et al. 1982). When infected plants are ordered in a cluster, there is a lower number of runs. A pathogen spreading from plant to plant would be expected to show an aggregation (clustering) of infected plants in parts of the field where initial infections occurred (Madden et al. 1982).

To determine whether WTVC disease tends to progress from an infection location down rows more frequently than across rows (which the plants are separated by bare ground alleys), we used ordinary runs analysis to index the degree of aggregation along each one-dimensional arrangement (down rows versus across rows) of squash plants in the experimental fields. The analysis was conducted per field at each rating date. Two separate analyses were performed, one for indexing the degree of aggregation of WTVC infected plants within rows, and one for comparing the frequency of aggregation of WTVC infection within vs across rows. For within row assessment, the sampling unit was individual plants. WTVC-infected plants were assigned a value of "1" and non-WTVC-infected plants were assigned a "0". The sampling unit for the second part was the quadrat. Quadrats with at least one infected plant were considered infected and assigned a value of "1". If none of the plants within a quadrat was infected with WTVC, it was assigned a value of "0". The sample size was reduced by performing the analysis using quadrats as sampling units, but this method allowed the comparison of the spatial distribution of WTVC-infected plants within and between rows in the experimental field. When the analysis was performed between rows, the experimental field was treated as two plots as there was an alley dividing the field. The first plot had 16 rows and the other had 14 rows. Hence, when ordinary runs analysis was performed between rows, there were 10 transects with 16 quadrats each in the first plot and 10 transects with 14 quadrats each in the other plot. When analyzed within rows, there were 30 rows with 10 quadrats each. Based on the arrangement of infected and non-infected sampling units along each one-dimensional arrangement, the number of runs was calculated. In ordinary runs analysis, a run is a succession of one or more plants with the same infection status (Madden et al. 1982; Turecheck et al. 2014). The total number of runs was compared to the expected number of runs that would be expected to occur by random chance for a given level of WTVC incidence. To test the null hypothesis of a random distribution of WTVC-infected plants, the Z-statistic was calculated. The Z-statistic will have a large negative value (< -1.64, P=0.05) if there is an aggregation of WTVC-infected plants (Madden et al. 1982).

Field-level patterns of whitefly and WTVC disease aggregation

The degree of disease aggregation in space and over time can provide valuable information about the patterns of disease spread. For example, an aggregated pattern of insect-transmitted disease may arise when an infective insect, with a short feeding-inoculation period, feeds and then moves to neighboring plants. Alternatively, the secondary spread of vectors from an initially infected plant into nearby healthy plants will also result in an aggregated distribution of infected plants. Conversely, a random pattern of infected plants suggests that the pathogen is not spreading from plant to plant within the field (Madden et al. 1982). We used spatial autocorrelation to index the degree of aggregation of whiteflies (traps and leaf flips) and WTVC-infected plants at multiple time points of the squash growing season.

The degree of spatial autocorrelation was indexed with Moran's *I*, a global estimate of aggregation, in the SAS software variogram procedure (proc variogram) (SAS Institute Inc, Cary, NC). Moran's *I* has a value ranging from -1 to +1. A value of -1 indicates a perfect negative autocorrelation, meaning all diseased plants are completely repelled from each other. Moran's *I* equal to 0 indicates perfect randomness (*e.g.* no spatial autocorrelation), and Moran's *I* equal to +1 indexes perfect positive autocorrelation (all disease is completely clustered, without absences in space). The null hypothesis will be that the infected plants are randomly distributed in the study area (Madden et al. 2007). The alternative hypothesis is that the infected plants are more spatially clustered than would be expected by chance alone. The null hypothesis will be rejected at a 95% level of significance (p<0.05).

Spatially-explicit patterns of WTVC changes over time

To determine whether there were general patterns in WTVC disease outbreaks and subsequent spread in squash fields over time, we used SADIE (Spatial Analysis by Distance IndicEs). The spatial analysis by distance indices is a local method used to detect locations within the squash fields that may drive the overall pattern of spatio-temporal disease progression. SADIE is a method of quantifying spatial patterns of organisms (Li et al. 2012; Perry 1998) whereby the analysis estimates how much redistribution of data is required for the original aggregated data set to attain a non-aggregated state. The degree of non-randomness is quantified by comparing the distance to regularity for the observed data set with distance to regularity for rearrangements of the observed data (Li et al. 2012). The ratio between the distance moved to achieve the regular pattern for the observed data and the arithmetic mean distance to regularity for randomized samples is called the index of aggregation (I_a). I_a values equal to 1 indicate randomly arranged counts, I_a greater than 1 indicates aggregation of counts or incidence and I_a less than 1 indicates regularity (Winder et al., 2019). Comparison of the observed minimum distance to regularity with the tails of the distribution of corresponding values from randomization simulations provides a test of the null hypothesis that the observed counts are arranged randomly, with a probability level, P_a. SADIE was implemented in R using the ‘sadie’ function in the ‘epiphy’ package (v0.3.4; Gigot 2018). The interpolated indices, predicted values of Perry’s index within a 2-dimensional grid calculated through local regression method (Loess), were plotted to create contour plots through the ‘levelplot’ function in the ‘lattice’ package in R (v0.10-10, Sarkar 2007). The contour plots show areas within the squash field where WTVC infected plants occurred as more abundant and densely populated disease patches (red to white areas) or gaps with more sparsely populated regions and lower disease abundance (green areas). Patches were defined as neighborhoods of consistently high counts while gaps are areas where low counts were observed (Winder et al. 2019). The range of WTVC incidence associated with the interpolated indices were calculated and imbedded in the contour plots. This was done by taking the minimum and maximum WTVC incidence within each level of interpolated index ±0.25.

Results

Occurrence of a mixed infection of CuLCrV and CYSDV among symptomatic plants

The symptoms of WTVC infection observed on squash plants were crumpling of leaves, stunting, chlorosis, and green streaking on fruits. Among samples of WTVC symptomatic plants, 96% from experimental fields and 91% from commercial fields tested positive to CuLCrV. The mixed infections of CuLCrV and CYSDV was detected on 40% and 56% of samples collected in the experimental and commercial squash fields, respectively.

Temporal relationships between abundance of dispersing whiteflies and WTVC disease incidence

In the 2019 experimental field, a positive correlation was detected between the number of adult whiteflies caught on traps at 8 DAP and the WTVC incidence taken at 22 DAP (r= .28, p= .025) and at 29 DAP (r= .41, p<.001) (Table 2.2, Fig. 2.3). The number of adult whiteflies caught on traps at 22 DAP had a negative correlation with the WTVC incidence taken at 29 DAP (*r*= -.22, *p*= .041). While correlation between WTVC incidence and whitefly counts taken at the same or after the WTVC assessment times have been detected, they were generally a weaker correlation and had no biological implication on WTVC incidence; hence were not presented.

In 2020, a positive correlation (*r*= .22, *p*= .034) was observed between initial whitefly counts on traps (11 DAP) and WTVC incidence at 18 DAP (Table 2.2, Fig. 2.4). Similar to what was observed in 2019, there was a negative correlation between the number of adult whiteflies caught on traps at 18 DAP and WTCV incidence at 25 DAP (*r*= -.54, *p*< .001) and at 32 DAP (*r*= -.57, *p*< .001).

Temporal relationships between abundance of whiteflies feeding on plants and WTVC disease incidence

In 2019, leaf-flip counts of whiteflies taken at 8 DAP had a positive correlation with the WTVC incidence observed at 15 (*r*= .29, *p*< .001), 22 (*r*= .33, *p*< .001), and 29 DAP (*r*= .32, *p*< .001) (Table 2.3, Fig. 2.3). Similarly, leaf-flip counts of whiteflies observed at 15 (*r*= .25, *p*< .001) and at 22 DAP (*r*= .12, *p*< .035) positively correlated with the WTVC incidence at 29 DAP.

The leaf-flip counts taken in the experimental field in 2020 also positively correlated with the observed WTCV incidence (Table 2.3, Fig. 2.4). Leaf-flip counts of whiteflies taken at 11 DAP had a positive correlation with the WTVC incidence observed at 18 (*r*= .15, *p*< .008), 25 (*r*= .24, *p*< .001), and 32 DAP (*r*= .20, *p*< .001). Leaf-flip counts of whiteflies taken at 18 DAP positively correlated with the WTVC incidence observed at 25 (*r*= .36, *p*= .008), and 32 DAP (*r*= .42, *p*< .001). Leaf-flip counts of whiteflies taken at 25 DAP had a positive correlation with the WTVC incidence observed at 32 DAP (r= .40, p <.001).

WTVC disease progression within and across rows

When performed based on the infection status of individual plants within a row, a shift from mostly random distribution to an aggregated distribution was observed in the experimental (Fig. 2.5A) and commercial squash fields (Fig. 2.5B). A random distribution ($Z \geq$ -1.64) of infected plants was detected in most rows when the WTVC incidence was between 1 to 20%. As the WTVC incidence increased (21-80%), an aggregated pattern (Z<-1.64) was observed more frequently than a random pattern especially in the commercial fields (Fig. 2.5B). In the experimental field, the number of rows with an aggregated WTVC infected plants was doubled from 8 rows, when WTVC incidence was between 1 - 20%, to 17 rows when the incidence was between 21 - 40% (Fig. 2.5A). The number of rows with a random distribution of WTVC infected plants has decreased to less than half (30 rows) when WTVC incidence was at 21- 41% incidence than when it was between 1-20% (55 rows) (Fig. 2.5A). Hence, the ratio of rows with aggregated over random distribution of infected plants increased as the WTVC incidence increased (Fig. 2.5). Aggregation of WTVC infection was detected more often within rows than between rows of squash in experimental fields (Table 2.4). When ordinary runs analysis was performed between rows of squash, an aggregated distribution was detected only at 18 and 25 DAP in 2020. When analyzed within rows of squash, an aggregated distribution was detected at 15, 22, and 29 DAP in 2019, and at 18, 25, and 32 DAP in 2020 (Table 2.4). It is possible that these differences in the patterns of aggregation between and down rows is due to the bare-ground, access alleys acting as semi-permeable barriers to whitefly dispersal.

Field-level patterns of whitefly and WTVC disease aggregation

The degree of disease aggregation throughout the study fields appeared to change with time, with the overall pattern of low levels of aggregation in the early stages of whitefly/disease colonization, increasing degree of aggregation as whitefly numbers and disease increased over time, and at the latest dates a decrease in the degree of aggregation as disease became abundant and whitefly abundance decreased.

Dispersing adult whiteflies (based on trap counts) had an aggregated distribution (Moran's $I > 0$, $P < .05$) in the 2019 and 2020 experimental field (Table 2.5). Aggregation of dispersing whiteflies was also detected in three out of the six commercial fields. In the 2020 experimental field and in one of the commercial fields (Squash 2.3, Table 2.5), a shift from random to aggregated distribution of dispersing whiteflies were observed. The spatial distribution of dispersing whiteflies remained to be random or aggregated throughout the growing season.

Aggregation of whitefly populations, based on the leaf-flip counts, was detected in all assessment periods in the experimental field in 2019 and 2020 (Table 2.6). In commercial fields, the aggregated pattern was detected during at least one assessment period in three out of six fields. In general, the spatial distribution of whiteflies feeding on squash plants remained to be random or aggregated throughout the study period.

The spatial distribution of WTVC infected plants at field-level followed a shift from a random towards an aggregated distribution as WTVC increased over time in the experimental and commercial fields as indicated by increasing Moran's *I* values (Table 2.7). In the 2019 experimental field, plants showing symptoms of WTVC were observed 15 days after planting (DAP) and were found to have an aggregated distribution (Moran's *I*=0.04 and *P*<.001). The spatial distribution of WTVC on the succeeding ratings in the 2019 experimental field was also aggregated. Whitefly feeding damage and WTVC infection resulted in severe stunting, silverleaf symptom, yellowing, wilting, and death of plants after 30 DAP in 2019.

In the 2020 experimental field, plants showing symptoms of WTVC were first observed at 11 DAP in the experimental field (Table 2.7). A random distribution (Moran's *I*=0.00 and *P*=.617) of WTVC was detected at 11 DAP. Succeeding ratings taken at 18, 25, and 32 DAP had aggregated distributions.

In commercial fields, one of the six fields (Squash 16) showed a shift from random distribution, during the first rating, to an aggregated distribution on the succeeding ratings (Table 2.7). Two fields showed an aggregated WTVC distribution based upon first rating and remained to have an aggregated distribution (Squash 6) or shifted to a regular distribution (Squash 5) as WTVC incidence reached close to 100%. Three out of six fields had random distribution of WTVC in all rating periods. In these fields, the WTVC incidence ranged from zero to 27% and did not aggregate. Out of 25 assessment periods, there was a 95% agreement between the spatial autocorrelation analysis and SADIE on the distribution of WTVC in squash fields.

Spatially-explicit patterns of changes in WTVC distribution over time

Results from the SADIE analysis showed the occurrence of one or more patches of WTVC infected plants in the squash fields (Fig. 2.6 & 2.7). Although new patches were formed, the patches merged forming a bigger patch of infected plants as the percent WTVC incidence increased over time. This resulted to a shift from a random towards an aggregated distribution as WTVC increased over time in the experimental and commercial fields as indicated by increasing *Ia* values (Table 2.7). In general, the WTVC incidence was higher among quadrats within patches and was lower in the gaps where infection occurred at a random or regular pattern (Fig. 2.6 & 2.7).

In the 2019 experimental field, a patch (*Ia*= 2.75 and *Pa*<.0001) of WTVC infected plants was observed on the southeastern edge of the field at 15 DAP (Fig. 2.6A, Table 2.7). As the incidence of WTVC increased over time, the clustering of infected plants formed two patches towards the western side of the field at 22 and 29 DAP. The initial occurrence of a patch of WTVC infected plants in the southeastern edge of the field and then spreading and forming a patch towards the western edge demonstrate an edge effect on the spread of WTVC by the whiteflies.

In the 2020 experimental field, a random (*Ia*= 1.05 and *Pa*=.34) occurrence of plants infected with WTVC was initially observed at 11 DAP in the field edges (south and northeast) similar to the edge associated colonization area observed in 2019 (Fig. 2.6B). By 18 DAP, the initial randomly distributed WTVC infected plants became two patches (*Ia*= 1.97 and *Pa*<.0001). As WTVC incidence progressed, the two merged to form a continuous, larger area of abundant and aggregated disease by 25 (*Ia*= 3.55 and *Pa*<.0001) and 32 DAP (*Ia*= 4.04 and *Pa*<.0001).

The pattern of spread of WTVC infected plants from patches that were observed during initial assessments was also seen in five out of six commercial fields. In general, smaller patches of WTVC infected plants was observed during the initial assessment in “Squash 6”, “Squash 16”, “Squash 4”, “Squash 5”, and “Squash 17” (Fig. 2.7B-E). As WTVC increased over time, the patches combined forming bigger patches of infected plants. Patches of WTVC infected plants initially occurring along the edge (along X-axis) of the field, as observed in experimental fields, were also observed in commercial fields such as in “Squash 4” and “Squash 17” (Fig. 2.7D and 2.7E). Only the side along the X-axis is considered as an edge in commercial fields because the other sides were also planted with squash. The pattern WTVC spread in “Squash 3” (Fig. 2.7A) deviated from patterns observed in experimental fields. In “Squash 3”, the patches observed during initial assessment did not converge, instead new smaller patches developed around the field.

Discussion

Whitefly transmit viruses in a persistent or semi-persistent manner. Depending on the virus and mode of transmission, whiteflies can acquire and transmit viruses within hours, remain viruliferous up to the duration of their life span, and can therefore feed and transmit the virus on multiple plants (Webb et al. 2007; Fiallo-Olive et al. 2020). When choosing a host to settle on, whiteflies prefer younger leaves where they can feed and oviposit (Liu and Stansly 1995). It only takes two to three weeks for whiteflies to complete their life cycle during optimal conditions, such as warmer temperatures and dry conditions during summer and fall in southern Georgia. Within only 1 day after turning adult, female whiteflies can oviposit and a new generation will emerge in about three weeks (Powell and Bellows 1992; Mansaray and Sundufu 2009).

In the field setting, plants are exposed to whitefly feeding upon emergence or right after transplanting. During this time, viruliferous whiteflies can feed and transmit viruses in squash plants. While the proportion of viruliferous whiteflies among the whitefly populations entering the field is unknown, as disease incidence increase and the whitefly population grows, the proportion of viruliferous whiteflies is also expected to increase. Similar to earlier reports (Turecheck et al. 2010; Kavalappara et al. 2021; Kuo et al. 2007), mixed infections of CuLCrV and CYSDV was common among symptomatic squash plants. Because mixed infections of whitefly-transmitted viruses are quite common, WTVC was used in this study as a collective term to refer to plants showing symptoms of virus infection. After inoculation, symptoms of WTVC show within 10 to 15 days post- inoculation (Gil-Salas et al. 2012; Akad et al. 2008). Assuming the conditions are optimal for whitefly development and the adult whiteflies oviposit while feeding and transmitting WTVC in squash plants, a new generation of whiteflies could develop on the WTVC infected plants. As the WTVC infected plant senesces, the new generation of whiteflies are likely to disperse and transmit the virus on non-infected plants thereby causing secondary infections. This cycle of secondary infection may repeat several times until the squash growing period is over if whiteflies are left unmanaged and when environmental conditions are favorable. Considering the length of time from whitefly-mediated virus transmission to symptom development and potential occurrence of secondary infections arising from new whitefly generations dispersing from WTVC infected plants, a lag between the whitefly occurrence and WTVC symptom expression is expected.

Consistent with the expected relationship between whitefly abundance and the time-lagged WTVC incidence, a positive correlation between initial whitefly counts and time-lagged WTVC incidence ratings was detected. Similar to the relationship between whitefly abundance and virus incidence observed in this study, a positive correlation between the number of whiteflies on the host plants and the virus incidence has been reported for other whitefly-transmitted viruses, such as cassava mosaic virus and tomato yellow leaf curl virus (Colvin et al. 2004; Anco et al. 2020). The positive correlation between whitefly counts and the time-lagged WTVC incidence suggests that the abundance of adult whiteflies or factors affecting whitefly populations during the early stages of crop development (i.e. seedling and vegetative stages) could be a good predictor of WTVC risk. In the tobacco-thrips-TSWV pathosystem for example, weather parameters that affect the population of thrips (*Frankliniella fusca*), including average winter temperatures and cumulative amount of rainfall in March, were found to be the most important predictors of TSWV in tobacco grown in North Carolina (Chappell et al. 2013; Morsello et al. 2010). The positive correlation between whitefly counts and the time-lagged WTVC incidence also adds more weight on the importance of effectively managing whiteflies early in the cropping season. In principle, because there is a positive relationship, application of pest management tactics that reduce whitefly populations, such as insecticide application or use of UV-reflective mulches, or insect-proof row covers, would also result in reduction of WTVC incidence.

The positive correlation between whitefly abundance and WTVC incidence may indicate that such vector-virus interactions could result in an aggregated distribution of vector as was observed in squash fields that had higher levels of WTVC incidences (Table 2.7). It has been established that non-viruliferous whiteflies are attracted to virus infected plants (Legarrea et al. 2015, Chesnais et al. 2022), this could potentially facilitate further virus acquisition and transmission to nearby healthy plants, which could eventually result in aggregated distribution. According to Thomas et al. (2001), insects can be highly aggregated even in a habitat where uniform conditions occur such as in a farm practicing a monoculture. Some of the factors that may cause insects to aggregate into patches include habitat disruption, microclimate, prey or predator occurrence, attraction to olfactory cues, a greater probability of mating, or competition with other species (Turchin 1989; Thomas et al. 2001, Vinatier et al. 2010). Although an aggregated distribution of whiteflies does not always occur (Table 2.5 & 2.6), as was observed in some of the commercial squash field surveyed in this study, occurrence of such should be considered when monitoring for whiteflies. The abundance of whiteflies in a field could be underestimated if the scouting were conducted in areas where lower numbers of whiteflies occur, when some areas in the field may have a higher number of whiteflies. Thus, scouting in multiple parts of the field is key in making an estimate of the whitefly abundance in each field. Moreover, whitefly counts taken by the leaf-flip method had a more consistent correlation with WTVC incidence ratings than counts taken on traps in this study. Studies comparing different sampling methods for whiteflies also showed that sticky traps were less efficient, inconsistent, and less precise in estimating of whitefly populations than the leaf flip method (Palumbo et al. 1995; Naranjo et al. 1995). Hence, whitely numbers taken through leaf-flip method may be a more reliable method to base management decisions than sticky card traps.

Studies on characterization of the temporal patterns and spatial distribution of whitefly-transmitted viruses at field level in watermelons and at a regional level tomatoes have been conducted in southwestern Florida, United States (Turecheck et al. 2014; Anco et al. 2021). In a watermelon field, an aggregation of squash vein yellowing virus infected plants was detected (Turecheck et al. 2014). A strong linear relationship between whitefly densities and TYLCV incidences in neighboring tomato fields extended up to 2.5 km for TYLCV and 5km for whiteflies (Anco et al. 2020). However, there is still a lack of information on the changes on the spatial distribution of whitefly-transmitted virus infected plants over the growing period. While some similarities in the spatial distribution of whitefly-transmitted viruses in squash may be expected due to the same vector and virus, differences in host crops, cropping systems, and weather (specially with freezing events during the winter) may influence the spatiotemporal patterns in Georgia.

The short-distance dispersal and host preference of whiteflies may explain the observed shift from a random to an aggregated distribution across different spatial scales, including at field level (two-dimensional scale) and within rows (one-dimensional scale) (Table 2.7, Figs. 2.3 - 2.5). Based on the random distribution of WTVC infected plants, it appears that primary infection would typically cause up to 20% WTVC incidence in squash fields (Fig. 2.5). As new generation of whiteflies emerges from initially infected plants, they are likely to feed and acquire the virus from the infected plant as they do not move long distances. By the time the nymphs reach an adult stage, they disperse within short distances into neighboring plants to feed and inoculate the viral pathogens (Sutterlin

2020) thereby leading to the secondary spread of WTVC. The infection arising from secondary spread of WTVC are observed when the virus incidences reaches over 20% as indicated by the occurrence of an aggregated distribution of WTVC infected plants. This phenomenon could have contributed to the observed shift into an aggregated distribution as the WTVC incidence increased over time. It has been previously reported that viruliferous whiteflies prefer non-infected plants while non-viruliferous whiteflies tend to feed on TYLCV infected plants (Legarrea et al. 2015). In another study, the mechanism behind the diminishing preference of viruliferous whiteflies to TYLCV infected plants was investigated. It was found that TYLCV caused neurodegeneration in the brain of viruliferous whiteflies leading to impairment of their senses of sight and smell (Wang et al. 2020). As a result, viruliferous whiteflies could not distinguish between infected and non-infected plants. With the diminishing preference to TYLCV infected plants, viruliferous whiteflies have greater chances of settling on non-infected plants and therefore are more efficient in spreading the virus. These vector-virus-host interactions would cause the occurrence of an aggregated distribution of WTVC infected plants, as was observed in this study. Therefore, the findings in this study conform to expectations considering the feeding behaviors and dispersal of the vector whiteflies.

The more frequent aggregation of infected plants within rows than between rows of squash suggests that the whiteflies are more likely to move to the nearest plant along a continuous canopy (within rows) than across a bare ground (between rows) (Table 2.4). This tendency of whiteflies to move along a path with least resistance within rows of plants leads to an aggregation of WTVC infected plants within rows of plants as the virus infection spreads over time. Insects moving along a path of least resistance (within rows) rather than between rows has also been reported on stink bugs in peanuts and soybeans as well as on leaf hoppers in corn (Panizzi et al. 1980; Tillman et al. 2009; Power 1992). Similar to what was observed in this study, occurrence of aggregation of virus infected plants within but not between rows has also been observed in other virus-plant pathosystems, such as potato virus S in potatoes (Lambert et al. 2007) and plum pox virus (PPV) in *Prunus* spp. (Gottwald et al. 2013; Dal Zotto et al. 2020). Therefore, in addition to the feeding preference of viruliferous and non-viruliferous whiteflies, another factor that contributes to the observed aggregation of WTVC infection is the tendency of whiteflies to move within rows of plants towards the nearest suitable plant.

The aggregation of WTVC infection occurring in both experimental and commercial fields (Table 2.7) suggests that the spatial patterns observed in fields where whiteflies were not managed (experimental) could also occur in commercial fields where management tactics against whiteflies were applied. The higher WTVC incidence and aggregation of infected plants in some commercial fields could also indicate an inadequate management of whiteflies, as was reported in other papers (Ally et al. 2019; Hilje et al. 2001; Gilbertson et al. 2011). One example of a management tactic used for managing insect-transmitted viruses that have an aggregated distribution is the removal of all PPV hosts (*Prunus* spp.) within 500m of PPV-infected trees, which contributed to the overall eradication of PPV in Pennsylvania, USA (Gottwald et al. 2013). The same tactic may also be used of managing WTVC in squash, where symptomatic plants including plants around it (specially plants within the same rows) will be removed to reduce sources of inoculum and slow the spread of WTVC in squash fields. However, to be effective in reducing losses to WTVC, removal of infected plants should be done as soon as infection is detected to limit the whiteflies from acquiring and spreading the virus. Some of squash production practices could also lead to lower levels of WTVC infection. For example, the “Squash 17” field was planted towards the end of September in 2020 (Table 2.1), by this time the whitefly populations were already declining due to colder temperature (Candian et al. 2021). With declining whitefly populations, the chances of dispersal and subsequent spread of WTVC is also reduced, which is likely the reason lower levels of WTVC infection were observed in this field (Table 2.7).

The edge effect on the colonization and spread of insect vectors and the viruses they transmit also influence the spatiotemporal patterns of insect-transmitted viruses. In onion fields, higher incidences of the thrips-transmitted iris yellow spot virus were observed in field edges and lower incidences near field centers (Gent et al. 2004). Fargette et al. (1985) also reported higher number of whiteflies and a higher incidence of cassava mosaic virus on the edges of cassava fields. Similar to the aforementioned studies, we found an edge effect on the distribution of WTVC in squash fields (Figs. 2.6 & 2.7). Patches of WTVC infected plants were detected near field edges during initial ratings, which combined to form bigger patches of WTVC infection in squash fields over time. Patches of WTVC infected plants occurring in field edges were seen to influence the overall spread of infection and spatial distribution of infected plants over time, especially when whiteflies were unmanaged as was observed in experimental fields. The edge effect on the distribution of WTVC could be associated with the dispersal behavior of whiteflies. While we do not have data to show this, we can speculate that whiteflies will potentially settle on the first host plant they find, which would be the field edges, after a dispersal event from another location. The edge-biased distribution is widespread among insect species in agricultural fields (Nguyen and Nansen 2018). In a winter wheat field, Winder et al. (1999) found a higher abundance of grain aphids (*Sitobion avenae* Fabricius) along field edges. In another study, higher counts of cabbage aphids (*Brevicoryne brassicae* L.) were found within 20 to 30m from field edges than further inwards into canola fields (Severtson et al. 2015). In winter wheat fields, *Amara* species, *Bembidion lampros* Herbst, as well as Carabidae and Lycosidae insects were predominantly found within 60 m of the field edge (Holland et al. 1999). Tillman et al. (2014) also found an edge effect on the colonization of stink bugs in corn and cotton fields that are next to woodlands. The occurrence of an edge effect on the distribution of whiteflies and WTVC should be accounted for when scouting, monitoring, and planning pest management strategies. When scouting WTVC infection in squash fields, a stratified sampling approach with a greater focus along field edges than field interiors can reduce scouting efforts in finding WTVC infected plants. Targeted scouting along field edges for whiteflies using the leaf-flip method could also facilitate earlier detection and application of management interventions against whiteflies.

