THE EFFECTIVENESS OF BIOLOGICAL CONTROL OF *FRANKLINIELLA OCCIDENTALIS* IN PREVENTION OF THE SPREAD OF TOMATO SPOTTED WILT VIRUS

by

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Abstract

A two-year greenhouse experiment was conducted to compare the relative effectiveness of biological control versus chemical control for western flower thrips, *Frankliniella occidentalis*, as a means of reducing the spread of *Tomato spotted wilt virus* (TSWV) on tomatoes. To compare efficacy of different thrips management tactics for reducing TSWV incidence, tomatoes were subjected to one of three treatments: 1) biological control based on weekly releases of the predatory mite, *Amblyseius cucumeris*, at a commercially-recommended rate, 2) a single chemical treatment with Conserve®, a spinosad formulation, or 3) no treatment. TSWV was introduced into the greenhouse either by starting with 20% of the crop already infected and releasing non-viruliferous thrips, or by making a single release of viruliferous thrips. Analyses were done among thrips management tactics for each virus introduction method to examine the cumulative number of weeks plants were infected, the weekly proportion of infected plants, and total marketable yield. The effects of different virus introduction methods were also compared.

A comparison of virus introduction methods showed that, among all plants, the average number of weeks they were infected by TSWV was significantly lower when virus was introduced through infected plants than by infected thrips. In addition, when virus was introduced by infected thrips, a significantly greater proportion of plants were infected in any given week than when virus was introduced on infected plants. Finally, crop yields were significantly lower when virus was introduced via infected thrips than on infected plants.

Among thrips management methods, plants were infected for significantly less time, and the proportion infected was lower in any given week, when biological or chemical control was applied compared to no thrips management. Tomato yields were not affected by thrips management tactic. There was no significant difference between biological and chemical control in the length of time that plants showed symptoms. However, the proportion of infected plants was marginally greater with biological control in weeks 4 and 5 than with chemical control; differences were not significant thereafter.

My findings suggest that inundative releases of biological control may provide as adequate a level of protection from TSWV as chemical control in commercial greenhouse tomato crops.

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Acknowledgements

Dedication

I would like to dedicate this to my husband, whose love, support, and patience made it possible for us to survive the trials and tribulations of graduate school. Thank you.

CHAPTER 1 - Introduction and Literature Review

Western Flower Thrips

Classification

Frankliniella occidentalis (Pergande), commonly known as the western flower thrips (WFT), is one of approximately 5,000 described species in the order Thysanoptera. WFT belongs to the family Thripidae which combined with the family Phlaeothripidae comprise almost 95% of all known species of Thysanoptera. WFT also is a member of the Thripinae, the only subfamily of Thysanoptera known to vector tospoviruses (Jones, 2005).

Distribution

WFT was first reported in western North America in 1895; but by the 1970s it had extended its distribution to the Midwest (Kirk, 2002). WFT was first observed in Kansas and Missouri in the early 1970s, and was found along the east coast of North America and many Canadian provinces by the early 1980s (Kirk et al., 2003). The incidence of tomato spotted wilt virus in several regions of the country may indicate an earlier presence of western flower thrips than had been recorded. In Europe the first records of WFT were found in the early 1980's on greenhouse African violet (Mantel et al., 1988). It is thought that the intercontinental spread of the WFT is linked to the movement of infested plant material between glasshouses and nursery businesses in both Europe and North America (Kirk et al., 2003). Since then, WFT has become a major worldwide pest of horticultural, agricultural and floricultural field and greenhouse crops, causing significant economic losses as a direct result of this insect's ability to transmit the tospovirus, *Tomato spotted wilt virus* (Jones, 2005).

Biology

The western flower thrips is capable of long-distance dispersal and can, during certain times of high population levels, be seen moving in large masses while carried on wind currents (Mound, 1983). Adults usually range from 0.5 to 1.5 mm in length, and have two pair of wings that bear long marginal (fringe) hairs. They also have a unique set of asymmetrical, hypognathus piercing-sucking mouthparts that are housed inside a mouthcone (Gerson, 2007, Joost et al., 2008). WFT has six life stages: egg, two larval instars, 'prepupa' (non-feeding larval stage), pupa, and adult. The time from egg to adult depends on temperature and the host plant. At 15°C the life cycle takes approximately 13 days; at 30°C it decreases to an average of 4.3 days (Van Driesche et al., 1998). Under suitable conditions, females lay 150-300 eggs during their lifetime and live 30-45 days (Cloyd, 2003).