An understanding of the shift from random to aggregated distribution and the edge effect on WTVC incidence are considerations for refining current management strategies. An example of a tactic that could be explored include planting zucchini squash (tolerant to whiteflies and CuLCrV) in the borders of the field which could help slow down the spread of disease and reduce yield losses from WTVC. Scouting and rouging virus-infected plants early in the growing season could also help reduce sources of inoculum for the secondary spread later in the season. Recently, detection of virus-infected plants through canines has been reported for the detection of watermelon plants infected with squash vein yellowing virus (Adkins et al. 2021). Canines can be trained to sniff out specific volatile organic compounds that are unique for disease infected plants, as shown for plum pox virus, little cherry disease, citrus canker, and citrus greening (Dininny 2019; Gottwald et al. 2019; Angle et al. 2016; Fuchs 2020). The use of canines can further help in the scouting, early detection, and rouging potential sources of inoculum early in the season. In principle, by reducing the number of randomly infected plants in the field early in the season, we can reduce the occurrence of aggregated WTVC infected plants arising from secondary spread later in the growing season. These examples of management tactics, when proven to work, are potential means of managing WTVC and whiteflies that growers can add in their pest management programs to reduce losses from WTVC infection.

Conclusions

The spatial distribution of WTVC infected plants shifted from a random pattern to an aggregated pattern as the disease incidence increased over time. The occurrence of explosive population of whiteflies and their movement within rows of plants after virus-acquisition from infected plants contributed to the aggregation symptomatic plants in squash fields. The abundance of whitefly populations positively correlated with WTVC incidence in squash fields. Hence, effective WTVC management should rely on reducing whitefly populations in squash fields. Our data indicate that WTVC infection generally initiates along the edges of the squash fields, which later get spread by viruliferous whiteflies more often within rows of squash than between rows of squash plants. The edge effect on the distribution of WTVC infected plants as well as the within row spread of infection need to be accounted for during scouting, monitoring, and planning pest management strategies. These findings can potentially be a basis for developing future studies on designing scouting and sampling patterns for early detection of WTVC infection, identifying effective pest management tactics and determining appropriate timing for applying each pest management tactic to reduce the impact of WTVC infection that often arise from primary and secondary spread by viruliferous whiteflies.

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Tables

Table 2.1. Production practices in experimental and commercial yellow squash fields in Georgia, USA included in this study.

Field	Field ID	County	Planting Date	Planting Material	Irrigation Type	Mulch Type
Experimental	FT 2019	Tift	3-Sep-2019	seed	overhead	bare ground
Experimental	FT 2020	Tift	3-Sep-2020	seed	overhead	bare ground
Commercial	Squash 3	Tift	10-Aug-2019	seedling	drip	white plastic
Commercial	Squash 4	Tift	21-Aug-2019	seedling	drip	white plastic
Commercial	Squash 5	Colquitt	6-Sep-2019	seed	overhead	bare ground
Commercial	Squash 6	Colquitt	20-Sep-2019	seed	overhead	bare ground
Commercial	Squash 16	Tift	17-Aug-2020	seedling	drip	black plastic
Commercial	Squash 17	Colquitt	28-Sep-2020	seed	overhead	bare ground

Table 2.2. Correlation matrix of whitefly counts from traps and WTVC incidence in experimental field trials conducted at the University of Georgia Black Shank Farm, Tifton, Georgia, USA in 2019 and 2020.

Virus incidence rating period	**Trap collection time (2019)**							
	8 DAP		15 DAP		22 DAP		29 DAP	
	r	p-value	r	p-value	r	p-value	r	p-value
15 DAP	0.2083	0.1043	0.0110	0.9200	0.0378	0.7295	0.1295	0.2238
22 DAP	0.2845	0.0250	0.1047	0.3376	-0.0251	0.8186	0.2909	0.0054
29 DAP	0.4145	0.0008	-0.2088	0.0537	-0.2203	0.0415	0.3778	0.0002

Virus incidence rating period	**Trap collection time (2020)**							
	11 DAP		18 DAP		25 DAP		32 DAP	
	r	p-value	r	p-value	r	p-value	r	p-value
11 DAP	-0.0115	0.9141	0.0350	0.7432	0.2223	0.0352	0.1310	0.2184
18 DAP	0.2242	0.0337	-0.1879	0.0762	-0.0226	0.8324	0.1359	0.2015
25 DAP	0.1730	0.1029	-0.5390	<.0001	-0.1002	0.3473	0.2034	0.0546
32 DAP	0.1803	0.0891	-0.5667	<.0001	-0.0919	0.3891	0.2644	0.0118

Table 2.3. Correlation matrix of whitefly counts from leaf-flips and WTVC incidence experimental field trials conducted at the University of Georgia Black Shank Farm, Tifton, Georgia, USA in 2019 and 2020.

Virus incidence rating period	**Leaf-flip counts assessment time (2019)**[a]							
	8 DAP		15 DAP		22 DAP		29 DAP	
	r	p-value	r	p-value	r	p-value	r	p-value
15 DAP	0.2871	<.0001	0.2775	<.0001	0.1348	0.0195	0.1640	0.0044
22 DAP	0.3336	<.0001	0.2492	<.0001	0.2393	<.0001	0.1875	0.0011
29 DAP	0.3172	<.0001	-0.0452	0.4359	0.1215	0.0354	0.1756	0.0023
Virus incidence rating period	**Leaf-flip counts assessment time (2020)**							
	11 DAP		18 DAP		25 DAP		32 DAP	
	r	p-value	r	p-value	r	p-value	r	p-value
11 DAP	0.0477	0.4108	0.0180	0.7562	0.1175	0.0419	0.1373	0.0174
18 DAP	0.1530	0.0079	0.2439	<.0001	0.2511	<.0001	0.1776	0.0020
25 DAP	0.2399	<.0001	0.3633	<.0001	0.3522	<.0001	0.1349	0.0194
32 DAP	0.2025	0.0004	0.4236	<.0001	0.4037	<.0001	0.2261	<.0001

[a] WTVC infected plants were not observed at 8 DAP so it was not included in the table.

Table 2.4. Detection of aggregated distribution of virus incidence in experimental field through ordinary runs analysis performed between rows versus across rows[a]

		Between rows (N=10/plot)[b]		**Within rows (N=30)**
YEAR	**RATING TIME**	Plot 1 16 quadrats/ row	Plot 2 14 quadrats/ row	10 quadrats/ row
2019	15 DAP	0	0	3
	22 DAP	0	0	3
	29 DAP	0	0	2
2020	11 DAP	0	0	0
	18 DAP	0	1	1
	25 DAP	1	0	2
	32 DAP	0	0	2

[a] As individual plants do not necessarily line up between rows, the ordinary runs analysis was performed based on presence or absence of infected plants per quadrat.

[b] Analysis for distribution across rows were done per plot because there is an alley in the middle of the field separating the two plots.

Table 2.5. Two-dimensional spatial distribution of adult whiteflies based on number of adults caught on traps in yellow squash fields based on spatial autocorrelation analysis per field and per rating period.

Squash field	Year	Rating period	Moran's I	Z	Pr > |Z	Distribution
Experimental field	2019	8 DAP	0.07	2.69	0.0071	Aggregated
		15 DAP	0.16	6.57	<.0001	Aggregated
		22 DAP	0.15	6.02	<.0001	Aggregated
		29 DAP	0.11	4.79	<.0001	Aggregated
Experimental field	2020	11 DAP	-0.03	-0.22	0.8249	Random
		18 DAP	0.20	8.25	<.0001	Aggregated
		25 DAP	0.01	1.11	0.269	Random
		32 DAP	0.11	4.89	<.0001	Aggregated
Commercial field (Squash 3)	2019	40 DAT	0.00	1.08	0.2795	Random
		54 DAT	0.03	2.24	0.0248	Aggregated
Commercial field (Squash 4)	2019	29 DAT	-0.01	0.95	0.3425	Random
		43 DAT	-0.02	0.56	0.5736	Random
		57 DAT	-0.01	0.80	0.4228	Random
Commercial field (Squash 5)	2019	65 DAP	-0.04	-0.08	0.9359	Random
Commercial field (Squash 6)	2019	55 DAP	0.12	5.40	<.0001	Aggregated
		69 DAP	0.12	5.27	<.0001	Aggregated
Commercial field (Squash 16)	2020	39 DAT	-0.08	-1.65	0.0998	Random
		53 DAT	-0.06	-0.74	0.4585	Random
Commercial field (Squash 17)	2020	39 DAP	0.02	2.02	0.0431	Aggregated
		51 DAP	-0.06	-1.00	0.318	Random

Table 2.6. Two-dimensional spatial distribution of adult whiteflies based on leaf-flip counts in yellow squash fields based on spatial autocorrelation analysis per field and per rating period.

Squash field	Year	Rating period	Moran's I	Z	Pr > |Z	Distribution
Experimental field	2019	8 DAP	0.10	16.42	<.0001	Aggregated
		15 DAP	0.06	9.80	<.0001	Aggregated
		22 DAP	0.07	11.87	<.0001	Aggregated
		29 DAP	0.07	11.87	<.0001	Aggregated
Experimental field	2020	11 DAP	0.04	6.66	<.0001	Aggregated
		18 DAP	0.09	13.95	<.0001	Aggregated
		25 DAP	0.05	9.31	<.0001	Aggregated
		32 DAP	0.03	6.14	<.0001	Aggregated
Commercial field (Squash 3)	2019	26 DAT	-0.03	0.13	0.8939	Random
		40 DAT	0.00	1.09	0.2754	Random
		54 DAT	0.00	1.30	0.1944	Random
Commercial field (Squash 4)	2019	15 DAT	0.01	1.44	0.1503	Random
		29 DAT	0.09	4.23	<.0001	Aggregated
		43 DAT	-0.09	-1.83	0.0672	Random
		57 DAT	0.01	1.47	0.1412	Random
Commercial field (Squash 5)	2019	48 DAP	0.12	5.22	<.0001	Aggregated
		65 DAP	0.03	2.25	0.0242	Aggregated
Commercial field (Squash 6)	2019	41 DAP	0.13	5.56	<.0001	Aggregated
		55 DAP	0.19	7.93	<.0001	Aggregated
		69 DAP	0.14	6.14	<.0001	Aggregated
Commercial field (Squash 16)	2020	25 DAT	0.01	1.67	0.0944	Random
		39 DAT	-0.04	-0.31	0.7585	Random
		53 DAT	-0.06	-0.99	0.3242	Random
Commercial field (Squash 17)	2020	25 DAP	-0.03	0.05	0.9582	Random
		39 DAP	-0.05	-0.70	0.4832	Random
		51 DAP	-0.05	-0.49	0.6273	Random

Table 2.7. Two-dimensional spatial distribution of WTVC in yellow squash fields based on spatial autocorrelation analysis and SADIE analysis based on WTVC incidence per field and per rating period.

Squash field	**Year**	**Rating period**	**Virus incidence**	**SPATIAL AUTOCORRELATION**				**SADIE**		
				Moran's *I*	***Z***	***Pr* > |*Z***	**Distribution**	***Ia***	***Pa***	**Distribution**
Experimental field	2019	15 DAP	4.19	0.04	11.10	<.0001	Aggregated	2.75	<.0001	Aggregated
		22 DAP	20.36	0.08	22.52	<.0001	Aggregated	3.55	<.0001	Aggregated
		29 DAP	40.31	0.11	30.50	<.0001	Aggregated	3.54	<.0001	Aggregated
Experimental field	2020	11 DAP	0.51	0.00	0.50	0.6175	Random	1.05	0.34	Random
		18 DAP	6.03	0.04	10.50	<.0001	Aggregated	1.97	<.0001	Aggregated
		25 DAP	34.74	0.12	31.70	<.0001	Aggregated	3.55	<.0001	Aggregated
		32 DAP	63.78	0.14	37.10	<.0001	Aggregated	4.04	<.0001	Aggregated
Commercial field (Squash 3)	2019	26 DAT	0.22	-0.02	-0.75	0.4553	Random	0.83	0.82	Random
		40 DAT	0.89	-0.02	-0.26	0.7973	Random	0.90	0.68	Random
		54 DAT	4.11	-0.02	-0.69	0.4898	Random	0.86	0.79	Random
Commercial field (Squash 4)	2019	15 DAT	0.11	-0.02	-0.50	0.6171	Random	0.88	0.72	Random
		29 DAT	1.41	-0.02	-0.44	0.6629	Random	0.85	0.88	Random
		43 DAT	10.77	0.00	0.86	0.3897	Random	1.15	0.18	Random
		57 DAT	27.44	0.01	1.88	0.0602	Random	1.20	0.11	Random
Commercial field (Squash 5)	2019	48 DAP	90.76	0.04	3.90	<.0001	Aggregated	1.44	0.01	Aggregated
		65 DAP	99.51	-0.02	-0.30	0.7613	Regular	1.05	0.36	Regular
Commercial field (Squash 6)	2019	41 DAP	40.84	0.08	7.54	<.0001	Aggregated	2.00	<.0001	Aggregated
		55 DAP	66.31	0.10	8.78	<.0001	Aggregated	2.35	<.0001	Aggregated
		69 DAP	78.91	0.11	9.08	<.0001	Aggregated	2.17	<.0001	Aggregated
Commercial field (Squash 16)	2020	25 DAT	16.48	0.01	2.04	0.0413	Aggregated	1.06	0.27	Random
		39 DAT	39.83	0.03	3.32	0.0009	Aggregated	1.42	<.0001	Aggregated
		53 DAT	51.49	0.05	4.99	<.0001	Aggregated	1.57	<.0001	Aggregated
Commercial field (Squash 17)	2020	25 DAP	0.37	-0.01	0.40	0.6928	Random	1.07	0.29	Random
		39 DAP	2.27	-0.02	-0.38	0.7015	Random	0.88	0.76	Random
		51 DAP	6.06	-0.01	0.40	0.6884	Random	1.13	0.18	Random

Figures

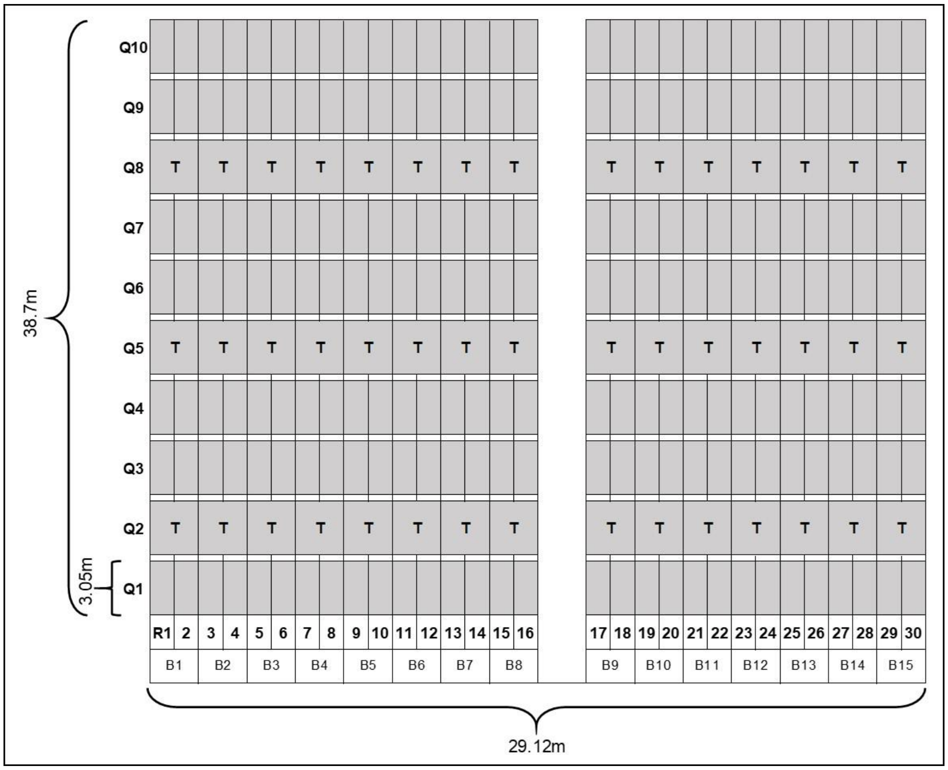


Figure 2.1. Experimental design in field trials conducted in UGA Black Shank Farm. Each gray cell represents a 0.91m x 3.05m quadrat; WTVC incidences were taken per quadrat. Legend: Q= quadrats, R = rows, T= indicates where traps were placed

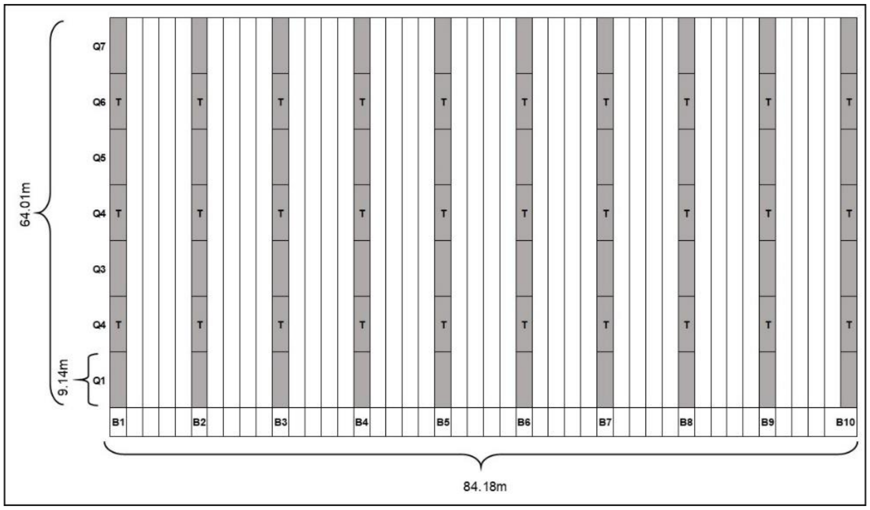
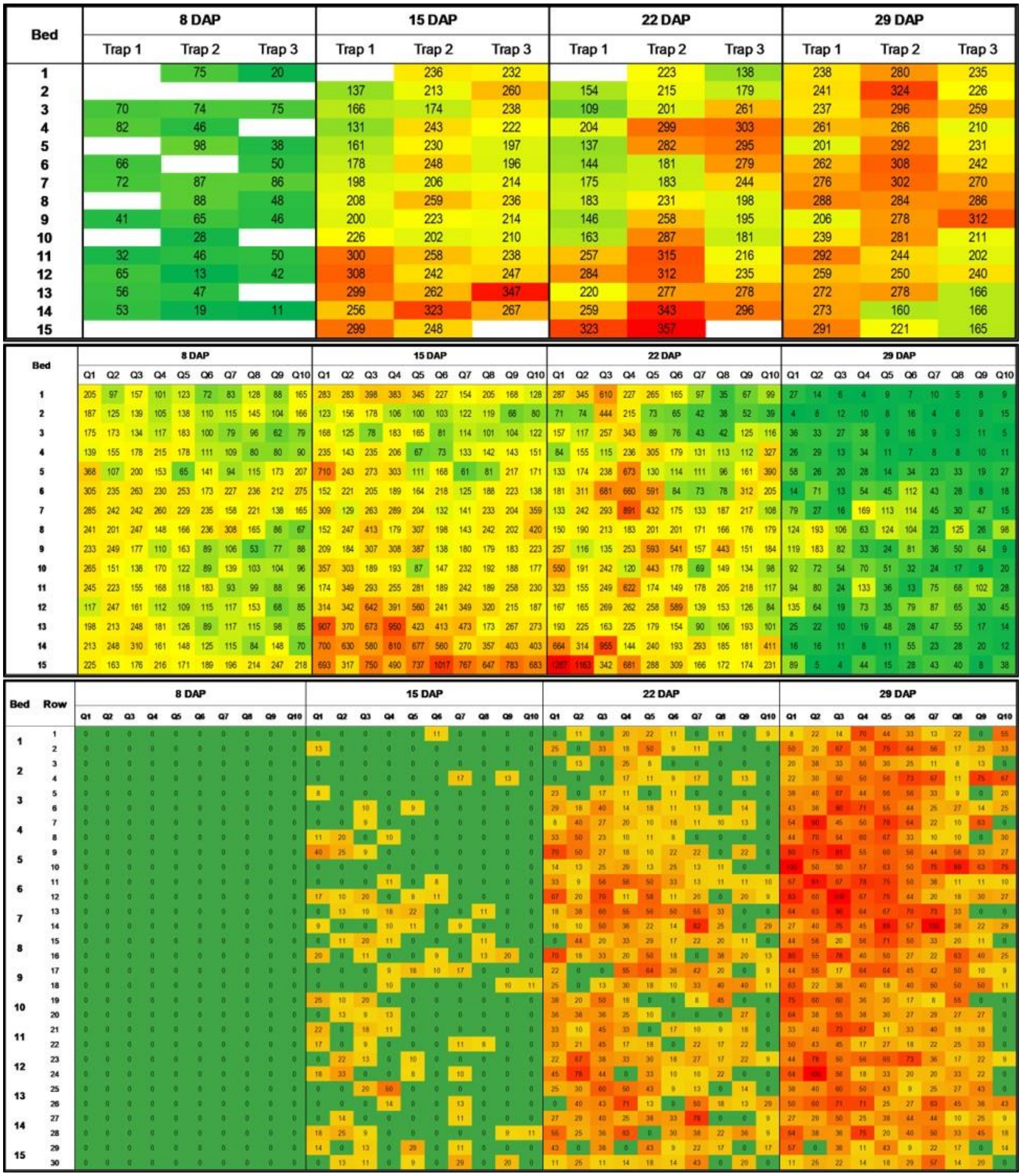


Figure 2.2. Study design for survey of commercial squash fields. Each gray cell represents a 1.83m x 9.14m quadrat. White columns represent 4 rows between each sampling row (gray columns); WTVC incidence was taken per quadrat. Legend: Q= quadrats, B = beds, T= indicates where traps were placed



Bed	8 DAP			15 DAP			22 DAP			29 DAP		
	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3
1		75	20		236	232		223	138	238	280	235
2				137	213	260	154	215	179	241	324	226
3	70	74	75	166	174	238	109	201	261	237	296	259
4	82	46		131	243	222	204	299	303	261	266	210
5		98	38	161	230	197	137	282	295	201	292	231
6	66		50	178	248	196	144	181	279	262	308	242
7	72	87	86	198	206	214	175	183	244	276	302	270
8		88	48	208	259	236	183	231	198	288	284	286
9	41	65	46	200	223	214	146	258	195	206	278	312
10		28		226	202	210	163	287	181	239	281	211
11	32	46	50	300	258	238	257	315	216	292	244	202
12	65	13	42	308	242	247	284	312	235	259	250	240
13	56	47		299	262	347	220	277	278	272	278	166
14	53	19	11	256	323	267	259	343	296	273	160	166
15				299	248		323	357		291	221	165

Bed	8 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	205	97	157	101	123	72	83	128	88	165
2	187	125	139	105	138	110	115	145	104	166
3	175	173	134	117	183	100	79	96	62	79
4	139	155	178	215	178	111	109	80	80	90
5	368	107	200	153	65	141	94	115	173	207
6	305	235	263	230	253	173	227	236	212	275
7	285	242	242	260	229	235	158	221	138	165
8	241	201	247	148	166	236	308	165	86	67
9	233	249	177	110	163	89	106	53	77	88
10	265	151	138	170	122	89	139	103	104	96
11	245	223	155	168	118	183	93	99	88	96
12	117	247	161	112	109	115	117	153	68	85
13	198	213	248	181	126	89	117	115	98	85
14	213	248	310	161	148	125	115	84	148	70
15	225	163	176	216	171	189	196	214	247	218

Bed	15 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	283	283	398	383	345	227	154	205	168	128
2	123	156	178	106	100	103	122	119	68	80
3	168	125	78	183	165	81	114	101	104	122
4	235	143	235	206	67	73	133	142	143	151
5	710	243	273	303	111	168	61	81	217	171
6	152	221	205	189	164	218	125	188	223	138
7	309	129	263	289	204	132	141	233	204	359
8	152	247	413	179	307	198	143	242	202	420
9	209	184	307	308	387	138	180	179	183	223
10	357	303	189	193	87	147	232	192	188	177
11	174	349	293	255	281	189	242	189	258	230
12	314	342	642	391	560	241	349	320	215	187
13	907	370	673	950	423	413	473	173	267	273
14	700	630	580	810	677	560	270	357	403	403
15	693	317	750	490	737	1017	767	647	783	683

Bed	22 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	287	345	610	227	265	165	97	35	67	99
2	71	74	444	215	73	65	42	38	52	39
3	157	117	257	343	89	76	43	42	125	116
4	84	155	115	236	305	179	131	113	112	327
5	133	174	238	673	130	114	111	96	161	390
6	181	311	681	660	591	84	73	78	312	205
7	133	242	293	891	432	175	133	187	217	108
8	150	190	213	183	201	201	171	166	176	179
9	257	116	135	253	593	541	157	443	151	184
10	550	191	242	120	443	178	69	149	134	98
11	323	155	249	622	174	149	178	205	218	117
12	167	165	269	262	258	589	139	153	126	84
13	193	225	163	225	179	154	90	106	193	101
14	664	314	955	144	240	193	293	185	181	411
15	1287	1163	342	681	288	309	166	172	174	231

Bed	29 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	27	14	6	4	9	7	10	5	8	9
2	4	8	12	10	8	16	4	6	9	15
3	36	33	27	38	9	16	9	3	11	5
4	26	29	13	34	11	7	8	8	10	11
5	58	26	20	28	14	34	23	33	19	27
6	14	71	13	54	45	112	43	28	8	18
7	79	27	16	169	113	114	45	30	47	15
8	124	193	106	63	124	104	23	125	26	98
9	119	183	82	33	24	81	36	50	64	9
10	92	72	54	70	51	32	24	17	9	20
11	94	80	24	133	36	13	75	68	102	28
12	135	64	19	73	35	79	87	65	30	45
13	25	22	10	19	48	28	47	55	17	14
14	16	16	11	8	11	55	23	28	20	12
15	89	5	4	44	15	28	43	40	8	38

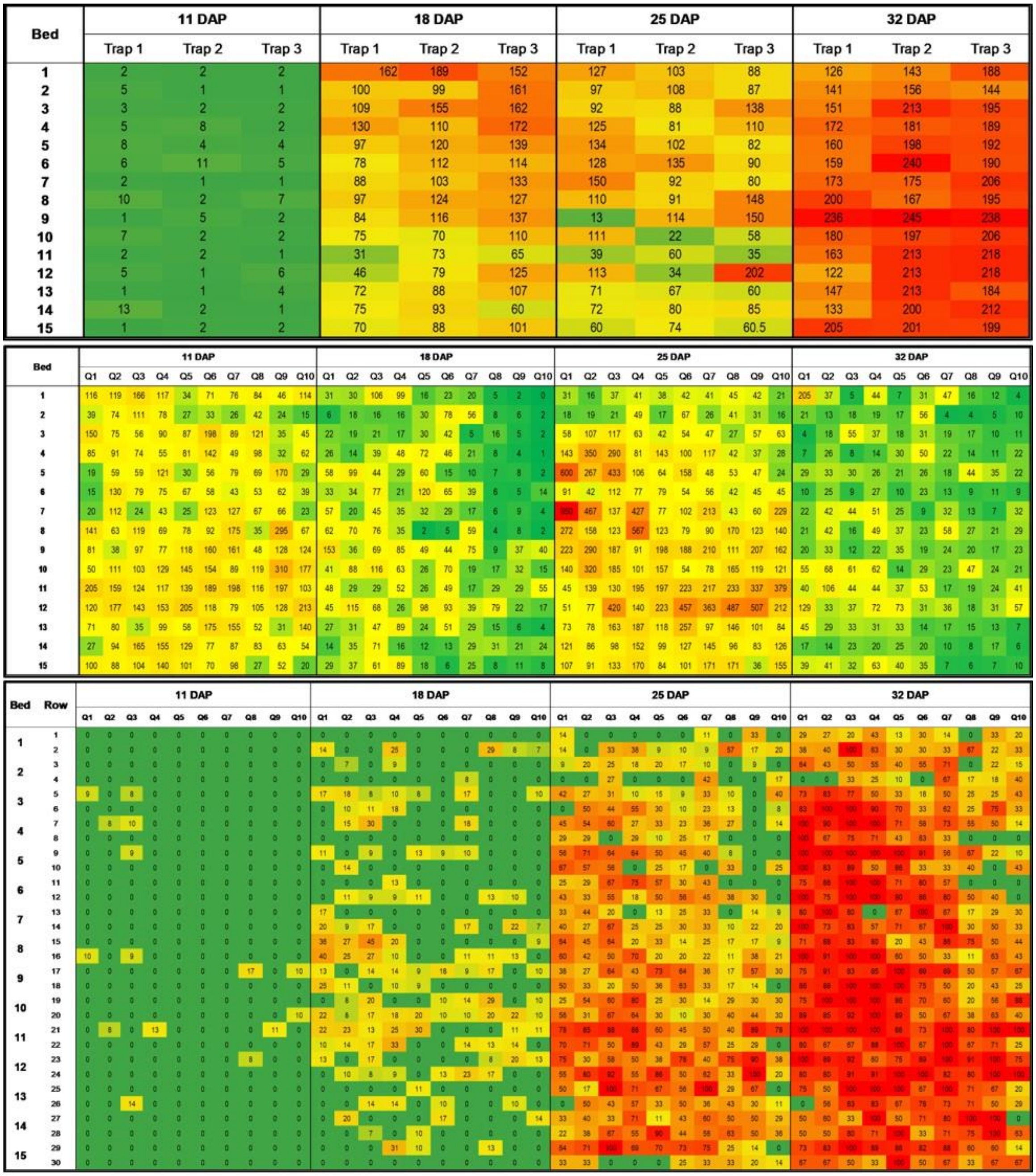
Bed	Row	8 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
2	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
3	5	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
4	7	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
5	9	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
6	11	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0
7	13	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0
8	15	0	0	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0	0	0
9	17	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0
10	19	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
11	21	0	0	0	0	0	0	0	0	0	0
	22	0	0	0	0	0	0	0	0	0	0
12	23	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0	0
13	25	0	0	0	0	0	0	0	0	0	0
	26	0	0	0	0	0	0	0	0	0	0
14	27	0	0	0	0	0	0	0	0	0	0
	28	0	0	0	0	0	0	0	0	0	0
15	29	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0

Bed	Row	15 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	0	0	0	0	0	11	0	0	0	0
	2	13	0	0	0	0	0	0	0	0	0
2	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	17	0	13	0
3	5	8	0	0	0	0	0	0	0	0	0
	6	0	0	10	0	9	0	0	0	0	0
4	7	0	0	9	0	0	0	0	0	0	0
	8	11	20	0	10	0	0	0	0	0	0
5	9	40	25	9	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
6	11	0	0	0	11	0	8	0	0	0	0
	12	17	10	20	0	8	11	0	0	0	0
7	13	0	13	10	18	22	0	0	11	0	0
	14	9	0	0	10	11	0	9	0	0	0
8	15	0	11	20	11	0	0	0	11	0	0
	16	20	0	11	0	0	9	0	13	20	0
9	17	0	0	0	9	18	10	17	0	0	0
	18	0	0	0	10	0	0	0	0	10	11
10	19	25	10	20	0	0	0	0	0	0	0
	20	0	13	9	13	0	0	0	0	0	0
11	21	22	0	18	11	0	0	0	0	0	0
	22	17	0	9	0	0	0	11	8	0	0
12	23	0	22	13	0	10	0	0	0	0	0
	24	18	33	0	0	8	0	10	0	0	0
13	25	0	0	20	50	0	0	0	0	0	0
	26	0	0	0	14	0	0	13	0	0	0
14	27	0	14	0	0	0	0	11	0	0	0
	28	18	25	9	0	0	0	0	0	9	11
15	29	14	0	13	0	29	0	11	0	0	0
	30	0	13	11	0	9	0	29	0	20	0

Bed	Row	22 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	0	11	0	20	22	11	0	11	0	9
	2	25	0	33	18	50	9	11	0	0	0
2	3	0	13	0	25	8	0	0	0	0	0
	4	0	0	0	17	11	9	17	0	13	0
3	5	23	0	17	11	0	11	0	0	0	0
	6	29	18	40	14	18	11	13	0	14	0
4	7	8	40	27	20	10	18	11	10	13	0
	8	33	50	23	10	11	8	0	0	0	0
5	9	70	50	27	18	10	22	22	0	22	0
	10	14	13	25	20	13	25	13	11	0	0
6	11	33	9	56	56	50	33	13	11	11	10
	12	67	20	70	11	58	11	20	0	20	9
7	13	18	38	60	56	56	50	55	33	0	0
	14	18	10	50	36	22	14	82	25	0	29
8	15	0	44	20	33	29	17	22	20	11	0
	16	70	18	33	20	50	18	0	38	20	13
9	17	22	0	0	55	64	36	42	20	0	9
	18	25	0	13	30	18	10	33	40	40	11
10	19	38	20	50	18	0	0	8	45	0	0
	20	36	36	36	25	10	0	0	0	27	0
11	21	33	10	45	33	0	17	10	9	18	0
	22	33	21	45	17	18	0	22	17	22	0
12	23	22	67	38	33	30	18	27	17	22	9
	24	45	78	44	0	33	10	10	22	0	0
13	25	25	30	60	50	43	9	13	0	14	0
	26	0	40	43	71	13	0	50	18	13	29
14	27	27	29	40	25	38	33	78	0	0	9
	28	56	25	36	63	0	30	38	22	36	9
15	29	43	0	38	0	43	9	22	17	0	17
	30	11	25	11	14	18	14	43	0	20	0

Bed	Row	29 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	8	22	14	70	44	33	13	22	0	55
	2	50	20	67	36	75	64	56	17	23	33
2	3	20	38	33	50	30	25	11	8	13	0
	4	22	30	50	50	56	73	67	11	75	67
3	5	38	40	67	44	56	56	33	9	0	20
	6	43	36	80	71	55	44	25	27	14	25
4	7	54	90	45	50	78	64	22	10	63	0
	8	44	70	54	60	67	33	10	10	0	30
5	9	80	75	91	55	60	56	44	58	33	27
	10	100	50	50	57	63	50	75	89	63	75
6	11	67	91	67	78	75	50	38	11	11	10
	12	83	60	100	67	75	44	20	18	30	27
7	13	64	63	90	64	67	70	73	33	0	0
	14	27	40	75	45	89	57	100	38	22	29
8	15	44	56	20	56	71	50	33	20	11	0
	16	80	55	78	40	50	27	22	63	40	25
9	17	44	55	17	64	64	45	42	50	10	9
	18	63	22	38	40	18	40	50	50	50	11
10	19	75	60	60	36	30	17	8	55	0	0
	20	64	38	55	38	30	27	29	27	27	0
11	21	33	40	73	67	11	33	40	18	18	0
	22	50	43	45	17	27	18	22	25	33	0
12	23	44	78	50	56	60	73	36	17	22	9
	24	64	100	58	18	33	20	20	33	22	0
13	25	38	40	60	50	43	9	25	27	43	0
	26	50	60	71	71	25	27	63	45	36	43
14	27	27	29	50	25	38	44	44	10	25	9
	28	64	38	36	75	20	40	50	33	45	18
15	29	57	0	38	11	43	9	22	17	0	14
	30	11	25	22	14	18	29	57	14	20	0

Figure 2.3. Heatmaps showing number of adult whiteflies[a] per 2cm² sticky trap (A), number of adult whiteflies in leaves (B), and WTVC incidence[b] (C) over time and space in the experimental field at the UGA Black Shank Farm in 2019. [a] Average number of adult whiteflies in eight 2cm^2 areas of a sticky trap (four in each side, front and back); [b] percent incidence of WTVC infected plants per 3.05m long rows of squash. Green areas had lower numbers of whiteflies and lower WTVC incidence. Red areas had higher whitefly counts and WTVC incidence



Bed	11 DAP			18 DAP			25 DAP			32 DAP		
	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3
1	2	2	2	162	189	152	127	103	88	126	143	188
2	5	1	1	100	99	161	97	108	87	141	156	144
3	3	2	2	109	155	162	92	88	138	151	213	195
4	5	8	2	130	110	172	125	81	110	172	181	189
5	8	4	4	97	120	139	134	102	82	160	198	192
6	6	11	5	78	112	114	128	135	90	159	240	190
7	2	1	1	88	103	133	150	92	80	173	175	206
8	10	2	7	97	124	127	110	91	148	200	167	195
9	1	5	2	84	116	137	13	114	150	236	245	238
10	7	2	2	75	70	110	111	22	58	180	197	206
11	2	2	1	31	73	65	39	60	35	163	213	218
12	5	1	6	46	79	125	113	34	202	122	213	218
13	1	1	4	72	88	107	71	67	60	147	213	184
14	13	2	1	75	93	60	72	80	85	133	200	212
15	1	2	2	70	88	101	60	74	60.5	205	201	199

Bed	11 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	116	119	166	117	34	71	76	84	46	114
2	39	74	111	78	27	33	26	42	24	15
3	150	75	56	90	87	198	89	121	35	45
4	85	91	74	55	81	142	49	98	32	62
5	19	59	59	121	30	56	79	69	170	29
6	15	130	79	75	67	58	43	53	62	39
7	20	112	24	43	25	123	127	67	66	23
8	141	63	119	69	78	92	175	35	295	67
9	81	38	97	77	118	160	161	48	128	124
10	50	111	103	129	145	154	89	119	310	177
11	205	159	124	117	139	189	198	116	197	103
12	120	177	143	153	205	118	79	105	128	213
13	71	80	35	99	58	175	155	52	31	140
14	27	94	165	155	129	77	87	83	63	54
15	100	88	104	140	101	70	98	27	52	20

Bed	18 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	31	30	106	99	16	23	20	5	2	0
2	6	18	16	16	30	78	56	8	6	2
3	22	19	21	17	30	42	5	16	5	2
4	26	14	39	48	72	46	21	8	4	1
5	58	99	44	29	60	15	10	7	8	2
6	33	34	77	21	120	65	39	6	5	14
7	57	20	45	35	32	29	17	6	9	4
8	62	70	76	35	2	5	59	4	8	2
9	153	36	69	85	49	44	75	9	37	40
10	41	88	116	63	26	70	19	17	32	15
11	48	29	29	52	26	49	17	29	29	55
12	45	115	68	26	98	93	39	79	22	17
13	27	31	47	89	24	51	29	15	6	4
14	14	35	71	16	12	13	29	31	21	24
15	29	37	61	89	18	6	25	8	11	8

Bed	25 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	31	16	37	41	38	42	41	45	42	21
2	18	19	21	49	17	67	26	41	31	16
3	58	107	117	63	42	54	47	27	57	63
4	143	350	290	81	143	100	117	42	37	28
5	600	267	433	106	64	158	48	53	47	24
6	91	42	112	77	79	54	56	42	45	45
7	850	467	137	427	77	102	213	43	60	229
8	272	158	123	567	123	79	90	170	123	140
9	223	290	187	91	198	188	210	111	207	162
10	140	320	185	101	157	54	78	165	119	121
11	45	139	130	195	197	223	217	233	337	379
12	51	77	420	140	223	457	363	487	507	212
13	73	78	163	187	118	257	97	146	101	84
14	121	86	98	152	99	127	145	96	83	126
15	107	91	133	170	84	101	171	171	36	155

Bed	32 DAP									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	205	37	5	44	7	31	47	16	12	4
2	21	13	18	19	17	56	4	4	5	10
3	4	18	55	37	18	31	19	17	10	11
4	7	26	8	14	30	50	22	14	11	22
5	29	33	30	26	21	26	18	44	35	22
6	10	25	9	27	10	23	13	9	11	9
7	22	42	44	51	25	9	32	13	7	32
8	21	42	16	49	37	23	58	27	21	29
9	20	33	12	22	35	19	24	20	17	23
10	55	68	61	62	14	29	23	47	24	21
11	40	106	44	44	37	53	17	19	24	41
12	129	33	37	72	73	31	36	18	31	57
13	45	29	33	31	33	14	17	15	13	7
14	17	14	23	20	25	20	10	8	17	6
15	39	41	32	63	40	35	7	6	7	10

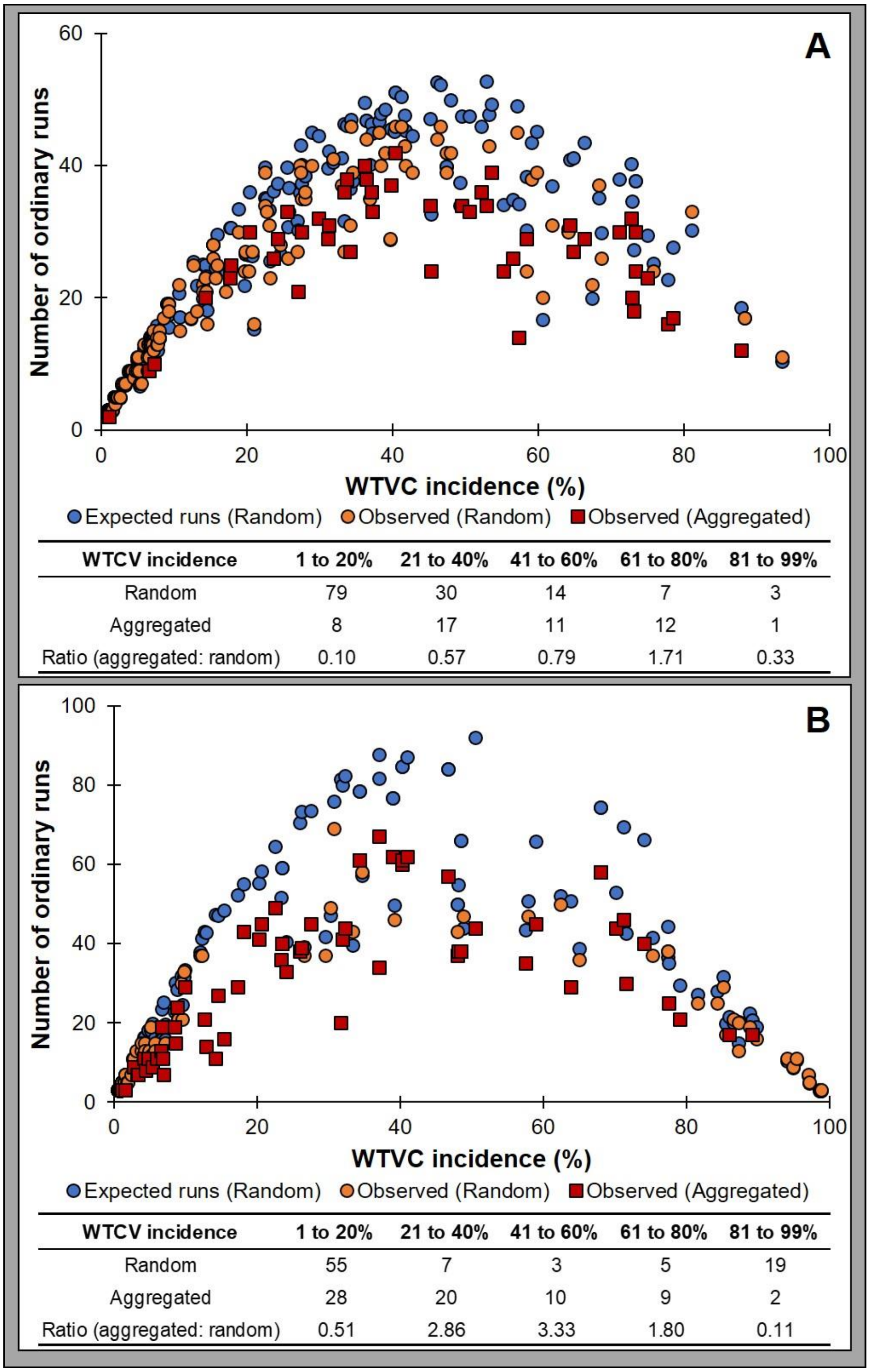
Bed	Row	11 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
2	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
3	5	9	0	8	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0
4	7	0	8	10	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
5	9	0	0	9	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
6	11	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0
7	13	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0
8	15	0	0	0	0	0	0	0	0	0	0
	16	10	0	9	0	0	0	0	0	0	0
9	17	0	0	0	0	0	0	0	17	0	10
	18	0	0	0	0	0	0	0	0	0	0
10	19	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	10
11	21	0	8	0	13	0	0	0	0	11	0
	22	0	0	0	0	0	0	0	0	0	0
12	23	0	0	0	0	0	0	0	8	0	0
	24	0	0	0	0	0	0	0	0	0	0
13	25	0	0	0	0	0	0	0	0	0	0
	26	0	0	14	0	0	0	0	0	0	0
14	27	0	0	0	0	0	0	0	0	0	0
	28	0	0	0	0	0	0	0	0	0	0
15	29	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0

Bed	Row	18 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	0	0	0	0	0	0	0	0	0	0
	2	14	0	0	25	0	0	0	29	8	7
2	3	0	7	0	9	0	0	0	0	0	0
	4	0	0	0	0	0	0	8	0	0	0
3	5	17	18	8	10	8	0	17	0	0	10
	6	0	10	11	18	0	0	0	0	0	0
4	7	0	15	30	0	0	0	18	0	0	0
	8	0	0	0	0	0	0	0	0	0	0
5	9	11	0	9	0	13	9	10	0	0	0
	10	0	14	0	0	0	0	0	0	0	0
6	11	0	0	0	13	0	0	0	0	0	0
	12	0	11	9	9	11	0	0	13	10	0
7	13	17	0	0	0	0	0	0	0	0	0
	14	20	9	17	0	0	0	17	0	22	7
8	15	38	27	45	20	0	0	0	0	0	9
	16	40	25	27	10	0	0	11	11	13	0
9	17	13	0	14	14	9	18	9	17	0	10
	18	25	11	0	10	9	0	0	0	0	0
10	19	0	8	20	0	0	10	14	29	0	10
	20	22	8	17	18	20	10	10	20	22	10
11	21	22	23	13	25	30	0	0	0	11	11
	22	10	14	17	33	0	0	14	13	14	0
12	23	13	0	17	0	0	0	0	8	20	13
	24	0	10	8	9	0	13	23	17	0	0
13	25	0	0	0	0	11	0	0	0	0	0
	26	0	0	14	14	0	10	0	0	10	0
14	27	0	20	0	0	0	17	0	0	0	14
	28	0	0	7	0	10	0	0	0	0	0
15	29	0	0	0	31	10	0	0	13	0	0
	30	0	0	0	0	0	0	0	0	0	0

Bed	Row	25 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	14	0	0	0	0	0	11	0	33	0
	2	14	0	33	38	9	10	9	57	17	20
2	3	9	20	25	18	20	17	10	0	9	0
	4	0	0	27	0	0	0	42	0	0	17
3	5	42	27	31	10	15	9	33	10	0	40
	6	0	50	44	55	30	10	23	13	0	8
4	7	45	54	60	27	33	23	36	27	0	14
	8	29	29	0	29	10	25	17	0	0	0
5	9	58	71	64	64	50	45	40	8	0	0
	10	67	57	56	0	25	17	0	33	0	25
6	11	25	29	67	75	57	30	43	0	0	0
	12	43	33	55	18	50	56	45	38	30	0
7	13	33	44	20	0	13	25	33	0	14	9
	14	40	27	67	25	25	30	33	10	22	20
8	15	64	45	64	20	33	14	25	17	17	9
	16	60	42	50	70	20	20	22	11	38	21
9	17	38	27	64	43	73	64	36	17	57	30
	18	50	33	20	50	36	63	33	17	14	0
10	19	25	54	60	80	25	30	14	29	30	30
	20	58	31	67	64	30	10	30	40	44	30
11	21	78	65	88	88	60	45	50	40	89	78
	22	70	71	50	89	43	29	57	25	29	0
12	23	75	30	58	50	38	78	40	75	90	38
	24	55	80	92	55	86	50	62	33	100	20
13	25	50	17	100	71	67	56	100	29	67	0
	26	0	50	43	57	33	50	36	43	30	11
14	27	33	40	33	71	11	43	60	50	50	29
	28	22	38	67	55	90	44	58	63	50	38
15	29	64	71	100	69	70	73	75	25	14	0
	30	33	33	0	0	0	25	33	33	20	14

Bed	Row	32 DAP									
		Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
1	1	29	27	20	43	13	30	14	0	33	20
	2	38	40	100	63	30	30	33	67	22	33
2	3	64	43	50	55	40	58	71	0	22	15
	4	0	0	33	25	10	0	67	17	18	40
3	5	73	83	77	50	33	18	50	25	25	43
	6	83	100	100	90	70	33	62	25	75	33
4	7	100	90	100	100	71	58	73	55	50	14
	8	100	67	75	71	43	83	33	0	0	0
5	9	100	100	100	100	100	91	56	67	22	10
	10	100	83	89	50	86	33	33	40	0	43
6	11	75	86	100	100	71	80	57	0	0	0
	12	100	75	100	100	80	86	80	50	40	0
7	13	80	100	80	0	67	100	67	17	29	30
	14	100	73	83	57	71	67	100	30	50	33
8	15	71	88	83	80	20	43	86	75	50	44
	16	100	91	100	100	60	50	33	11	63	43
9	17	75	91	83	85	100	89	89	50	57	67
	18	86	88	100	100	100	75	50	20	43	25
10	19	75	100	100	100	86	70	80	20	56	86
	20	89	85	92	100	89	50	67	38	63	40
11	21	100	100	100	100	88	73	100	80	100	100
	22	80	67	67	88	100	67	100	67	71	25
12	23	100	89	92	80	75	89	100	91	100	75
	24	80	80	91	91	100	100	82	80	100	100
13	25	75	50	100	100	100	67	100	71	67	20
	26	0	56	83	83	67	78	73	71	50	29
14	27	50	60	33	100	50	71	80	100	100	0
	28	50	50	80	71	100	33	71	75	100	83
15	29	73	83	100	89	88	82	88	80	60	14
	30	67	67	50	33	100	50	67	33	67	67

Figure 2.4. Heatmaps showing number of adult whiteflies[a] caught on sticky trap (A), number of adult whiteflies in leaves (B), and WTVC incidence[b] (C) over time and space in the experimental field at the UGA Black Shank Farm in 2020. [a] Average number of adult whiteflies in eight 2cm^2 areas of a sticky trap (four in each side, front and back); [b] percent incidence of WTVC infected plants per 3.05m long rows of squash**.** Green areas had lower numbers of whiteflies and lower WTVC incidence. Red areas had higher whitefly counts and WTVC incidence



WTCV incidence	1 to 20%	21 to 40%	41 to 60%	61 to 80%	81 to 99%
Random	79	30	14	7	3
Aggregated	8	17	11	12	1
Ratio (aggregated: random)	0.10	0.57	0.79	1.71	0.33

WTCV incidence	1 to 20%	21 to 40%	41 to 60%	61 to 80%	81 to 99%
Random	55	7	3	5	19
Aggregated	28	20	10	9	2
Ratio (aggregated: random)	0.51	2.86	3.33	1.80	0.11

Figure 2.5. Analysis of one-dimensional spatial distribution of WTVC per row of plants based on ordinary runs analysis in experimental (A) and commercial (B) squash fields. The graph for the experimental field (A) comprised data collected in field trials conducted in 2019 and 2020 fall growing seasons. The plot of commercial fields (B) includes data collected from all six commercial squash fields that were surveyed in 2019 and 2020