Studies on the life history of WFT on different host plants have been done by Zhang et al. (2007), van Rijn et al. (1995), and Gaum et al. (1994). Zhang et al. (2007) compared host plant suitability of five different greenhouse-grown vegetable crops: cabbage (*Brassica oleracea* L. var. Jingbeng), cucumber (*Cucumis sativus* L. var. Zhongnong 8), capsicum (*Capsicum annuum* L. var. Zhongjiao 5), kidney bean (*Phaseolus vulgaris* L. var. Gonggeizhe) and tomato (*Lycopersicon esculentum* M. var. zhongza 9). Host plant suitability was assessed by comparing WFT developmental times and potential population growth. Zhang et al. (2007) found that there were significant differences in development. At 27 ± 1°C the western flower thrips had a developmental time of 12.91 ± 0.04 days (egg to adult) on tomato, which was longer than on the other four host plants. Gaum et al. (1994) estimated the minimum thermal threshold of WFT on English cucumber to be 9.4°C, and on chrysanthemum McDonald et al. (1998) estimated a thermal constant of 268 degree-days above a threshold temperature of 7.9°C for complete development.

Pest status

In field crops WFT are able to survive in regions with mild winters and overwinter in soil or on weed hosts. They cannot survive extremely harsh winters and therefore do not overwinter outdoors in the colder regions of North America (McDonald et al. 1997); but they can survive and reproduce continuously in the ideal conditions many greenhouse crops provide, making them a leading year-round economic pest for the greenhouse industry. Since their introduction in the 1980s, the WFT has become the number one pest in European greenhouses (van Lenteren, 1999).

The western flower thrips is considered to be a serious economic pest due to the damage they cause either by direct feeding or oviposition, or indirect effects such as the transmission of plant viruses. WFT oviposition can occur directly on the fruit in a tomato crop resulting in dimples across the surface of the fruit causing cosmetic damage and economic loss in a highly competitive market (Salguero Navas et al., 2002). Feeding damage occurs as the piercing-sucking mouth parts are inserted into individual plant cells to probe and consume the contents of the cell. Cells that have had their content extracted from feeding die causing deformed plant growth, deformation of flowers, or silvered flecking or streaks on the plant's epidermal layer.

Feeding may also lead to indirect damage when WFT transmit viruses in the genus *Tospovirus*. Tospoviruses are exclusively transmitted by thrips and cause devastating diseases of many economically important crops worldwide (Maris et al., 2004). The WFT is currently considered the most efficient vector of these viruses (German et al., 1992, Ullman et al., 1993). In a study done by Wijkamp et al. (1995), the competence of six different populations of thrips species were tested for their ability and efficiency to transmit four different tospoviruses. They found that *F. occidentalis* was the only species that was able to transmit all four of the tospoviruses tested, although five of the different species of thrips were shown to vector TSWV.

The greatest threat comes from their global distribution and capacity to vector tospoviruses (Kritzman et al., 2002, Ullman et al. 1997).

In 1935 the species later recognized as *F. occidentalis* was first reported as a vector of the tospovirus, *Tomato spotted wilt virus* (TSWV) in the United States (Gardener et al., 1935). Thrips transmit TSWV by injecting virions from their salivary glands into the plant during feeding. Joost et al. (2008) suggests that altering the feeding behavior could change their ability to vector the virus. WFT is found predominantly in flowers feeding on pollen (Cho et al., 2000). The study conducted by Joost et al. (2008) measured the response to plant age and leaf age, using the probing and settling behavior as the indicator for two different thrips species, *F. occidentalis* and *F. fusca*. It was found that both plant and leaf age can have species-specific effects on the probing and settling behaviors of thrips. The WFT was found to be less sensitive to leaf and plant quality than *F. fusca*, but this may be because the primary and preferred food source for the WFT is pollen and not leaves. Plant and leaf age did not have a strong effect on *F. occidentalis*, but they did have a significant effect on the probing and settling behavior of the *F. fusca*.