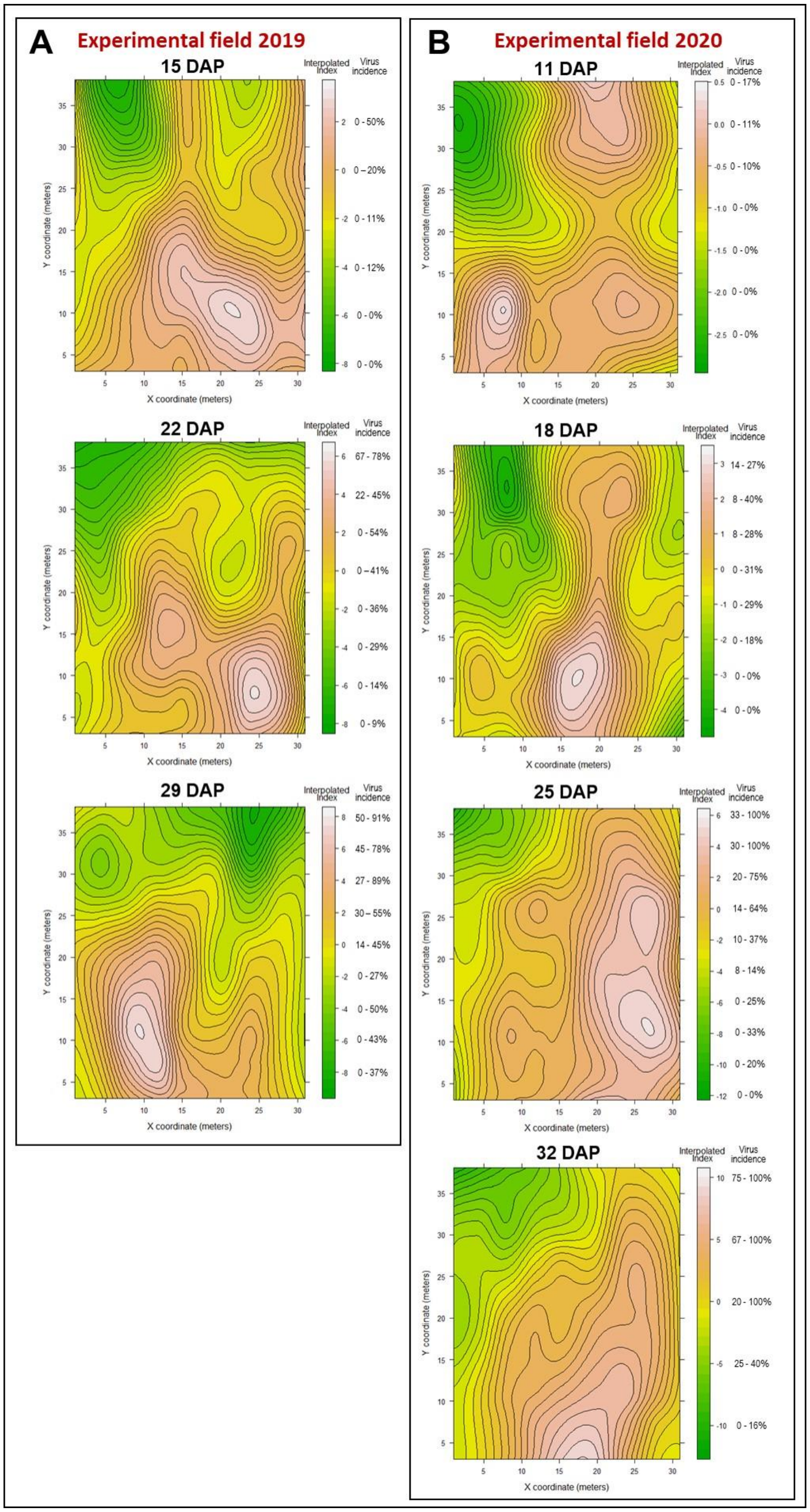
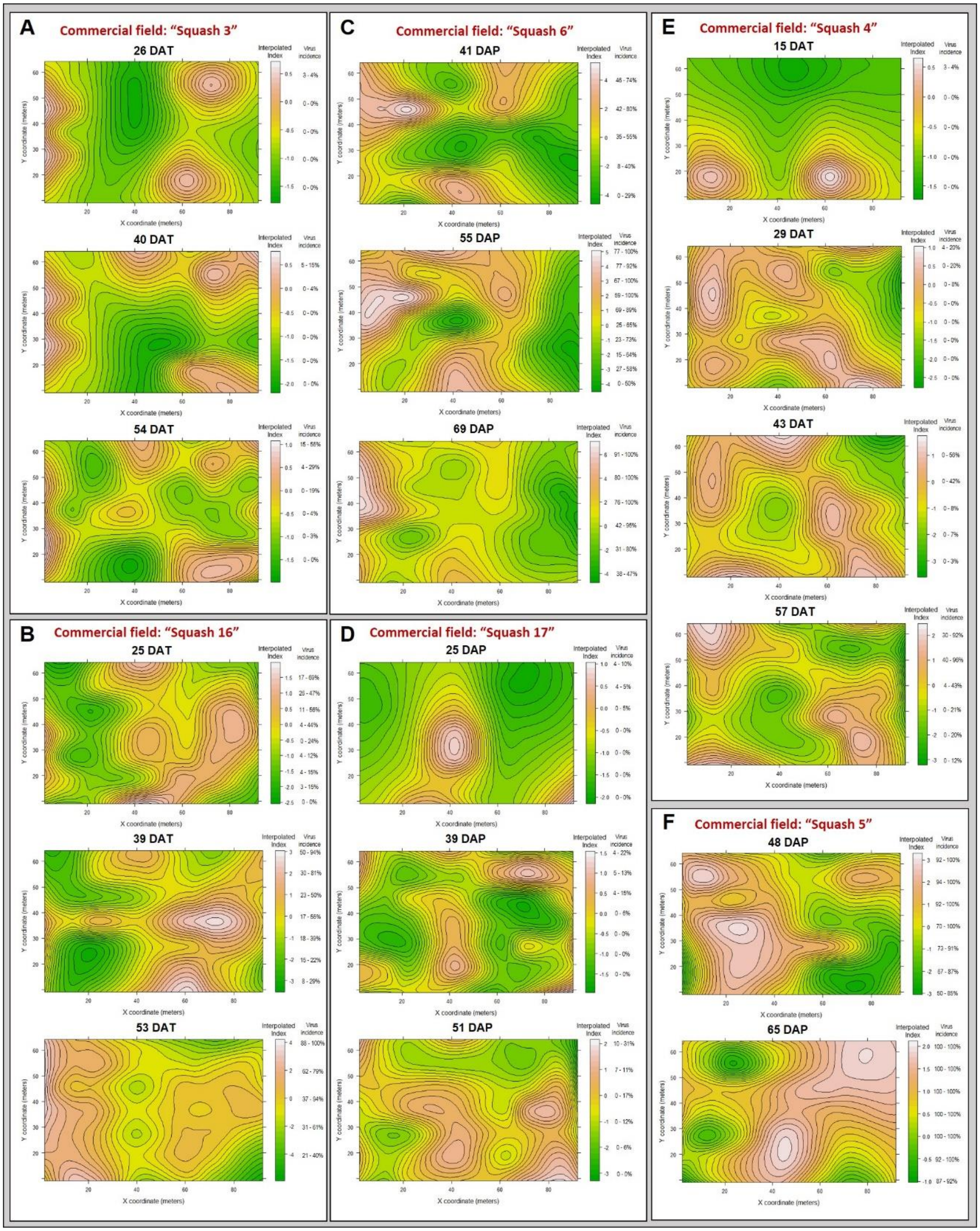


Figure 2.6. Contour maps of showing areas within the experimental field where WTVC infected plants occurred as patches (red to white areas) or gaps (green areas) based on the interpolated index from the SADIE analysis



A Commercial field: "Squash 3"

26 DAT

40 DAT

54 DAT

B Commercial field: "Squash 16"

25 DAT

39 DAT

53 DAT

C Commercial field: "Squash 6"

41 DAP

55 DAP

69 DAP

D Commercial field: "Squash 17"

25 DAP

39 DAP

51 DAP

E Commercial field: "Squash 4"

15 DAT

29 DAT

43 DAT

57 DAT

F Commercial field: "Squash 5"

48 DAP

65 DAP

Figure 2.7. Maps of clustering indices with interpolated landscape and contours showing areas within commercial squash fields where WTVC infected plants occurred as patches (red to white areas) or gaps (green areas) based on the interpolated index from the SADIE analysis

CHAPTER 3

ASSESSMENT OF PRICKLY SIDA AS A POTENTIAL INOCULUM SOURCE FOR SIDA GOLDEN MOSAIC VIRUS IN COMMERCIAL SNAP BEAN FARMS IN SOUTHERN GEORGIA[1]

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[1] Codod, C.B., Severns, P.M., Sparks, A.N., Jr., Srinivasan, R., Kemerait, R.C. Jr. and Dutta, B. To be submitted to *Plant Disease*.

Abstract

Sida golden mosaic virus (SiGMV), an obligate pathogen that infects snap beans, is known to infect prickly sida (*Sida spinosa* L.), which is a common weed in agricultural farms in Georgia. Prickly sida has also been reported as a suitable host of sweetpotato whitefly (*Bemisia tabaci*), the vector of SiGMV. Despite being a host for both SiGMV and its vector, the role of prickly sida as a reservoir and inoculum source for SiGMV in snap bean farms has not been evaluated. This study was conducted to document the occurrence of SiGMV-infected prickly sida plants and to assess its potential role as a reservoir and a source of SiGMV inoculum in snap bean farms. A survey of 17 commercial snap bean farms conducted in spring 2021 confirmed the presence of SiGMV-infected prickly sida in southern Georgia. In fall 2021 and 2022, on-farm field trials were conducted in four commercial farms where SiGMV-infected prickly sida plants were documented earlier as a part of survey in spring 2021. The spatial distribution and temporal patterns of adult whiteflies and SiGMV on snap bean were determined and compared between two macroplots (13.7 m x 30.5 m), ‘with prickly sida’ or ‘without prickly sida’, that were at least 232 m apart in each farm. Snap bean macroplots ‘with prickly sida’ were adjacent to non-cultivated margins where SiGMV-infected prickly sida were detected. We did not observe any consistent differences in counts of adult whiteflies between macroplots with or without prickly sida in the four commercial farms. SiGMV infection was detected earlier and with higher incidences in snap bean macroplots with prickly sida compared to macroplots without prickly sida. An apparent disease gradient was observed in two of the four farms. Higher SiGMV incidences were observed on the

edges of macroplots with prickly sida. These findings indicate prickly sida as a potential natural reservoir and a source for SiGMV spread in snap bean farms in southern Georgia.

Introduction

Plant insect pests and pathogens with wide-host range thrive in the natural environment where resources spatially and temporally fluctuate in abundance (Duffus 1971; Freeman and Aftab 2011). In the absence of a preferred and dominant crop host plant, polyphagous insect pests can feed and reproduce in non-crop plants as alternative hosts in the same landscape (Fermin 2018). The American Phytopathological Society defines alternative hosts as ‘a plant other than the main host that a parasite can colonize; alternative hosts are not required for completion of the developmental cycle of the parasite’ (D’Arcy et al. 2001). Alternative plant hosts can serve as an inoculum source and can potentially act as a green bridge for phytopathogens between cropping seasons (Freeman and Aftab 2011; Legarrea et al. 2020; Nischwitz et al. 2012; Srinivasan and Alvarez 2008; Srinivasan et al. 2014). With a wide host range, polyphagous pests and phytopathogens with alternative host species persist in the landscape throughout the year where they build up their populations, and later colonize recently emerged crops (Parry et al. 2019). Identification and verification of non-crop hosts that harbor arthropod pests and serve as inoculum sources can help refine tactics for managing insect pests and plant pathogens.

Sweetpotato whitefly (*Bemisia tabaci* Gennadius) has a wide host range, including vegetables, row crops, and weeds (Evans 2007; Simmons et al. 2008). In the southeastern US, diverse hosts of this pest include economically important crops such as squash (*Cucurbita pepo* L.), tomato (*Solanum lycopersicum* L.), snap bean (*Phaseolus vulgaris* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), cotton (*Gossypium hirsutum* L.), peanuts (*Arachis hypogaea* L.), and soybean (*Glycine max* L.). Apart from these crop-hosts the whitefly can also colonize weed hosts including common cocklebur (*Xanthium strumarium* L.), common mallow (*Malva neglecta* Wallr.), prickly sida (*Sida spinosa* L.), lantana (*Lantana camara* L.), and small flower morningglory (*Ipomoea purpurea* (L.) Roth) (Barman et al. 2022). With their ability to persist year-round by moving from one host to another, whiteflies can establish high populations that can potentially devastate economically important host crops such as snap bean.

Sida golden mosaic virus (SiGMV) is one of the whitefly-transmitted viruses impacting snap bean (*Phaseolus vulgaris*) in the southeastern US (Durham et al. 2010). In southern Georgia, snap bean is grown in the spring and the fall growing seasons from March to June and September to November, respectively (Sparks et al. 2018) with a four-month crop-free period. SiGMV is an obligate pathogen, which requires an alternative/weed host to survive between cropping seasons. Weeds growing near commercial farms could serve as alternative hosts, natural reservoirs, and sources of inoculum for obligate pathogens between cropping seasons (Nischwitz et a. 2012; Wyka and Broders 2023). Potential alternative hosts for SiGMV in Georgia included hollyhock (*Alcea rosea* L.), marshmallow (*Althaea officinalis* L.), okra (*Abelmoschus esculentus* (L.) Moench), country mallow (*Sida cordifolia* L.), prickly sida (*Sida spinosa* L.), and tobacco (*Nicotiana tabacum* L.) (Gautam et al. 2023). Among the potential SiGMV alternative hosts, prickly sida is one that has been reported to occur as a common weed in agricultural farms in southeastern US (Webster and MacDonald 2001).

Prickly sida belongs to the mallow family, *Malvaceae*, which is a summer annual in temperate regions but occurs as a perennial in tropical regions (Mohler et al. 2021). Prickly sida is one of the most troublesome weeds in corn, tobacco, and vegetable farms in Georgia (Webster and MacDonald 2001). As it is frequently found in proximity to important food and fiber crops, it could potentially serve as a natural reservoir and inoculum source for SiGMV between snap bean cropping seasons in commercial snap bean farms. Although it is known that SiGMV infects both snap beans and prickly sida (Roye et al. 1997; Durham et al. 2010; Gautam et al. 2023), the role of prickly sida in the prevalence and spatiotemporal spread of SiGMV on snap beans is still not well understood. Hence, the objective of this study is to evaluate the occurrence of prickly sida as an inoculum source for the whitefly-transmitted SiGMV in commercial snap bean farms and to assess its influence on the spatiotemporal spread of the pathogen.

Materials and methods

Natural occurrence of SiGMV-infected prickly sida in commercial snap bean farms in southern Georgia

The margins of commercial snap bean farms (*n*= 17) were scouted for the presence of prickly sida and once found, leaf samples were tested to determine their SiGMV infection status. Farm margins referred to non-cultivated areas that often served as driveways along the edges of the farm. The survey was conducted in spring (March to May) 2021. Leaves were collected arbitrarily from prickly sida plants (*n*= 10 symptomatic or asymptomatic plants/location) growing along the farms' margins. In farms where prickly sida showing symptoms of SiGMV were found, leaf samples from both symptomatic and asymptomatic plants were collected. In some farms, symptomatic prickly sida were not found, hence, only asymptomatic samples were collected. All samples were stored in plastic ziplock bags (Qosina Corp, Ronkonkoma, NY) and transported on ice to the laboratory in Tifton, where they were stored at -20°C.

DNA was extracted from the prickly sida samples using the DNeasy® Plant Mini Kit (QIAGEN, Germantown, Maryland) following the manufacturer's protocol. A 1 ng/µl of DNA per sample was used as a template with primer pair (SiGMVF and SiGMVR) that target a 574 bp region of AV1 gene in SiGMV (Gautam et al. 2023). The PCR cycle involved an initial denaturation step for 2 min at 95°C followed by 40 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and ending with a final extension of 72°C for 10 min (Gautam et al. 2023). PCR products were visualized using agarose gel (1%) electrophoresis.

Assessment of whitefly abundance and SiGMV incidence in snap bean plots with and without prickly sida

An on-farm study was conducted to assess if the occurrence of SiGMV-infected prickly sida in non-cultivated farm margins influences the spatial and temporal patterns of SiGMV in commercial snap bean farms. Of the 17 commercial farms surveyed for the presence of prickly sida, four farms (two in each year) were selected and used as sites for the on-farm study during the fall seasons in 2021 (*n*= 2) and 2022 (*n*= 2) (Fig. 3.1A). These four farms were selected for the on-farm study as they were planted with snap bean during both spring and fall seasons. Most of the farms surveyed for the presence of prickly sida were only planted with snap bean in the spring season. The snap bean variety was the same in all four farms and they were planted between 23rd August to 8th September in 2021 and 2022 (Table 3.1). Moreover, these farms had prickly sida (also infected with SiGMV) on one side of the farm whereas absent in other side. This allowed comparison of the spatiotemporal spread of SiGMV infection in snap bean plants within macroplots with or without prickly sida. A macroplot is defined as an area larger than 2 m^2, requiring the observer to move around within them in order to obtain estimate (Mitchell et al. 1988).

In this study, a macroplot consisted of 15 rows (13.7 m total width) of snap beans measuring 30.5 m long. Each 30.5 m long row was divided into 10 quadrats measuring 3.1 m long resulting in a total of 150 quadrats per macroplot. In each snap bean farm, there were two macroplots. One macroplot, hereby termed as ‘plot with prickly sida’, was adjacent to the margin of the farm with live SiGMV symptomatic prickly sida plants (Fig. 3.1B). The distance between the edge of the macroplot to the farm margin where SiGMV-infected prickly sida was found was between 7 m to 12 m (Table 3.1). Prickly sida was not present in the margin of the farm adjacent to the other macroplot, hence was termed as ‘plot without prickly sida’. The distance between the two macroplots ranged from 232 m to 475 m depending on the farm's size (Table 3.1).

Prickly sida plants on the farm margins were assessed for SiGMV symptoms and presence of adult whiteflies at a seven-day interval for 4-5 weeks. Within a 30 m section of the snap bean farm margin, adjacent to the macroplot, arbitrarily selected prickly sida plants (*n*= 60) were scored as symptomatic or asymptomatic. The prickly sida plants were also inspected for the presence or absence of adult whiteflies by gently flipping the leaves and checking the abaxial side. Leaves from symptomatic prickly sida (*n*= 34) were collected and tested to confirm SiGMV infection through PCR following published protocols (Gautam et al. 2023).

Within each macroplot, snap bean plants were visually assessed for SiGMV infection based on presence of symptoms per quadrat at a seven-day interval until harvest maturity. Ratings were taken four to five times per farm between 14 to 54 days after planting (DAP). In each quadrat, the number of symptomatic plants was recorded during each assessment period. The approximate plant population per quadrat was determined by counting the number of plants within a 1m row and then multiplying this value by the total length of the quadrat (3.05 m). The percent SiGMV incidence was calculated by dividing the number of infected plants by the total number of plants and then multiplying it by 100. Leaf samples from symptomatic snap bean plants (*n*= 50) were collected from each farm and tested to confirm the presence of SiGMV using a PCR assay following a protocol by Gautam et al. (2023).

The number of adult whiteflies was counted by gently flipping the leaves of snap beans and then checking the abaxial side of the leaves. The number of adult whiteflies on snap bean leaves was assessed repeatedly at a seven-day interval. Whitefly counts were performed in the 1st, 3rd, 5th, 7th, and 9th quadrats (two leaves/ quadrat) of the 2nd, 5th, 8th, 11th, and 14th rows of each macroplot. At each rating time, whitefly counts were taken from 50 leaves per macroplot.

Spatial patterns of whitefly and SiGMV infected plants over time in snap bean farms

<u>Weather conditions.</u> The daily maximum temperature, minimum temperature, and amount of precipitation were retrieved from the UGA Weather Network (Ponder Farm

weather station, http://www.georgiaweather.net/mindex.php?variable=HI&site=TYTY). The Ponder Farm weather station, located at Ty Ty, Georgia, was the closest station to all four snap bean farms where field trials were conducted in 2021 (Farms 1 and 2) and 2022 (Farms 3.3 and 3.4). The mean of daily minimum (C) and maximum (D) temperatures were calculated per month. The number of days with precipitation and the cumulative amount of precipitation were also calculated per month.

Whitefly and SiGMV incidence on prickly sida in commercial farms. The percent SiGMV incidence in prickly sida was calculated by dividing the number of SiGMV symptomatic prickly sida over the total number of plants assessed (*n*= 60) and then multiplied by 100. As growers regularly mow the margins of the farms to manage the weeds (including prickly sida), prickly sida plants were arbitrarily selected at each assessment period. For these reasons, the mean SiGMV incidence over four assessment periods was calculated per farm and presented in this manuscript. Similarly, the percentage of prickly sida infested with at least one adult whitefly was calculated and the mean percent whitefly-infested prickly sida over a four assessment periods was determined for each farm.

Whitefly abundance and SiGMV incidence. A heat map showing adult whitefly counts per macroplot over time was created using the ‘ggplot’ function in the ‘ggplot2’ package in R (Wickham 2016; R Core Team 2020). As the number of whiteflies were counted on two leaves per quadrat, the mean number of whiteflies per leaf was used for the heat maps at each assessment period for each macroplot. Similarly, the ‘ggplot’ function was used to create a heatmap of the incidence of SiGMV as it changed over each assessment time in each macroplot.

SiGMV incidences at different distances from farm edges near SiGMV infected prickly sida

Final SiGMV incidences in snap beans for each farm were used to assess if a disease gradient from the edges of the macroplots emerged when snap beans were adjacent to SiGMV-infected prickly sida. The final ratings were taken between 42 to 54 days after planting in some cases it coincided with harvest maturity, depending on the farm. The mean SiGMV incidence was calculated for each distance from the edge of the macroplot using the ‘aggregate’ function in R (R Core Team 2020). The distance from farm edge ranged from 3.1 m to 30.4 m or 0.9 m to 13.7 m depending on the orientation of the macroplot relative to the farm margin where SiGMV-infected sida were present. A scatter plot was created to show mean SiGMV incidences at varying distances to the farm edge adjacent to SiGMV-infected sida. To describe a potential disease gradient with respect to the presence of SiGMV-infected sida, a smoothed trendline was added in the scatterplot using the ‘geom_smooth’ function and ‘loess’ method in the ‘ggplot2’ package in R (Wickham 2016; R Core Team 2020).

Comparison of temporal progress of adult whiteflies and SiGMV infection in snap bean macroplots with and without an inoculum source

<u>Adult whitefly abundance.</u> A line graph showing the temporal pattern of whitefly abundance was created for each farm by plotting the mean number of adult whiteflies per plot per assessment period (number of days after planting). The mean number of adult whiteflies was calculated using the ‘aggregate’ function in R (R Core Team 2020).

To compare the whitefly abundance between the two macroplots (with or without prickly sida), the area under the curve (AUC) for whitefly abundance over time was calculated using the ‘audpc’ function in the ‘epifitter’ package in R (Alves and Del Ponte 2021). A pairwise comparison between the area under the whitefly abundance curve for the two macroplots was performed using the ‘Wilcoxon sum rank test’ through the ‘wilcox.test’ function in R (R Core Team 2020) as the data were not normally distributed. The Wilcoxon sum rank test, also called Mann-Whitney U test, is a non-parametric version of the t-test, which tests for differences between two groups (Mann and Whitney 1947; McKnigh and Najab 2010; Wilcoxon 1945).

SiGMV incidence. The mean SiGMV incidences per macroplot was calculated using the ‘aggregate’ function in R (R Core Team 2020). The mean SiGMV incidences were plotted in a line graph using the ‘ggplot’ function in the ‘ggplot2’ package in R (Wickham 2016; R Core Team 2020).

The area under the disease progress curve (AUDPC) was calculated based upon the SiGMV incidence ratings per quadrat. AUDPC values were calculated in R using the ‘audpc’ function in the ‘epifitter’ package in R (Alves and Del Ponte 2021). The AUDPC values in the two macroplots were compared using the ‘Wilcoxon sum rank test’ through the ‘wilcox.test’ function in R (R Core Team 2020).

Results

Natural occurrence of SiGMV-infected prickly sida in commercial snap bean farms in southern Georgia

In our sampling during the spring season of 2021, prickly sida was found in 94% of the farms surveyed (Table 3.2). These weeds were usually found in non-cultivated margins of snap bean farms (within 7-12 m of the farm edge) and when tested for the presence of SiGMV through PCR, 47% of the surveyed farms had SiGMV-infected prickly sida (Table 3.2). The symptoms observed on SiGMV-infected prickly sida were characterized by golden mosaic on leaves and general stunting of plants. Among the symptomatic samples, 88% tested positive for the virus. Conversely, only 2% of the asymptomatic prickly sida samples were positive for SiGMV.

Spatial patterns of whitefly and SiGMV infection in fall-grown snap beans

Weather conditions. During the months (June, July, August) prior to planting the fall season snap bean crop, there was a higher number of rainy days in 2021 (14 - 22 rainy days) than in 2022 (5 - 19 rainy days) (Supplementary Fig. S1A). During the growing season (September and October), the number of rainy days and the total amount of rain were higher in 2021 (7 - 10 rainy days, 6.7 - 9.6 cm) than in 2022 (4 - 7 rainy days, 4.4 - 8.7 cm) (Supplementary Fig. S3.1A and B).

The minimum and maximum temperatures were lower by 0.7 - 3.3 °C in 2021 than in 2022 during the months of April to June (Supplementary Fig. S3.1C and D). The temperatures had lesser variation (0 - 1.1 °C) during the months of July to September (Supplementary Fig. S3.1C and D).

Whitefly and SiGMV incidence on prickly sida in commercial farms. The percentage of prickly sida (*n*= 60) that had at least one adult whitefly was highest in Farm 4 (36%), followed by Farm 2 (27%), Farm 1 (16%), and Farm 3 (15%) (Fig. 3.2). The percent SiGMV incidence in prickly sida varied considerably among four commercial farms [Farm 1: 13%; Farm 2: 7%; Farm 3: 18% and Farm 4: 49%] (Fig. 3.2). The presence of SiGMV was confirmed in 91% of leaf samples (*n*= 34) from symptomatic prickly sida plants through PCR assay in these four commercial farms.

Spatial patterns of whitefly and SiGMV in macroplots with and without prickly sida. In Farm 4, higher SiGMV incidences occurred in areas where higher adult whitefly counts were observed in the macroplot with prickly sida (Fig. 3.3). The occurrence of SiGMV and adult whitefly hotspots in the same area within the macroplot was not observed in the other three farms (Fig. 3.4-3.6).

Between macroplots, there were greater numbers of quadrats with SiGMV symptomatic snap beans in macroplots with prickly sida compared with those without prickly sida in all four farms (Fig. 3.3-3.6). SiGMV symptomatic snap beans were observed earlier in macroplots with prickly sida in Farm 3 (one week) and Farm 1 (two weeks) (Fig. 3.4 & 3.5). In Farm 2, SiGMV symptomatic snap beans were not observed in the macroplot without prickly sida but were observed in two quadrats starting at 28 DAP in the macroplot with prickly sida (Fig. 3.6). In Farm 4, SiGMV symptomatic snap bean plants were observed in both macroplots during the first assessment period at 26 DAP; however, symptomatic plants were observed in four quadrats in the macroplot with prickly sida compared with one quadrat in the other macroplot (Fig. 3.3).

Higher SiGMV incidences in snap beans were observed among quadrats closer to the farm margins with prickly sida (Fig. 3.3B & 3.6B). The higher incidence of SiGMV in snap beans towards the farm margin was not observed in the other two farms (Fig. 3.4B & 3.5B).

Confirmation of SiGMV infection in symptomatic snap beans. Fifty leaf samples from symptomatic snap beans were collected in Farm 4 (*n*= 20), Farm 3 (*n*= 10), Farm 1 (*n*= 10), and Farm 2 (*n*= 10) farms. SiGMV infection was confirmed in 84% of the leaf samples tested through SiGMV-specific PCR assay.

SiGMV incidences at different distances from farm edges near SiGMV-infected prickly sida

The smoothed loess trendline representing the mean SiGMV incidences as distance increases from the farm edge inward suggested a consistent decrease in SiGMV incidences from the edge until the average SiGMV incidence approached 0% by 12 m away from the edge (Fig. 3.7A: Farm 2 & Fig. 3.7B: Farm 4). This pattern of decreasing SiGMV incidences from the farm edge was observed in two out of four farms.

Comparison of temporal progress of adult whiteflies and SiGMV infection in snap bean macroplots with and without an inoculum source

In Farm 2 and 4, the mean number of adult whiteflies and the AUC values for their counts were higher in the macroplots with prickly sida than in plots without prickly sida (Fig. 3.8). Coincidentally, it was also in Farm 2 and Farm 4 where a disease gradient with higher SiGMV incidences were observed in snap beans closer to SiGMV-infected prickly sida (Fig. 3.7A: Farm 2 & Fig. 3.7B: Farm 4). In contrast, the number of adult whiteflies were higher in plots without prickly sida in Farm 3 and Farm 1.

While the mean SiGMV incidences were quite low (less than 1%) in all four farms, higher levels of SiGMV incidences were observed over time in plots with prickly sida than in plots without prickly sida (Fig. 3.9A). The AUDPC values were significantly higher in plots with prickly sida than in plots without prickly sida in farms 1, 2, and 4 (Fig. 3.9B).