Tomato Spotted Wilt Virus

History and classification

TSWV was first described as 'spotted wilt' disease of tomato in Australia in 1915 (Brittlebank, 1919). It was not until the early 1930s that the virus became known as tomato spotted wilt virus (Gardener et al., 1935). Milne and Francki (1984) were the first to recognize characteristic similarities between TSWV and a family of viruses that usually infect vertebrates, which later led to the inclusion of the *Tospovirus* genus into the family *Bunyaviridae*. There are over 300 members in the family Bunyaviridae, most of which are vectored by arthropods and cause serious animal or human illness (Ullman et al., 1995a).

Tospoviruses are the only plant-infecting members of the family Bunyaviridae. Most

viruses in this family have been shown to replicate in the arthropod host, as is the case for

TSWV and the nine different species of thrips that are currently the only known vectors (German

et al., 1992, Ohinshi et al., 2001, Ullman et al., 1993). Tomato spotted wilt virus is considered to

be the type member of the *Tospovirus* genus, and, as such, is used in the classification process to

identify new species that were once recognized as TSWV, but are now able to be distinguished

as separate species as technology in the field of plant pathology continues to advance.

Characteristics of TSWV and other tospoviruses

Tospoviruses are quasi-spherical in shape with a diameter of 80-100 nm (German et al.,

1992). Virions are enveloped by a membrane acquired from the host during maturation (Ullman

et al., 1995). They are characterized by a tripartite single strand RNA genome with the two

ambisense segments designated as small (S) and medium (M), and a large (L) negative-strand

segment, all of which are known to encode six proteins from five open reading frames (Murphy

et al., 1995). The four structural proteins consist of a putative RNA dependent RNA polymerase

(L), two glycosylated membrane proteins (G1 and G2) and a nucleocapsid protein (N) (Ullman et

al., 1995a, Chu et al., 2001). The viral nucleocapsid protein (N) and a nonstructural protein

(NS_s) are encoded by the S RNA of TSWV (Ullman et al., 1995a). The L RNA encodes the L

protein (de Haan et al., 1991) and the M RNA encodes a precursor to the two glycoproteins that

are associated with the viral envelope or membrane (Law et al., 1992). The two nonstructural

proteins have been used as indicator markers of virus infection in the WFT as they are detectable

in infected cells or tissue of the insect (Sherwood et al., 2001).

Symptoms

Virus symptoms expressed by the plant typically include leaf curling, bronzing, concentric ring spots, necrotic streaks, mottling, chlorosis, or lesions. Virus symptoms on tomato fruits may include pale or yellowed skin color, concentric ring spots, uneven blotchy skin color, or even fruit deformity. Mild symptoms in green unripe fruit may consist of pale green or white spots and blotches, which may remain white after ripening or may become pale red or yellow (Allen et al., 1986).

The WFT-TSWV-Plant Interaction

Transmission

The complex interaction between the plant, the thrips and the TSWV requires a constant exchange, by continuous passage, of virus between plant and thrips. Successful virus movement within plant and insect tissues must occur for this system to be maintained (Ohnishi et al., 2001). Plant virus acquisition and transmission occur during the feeding process. Western flower thrips have piercing-sucking mouth parts that are used to remove plant cell fluid. The mouth parts are composed of one mandibular stylet and two maxillary stylets. The left, single mandible is used to punch a hole in the feeding substrate, and the enclosed single feeding-salivary channel is made up of two maxillary stylets with a sub-apical aperture (Mound et. al., 1995). In the process of feeding, saliva is injected into the plant creating the potential for the virus to be transmitted to the plant when it is present in the saliva of the thrips. This makes the process of virus transmission exclusively circulative-propagative (German et al., 1992, Ullman et al., 1993, Ullman et. al., 2002).

Wijkamp et al. (1996) preformed a test to quantify the transmission of TSWV by the WFT by determining the median acquisition access period (AAP_{50}) and median inoculation

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access period (IAP_{50}). Their results showed that TSWV could be acquired and transmitted efficiently by *F. occidentalis* in a period of 5 minutes. The AAP_{50} of larvae that acquired TSWV from *Impatiens* plants was 106 minutes with an optimum AAP of 21.3 hours. The IAP_{50} of larvae that acquired TSWV from petunia was 58 minutes or 137 minutes when acquired from *Datura stramonium*. The IAP optimum was 42.7 hours. Wijkamp et al (1996) states that the major factors that ultimately determine the spread of the TSWV are both time spent on the host plant and the period required by the vector to acquire or inoculate the virus. Transmission efficiency may also need to be determined at a population level as well. In a study conducted by van de Wetering et al. (1999), efficiency of TSWV transmission was analyzed using two different tospoviruses and 14 populations of WFT collected at 14 different locations in a variety of crops. Their study found that all of the populations of *F. occidentalis* were competent vectors, but the transmission efficiency, expressed as the percentage of adults that were transmitters, ranged from 18 to 75%. This suggests that an improved understanding of the complex tospovirus-thrips interactions is needed, not only at an individual organism level, but at the population level as well.

The movement and replication process in which the virus moves throughout the plant is fairly well understood, but the exact process by which virus movement occurs within the thrips is still unclear. It is generally accepted that WFT are only able to transmit the TSWV if it is acquired at the earliest larval stage, as the potential for the insect to facilitate virus transmission from virus ingestion rapidly decreases as the thrips matures (van de Wetering et al., 1996, de Assis Filho et al., 2004, Ullman et al., 1996). It has been reported that thrips, while able to acquire the virus at the adult stage, are not able to transmit the virus (Nagata et al., 2002, Ohnishi et al., 2001).

Successful virus transmission requires the virus to migrate from the midgut to eventually translocate to the salivary glands. Virus is first found in the midgut epithelial cells after initial ingestion and migration through the apical membrane, where it then migrates to the surrounding visceral and longitudinal muscular cells of the midgut (Ullman et al., 1993). As the virus replicates it then must move across the basal lamina (BL), circulate in the hemocoel, and enter the salivary glands, passing through the cell membrane where it can also potentially replicate and be transmitted to new plant material through saliva injection during feeding (German et al., 1992). Currently the exact mechanism and pathway in which TSWV crosses the midgut BL from the midgut lumen, fat body membrane, or salivary gland BL during the acquisition access feeding period during the early larval stages has not been determined (Ohnishi et al. 2001).

Management problems

The complex task of controlling the spread of the TSWV is hindered by several major factors, including the broad host range of both vector and virus. Over 650 plant species are known to be susceptible to the TSWV, and these include agricultural and ornamental crops as well as weed hosts. This wide host range has created a broad array of ecological niches for its insect vectors (German et al., 1992, Cho et al. 1988). Different types of plants can exhibit TSWV symptoms in a variety of ways after becoming infected. Symptoms can be seen in the fruit of the plant and show little effect on the foliage; at other times severe necrotic symptoms occur which may kill the plant (Jones, 2005). Ullman et al. (1995b) states that the "thrips vectors and the viruses making up the *Tospoviruses* have large overlapping host ranges that make management of virus spread one of the greatest challenges facing agriculture and ornamental industries today."

Effects on biology

Preference and suitability of WFT for TSWV-infected versus noninfected pepper plants were analyzed by Maris et al. (2004) using female thrips behavior, oviposition and subsequent development of progeny as criteria. Their results showed that TSWV-infected plants were more attractive for feeding and oviposition than uninfected plants. Considerably more eggs were produced on infected plants than noninfected plants. This has strong implications for TSWV epidemics as the wingless first instar larvae typically feed on the plants where their eggs were oviposited creating an acquisition access feeding period (AAP) for the larvae to ingest the virus (Terry, 1997). Therefore the attraction of thrips to oviposit on TSWV-infected plants may partially determine the proportion of TSWV vectors and consequently the level of TSWV epidemics (Chaisuekul et al., 2005).