Discussion

In seasonal cropping systems, there are times when crop hosts of pests and pathogens are scarce such as between harvest of spring crop and planting of fall crop **(**Kennedy and Storer 2000). During these temporal breaks in host crop availability, pests and pathogens infest alternative hosts, such as weeds, until a suitable cultivated host crop becomes available (Hewings and Eastman 1995; Rashidi et al. 2021). Weeds are known for their potential as alternative hosts and serving as natural reservoirs and inoculum sources for pathogens affecting crops (Altieri 1988; Barreto et al. 2013; Kennedy and Storer 2000; Lovelock et al. 2023; Srinivasan et al. 2012a; Van Emden 1965). For insect-transmitted plant viruses, a plant is considered a significant source of inoculum if it is a suitable host for both the insect vector and the virus; the vector can acquire the virus from the plant; and the plant must be present at a time that would complement disease cycles (Culbreath et al. 2003). In this study, we assessed the potential of a commonly occurring weed, prickly sida, as a potential inoculum source for the whitefly-transmitted SiGMV in commercial snap bean farms.

Prickly sida is a known host for SiGMV and its vector, the sweetpotato whitefly (Barman et al. 2022; Gautam et al. 2023). Studies conducted in Georgia have documented the prevalence of prickly sida as weeds in agricultural farms (Barman et al. 2022; Webster and MacDonald 2001). Being a host for both whiteflies and SiGMV, and its prevalence in the farmscape, prickly sida could potentially serve as a natural reservoir and a source of inoculum for SiGMV in snap bean farms. We evaluated the occurrence of natural SiGMV infection in prickly sida adjacent to commercial snap bean farms and assessed if the spatial distribution and temporal patterns of disease caused by SiGMV in snap beans were associated with the presence of SiGMV-infected prickly sida along farm margins. These aspects were never been studied earlier in this pathosystem under the natural settings. The ecosystem in southern Georgia provided a unique opportunity to assess these aspects as the vector, virus, host and alternative host occur together. We first established that SiGMV infection occurs naturally in prickly sida plants growing along farm margins during our commercial field surveys (*n*= 17 fields; Spring 2021). The SiGMV-infected prickly sida was common in these commercial snap bean farms during the spring season. Similar to whitefly-transmitted viruses affecting cucurbits (Adeleke et al. 2022; Candian et al. 2021), SiGMV is not a concern during the spring season because of low whitefly populations. However, the detection of SiGMV-infected prickly sida during spring season is of considerable importance in SiGMV epidemiology. It indicates the potential persistence of SiGMV within the vicinity of commercial snap bean farms during spring season and emerge as a potential source of inoculum in the fall growing season.

Crop hosts of whitefly are grown throughout the year in southern Georgia, which allows the whiteflies to persist and remain active year-round (Sparks et al. 2018). However, whitefly populations do not begin to build until late July and often reach higher populations in September (Barman et al. 2019; Candian et al. 2021; Srinivasan et al. 2012b). The period of higher whitefly populations coincides with the planting of fall grown vegetable crops including snap beans in southern Georgia. As the farm is cleared of weeds and residues from previous crops, adult whiteflies would likely disperse and seek refuge on weed hosts such as prickly sida that grow in non-cultivated farm margins or in some cases can encroach and infest neighboring field with crop host. Whiteflies are known to show preference for virus infected plants particularly those expressing symptoms (Legarrea et al. 2015; Ontiveros et al. 2022). Hence, it is possible for adult whiteflies to preferentially settle on SiGMV-infected prickly sida, which typically show a golden mosaic symptom. After settling, whiteflies may feed, acquire the virus, and reproduce on infected prickly sida during the period when the farms are being prepared for fall grown vegetable crops including snap beans. The viruliferous whiteflies may disperse from prickly sida and transmit SiGMV to snap bean plants in the farm.

In on-farm studies conducted during the fall growing season, adult whiteflies and SiGMV incidences (up to 49%) were observed in prickly sida growing along the non-cultivated margins of snap bean farms. The occurrence of whiteflies in prickly sida (up to 36%) confirm their colonization in naturally occurring prickly sida within the vicinity of snap bean farms. The high incidences of SiGMV in prickly sida also indicates the spread of SiGMV by adult whiteflies in prickly sida plants that were prevalent along farm margins. When feeding on SiGMV-infected prickly sida, whiteflies produce higher number of eggs and have faster egg-to-adult development time (Gautam et al. 2023). The greater fecundity and shorter developmental time in infected prickly sida may contribute to the spread of SiGMV in the farmscape and into snap bean farms as it also could help in the proliferation of viruliferous adult whiteflies.

Adult whitefly counts were higher on snap bean plants within macroplots with prickly sida than those on macroplots without prickly sida in farms 2 and 4 (Fig. 8). This pattern, however, was not observed in the other two farms. The possible explanation for this inconsistency in adult whitefly counts could be the slope of the farms. While these farms had the same snap bean variety and planted about the same time, farms 2 and 4 were sloping downwards in the macroplots with prickly sida and there were tree lines at the margins of these farms. The downward slope and the presence of tree lines may have contributed to inadequate insecticide application coverage towards edges of the farms close to the tree lines because insecticides were applied using crop dusters (agricultural airplanes) in these snap bean farms. Hence, the observed higher number of adult whiteflies in macroplots with prickly sida in farms 2 and 4 could have been an effect of the inadequate insecticide application coverage.

When symptoms of SiGMV were first observed in Florida snap bean farms, up to 100% of the snap beans along the edge of the farm were symptomatic (Durham et al. 2010). This within-farm pattern of disease distribution agrees with observations made in this study where higher SiGMV incidences were observed along farm edges adjacent to the noncultivated margins with prickly sida. The presence of SiGMV-infected prickly sida also seemed to be associated with the earlier occurrence and higher incidence of SiGMV infection in commercial snap bean farms in 2021 and 2022. Despite differences in weather conditions prior to planting and during the fall growing seasons in 2021 and 2022, the results from this study consistently exhibited that the presence of SiGMV-infected sida appears to influence the spatial distribution and temporal patterns of SiGMV infection in snap beans. The results from our study implicate prickly sida plays as a natural reservoir and a source of SiGMV inoculum for snap beans between cropping seasons. It is therefore important to consider managing prickly sida prior to planting as a part of an integrated pest management program to reduce inoculum sources for the whitefly-transmitted SiGMV in snap bean farms.

Host plant resistance is considered the most cost-effective management tactic for whitefly-transmitted viruses (Lapidot et al. 2014; Simmons and Riley 2021). A recent field screening study reported some *Phaseolus* genotypes with moderate- to high-levels of resistance against SiGMV and another whitefly-transmitted virus, cucurbit leaf crumple virus (Agarwal et al. 2021). However, these *Phaseolus* genotypes need to be further evaluated for desirable agronomic traits before future deployment. In terms of managing prickly sida, growers manage weeds using herbicides within the farm prior to planting. Based on field experiments conducted in Stoneville, Mississippi, the herbicides that were found most effective in managing prickly sida were Roundup Max (Glyphosate; Monsanto, St. Louis, MO), Enlist Duo (2,4-D Choline+Glyphosate; Corteva, Indianapolis, IN), Aatrex (Atrazine; Syngenta, Wilmington, DE) tank-mixed with Capreno (Thiencarbazone+Tembotrione; Bayer, Pittsburgh, PA), and Aatrex tank-mixed with Corvus (Thiencarbazone+Isoxaflutole; Bayer, Pittsburgh, PA), which provided greater than 95% control of prickly sida (Bararpour et al. 2023). Growers also mow weeds, including prickly sida, around the non-cultivated farm margins regularly to clear driveways to allow access during farm operations. However, prickly sida growing under tree lines and along non-cultivated driveways tend to escape preplant herbicide applications and mowing.

Prickly sida is locally termed ‘iron weed’ because established plants have woody stems and do not easily get mowed by the mechanical weeder (Huff and Rhodes 2017). Based on field observations from this study, even if the prickly sida shoots are cut, re-sprouting often occurs from the crown if the roots remain intact. According to Mohler et al. (2021), prickly sida could be effectively managed through plowing as seedling emergence declines rapidly with greater depths. Prickly sida seeds buried deeper than 5 cm resulted in high seed mortality and reduced seedling emergence (Smith et al. 1992). The lack of tillage in non-cultivated farm margins may therefore contribute to the prevalence of prickly sida. In such cases, spot application of herbicides where patches of prickly sida grow may help in reducing its population and subsequently may aid in reducing potential inoculum source for SiGMV in snap bean farms. By effectively reducing sources of inoculum within the vicinity of the farm, through plowing or herbicide application to manage prickly sida growing along farm margins, the prevalence of SiGMV in snap beans may potentially be reduced.

Inoculum sources other than prickly sida growing within the farm margins, could also influence the prevalence and spatiotemporal patterns of SiGMV infection in snap bean farms. This can be inferred from the observed results, which showed that in some cases infection also occurred in macroplots on farm edges without prickly sida. The SiGMV-viruliferous whiteflies may also come from other sources such as those experimental weed hosts identified by Gautam et al. (2023) including hollyhock, marshmallow, and country mallow. Additionally, viruliferous whiteflies may also immigrate from snap bean farms that could be farther away or from other SiGMV crop hosts such as okra and tobacco (Gautam et al. 2023). Adult whiteflies disperse and may take on short or long-distance flights especially when the host plants are being disturbed such as during crop defoliation, mowing of weeds, and crop harvest (Kennedy and Storer 2000; Riley and Ciomperlik 1997). Thus, it should be recognized that managing prickly sida growing along farm margins is unlikely to be a ‘silver bullet’ but could potentially be a part of an integrated pest management program for managing SiGMV in snap bean farms.

In conclusion, SiGMV infection in prickly sida occurs naturally and is associated with the prevalence, spatial distribution, and temporal patterns of disease caused by SiGMV in snap bean farms. Prickly sida may be considered a significant source of inoculum for the whitefly-transmitted SiGMV following the previously reported criterion for the identification of significant inoculum sources for the thrips-transmitted tomato spotted wilt virus (Culbreath et al. 2003). Prickly sida has been reported as a host for whitefly and SiGMV and also been demonstrated as a suitable host that supports whitefly reproduction (Barman et al. 2022; Gautam et al. 2023). SiGMV acquisition and inoculation by adult whiteflies from infected prickly sida to snap beans as well as back-transmission of SiGMV from snap beans to prickly sida has been demonstrated (Gautam et al. 2023). Results from this study affirm that prickly sida were present at a time, which complements the disease cycle. This is supported by the fact that SiGMV infected prickly sida occurred within the vicinity of the farm and potentially served as a natural reservoir during and between snap bean growing seasons. Thus, prickly sida does fit the definition of a significant source of inoculum for SiGMV.

Acknowledgements

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Tables

Table 3.1. Description of commercial snap bean farms used as study sites in fall 2021 and 2022 in southern Georgia

Year	Farms[a]	Planting date	Farm size	Variety	Distance between macroplots[b]	Distance to SiGMV infected prickly sida[c]
2021	Farm 1	Sept. 7, 2021	35 ac	Prevail	302 m	7 m
2021	Farm 2	Aug. 23, 2021	28 ac	Prevail	232 m	10 m
2022	Farm 3	Sept. 8, 2022	30 ac	Prevail	329 m	8 m
2022	Farm 4	Sept. 3, 2022	31 ac	Prevail	475 m	12 m

[a] All four snap bean farms were located in Worth county in southern Georgia

[b] Distance (meters) between the macroplot with prickly sida to the macroplot without prickly sida

[c] Distance from macroplot to farm margin where SiGMV-infected prickly was found

Table 3.2. Natural occurrence of SiGMV-infected prickly sida in commercial snap bean farms during spring season in southern Georgia. 'Not found' indicates that prickly sida was not found along the farm margins. 'No sample' indicates that prickly sida plants showing symptoms of SiGMV infection were not found in the farm margins.

County	**Location**	**Prickly sida**	**Number of samples that tested positive for SiGMV**		**Symptoms**
			Asymptomatic samples	**Symptomatic samples**	
Worth	Hogan Rd	Present	0% (0 of 7)	100% (3 of 3)	Mosaic
Worth	Brown Cemetery Rd	Present	0% (0 of 9)	0% (0 of 1)	Mosaic
Worth	Sumner Rd	Present	0% (0 of 8)	100% (2 of 2)	Mosaic
Worth	Hogan Rd	Present	0% (0 of 5)	60% (3 of 5)	Mosaic, stunting
Worth	Cotton Rd	Present	0% (0 of 10)	No sample	No sample
Worth	Cotton Rd	Present	0% (0 of 5)	100% (5 of 5)	Mosaic, stunting
Tift	Hwy 82	Present	0% (0 of 5)	100% (5 of 5)	Mosaic
Tift	Coarsey Lane	Present	0% (0 of 9)	100% (1 of 1)	Mosaic
Tift	Hobbs Walker Rd	Present	0% (0 of 10)	No sample	No sample
Tift	Upper Ty Ty Rd	Not found	Not found	Not found	Not found
Tift	Little River Rd	Present	0% (0 of 10)	No sample	No sample
Tift	Lower Brookfield	Present	0% (0 of 10)	No sample	No sample
Sumter	Americus Dawson Rd	Present	33% (2 of 6)	100% (4 of 4)	Mosaic
Sumter	District Line Rd	Present	0% (0 of 10)	No sample	No sample
Sumter	US Hwy 280	Present	0% (0 of 10)	No sample	No sample
Sumter	Boo Cosby Rd	Present	10% (1 of 10)	No sample	No sample
Sumter	Ellis Rd	Present	0% (0 of 10)	No sample	No sample
Percent occurrence of prickly sida[a]		**94%**	**2.24%[c] (3 of 134)**	**88.46%[c] (23 of 26)**	
Percent occurrence of SiGMV[b]		**47%**			

[a] Percentage of farms surveyed (*n*= 17) with prickly sida

[b] Percentage of farms surveyed with SiGMV-infected prickly sida based on result of PCR assay

[c] Percentage of samples where SiGMV was detected through PCR assay

Figures

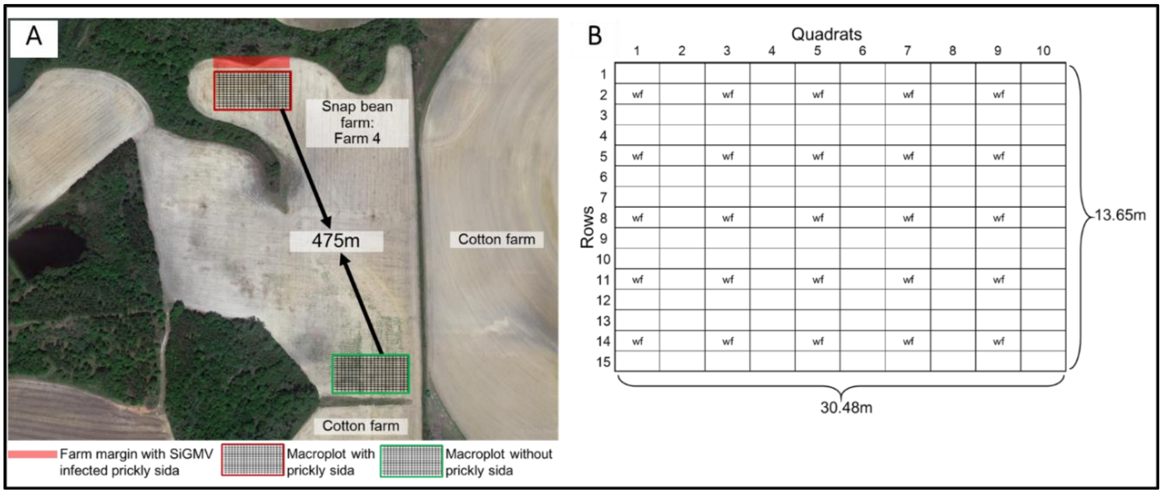


Figure 3.1. Experimental design for assessing the spatial and temporal patterns of whitefly and SiGMV in commercial snap bean farms. Two macroplots were set up; one was adjacent to farm margin where SiGMV-infected prickly sida plants were detected (indicated by red bar) and one where prickly sida was not found (A). Each macroplot was composed of 15 rows of snap beans (13.7 m wide) that were 30.5 m long (B). Each cell represents a 0.9 m x 3.1 m quadrat. SiGMV incidence ratings were taken in each quadrat. Cells with ‘wf’ indicate quadrats where whitefly counts were taken.

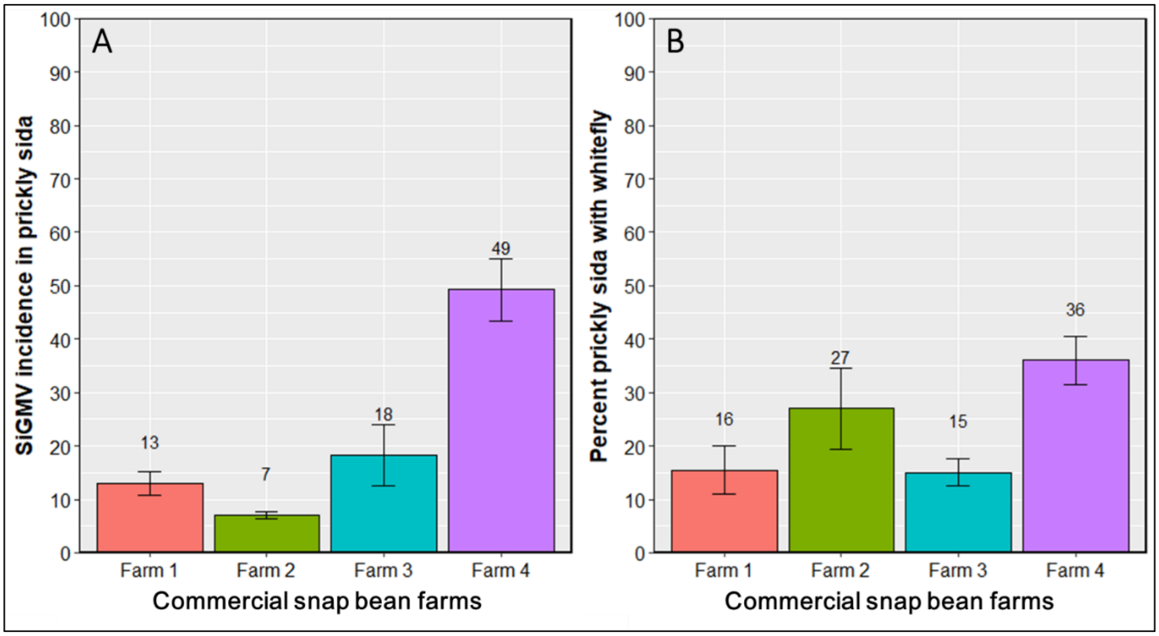


Figure 3.2. Mean ± standard errors of percent prickly sida with adult whitefly (A) and SiGMV symptoms (B) along the margins of four snap bean farms. The sample size was 60 arbitrarily selected prickly sida plants per farm.

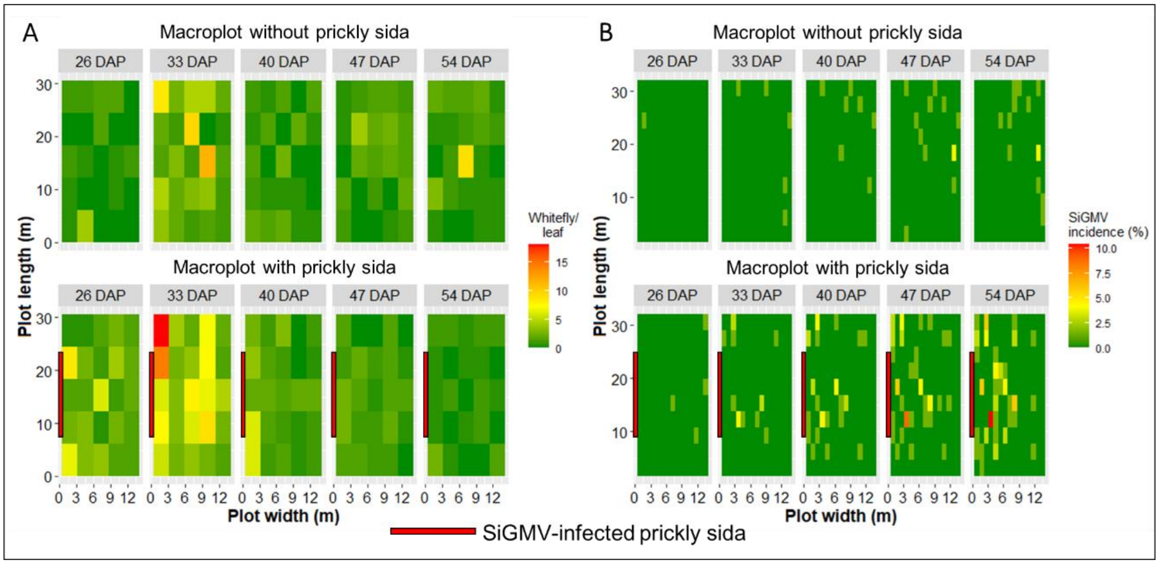


Figure 3.3. Heatmaps of adult whitefly counts on snap bean leaves (A), and SiGMV incidences (B) over space and time in macroplots with or without prickly sida in Farm 4. Red bars indicate farm margin with SiGMV-infected prickly sida. Areas within the macroplots with lower numbers of adult whiteflies and SiGMV incidences were indicated by green color. Dark red areas had higher numbers of adult whitefly and SiGMV incidence.

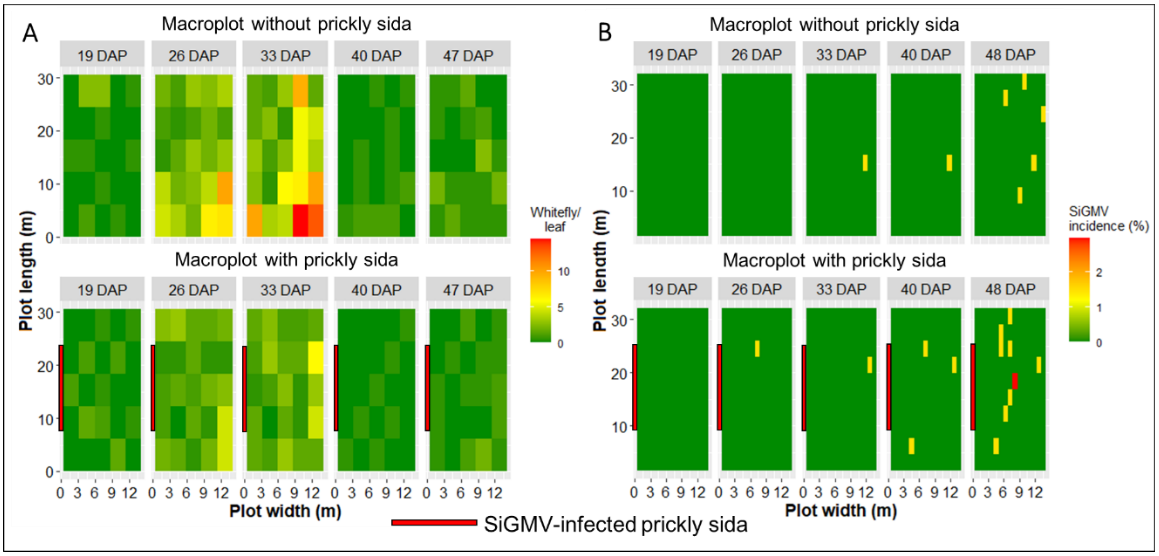


Figure 3.4. Heatmaps of adult whitefly counts on snap bean leaves (A), and SiGMV incidences (B) over space and time in macroplots with or without prickly sida in Farm 3. Red bars indicate farm margin with SiGMV-infected prickly sida. Areas within the macroplots with lower numbers of adult whiteflies and SiGMV incidences were indicated by green color. Dark red areas had higher numbers of adult whitefly and SiGMV incidence.

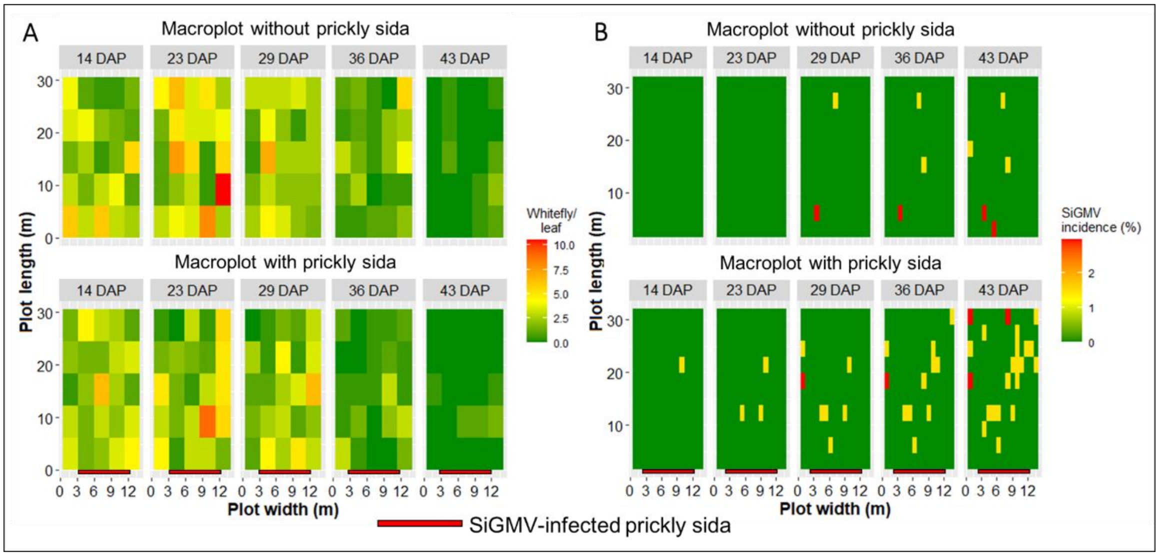


Figure 3.5. Heatmaps of adult whitefly counts on snap bean leaves (A), and SiGMV incidences (B) over space and time in macroplots with or without prickly sida in Farm 1. Red bars indicate farm margin with SiGMV-infected prickly sida. Areas within the macroplots with lower numbers of adult whiteflies and SiGMV incidences were indicated by green color. Dark red areas had higher numbers of adult whitefly and SiGMV incidence.