The tospoviruses are able to infect hundreds of plant species and are known to cause devastating damage, making them one of the most economically-important groups of plant pathogens worldwide (Sherwood et al., 2001). The epidemiology of TSWV, though still not fully understood, must take into consideration major influential factors. Within greenhouses the most influential of these factors are host plant species, the physical environment, and pest control (Broadbent et al., 1995).

Management of WFT

Frankliniella occidentalis is considered a difficult pest to regulate or manage in a greenhouse system. The typical cryptic behavior of the WFT makes it difficult to target with chemical control, and because there are few available registered pesticides that are effective, most are not compatible with biological control agents (Steiner, 1990). Yet, insecticides are the main control measure used for WFT by growers in many major greenhouse markets (Buitenhuis

et al., 2006). WFT are often aggregated within crops creating pest control problems in both the

field and greenhouse (Mound et al., 1995). Higgins (1992) found that 84-95% of adults that

were in flowers were female, and that greater than 85% of the larvae were found on leaves and

not flowers. By determining the distribution characteristics of the WFT population and using

spatial analysis of diverse TSWV infections scenarios proper integrated pest management

strategies can be designed (Coutts et al., 2004, Steiner, 1990).

The high reproductive potential as well as the short generation time of the WFT, coupled

with the improper use of pesticides, has resulted in the development of insecticide resistance to

several major classes of chemicals (Broadbent et al., 1997). Coutts et al. (2005) found that

drenching healthy seedlings with systemically active neonicotinoid insecticides just before

transplanting was an effective thrips control method. Currently a spinosad, a biorational or

reduced-risk insecticide derived from a soil microorganism, is the most frequently used pesticide

(Van Driesche et al., 2006), and has been used with success in reducing thrips populations. To

reduce the risk of resistance developing to this chemical control, other options such as the use of

predators (Van Driesche et al., 2006) or resistant plant varieties (Ullman et al., 1995b) must be

investigated. However, because of the highly competitive market for greenhouse-grown

vegetables and ornamentals, pest damage is not tolerated and alternative thrips management

methods will need to be highly effective.

Nault et al. (2003) states that often the decision to spray is based on the abundance of

total thrips observed rather than known economic thresholds, abundance of thrips species known

to transmit TSWV, or those responsible for direct fruit damage. Identifying the species

composition could provide growers with research-based information upon which to make control

decisions. Parajulee et al. (2006) conducted a study in which they found that visual counts of

WFT gave a reasonable estimate of adult thrips populations. However, there was a significant difference in the number of larvae found using a washing technique on the plant. Jones (2004) proposed that an integrated disease management strategy for TSWV in field crops must incorporate pest monitoring, a wide range of phytosanitary and agronomic control measures, TSWV-resistant cultivars, and appropriate insecticides for the success of cropping systems that are currently facing the devastation from the WFT/ TSWV epidemic.

Before an IPM program can be developed using reduced-risk insecticides it is important to assess if the use of biorational chemicals are a viable and acceptable pest management strategy to incorporate with specific biological control programs (Cloyd, 2006). Results of a study conducted by Van Driesche et al. (2006), showed that the use of spinosad may not be compatible with releases of *Amblyseius cucumeris* (Oudemans) (Acarina: Phytoseiidae) in a crop system. They found that fresh residues (2h) of the spinosad on plant material was not toxic to motile stages of the predatory mite, but that it did affect the oviposition over a two- to three-day period by 48% the second day and 76% the third day. These results suggest that if used conjunctively with a chemical application of the spinosad in an IPM program, predatory mite population growth would be inhibited which would not allow them to effectively suppress WFT populations without supplemental releases of the mites being made. When using reduced-risk insecticides, accurate timing would enable the grower to achieve effective WFT control, while inflicting minimal damage to biological control agents (Jones et al., 2005).

The total world area covered by greenhouses is minimal when compared to the total land area used for agriculture, horticulture, and ornamental crops worldwide; yet the use of biological control is more prevalent in these protected structures than in field crops. Greenhouses provide a unique environmental setting by allowing conditions such as temperature and relative humidity

to be tightly controlled. This ultimately creates opportunities for the use of biological control agents that are sensitive to these conditions (Paulitz et al., 2001). Ideal growing environments that favor high crop productivity may also be the same conditions that favor pest infestation, increase disease spread, or promote efficacy of a biological control agent. For this reason it is vital to understand the dynamics of each of these relationships for the development of effective WFT/TSWV management programs. The sole use of biological control agents in greenhouses may not be sufficient to control phytophagous insect populations like the western flower thrips (van Lenteren et al., 1999, Van Driesche et al., 2006).

The use of phytoseiid mites as biological control agents started with observations of mites preying on *Thrips tabaci* (Lineman) in greenhouse crops (MacGill, 1939; Woets, 1973). *Neoseiulus barkeri* (Hughes) (=*A. mckenziei*) was the first predatory mite specifically used as a biological agent for management of thrips populations (Ramakers, 1980). The introduction of *A. cucumeris* (Oudemans) was ultimately more successful than the *A. mckenziei* and is still the most widely used predatory mite chiefly due to its large scale commercial availability (de Klerk et al., 1986; Messelink et al., 2006). Messelink et al. (2006) determined that commercial availability and costs related to production of other predatory mite species continue to be primary limiting factors.

Messelink et al. (2006) evaluated ten predatory mite species on greenhouse cucumber for control of WFT and found that *A. cucumeris* was less effective in greenhouse crops that did not produce enough pollen. They also found that insecticides were required in order to make a clean start at the beginning of each planting for a greenhouse crop. Integrating a biological control program into a greenhouse system requires a strategic approach as well as a good understanding of the interaction between plant, pest and natural enemy (de Courcy Williams, 2001). Devising a

reliable and cost effective pest management program without the essential understanding of these interactions is fundamentally infeasible (Parrella et al., 1992).

The concept of integrated pest management (IPM) was first introduced in 1959, and has since become essential in systems where pesticides are no longer effective or cannot be used and where use of natural enemies for pest control in a crop is preferred (Hajek 2004). Using several different types of pest management may be necessary to create a thrips management strategy that will ultimately be more effective than a single strategy of chemical control for a greenhouse crop. Shipp and Wang (2003) performed a study that evaluated *A. cucumeris* and *Orius insidiosus* (Hemiptera: Anthocoridae) for control of *F. occidentalis* on greenhouse tomatoes. Based on their results, inundative releases of *A. cucumeris* at a rate of 1 sachet (1000 mites) per plant at intervals of four weeks adequately controlled WFT on greenhouse tomatoes. Shipp and Wang (2003) stated that effective control was not attained until the fifth week after initial biological control implementation as the predatory mites required time to disperse throughout the crop. They were able to demonstrate successful economic control of WFT with the use of *A. cucumeris* as the biological control agent. Shipp and Wang (2003) also concluded that the predatory mites should be introduced before thrips densities exceeded 50-75 adult and larval thrips per plant to protect tomato fruits from direct damage.

In a second study, Shipp and Wang (2006) evaluated *Dicyphus hereperus* (Heteroptera: Miridae) for its ability to control WFT on greenhouse tomato. This predator was capable of completing development and reproducing on tomatoes with WFT as its main food source. However, it did show a tendency to feed on the tomato fruit which tended to correlate with the availability of prey. In this study the authors concluded that *D. hesperus* showed potential to be an effective biological control agent for WFT in a tomato greenhouse crop, but required a higher

release rate than the ≈ 0.1:10 predator: prey ratio that was used for their study to successfully suppress WFT populations below the economically acceptable level (75 thrips per plant) within a reasonably short period of time.

A study done by de Courcy Williams (2001) looked at the effectiveness of *A. cucumeris* releases in controlling WFT in a cyclamen crop. The author states that high inoculative releases of the predatory mite early in the flowering cycle should provide adequate WFT management when coupled with frequent low supplemental releases of the predatory mite. This study also showed that the introduction rate had a significant effect on the level of control of WFT and that higher release numbers gave better control. That is, lower introduction rates of *A. cucumeris* resulted in a delay in thrips control in the cyclamen crop, making frequent inundative releases of high numbers of predatory mites essential for providing preventative control.