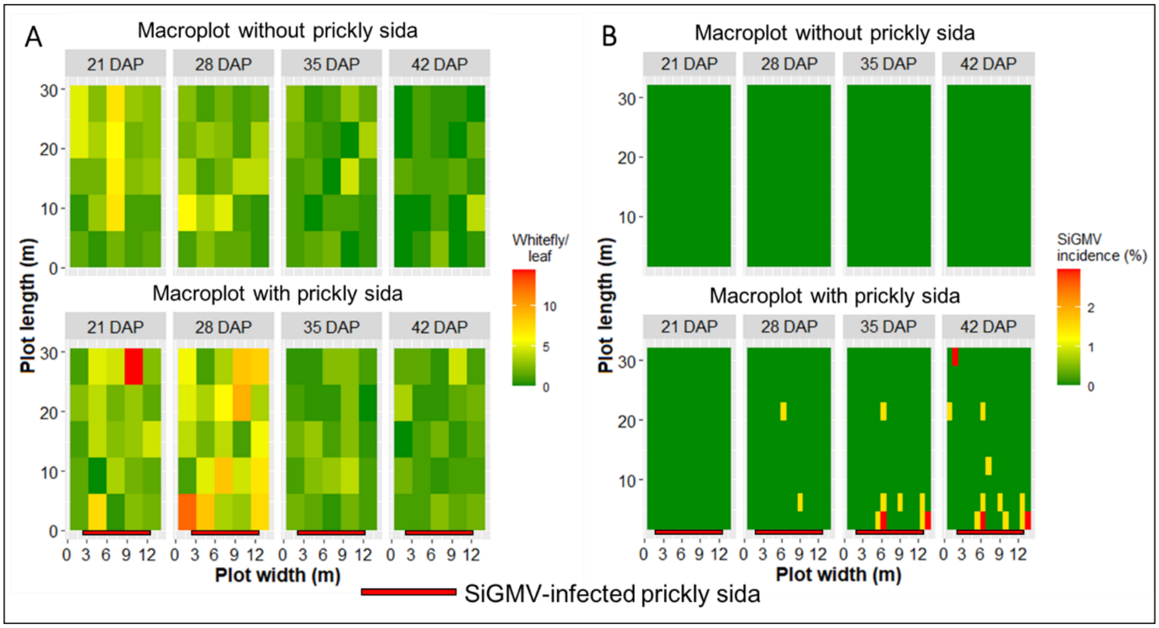


Figure 3.6. Heatmaps of adult whitefly counts on snap bean leaves (A), and SiGMV incidences (B) over space and time in macroplots with or without prickly sida in Farm 2. Red bars indicate farm margin with SiGMV-infected prickly sida. Areas within the macroplots with lower numbers of adult whiteflies and SiGMV incidences were indicated by green color. Dark red areas had higher numbers of adult whitefly and SiGMV incidence.

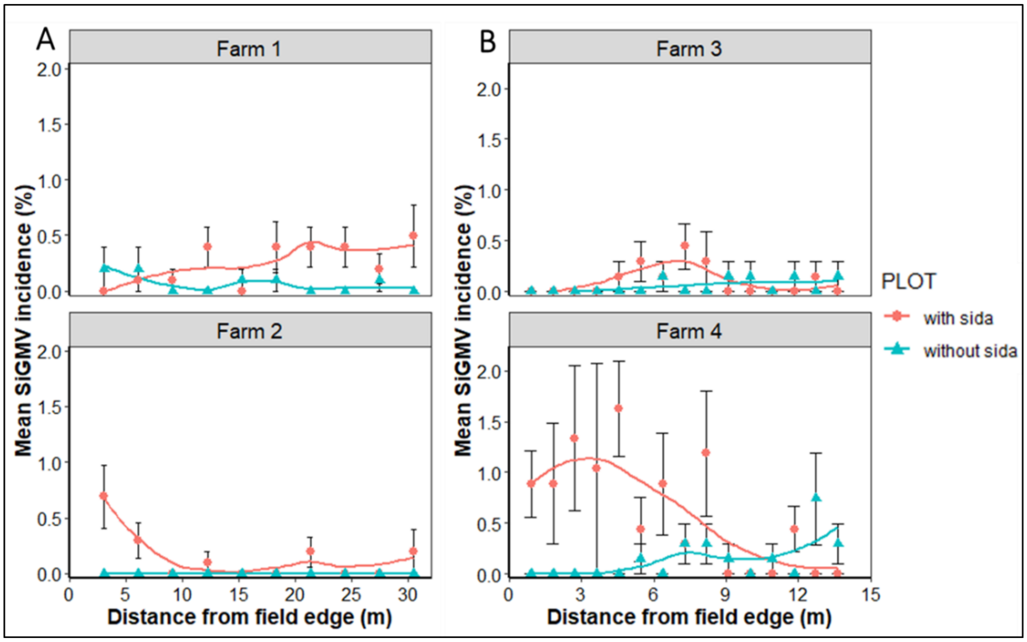


Figure 3.7. Mean and standard errors of SiGMV incidences across different distances from farm edge with or without prickly sida in four study sites. Distances from farm edge (x-axis) vary depending on the orientation of the rows relative to the farm margin with prickly sida. In Farm 1 and Farm 2, where the rows of snap beans were perpendicular to the farm margin, the distance ranged from 3-30m. In Farms 3 and 4, where the rows of snap beans were parallel to the farm margin, the distance from farm edge ranged from 1-14m.

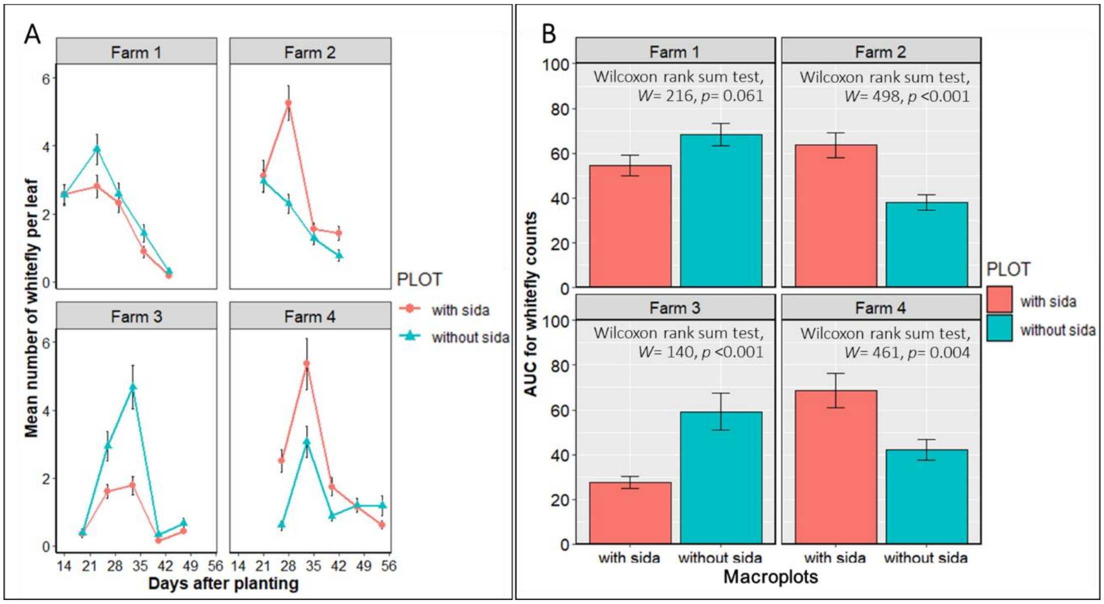


Figure 3.8. Mean and standard errors of adult whitefly count over time (A) and area under the curves for whitefly counts (B) in snap bean macroplots with or without prickly sida. *P*-values <0.05 from Wilcoxon rank sum test indicate significant difference in AUC values between the two macroplots.

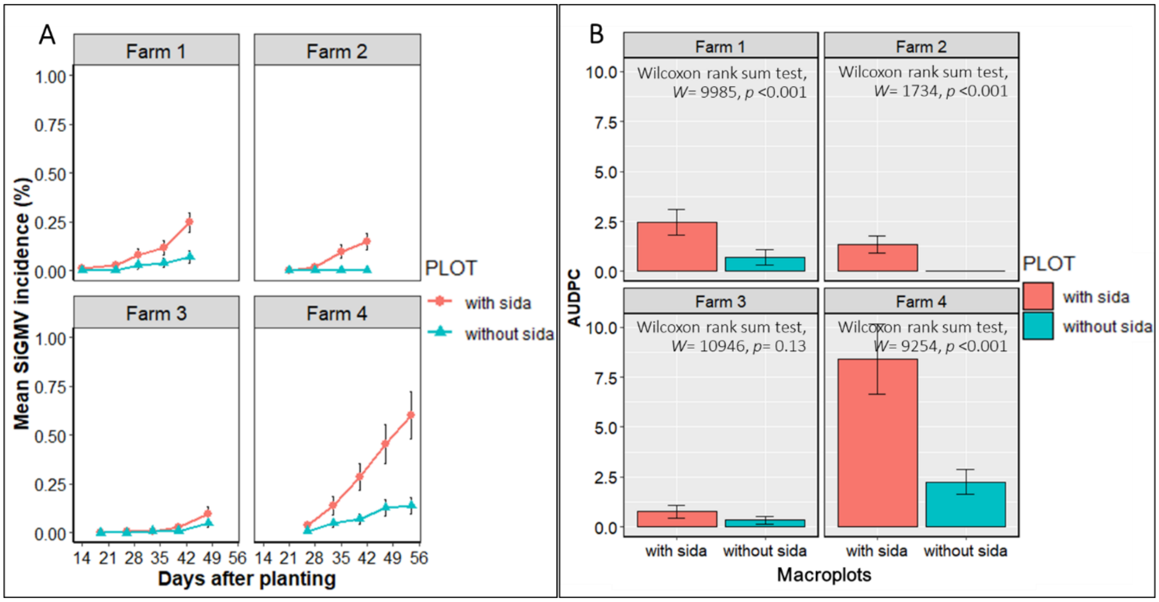
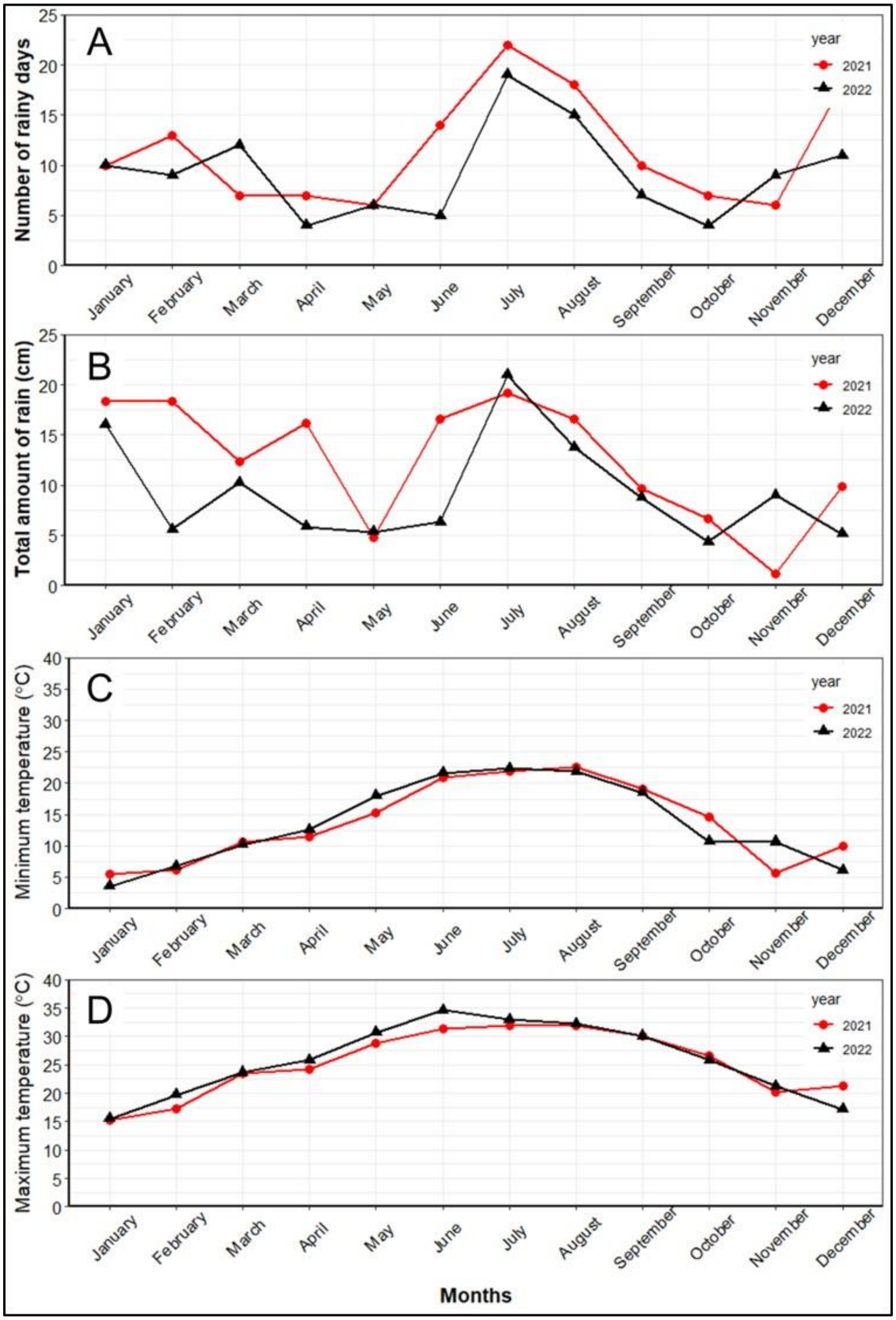


Figure 3.9. Mean and standard errors of SiGMV incidences over time (A) and area under the disease progress curves (B) in snap bean macroplots with or without prickly sida. *P*-value <0.05 from Wilcoxon rank sum test indicate significant difference in AUDPC values between the two macroplots.

Supplementary Figures



Supplementary Figure S3.1. Summary of weather conditions in Worth county (location of snap bean farms) in 2021 and 2022. The number of rainy days (A) and total amount of rain (B) were calculated per month. The minimum (C) and maximum (D) temperatures represent the mean of daily temperatures per month. Red lines with circle points represent weather data for 2021. Black lines with triangle points represent weather data for 2022.

CHAPTER 4

UTILIZING RANDOM FOREST ANALYSIS TO DEVELOP A RISK ASSESSMENT INDEX FOR WHITEFLY-TRANSMITTED VIRUSES AFFECTING FALL-GROWN SQUASH IN SOUTHERN GEORGIA[1]

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Abstract

Managing viruses transmitted by the sweetpotato whitefly (*Bemisia tabaci* Gennadius) is challenging because no single tactic provides adequate and consistent efficacy against these pathogens. The whitefly-transmitted viruses commonly occur as a mixed infection in squash plants as a result they have been collectively termed as "whitefly-transmitted virus complex" (WTVC) in this paper. While some tactics for managing WTVC are available in squash, which sometime are used in combination; however, relative impact of each management tactic in reducing disease severity is not known. Utilizing multiple field studies across three years (2020-2022), we determined the relative impact of different management tactics on WTVC in squash. Based on random forest analysis, important management tactics were planting date, and row cover followed by the type of squash, planting material, and plastic-mulch type. Further, utilizing the relative impact of each management tactic a risk assessment index was developed against WTVC. Spearman correlation analysis indicated a strong positive correlation between the cumulative risk point values and the standardized AUDPC values in both training and testing data sets. The risk index was validated through data collected in commercial squash fields. Findings from this study demonstrated the use of random forest analysis for determining relative impact of WTVC management tactics and utilizing it for the development of a risk index.

Introduction

The sweetpotato whitefly (*Bemisia tabaci* Gennadius) and the viruses transmitted by it have emerged as a global threat to the production of a wide range of crops over the last 20 years (Chen et al. 2004; Navas-Castillo et al. 2011). The whitefly-transmitted viruses affecting summer squash (*Cucurbita pepo*) in Georgia include the cucurbit leaf crumple virus (CuLCrV), cucurbit yellow stunting disorder virus (CYSDV), and cucurbit chlorotic yellows virus (CCYV) (Gadhave et al. 2018; Kavalappara et al. 2021; Srinivasan et al. 2020). The diseases caused by the aforementioned viruses were collectively termed as the “whitefly-transmitted virus complex” (WTVC) in this study as they usually occur as mixed infections in squash fields (Codod et al. 2022; Gautam et al. 2020; Kavalappara et al. 2021).

During the spring season in Georgia, the WTVC is not a concern in squash crops (*Cucurbita pepo*), such as yellow squash and zucchini as the whitefly populations are generally low during that period (Candian et al. 2021). However, during the fall growing season, especially in a dry and warm year, whitefly populations can reach an overwhelming level resulting in subsequent increased transmission and prevalence of WTVC. Such situations can lead to significant yield losses in squash. In fall 2017, an estimated 35% reduction in squash crop value amounting to $38 million occurred due to diseases caused by whitefly-transmitted viruses (Little et al. 2019) in Georgia. In another report, a squash grower reported up to 76.8% in yield reduction in 2017 compared to 2015 (Hale 2018) in Georgia. These examples demonstrate the threat and seriousness of whitefly and WTVC in growing squash in areas where warm and dry conditions occur such as in southern Georgia.

Management of WTVC in squash is challenging as host resistance to CuLCrV, CCYV, and CYSDV in yellow squash and zucchini cultivars is not available (Candian et al. 2021). Tactics for managing whiteflies and the viruses they transmit in squash include the use of UV-reflective mulch, insect-proof row cover, and insecticides; however, the benefits from these tactics have been limited to moderate in reducing WTVC severity (Frank and Liburd 2005; La Tora et al. 2022; Natwick and Durazo 1985; Nyoike and Liburd 2010). Moreover, there is currently no information on whether one tactic impacts WTVC severity more than the others and how this information could be used to create an integrated pest management tool for growers.

Growers currently rely on insecticide programs to manage whitefly populations (LaTora et al. 2022; Luckew et al. 2022). Through effective management of whiteflies, growers indirectly manage WTVC by reducing the number of whiteflies that may spread the WTVC. While several insecticides are effective against whiteflies, most are active on immature stages with limited efficacy on adults (Sparks et al. 2018). Furthermore, management with foliar applied insecticide is challenging as adults and immatures frequently escape contact as they infest and feed on the underside of leaves (Palumbo and Coates 1996). Development of resistance to commonly used insecticides can also be an issue (McAuslane and Smith 2018). Optimal conditions for establishment of high whitefly populations during the fall growing season could also make insecticide-based management inadequate. Hence, implementation of an integrated pest management program by combined application of tactics in addition to using insecticides should be considered.

The occurrence of WTVC impacting yellow squash production was not the first insect- transmitted pathogen to become a major concern in crop production in the southeastern U.S. The establishment of tomato spotted wilt virus (TSWV) transmitted by thrips (*Frankliniella occidentalis* and *F. fusca*) has occurred throughout the southeastern peanut growing region since the late 1980s and has caused significant yield losses in peanuts (Bertrand 1998; Culbreath and Srinivasan 2011). No single disease management tactic provided adequate and consistent management of TSWV in peanuts. However, integration of multiple pest management tactics resulted in effective management and reduction (Culbreath and Srinivasan 2011). The development and use of the spotted wilt risk index (Brown et al. 2005), now refined as ‘PEANUT Rx’ (Kemerait et al. 2013) has enhanced the adoption of genetic, chemical, and cultural practices for management of TSWV in peanuts. The PEANUT Rx considers various production practices and pest and disease management inputs as risk-mitigation factors, which influence TSWV risk (Brown et al. 2005; Chappell et al. 2020; Culbreath and Srinivasan 2011). Index values for PEANUT Rx were based on results from numerous field studies, which estimates the average reduction in TSWV incidence associated with each risk mitigation practice (e.g., peanut variety, tillage, planting date, plant density and row spacing, herbicide and insecticide use) (Brown et al. 2005; Culbreath et al. 2013; Hurt et al. 2006; Marois and Wright 2003). For any given suite of risk mitigation practices that a grower plans to implement, the sum of risk point values associated with each practice is used to obtain an overall estimate of risk. PEANUT Rx provided a method by which growers can assess the relative risk of TSWV in a particular peanut field based on the overall production practices employed. Assessment of risk also allowed growers to identify and adjust the combination of TSWV-suppressive factors that best apply to their situation (Brown et al. 2005). The wide adoption of PEANUT Rx by peanut growers has contributed to the area-wide management of TSWV in the southeastern US.

The concept of using a risk index to facilitate the implementation of integrated pest management (IPM) practices of an insect-transmitted virus has also been developed for the management of the whitefly-transmitted tomato yellow leaf curl virus (TYLCV) in tomatoes (https://site.caes.uga.edu/whiteflies-tylcv/risk-index/) by the University of Georgia College of Agricultural and Environmental Sciences (n.d.). The same concept could also be applied to the WTVC in squash. While the pathosystems are different in terms of insect-vectors and hosts, there are similarities between the thrips-transmitted TSWV and whitefly-transmitted TYLCV and WTVC. Each pathosystem requires the presence of insect vectors (whitefly for WTVC and TYLCV, and thrips for TSWV) for the spread of the pathogen in the natural environment (Navas-Castillo et al. 2011; Riley et al. 2011; Ullman et al. 1997). The WTVC and TSWV pathosystems are also similar in terms of the seasonality of the vectors. In Georgia, thrips populations gradually increase until they reach their highest peak in the month of May, after which populations drop drastically (Wells et al. 2002). Similarly, whitefly populations in southern Georgia gradually increase and reach their peak around mid-September, and then reduce drastically in October (Barman et al. 2019; Candian et al. 2021; Srinivasan et al. 2012). With the similarities between WTVC, TYLCV, and TSWV pathosystems, the use of an integrated pest management tactic with the aid of a risk index might also be effective for the management of the WTVC in squash.

Both the PEANUT Rx and the risk index for TYLCV in tomatoes were developed through an ‘expert system’. An ‘expert system’ is where risk values were manually assigned to each risk mitigation practice depending on their relative effect on disease intensity as observed in numerous field studies (Brown et al. 2005; University of Georgia

College of Agricultural and Environmental Sciences, n.d.). However, lack of information on the relative effect of risk mitigation tactics, and whether one tactic has a greater influence on WTVC than others may cause bias in the manual assignment of risk point values. Utilization of methods for estimating the relative effect of each risk mitigation tactic, such as random forest, may be useful for estimating risk point values that reflect the relative influence of risk mitigation tactics on disease intensity based on available data.

Random forest is a machine learning algorithm, which combines the results from multiple decision trees into a single output (Breiman 2001; Cutler et al. 2012). Random forest can be used for either a categorical or a continuous response variable (Breiman 2001; Cutler et al. 2012; Naghibi et al. 2016). It is known for its robust performance across a wide range of data sets, high prediction accuracy, and ability to avoid overfitting (Jing et al. 2015; Wang et al. 2019). Random forest is a popular method for estimating the importance of predictor variables. It is based on the levels of accuracy in prediction that are impacted when a certain variable is removed in the model (Cutler et al. 2007; Fernandez-Delgado et al. 2014; Yahsi et al. 2019). Such measure of variable importance may be utilized as a basis for estimating risk point values in developing a risk assessment index similar to the PEANUT Rx.

Hence, in this manuscript we estimated the relative influence of different tactics on the standardized area under disease progress curve values for the WTVC in squash through random forest. Later, we also developed a risk assessment index based upon the relative influence of different tactics and validated it in commercial squash production fields in southern Georgia.

Materials and methods

Data generation from field trials

Multiple field trials (*n*= 14) assessing different tactics for managing WTVC in squash were conducted in southern Georgia during the fall growing seasons from 2020 to 2022 (Table 4.1). The production practices and pest management tactics investigated included mulch types, planting dates, row covers, insecticides, planting materials, and types of squash. Field trials that assessed the effect of a single tactic were arranged in a randomized complete block design. Field trials assessing two to three tactics were arranged in a split-plot and split-split plot design, respectively. Squash was planted on raised beds at 0.3 m spacing between plants. The length of rows ranged from 4.6 to 6.1 m, except for the field trial conducted in a commercial field where length of rows was 30.5 m. The squash varieties used in these field trials included ‘Delta’, ‘Gold Prize’, ‘Gold Star’, ‘Lioness’, ‘Paycheck’, and ‘Payload’; none of them had resistance against CCYV or CuLCrV, or CYSDV.

Visual assessment of WTVC was based on symptom expression in field trials. Each plant within the sampling plot was scored as “0” or “1” if it was asymptomatic or “1” if it was symptomatic to WTVC. Symptoms caused by the WTVC included leaf crumpling, stunting, chlorosis, and occurrence of green streaks in the fruits. The percentage incidence of WTVC was calculated by dividing the number of symptomatic plants by the total number of plants per plot then multiplying by 100. The WTVC incidences were assessed at a weekly interval for four to six times starting at 14 to 21 days after planting until the termination of the field trials.

The incidences of WTVC over time were used to calculate the area under disease progress curves (AUDPC) through the ‘audpc’ function in the ‘agricolae’ package in R (de Mendiburu 2019). The AUDPC values were calculated for each replicated plots for each treatment in each field trial and were standardized by dividing the AUDPC over the number of days between the initial and the final rating. The standardized values of AUDPCs were termed as ‘standardized AUDPC’ (sAUDPC) in this paper.

Standardized AUDPC values from the field trials were compiled into a dataset of 1000 observations. The risk mitigation tactics evaluated include planting date, mulch type, row cover usage, type of squash, and planting material. These tactics were used as predictors for sAUDPC values (Table 4.2).

Four categories of planting dates were created relative to when the peak of whitefly population occurs in southern Georgia. Based upon previous reports and field observations, the peak of whitefly population occurs towards the end of August to mid-September (Barman et al. 2019; Srinivasan et al. 2012). Thus, planting dates were categorized as “before August 11”, “August 11 to 25”, “August 26 to September 20”, and “after September 20”. These time periods represented periods before the population peak, period leading to the peak, period at peak population level, and period after the peak population level has already passed/occurred. The categories for planting dates were based upon current climatic and temporal patterns of whitefly populations. This categorization could be shifted if future conditions deviate from the conditions under which the data for this risk index was collected.

The other predictor variables were also categorical. The types of squash included in this study were zucchini (*C. pepo* var. *cylindrica*) and yellow squash (*C. pepo* var.

retocollis). The types of plastic mulch were either white plastic, or UV-reflective plastic. In some cases as a common practice in southern Georgia, black plastic mulch is often painted with white paint, which was also considered as white plastic mulch in this manuscript. Row cover treatments were either with or without cover. The row covers used were made of lightweight polypropylene fabrics with a mesh size (0.35 mm^2) small enough to prevent whiteflies and other insects from getting access to the crop without excluding light, air, and rainfall (Agribon ®; Johnny's Selected Seeds, Winslow, ME). The row covers were placed either directly on top of the plants or over wire support hoops (Johnny's Selected Seeds, Winslow, ME) to form tunnels that enclosed the squash plants. Row covers were placed immediately after planting and removed at-flowering, approximately at three-weeks after planting. Planting materials were either seeds or seedlings.

Detection of CuLCrV, CYSDV and CCYV single or mixed infection

Leaf samples (*n*= 54) from squash plants displaying symptoms of WTVC infection were collected from the field trials and tested through virus-specific PCR assays to confirm the presence of CuLCrV or CYSDV, or CCYV single infection or mixed infection with either two or three viruses. DNA and RNA were extracted using the DNeasy® Plant Mini Kit and RNeasy® Plant Mini Kit (QIAGEN, Germantown, Maryland), respectively, following the manufacturer's protocol. CuLCrV infection was tested using primers 3FAC3 and 5RAC1 targeting the 525 bp common region of AC3, AC2 and AC1 genes of CuLCrV DNA-A component (Gadhave et al. 2020). PCR was performed in a thermocycler (5 min at 94 °C followed by 40 cycles of 94 °C for 1 min, 54 °C for 30 s, 72 °C for 1 min, and a final extension of 72 °C for 10 min) and the amplified products were visualized using agarose gel (1%) electrophoresis.

RNA extracted from the samples were subjected to cDNA synthesis using iScript^{TM} cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA). CYSDV infection was tested using primers, CYSCPf and CYSCPr, which targeted a 755 bp of the coat protein gene (Rubio et al. 2001). The cDNA was amplified through PCR using a previously published protocol (5 min at 94 °C followed by 40 cycles at 94 °C for 30 s, 50 °C for 45 s, 72 °C for 2 min, and a final extension at 72 °C for 5 min) (Gautam et al. 2020).

The presence of CCYV was tested using primers, CCYV-RDRP-1515F and CCYV-RDRP-1515R, targeting a 953-nt segment of CCYV RNA1 (Kavalappara et al. 2021). The PCR conditions for CCYV involved initial denaturation at 95 °C for 2 min, followed by 35 cycles each of 95 °C for 30 s, 62 °C for 30 s, 72 °C for 1 min, and final extension of 72 °C for 5 min (Kavalappara et al. 2021).

Estimating the relative influence of risk mitigation tactics on WTVC

The data set of sAUDPC values and predictor variables (*n*= 1000) was split into a training (70% of the data set) and testing (30% of the data set) data sets. The ‘set.seed’ function in R was used when splitting the data so that all combinations of the five predictor variables present in the training set were also present in the testing set. The training data set was used for developing the risk index. The testing data set was used exclusively for testing the risk index developed based on the training data set.

A random forest analysis was performed on the training data set to determine the relative influence of each risk mitigation tactic as predictor of sAUDPC. The predictor variables used in the analysis were planting date, mulch type, row cover, type of squash, and planting material (Table 4.2). Data were analyzed with the “randomForest” package in R (Liaw and Wiener 2002). The random forest analysis was performed by creating 1,000 trees (ntree), each with three variables (mtry). The ‘percentage increase in mean square error’ (%IncMSE) values were calculated using the ‘importance’ function in the “randomForest” package. The %IncMSE is a measure of the relative influence of variables as predictors of a response variable. Predictor variables with larger %IncMSE values account for more variation on the response variable (Cutler et al. 2007; Yahsi et al. 2019). The %IncMSE values were summed for all predictor variables. The percentage influence of each risk mitigation tactic as predictor of sAUDPC was then calculated by dividing the %IncMSE value over the total for all variables followed by multiplying with 100 (Table 4.3).

Development of a risk assessment index for WTVC

(i) Calculation of maximum risk point value per variable.

The percent influence in the random forest model for each predictor variable was used as a basis for estimating and weighing the ‘maximum risk point value for the risk index’. The ‘maximum risk point value for the risk index’ was defined as the maximum value possible when the risk points associated with the five predictor variables in the risk index were summed. Two conditions were set for estimating the maximum risk point value for the risk index. The first condition was to use a number that is a multiple of five to simplify the calculation of total risk point values (Brown et al. 2005). The second condition entailed that when the value was multiplied by the percent influence of the least important predictor variable, the product should be no less than ten. In doing so, the categories under the previously mentioned predictor variable associated with higher sAUDPC value had a risk value of at least ten points while the other category had five points. After determining the maximum risk point value for the risk index, it was multiplied with the percent importance of each predictor variable and then rounded to the nearest multiple of five to estimate the maximum risk point for each predictor variable.

(ii) Calculation of risk point values for each category under each variable. The category that had the highest mean sAUDPC value was assigned the maximum risk point value possible for that predictor variable. The risk point values for the other categories were estimated using the formula: Risk point value for category “A” of variable “X” = (mean sAUDPC for category A/ mean sAUDPC for category with highest risk value in variable X) * maximum risk point value for variable X. ‘X’ can be any of the five predictor variables (Table 4.2). For example, as the sAUDPC value was highest for ‘without row cover’ category, it was assigned with the maximum risk point value for the predictor variable ‘row cover’. The risk point value for ‘with row cover’ category was estimated by dividing the mean sAUDPC for ‘with row cover’ over the mean sAUDPC for ‘without row cover’ and then multiplied with the maximum risk point value for row covers. The risk point values were rounded to the nearest multiple of five.

(iii) Defining risk categories. For each combination of the five predictor variables, the risk point values were summed. The summed risk point values were categorized into three levels of risks: ‘low-risk’ or ‘moderate-risk’, or ‘high-risk’. Risk levels were categorized following the method used for defining risk levels in ‘PEANUT Rx’ with some modifications (Brown et al. 2005). The range for a low-risk level was defined as unattainable when yellow squash is used except when planted on low-risk planting dates (before 11th Aug or after 20th Sept) and low-risk categories were chosen in the other predictor variables. The range for a high-risk level is defined as unattainable when yellow squash is planted on low-risk planting dates (before 11th Aug or after 20th Sept) except if high-risk categories were chosen in all other predictor variables. Moderate-risk consists of all combinations of the five predictor variables that resulted in risk point values between the low- and the high-risk categories.

Testing the risk index on training and testing data sets

The cumulative risk point values were calculated by calculating the sum of the risk point values associated with the type of squash, planting date, mulch type, use of row cover, and planting material. A Spearman correlation analysis was performed between the cumulative risk point values and the sAUDPC values in the training data set. A scatterplot was generated using the ‘ggscatter’ package in R (Kassambara 2020). To visualize the trend of correlation between the cumulative risk point values and sAUDPC a regression line was added in the scatter plot through the ‘reg.line’ function. Similarly, a spearman correlation analysis was also performed, and a scatter plot was generated to visualize the correlation between the cumulative risk point values and the sAUDPC in the testing data set.

Correlating yield with risk point values

The correlation between yield and cumulative risk point values was estimated using yield data taken from a three-factorial study conducted in the 2021 and 2022 fall growing seasons. The study had three planting dates, two types of mulch, and with or without row cover. The planting dates were 1st week of August, 3rd week of August, and 2nd week of September. The types of mulch used were UV-reflective and white plastic mulch. There were 12 different combinations of planting date, mulch type, and row cover treatments.

The yield was measured by harvesting and classifying yellow squash fruits as marketable or non-marketable. Fruits were considered non-marketable when they exhibited virus symptoms, such as green streaking and wrinkling, or distorted shape due to poor pollination or other causes. Fruits were harvested at a two to three-day interval. Fruits exhibiting symptoms of WTVC infection and severely distorted shape were considered non-marketable. Symptoms of WTVC infection on fruits includes green streaking and wrinkling. The number of marketable fruits were recorded during each harvesting time per plot. The sum of marketable fruits over four harvest times were calculated. A Spearman correlation analysis was performed to test whether the number of marketable fruits correlated with the risk point values.

Risk index validation in commercial squash fields

Twenty-two commercial squash fields were surveyed during the fall growing season in 2022. A stratified sampling method was used in assessing WTVC in commercial fields. Each field was divided into nine strata. Within each stratum, two rows were selected arbitrarily and visually assessed for presence or absence of WTVC symptoms. Fifty continuous plants per row were scored for presence or absence of WTVC symptoms. One rating per field was conducted between 35 to 45 days after planting. Information on the type of squash, planting date, type of mulch used, use of row cover, and planting material used in each field were recorded. Because only one WTVC incidence rating was taken in the fields surveyed, the WTVC incidences were used for validating the risk index. The mean WTVC incidence per field was calculated by taking the sum of WTVC incidences among all 18 rows, divided by 18, and then multiplied by 100. A Spearman correlation analysis was performed to test whether the WTVC incidences correlated with the cumulative risk point values in the commercial squash fields.

Results

Detection of CuLCrV, CCYV, and CYSDV single or mixed infection

Among samples displaying symptoms of WTVC infection, 98.2% were positive for CuLCrV, 53.7% for CCYV, and 61.1% for CYSDV. Of the 54 samples, 44.5% had mixed infection (with two viruses in different combinations or three viruses). Specifically, 18.5% were positive for both CuLCrV and CCYV but were negative for CYSDV, while 25.9% were positive for CuLCrV and CYSDV but were negative for CCYV. As 98.2% of the samples were CuLCrV positive, the percentage of samples that were infected by both CCYV and CYSDV but negative to CuLCrV could not be determined. Mixed infection of all three viruses (CuLCrV, CCYV, and CYSDV) was detected in 33.3% of the samples collected from field trials. Overall, mixed infection by two (44.5%) or three (33.3%) of the whitefly transmitted viruses was typical and was detected in 77.8% of the samples tested in this study.

Estimating the relative influence of risk mitigation tactics on WTVC

Planting date had the greatest relative influence (39.7%) on the sAUDPC values of WTVC among the five risk mitigation tactics included in this study (Table 3). The use of row cover was the second most influential risk mitigation tactic (29.9%) followed by type of squash (12.7%), planting material (10.8%), and plastic-mulch type (7.0%).

Development of a risk assessment index for WTVC

<u>(i) Calculation of maximum risk point value per variable.</u> The maximum risk point value for the risk index was set at 150 points (Table 4.4). The maximum risk point values for the predictor variables were obtained by multiplying the percent influence by the 150 and rounding to the nearest multiple of five [planting date (60), use of row cover (45), type of squash (20), planting material (15), and plastic-mulch type (10)] (Tables 4.3 and 4.4).

<u>(ii) Calculation of risk point values for each category under each variable</u>

Planting date. Planting squash before or after the period when the peak of whitefly populations typically occurs contributed to reducing risk to WTVC. Squash planted before (before 11th of August) or after (after 20th September) the period when peak whitefly population occurs had lower sAUDPC values and risk point values (5 for the former and 30 for the later) (Table 4.4). Planting squash during the period immediately prior to the peak for whitefly population, 11th to 25th August, and during the period when the peak population occurs, 26th August to 20th September, resulted in higher WTVC incidences. Higher risk values of 55 and 65 points were therefore associated with these planting dates (Table 4.4).

Row cover. The use of row covers resulted in lower mean sAUDPC values compared to squash plots that were without row covers (Table 4.4). Consequently, the risk point value of 45 was assigned for the variable where squash were grown without row covers and 10 points were assigned for squash plants that were grown with row covers.

Type of squash. Among the two types of squash included in the risk index, yellow squash was more susceptible to WTVC. Yellow squash had a mean sAUDPC value of 26.4 and the assigned risk point value was 20 points (Table 4.4). Zucchini squash had a mean sAUDPC value of 16.6 with an assigned a risk point value of 15 points.

Planting material. The sAUDPC values for the type of planting materials used differed. Plots where seedlings were used as planting material (sAUDPC=21.7) had a lower sAUDPC value compared to plots where seeds were used as a planting material (sAUDPC=26.5) (Table 4.4). Hence, the risk values for the two types of planting materials were assigned as 10 points for seedlings and 15 points for seeds.

Plastic-mulch type. Plots that had UV-reflective plastic had a lower mean sAUDPC value (17.6) compared to plots with had white plastic (27.6) (Table 4.4). The risk point value for UV-reflective plastic mulch was assigned as 5 points while white plastic mulch with 10 points.

(iii) Defining risk categories. The risk to WTVC was categorized as low, moderate, or high (Table 4.4, Fig. 4.1). Low-risk was defined as combinations of planting date, squash type, planting material, row cover, and mulch type that results to a cumulative risk point value of 75 or lower (Table 4.4). Cumulative risk point values between 80 to 115 were categorized as moderate-risk. Combinations of the predictor variables that lead to risk point values greater than 115 were categorized as high-risk.

Testing the risk index on training and testing data sets

A significant positive correlation (r= 0.58, p< .001) between risk point values and the sAUDPC values was detected in the training dataset from which the risk index was developed (Fig. 4.2A). Consistent with result observed in the training data, there was a significant positive correlation (r= 0.58, p< .001) between risk point values and the sAUDPC values in the testing data set, which was a subset of the dataset that was used only for testing the risk index (Fig. 4.2B). Plots assigned as lower-risk levels had low sAUDPC values in both the training and testing data sets. The sAUDPC values were variable in the moderate and high-risk categories. However, sAUDPC values were generally higher on the high- than the moderate-risk situations.

Correlating yield with risk point values

A significant negative correlation between the number of marketable fruits and risk point values was detected (Fig. 4.3). Higher numbers of marketable fruits were harvested among low-risk plots in general. In some of the high-risk treatments, the squash plants were not able to produce any marketable fruits as was observed in 2022.

Risk index validation in commercial squash fields

Among the commercial fields surveyed, differences in planting dates, mulch type, and squash type created different risk levels for WTVC (Table 4.5). Seedlings were used as planting material and row cover was not used in all the surveyed fields. The risk point values and the WTVC incidences in commercial fields had a significant positive correlation (Fig. 4.4). Lower WTVC incidences were observed among fields that had moderate-risk levels. While lower levels of WTVC incidences were observed in some fields with high-risk levels. Interestingly, the field with highest WTVC incidence (44%) was among the fields with high-risk level identified according to our risk-index.

Discussion

This study utilized a unique approach for developing a risk assessment index for disease caused by WTVC in fall-grown squash in Georgia. The relative influence of each pest management tactic from random forest analysis was used to estimate risk point values that aided in developing a risk assessment index for WTVC in squash. This approach deviated from procedures used to create the PEANUT Rx risk index, which was through manual assignment of risk points based on an expert system (Brown et al. 2005). Here, random forest, which is a machine learning tool, was chosen as a method for estimating the relative influence of pest management tactics as it enables users to utilize data containing both categorical and continuous variables (Naghibi et al. 2016). Unlike parametric procedures, the random forest analysis does not require normal distribution and accounts for complex interactions between predictor variables (Sapir-Pichhadze and

Kaplan 2020). For these reasons, random forest has become a popular method for developing predictive models in various fields including medicine, ecology, environmental science, stock market, banking, and e-commerce (Buskirk 2018; Cutler et al. 2007; Sadler et al. 2018; Yang et al. 2020). Unlike other studies, which used the random forest to make a predictive model, we used it to develop a simpler model in the form of a risk assessment index. Following the definition by Brown et al. (2005), a risk assessment index "combines known factors influencing disease severity into a comprehensive but simple estimate of risk for a given production plan. It assigns a relative numeric weightage to each factor so that an overall level of risk can be estimated". Numeric values were assigned so that higher point totals were indicative of "higher levels of risk". A risk index was perceived to be simpler and easier to use as it only requires the summation of risk point values to get a risk prediction. Once a set of risk mitigation tactics are selected, the user of a risk assessment index could interpret the cumulative risk point values to different risk levels as low, moderate, or high. This format would permit distribution and use of risk index in both print and digital application format. Predictive models, such as those developed in other studies, would require running the random forest model in a computer to get a prediction.

Estimation of the relative influence of management tactics on the WTVC through random forest was critical in estimating risk point values based on available data. Random forest calculates the relative influence of predictors based on the changes in prediction error as one predictor is removed from the model (Cutler et al. 2012). Another feature of random forest is its ability to capture and account for interactions between the predictors (Cutler et al. 2012). If such interactions occur, the variables involved would show up as important since removal of one would impact the predictive power of the interaction (Cutler et al. 2012). Using random forest, relative influence of each risk mitigation tactic was determined along with their potential variable interactions on the estimation of risk point values, which was later used for developing a risk index. Results from the random forest revealed that some risk mitigation tactics may influence the WTVC more than the others. This information would be useful in designing integrated pest management programs as it aids in determining relative importance of each tactics, which will eventually aid growers in taking informed decisions.

Among five variables used as predictors of the sAUDPC values for WTVC in the random forest analysis, the date of planting and the use of row cover were found to be the two most influential risk mitigation tactics. Planting squash before or after the peak of whitefly populations resulted in reduced incidence levels of WTVC. This reiterates the importance of avoidance as a component of integrated pest management programs against the WTVC. Adjustment of planting dates to avoid pests and pathogens is a tactic that has been reported to work effectively in managing pests of different crops such as cotton (Kerns et al. 2019; Parajulee et al. 2011) and peanut (Brown et al. 2005; Chappell et al. 2020; Shokes et al. 1982). Although lower levels of WTVC were also observed for squash planted after the peak of whitefly populations (after 20th September); the fact that these plants will be at risk of frost damage that may coincide with harvest maturity makes it impractical. Also, due to the gradual decrease in air temperature from early October, general phenology of the crop may get affected that may translate to reduced yield.

Ideally, planting squash during a high-risk period should be avoided. However, if market demand requires planting between 11th to 25th August or 26th August to 20th

September, use of tactics that reduce risk should be considered. In addition to adjusting planting dates to avoid whiteflies and WTVC, type of planting materials like seedlings as opposed to direct seeding may reduce the exposure time of squash plants to whiteflies. Squash transplants are usually grown in a greenhouse for at least 10 days prior to being planted in the open field. With direct seeding, seedlings emerge four days after planting, at which point they are susceptible to whitefly feeding. Thus, squash grown from direct seeding is exposed to whitefly feeding and virus infection for at least more than six days during early phase of plant development than when transplants are used.

The use of insect-proof row covers is another tactic that can reduce exposure time during a period from seedling to vegetative stage to whitefly feeding and subsequent transmission of WTVC. Row covers are spun-bonded polypropylene fabrics that are permeable to rain and air but serve as barrier to exclude insects from feeding on the plants (Lilley and Sanchez 2016; Hanna et al. 2018). While this is new to squash growers, the use of covers in crop production is not uncommon for the growers in the southeastern US. Row covers are being used to protect strawberries and other crops from frost damage during the early spring season (Himelrick et al. 2001; Hochmuth et al. 2018). Growers who use row covers on strawberries and other crops for frost protection may be able to adopt row covers for managing WTVC in squash.

The practice of using row covers as an insect pest management tactic in cucurbit crops has been demonstrated to be effective against whiteflies, pickleworm (*Diaphania nitidalis* Stoll), melonworm (*Diaphania hyalinata* L), aphids (*Myzus persicae* Sulzer), and cucumber beetles (*Diabrotica undecimpunctata* Barber) (Cline et al. 2008; LaTora et al. 2022; Natwick and Durazo 1985; Perring et al. 1989; Rojas et al. 2011). Previous studies also indicated reduction in aphid and whiteflies populations and subsequent reduction in virus transmission utilizing row covers (Natwick and Durazo 1985; Perring et al. 1989; Ibarra et al. 2001; LaTora et al. 2022). These reports corroborate our observations on the effectiveness of row covers as one of the most important predictors of risk to WTVC in squash. The row cover is therefore an important tactic that can be used to reduce risk, especially when planting dates are driven by market demand.

The "type of squash" was the third most important predictor of WTVC in squash. Greater risk is associated with using yellow squash compared with zucchini squash. As whitefly feeding and the WTVC infection has greater impact on the yield of yellow squash than zucchini (Candian et al. 2021), growing later would be a viable alternative in areas where significant losses due to WTVC is a concern. If market demands require yellow squash to be grown, it is logical to use tactics and production practices that reduce risk to WTVC such as using UV-reflective mulch and row covers, and planting during a low-risk period. Planting yellow squash (risk point value: 20 points) prior to 11th August (risk point value: 5 points) using seedlings as planting material (risk point value: 10 points) on UV-reflective mulch (risk point value: 5 points) and with row cover (risk point value: 10 points) results in a low-risk level (cumulative risk points= 50 points). Ideally, application of these tactics may aid in reducing risk against the WTVC and associated yield losses in squash.

The use of UV-reflective mulch for management of insect pests and insect-transmitted viruses has been reported in multiple studies (Nyoike and Liburd 2010; Simmons et al. 2010; Frank and Liburd 2005). Reflection of light by UV-reflective mulch repels whiteflies and disrupts their visual cues in searching and landing on a plant

(Antignus 2012). Light reflection coming from the ground with the use of reflective mulching sheets disturbs the normal flight orientation of flying insects that maintains their horizontal orientation by perceiving sunlight on their dorsal side during flight (Shimoda and Honda 2013). The amount of UV-reflectance is highest when squash plants are small and decreases as the plant grows and covers wider area of the plastic mulch. While the repellent effect of the UV-reflective mulch decreases over time, reduction of whitefly infestation in plots with UV-reflective plastic early in the season may be enough to delay virus infection and reduce the overall impact of WTVC in the squash crop.

In southern Georgia, growers tend to use white plastic mulch for growing vegetables in the fall season. Black plastic mulch is used for spring crops to retain heat in the soil to promote crop growth. The growers reuse the plastic mulch for their fall crop by painting them white. One way to encourage growers into using UV-reflective mulch might be to encourage the use of silver paint instead of white paint when reusing plastic mulch for growing crops in the fall season. Another option would be to lay a new set of UV-reflective plastic for the fall growing season and then paint the plastic with black color for the spring crop.

As none of the currently available pest management tactics individually provides adequate and consistent level of control to WTVC, a risk assessment index was developed to serve as a guide in the implementation of IPM strategies. The risk point values correlated positively with the sAUDPC values and WTVC incidences. This positive correlation indicates the potential of the risk assessment index to be used for identifying and avoiding high-risk situations that could lead to significant yield losses. With the risk index, growers who determine that their suite of squash production programs results in high-risk levels may consider planting before 11th August or after 20th September. Other considerations would include row cover, UV-reflective mulch, and planting zucchini instead of yellow squash to reduce their risk to WTVC. However, obvious variability of sAUDPC values were observed between moderate- and high-risk situations. This variation may indicate that one or more risk mitigation tactic may influence the risk to WTVC on a greater or lesser scale than was reflected in the current index. The variability within the moderate- and high-risk situations may also indicate that other factors may need to be considered. Additional factors that could influence the prevalence of WTVC in squash but were not tested in this study may include distance to the nearest cotton field, use of an insecticide spray program, choice of previous crop planted in the field, amount and frequency of rainfall, and number of freeze events during the winter months. These factors might influence the abundance of whiteflies that could infest and potentially spread WTVC in squash fields. These concerns will be addressed as more field studies will be conducted for further refinement of the risk index.

As the risk index will be used to identify and avoid situations that result in high-risk of losses due to WTVC, a correlation between yield and risk point values was also performed. The negative correlation between risk point values and the number of marketable fruits indicated that the risk index is not only a good predictor of risk to WTVC but also appears to account for the impact of WTVC on squash yield. Due to high WTVC incidences associated with high-risk levels, greater yield losses also occurred. The WTVC infection and whitefly feeding associated damage can also result in the production of non-marketable fruits and in such cases, the yield might not justify the cost of harvesting.

While the idea of developing a risk index to facilitate the implementation of IPM programs for WTVC in squash was based upon PEANUT Rx, there were some distinctions between these two risk indices. The method at which risk point values were estimated for each risk mitigation tactic was different for the WTVC risk index (random forest) and PEANUT Rx (expert system). There were more categories within each risk mitigation tactic in PEANUT Rx (Brown et al. 2005) than the WTVC risk index which had mostly two categories (Table 4). As resistance to TSWV were available, peanut variety was one of the risk mitigation tactics in PEANUT Rx. In place of variety, the type of squash (zucchini or yellow squash) was used as a risk mitigation tactic in the WTVC risk index as no squash variety was resistant to WTVC. Despite these differences, the risk assessment index for WTVC in squash was developed in this study to serve the same purpose as the PEANUT Rx, to be used for assessing risk based on suite of risk mitigation tactics that were selected by the grower. Similar to the original TSWV risk index, the risk index developed in this study is not perfect but could be modified and improved as more research will be conducted.

Results from this study demonstrated the potential use of random forest in developing a risk assessment index. This analytical tool aided in determining risk point values based on the relative importance of risk mitigation tactics of WTVC in squash. The use of random forest was critical in avoiding potential bias in estimating risk point values arising from lack of data to support manual assignment of risk values. More importantly, the utilization of the relative importance of risk mitigation tactics from random forest helped to develop a simpler risk assessment index, as opposed to a predictive model as demonstrated in this study. The risk assessment index developed here may potentially be used to identify combination of risk mitigation tactics that results in lower WTVC levels in fall-grown squash in Georgia. This in time may facilitate implementation of an integrated pest management program for WTVC in squash.

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Tables

Table 4.1. List and description of the field trials where data were generated for the development of the risk assessment index for whitefly-transmitted virus complex in squash

Field trial	Planting date	Mulch type	Squash type	Row cover	Planting material
Assessment of the effect of different mulch types on number of whiteflies and WTVC incidence	8/20/2020	white plastic, UV-reflective	yellow	without cover	seeds
Effect of planting dates on WTVC incidence on yellow squash and zucchini squash	8/17/2020, 8/31/2020, 9/15/2020, 9/30/2020, 10/15/2020	white plastic	yellow, zucchini	without cover	seedlings
Evaluation of various insecticides, including stylet oil and row cover, on whitefly population and WTVC	9/10/2020	white plastic	yellow	with cover, without cover	seeds
Evaluation of insecticides on whitefly-transmitted WTVC in squash across multiple planting dates	6/29/2020, 7/6/2020, 7/13/2020, 7/20/2020, 7/27/2020, 8/3/2020, 8/10/2020, 8/17/2020, 8/14/2020, 8/31/2020	white plastic	yellow	without cover	seeds
Assessment of UV-reflective mulch and row covers for management of whiteflies and whitefly-transmitted WTVC in yellow squash.	9/9/2020	white plastic, UV-reflective	yellow	with cover, without cover	seedlings
Evaluation of insecticides on whitefly-transmitted WTVC in squash across multiple planting dates	7/19/2021, 7/26/2021, 8/2/2021, 8/9/2021, 8/16/2021, 8/23/2021, 8/30/2021, 9/7/2021	white plastic	yellow	without cover	seeds
Assessment of planting dates, mulch types, and row cover for the management of WTVC in yellow squash	8/2/2021, 8/23/2021, 9/14/2021	white plastic, UV-reflective	yellow	with cover, without cover	seeds
Simulation of different risk levels using various combinations of squash type, planting date, mulch type, planting material, and row cover	8/16/2021, 9/6/2021, 9/27/2021	white plastic, UV-reflective	yellow, zucchini	with cover, without cover	seeds, seedlings

Assessment of UV-reflective mulch and row covers for management of whiteflies and whitefly-transmitted WTVC in yellow squash.	9/13/2020	white plastic, UV-reflective	yellow	with cover, without cover	seedlings
Evaluation of insecticides on whitefly-transmitted WTVC in squash across multiple planting dates	7/25/2022, 8/1/2022, 8/8/2022, 8/15/2022, 8/22/2022, 8/29/2022, 9/6/2022, 9/12/2022	white plastic	yellow	without cover	seeds
Effect of squash planting material on WTVC incidence (Experiment A)	8/7/2022, 8/21/2022	white plastic	yellow	without cover	seeds, seedlings
Effect of squash planting material on WTVC incidence (Experiment B)	8/22/2022, 10/3/2022	white plastic	yellow	without cover	seeds, seedlings
Assessment of planting dates, mulch types, and row cover for the management of WTVC in yellow squash	8/2/2022, 8/23/2022, 9/13/2022	white plastic, UV-reflective	yellow	with cover, without cover	seeds
Simulation of different risk levels using various combinations of squash type, planting date, mulch type, planting material, and row cover	8/16/2022, 9/6/2022, 9/27/2022	white plastic, UV-reflective	yellow squash, zucchini	with cover, without cover	seeds, seedlings

Table 4.2. List and description of risk mitigation tactics used in the random forest analysis as predictors of standardized area under disease progress curve (sAUDPC) values for whitefly-transmitted virus complex in squash

Predictor Variables	**Variable Description**
Type of squash	The type of squash was either yellow squash or zucchini squash.
Use of row cover	The use of row covers was categorized as with or without row cover. Row covers are insect proof mesh installed over the plants immediately after planting and removed at flowering (approximately 20 days after planting). Row covers were either single row cover that only covered single rows of squash or floating cover that covered up to six rows of squash.
Planting material	Squash was either direct seeded or transplanted using greenhouse grown seedlings.
Planting date	This variable combines the date of planting and whitefly pressure, relative to when peak whitefly population occurs, to create a predictor associated with vector activity. Planting dates were categorized as “before August 11”, “August 11 to 25”, “August 26 to September 20”, and “after September 20” to represent periods before the population peak, period leading to the peak, period when peak population occurs, and period after the peak of whitefly population has occurred.
Mulch type	The types of mulch in this study were white plastic and UV-reflective plastic.