Matsuura et al. (2006) performed a study using verbena cvs. Pink Parfait and /or Fancy Parfait as a trap crop to suppress TSWV transmission in chrysanthemums by reducing the occurrence of WFT in the main crop. They concluded that some economic loss would occur as a result of greenhouse production space lost to the trap crop (i.e., 17%-25% of the chrysanthemum crop space was used for the verbena in this study). It was also noted that verbena has the potential to be very effective at preventing WFT infestation, and TSWV infection in chrysanthemums, particularly when combined with other thrips management techniques.

Several studies have demonstrated that early suppression of the WFT is critical for management of TSWV in both field and greenhouse tomato crop production. Aramburu et al. (1997), Moriones et al. (1998), Nault et al. (2002), and Chaisuekul et al. (2003), all found that plants that exhibited virus symptoms earlier produced lower yields than those in which symptoms developed later. Early symptomatic plants also showed more severe foliage and fruit

TSWV symptoms after inoculation. Aramburu et al. (1997) characterized the initial 0-60 day time period directly following transplanting as the critical period that should be focused upon when developing control strategies for management of TSWV in field tomatoes. Nault et al. (2002) found thrips injury to be greatest in the spring tomato crop, but that tomato yield was reduced in both the spring and fall crops. Moriones et al. (1998) found that the greatest overall reduced plant growth was found in the plants with the earliest signs of TSWV. Research from these studies indicates that the prevention of thrips inoculation at the early growing stages of the season needs to be emphasized in TSWV management programs to reduce the impact of TSWV on yield.

In the present study, my research focused on two different methods of virus introduction into the greenhouse crop: through introduction of viruliferous thrips or through the introduction of inoculated plants. Separate thrips pest management methods using the biological control agent *Amblyseius cucumeris* and the spinosad, Conserve®, also were assessed for their relative ability to manage TSWV in the tomato crop. Crop yield and percentage of infected plants at the end of each turn were the primary measures of efficacy. My objectives were to determine whether or not *A. cucumeris* would affect the population of established western flower thrips, to assess if there were differences in the amount of TSWV spread between biological and chemical control of western flower thrips, and to determine if there were differences in the amount of TSWV spread between the two types of virus introduction on the greenhouse crop. To be able to control TSWV through crop management requires an extensive knowledge of TSWV epidemiology in the affected regions (Moriones et al. 1998) for both field and greenhouse crops. This study was designed to compare the efficacy of different thrips management strategies for management of TSWV, in hopes that information from this experiment would help to further our

knowledge and understanding of the complex relationship of *Frankliniella occidentalis* and the

Tomato spotted wilt virus in a greenhouse tomato crop, and to develop alternative management

options.

CHAPTER 2 - The Effectiveness of Biological Control of *Frankliniella occidentalis* in Prevention of the Spread of Tomato Spotted Wilt Virus

Abstract

Western flower thrips, *Frankliniella occidentalis*, is a serious economic pest of ornamental and horticultural crops primarily because it can transmit tospoviruses. Preventative insecticide applications continue to be the primary thrips control used in commercial greenhouses; but reliance on this single tactic raises a variety of issues that point to the need for alternative management options. Therefore, I conducted experiments in greenhouses to compare the relative effectiveness of biological control versus chemical control of western flower thrips as a means of reducing the spread of *Tomato spotted wilt virus* (TSWV) on tomatoes grown under commercial conditions. Tomatoes were subjected to one of three treatments: 1) biological control based on weekly releases of the predatory mite, *Amblyseius cucumeris*, at a commercially-recommended rate, 2) a single chemical treatment with Conserve®, a spinosad formulation, or 3) no treatment. TSWV was introduced into a greenhouse either by starting with 20% of the crop already infected, or making a single release of viruliferous adult thrips. The average number of weeks plants were infected by TSWV was significantly lower in greenhouses in which virus was introduced through infected plants than those into which infected thrips were released. In addition, when virus was introduced by infected thrips, a significantly greater proportion of plants were infected, and crop yields were lower, than when virus was introduced

on infected plants. Among thrips management methods, plants were infected for significantly less time, and the proportion infected was lower in any given week, when biological or chemical control was applied compared to no thrips management. Although there was no difference between biological and chemical control in the length of time that plants showed symptoms, the proportion of infected plants was marginally greater with biological control in weeks 4 and 5 than with chemical control; differences were not significant thereafter. Tomato yields were not affected by thrips management tactic. These findings suggest that inundative releases of biological control may provide as good a level of protection from TSWV as chemical control in commercial greenhouse tomato crops.