Table 4.3. Ranking of risk mitigation tactics and estimated maximum risk point values for each tactic based upon the percentage increase in mean square error calculated through random forest analysis.

Predictor variables	**Percentage increase in mean square error**	**Percent influence**[a]	**Maximum risk value per variable**[b]
Planting date	165.52	39.68	60
Use of row cover	124.52	29.85	45
Type of squash	52.80	12.66	20
Planting material	45.09	10.81	15
Mulch type	29.18	6.99	10

[a] Calculated based upon percentage increase in mean square error

[b] Calculated by multiplying a base value of 150 to the percent importance of each risk mitigation tactic; 150 is the maximum risk point value attainable in the risk index; values were rounded to the nearest multiple of five

Table 4.4. The whitefly-transmitted virus complex risk assessment index containing the risk mitigation tactics, mean standardized area under disease progress curve (sAUDPC) values, and risk point values for each category under each predictor variable.

Predictor variables	**Categories**	**Mean sAUDPC**[a]	**Risk point value**[b]
Planting date			
	before August 11	4.57	5
	August 11 to 25	35.50	55
	August 26 to September 20	38.54	60
	after September 20	20.03	30
Row cover			
	with row cover[c]	8.13	10
	without row cover	30.66	45
Squash type			
	zucchini squash	16.55	15
	yellow squash	26.44	20
Planting material			
	seedling	21.67	10
	seed	26.54	15
Mulch type			
	UV-reflective plastic	17.56	5
	white plastic	27.63	10
Interpretation of summed risk point values			
	Low Risk		**45 - 75**
	Moderate Risk		**80 - 115**
	High Risk		**120 - 150**

[a] Mean sAUDPC values were calculated per category across multiple studies

[b] Values were rounded to the nearest multiple of five

[c] Row covers are insect-proof netting that are installed immediately after planting and removed at flowering stage

Table 4.5. List of commercial squash fields surveyed for validation of the whitefly-transmitted virus complex (WTVC) risk assessment index

Predictor variables for WTVC in squash					**Risk point values**					**Total risk point values**	**Risk level**	**Mean WTVC incidence**[a]
Squash type	**Planting date**	**Row Cover**	**Planting material**	**Mulch type**	**Squash type**	**Planting date**	**Row Cover**	**Planting material**	**Mulch type**			
zucchini	Before August 11	without	seedlings	white	15	5	45	10	10	85	Moderate	0.00
zucchini	Before August 11	without	seedlings	white	15	5	45	10	10	85	Moderate	6.67
zucchini	Before August 11	without	seedlings	white	15	5	45	10	10	85	Moderate	1.92
yellow	Before August 11	without	seedlings	white	20	5	45	10	10	90	Moderate	0.44
yellow	Before August 11	without	seedlings	white	20	5	45	10	10	90	Moderate	2.11
yellow	Before August 11	without	seedlings	white	20	5	45	10	10	90	Moderate	7.89
yellow	Before August 11	without	seedlings	white	20	5	45	10	10	90	Moderate	0.88
yellow	Before August 11	without	seedlings	white	20	5	45	10	10	90	Moderate	0.33
yellow	After September 20	without	seedlings	white	20	30	45	10	10	115	Moderate	20.56
zucchini	August 11 to 25	without	seedlings	UV-reflective	15	55	45	10	5	130	High	3.22
zucchini	August 11 to 25	without	seedlings	UV-reflective	15	55	45	10	5	130	High	1.11
zucchini	August 26 to September 20	without	seedlings	UV-reflective	15	60	45	10	5	135	High	4.67
yellow	August 11 to 25	without	seedlings	UV-reflective	20	55	45	10	5	135	High	3.67
yellow	August 11 to 25	without	seedlings	UV-reflective	20	55	45	10	5	135	High	6.11
zucchini	August 26 to September 20	without	seedlings	white	15	60	45	10	10	140	High	20.33

yellow	August 26 to September 20	without	seedlings	UV-reflective	20	60	45	10	5	140	High	7.11
yellow	August 11 to 25	without	seedlings	white	20	55	45	10	10	140	High	1.78
yellow	August 11 to 25	without	seedlings	white	20	55	45	10	10	140	High	2.00
yellow	August 11 to 25	without	seedlings	white	20	55	45	10	10	140	High	10.77
yellow	August 11 to 25	without	seedlings	white	20	55	45	10	10	140	High	39.83
yellow	August 26 to September 20	without	seedlings	white	20	60	45	10	10	145	High	8.11
yellow	August 26 to September 20	without	seedlings	white	20	60	45	10	10	145	High	44.00

[a] Mean WTVC incidences were calculated by taking the mean among 18 rows of squash selected through a stratified sampling method

Figures

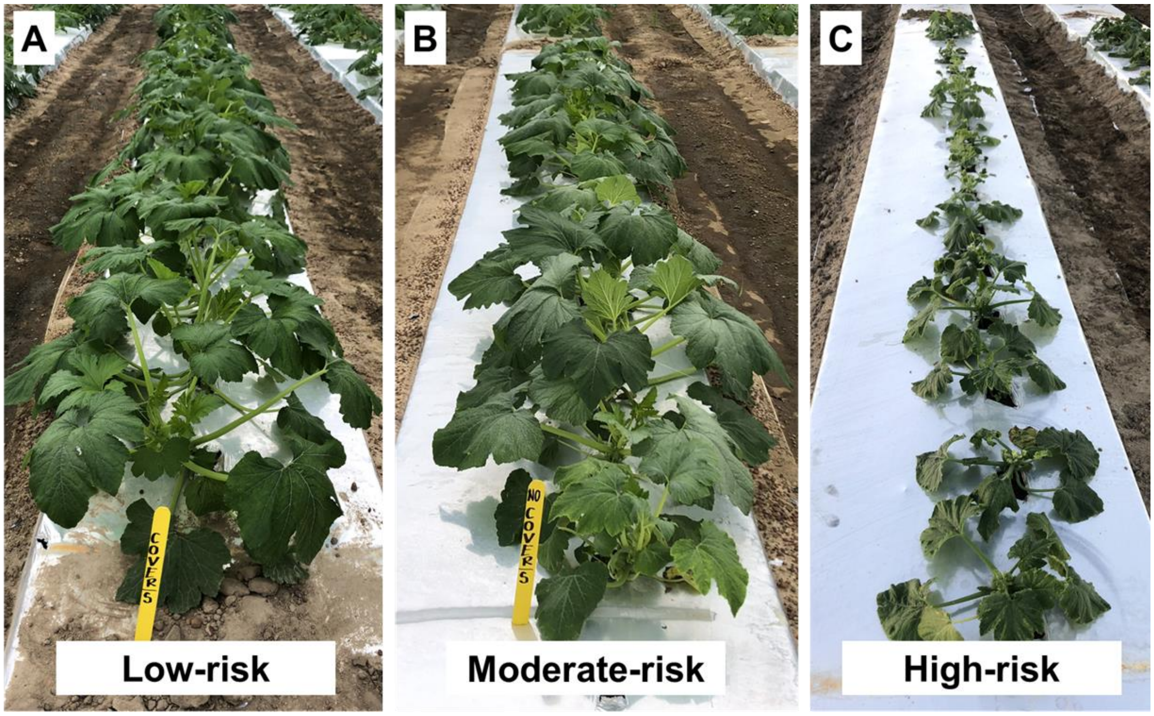

Figure 4.1. Yellow squash plots showing the occurrence of whitefly-transmitted virus complex (WTVC) infection in three levels of risk to WTVC based upon the risk assessment index. The low-risk plot consisted of yellow squash planted on August 2, 2022 on a UV-reflective plastic and with a row cover (A). The moderate-risk plot was planted on August 2, 2022 on a UV-reflective plastic and without a row cover (B). The high-risk plot was planted on September 13, 2022 on a UV-reflective plastic and without a row cover (C). Pictures were taken at 21 (A and B) and 28 (C) days after planting.

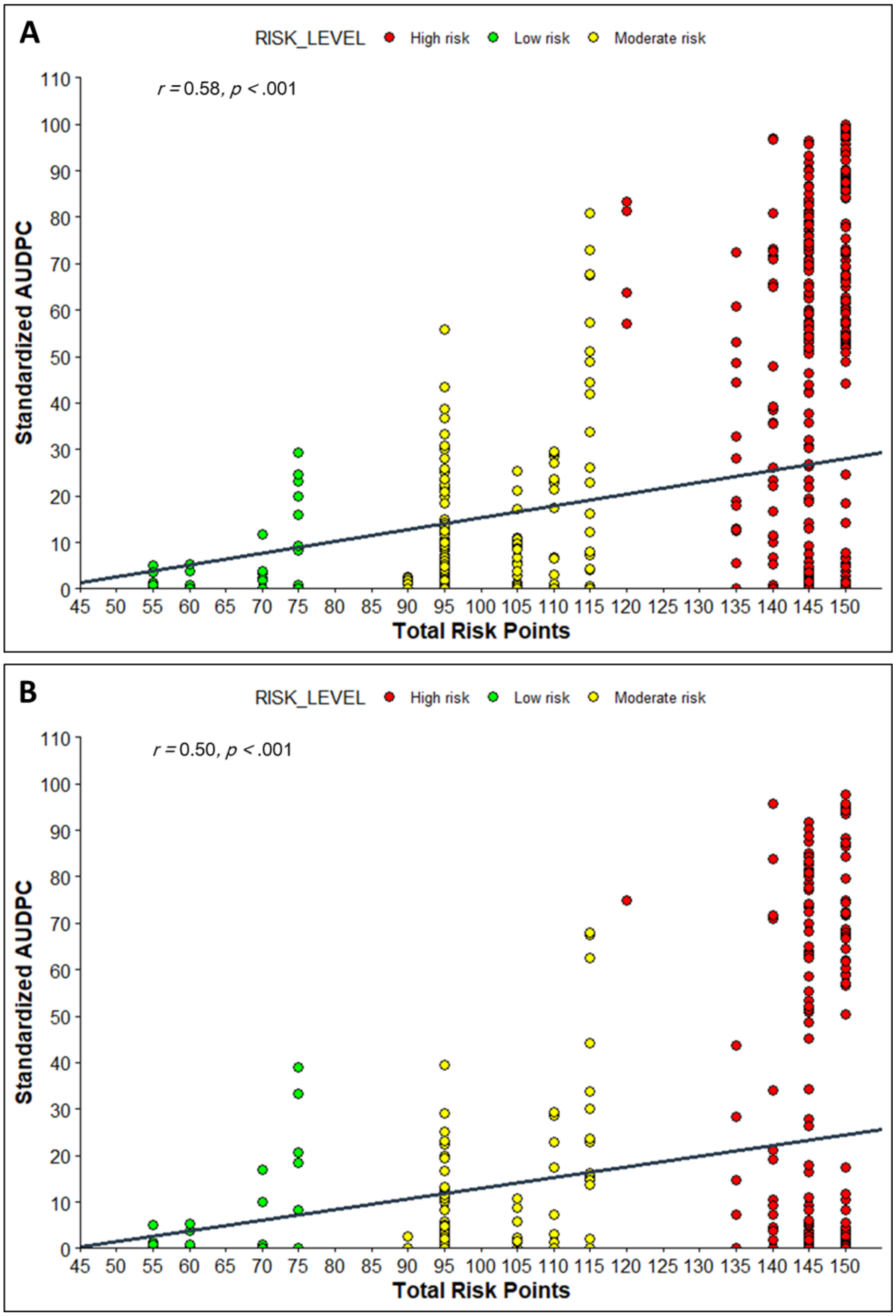

Figure 4.2. Positive correlation between risk point values and standardized area under disease progress curve (sAUDPC) values in the training (A) and testing (B) data set

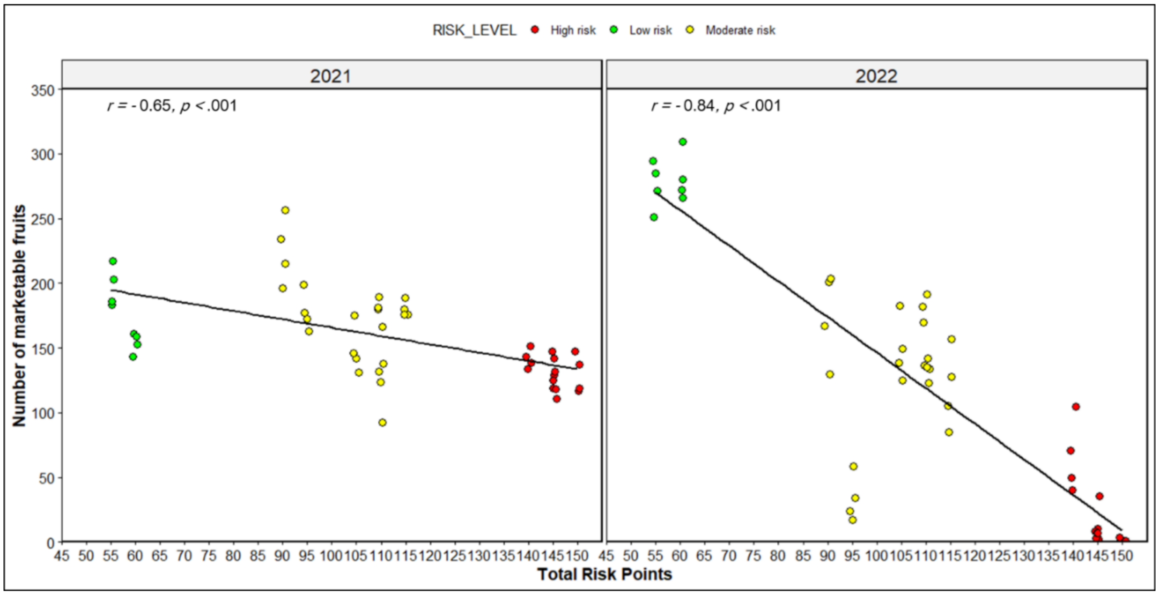

Figure 4.3. Negative correlation between the risk point values and the number of marketable fruits of yellow squash per plot in a three-factorial field trial. The field trial consisted of three planting dates, two types of plastic mulch, and with or without row cover.

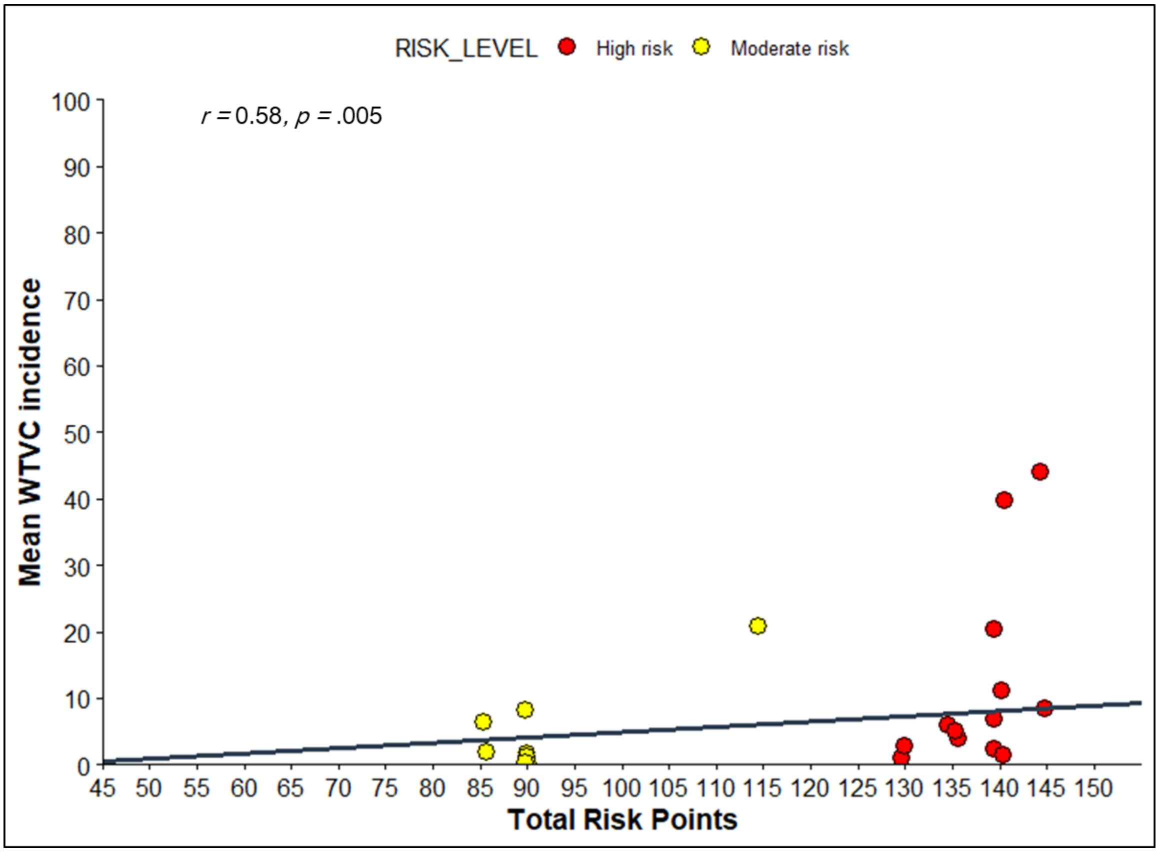

Figure 4.4. Validation of the whitefly-transmitted virus complex (WTVC) risk assessment index in commercial squash fields; mean WTVC incidences were calculated by taking the mean WTVC incidences among 18 rows of plants selected through a stratified sampling method

CHAPTER 5

CONCLUSIONS

Whitefly-transmitted viruses have emerged as a primary concern for fall-grown vegetables in southern Georgia. Growers rely on managing whiteflies, the insect vector, through insecticides to minimize the impact of whitefly-transmitted viruses in their crops. Growers typically apply insecticides two to three times a week to manage whiteflies. With the frequency of application, there are concerns of resistance development against the commonly used insecticides by whiteflies. Growers are also concerned that the high whitefly populations occurring during the fall growing season could overwhelm the efficacy of insecticides. Hence, this study was conducted to better understand the spatiotemporal patterns of whiteflies and whitefly-transmitted viruses impacting squash and snap beans to identify where and when the disease occurs in the fields. Such information may be used in improving integrated pest management programs. A risk assessment index for the disease caused by the whitefly-transmitted virus complex (WTVC) in squash was developed in this study. Such an index helps to quantify the impact of factors that influence the intensity of WTVC in squash fields and may be used to determine combinations of different tactics that reduce risk to WTVC. By helping to identify combinations of tactics that reduce risk to WTVC, the use of risk assessment index could facilitate the implementation of an integrated pest management program for WTVC in squash.

The spatial distribution of WTVC infected squash plants shifted from a random pattern to an aggregated pattern as the disease incidence increased over time. The occurrence of rapid increase in the size of the population of whiteflies and their movement from virus-infected to healthy plants contributed to the aggregation of symptomatic plants in squash fields. Whitefly abundance positively correlated with WTVC incidence in squash fields. Areas within the field, particularly along field edges, where higher numbers of whiteflies were observed had higher virus incidences. WTVC infection initiated along the edges of the squash fields, which was later spread by viruliferous whiteflies more often within rows of squash than between rows of squash plants.

In snap bean fields, similar patterns of higher incidence of the whitefly-transmitted SiGMV (Sida golden mosaic virus) along field edges was observed particularly on macroplots adjacent to field margins where SiGMV-infected prickly sida plants were growing. The presence of SiGMV-infected prickly sida along field margins was associated with earlier and increased levels of disease occurrence in snap beans. Prickly sida is a host for both whitefly and SiGMV, it supports whitefly reproduction, whiteflies can acquire and transmit SiGMV from prickly sida to snap beans and vice versa, and it occurs within the vicinity of the fields where snap beans are grown commercially. Hence, prickly sida potentially serves as a natural reservoir between snap bean growing seasons and as a source of SiGMV inoculum during the growing season.

Spatial distribution, particularly the prevalence of infection along field edges and areas close to potential inoculum sources are important observations. Such are considerations for designing scouting and sampling patterns for early detection of virus infection, for identifying effective pest management tactics and for determining appropriate timing for applying each pest management tactic to reduce the impact of disease caused by whitefly-transmitted viruses.

As no single tactic provides adequate and consistent control of whitefly-transmitted viruses in squash, implementation of an integrated pest management program should be considered. A risk assessment index was developed in this study for the disease caused by WTVC in squash. As there were limited data to support manual assignment of risk point values, a novel approach to developing a risk index was implemented by using random forest to estimate the importance of each variable as predictor of WTVC. Planting date and row cover were the most important predictors followed by type of squash, planting material, and plastic-mulch type. Spearman correlation analysis indicated a strong positive correlation between the cumulative risk point values and the sAUDPC values for both the training and testing data sets. The risk index was validated through a survey of commercial fields. The risk index developed in this study is a promising tool that may be used to identify and avoid high-risk situations, reducing the impacts of WTVC in squash.

Overall, the findings from this study provide information on the epidemiology and management of whitefly transmitted viruses affecting squash and snap beans which are two of the major vegetables produced in southern Georgia. The spatiotemporal patterns of diseases are important epidemiological information that may be used in designing methods for early detection and targeted management of diseases. The risk index developed in this study may be used as a working model for predicting risk of WTVC in squash and may be further improved as new tactics are identified and as new data are generated.

APPENDIX A

SPATIOTEMPORAL PATTERNS OF SIGMV INFECTION IN SNAP BEAN PLOTS WITH AND WITHOUT SIGMV INFECTED PRICKLY SIDA IN EXPERIMENTAL FIELD

Rationale

Higher levels of SiGMV incidences were observed in on-farm trials in southern Georgia. This experiment was conducted at the UGA Black Shank Farm in an attempt to replicate the spatiotemporal patterns of SiGMV in snap beans as observed in the on-farm study presented in Chapter 3.

Materials and Methods

Two plots of snap beans (cv. 'Caprice') were planted on 6th October 2022. The plots were 440m apart from each other. Each plot had 12 rows that are 45.7m long. Each row was divided into 3.1m (10ft) long quadrats and marked with field flags. In one of the plots, eight SiGMV-infected prickly sida plants placed in clay pots were placed on one edge of the plot and was termed 'plot with SiGMV infected prickly sida'. No prickly sida was placed or growing near the other plot hence was termed 'plot without prickly sida'.

The incidence of disease caused by SiGMV was assessed per quadrat at a seven-day interval. Whitefly abundance was assessed by counting the number of adult whiteflies in snap bean leaves. The number of adult whiteflies were counted in two leaves per quadrat. Heatmaps showing whitefly abundance and SiGMV incidences over space and time were created per sampling plot over time.

Results and Discussion

Higher SiGMV incidences at 43 DAP (days after planting) were observed in areas within the snap bean plots where higher numbers of adult whiteflies were observed at 21 DAP (Fig. 5.1). This corroborates the observed correlation of whitefly abundance and incidence of whitefly transmitted viruses in squash as observed in Chapter 2. In both the plot with prickly sida and plot without prickly sida, there is a noticeable aggregation of SiGMV-infected plants close to field edges (Fig. 5.1). However, in contrast to the hypothesized higher levels of SiGMV in the plot with prickly sida, higher SiGMV incidence was observed in the plot without prickly sida. While not proven, one possible explanation to the higher SiGMV incidence in the plot without prickly sida could be the location and vegetation around the two plots. The plot with SiGMV-infected sida was located near plots planted with other host crops, squash and broccoli; while no other host crops were growing near the plot without sida. It is possible that whiteflies preferred to settle on the squash and broccoli plants over the snap beans in the plot with prickly sida. This could be evidenced by noticeably higher numbers of adult whiteflies observed on snap beans within the plot without prickly sida, particularly at 14 and 21 DAP.

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Figure

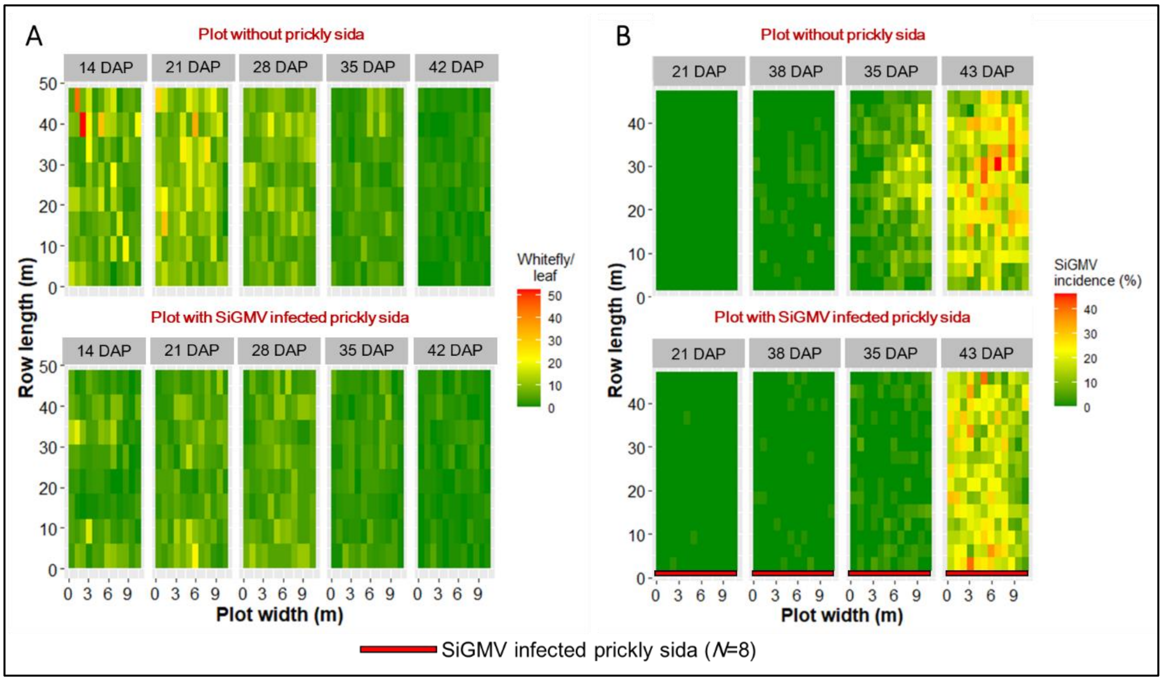

Figure 5.1. Heatmaps of adult whitefly counts on snap bean leaves (A), and SiGMV incidences (B) over space and time in plots with or without SiGMV infected prickly sida in experimental field. Dark green indicates areas in the plots with lower numbers of adult whiteflies and SiGMV incidences. Dark red areas had higher numbers of adult whitefly and SiGMV incidence.