Introduction

The western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) is an important worldwide pest of a wide variety of ornamental and horticultural crops in greenhouses as well as broad range of horticultural and agronomic crops grown in the field (Ullman et al., 1995; van Lenteren, 1999; Jones, 2005). WFT is considered a serious economic pest primarily because of its ability to transmit the tospoviruses. WFT is currently understood to be the most efficient vector of Tomato spotted wilt virus (TSWV) and four other tospoviruses: *Impatiens necrotic spot virus*, *Tomato chlorotic spot virus*, *Chrysanthemum stem necrosis virus*, and *Groundnut ringspot* virus (Allen & Broadbent, 1986; German et al., 1992; Ullman et al., 1993, 2002; Wijkamp et al., 1996).

Tospoviruses are the only plant-infecting members of the family *Bunyaviridae*. It is generally accepted that western flower thrips are only able to transmit the TSWV if it is acquired at the earliest larval stage, as the potential for the insect to facilitate virus transmission from virus ingestion rapidly decreases as the thrips mature (Ullman et al., 1996, van de Wetering et al., 1996, de Assis Filho et al., 2004). Once acquired, virus particles replicate inside the thrips bodies, thus making the transmission process exclusively circulative-propagative (German et al., 1992, Ullman et al., 1993, Ullman et. al., 2002). Infected adult WFT transmit TSWV as saliva is injected into the plant during feeding. Virions continue to replicate upon entering the plant cells. Thus, the major factors that ultimately determine the spread of TSWV are both time spent on the host plant and the period required by the vector to acquire or inoculate the virus (Wijkamp et al., 1996).

Preventative insecticide applications continue to be the primary, if not sole, thrips control measure used in commercial greenhouses (Buitenhuis et al., 2006). Currently spinosad, a reduced-risk biorational insecticide derived from a soil microorganism, is the most frequently used pesticide; it has been used with success to reduce thrips populations (Van Driesche et al., 2006a).   However, WFT is a difficult pest to regulate or manage in greenhouse systems because of its cryptic behavior, which makes detection and targeting chemical control difficult (Steiner, 1990). Moreover, chemical applications may be limited by restrictions on frequency of use combined with short-residual time and possible incompatibility with biological control agents used for thrips (Jones et al 2005, Cloyd, 2006, Van Driesche et al., 2006).   In addition, this single-tactic strategy, combined with the high reproductive potential and short generation time of WFT, has resulted in the development of insecticide resistance in WFT to several major classes of chemicals including spinosads (Broadbent and Pree, 1997; Bielza et al., 2007, 2008).   In light of these and other issues associated with chemical pesticide use, there is a need to develop alternative management options.

One non-pesticide management tool for managing TSWV could be biological control of WFT (Van Driesche et al., 2006b). Biological control has been used widely and successfully to manage greenhouse pests, including western flower thrips, on a variety of food and ornamental crops (Hussey and Scopes, 1985, Botto and Lanteri, 1999, Bolkan et al., 2001, Paulitz et al., 2001, Cloyd et al., 2003). Specifically, the use of phytoseiid mites (Acari: Phytoseiidae) as biological control agents for thrips started with observations of phytoseiids attacking *Thrips tabaci* (Lineman) in greenhouse crops (MacGill, 1939; Woets, 1973). *Amblyseius cucumeris* has been the most widely-used predatory mite for use against WFT, chiefly due to its large-scale commercial availability (de Klerk et al., 1986; Messelink et al., 2006). Shipp and Wang (2003)

suggested that *A. cucumeris* can be used to successfully control WFT on tomatoes. However, that study did not consider the role of WFT as a vector of tospoviruses, and it is not known whether biological control of WFT helps control TSWV in greenhouse-grown tomatoes. Early suppression of the WFT is critical for management of TSWV in both field and greenhouse tomato crop production (Aramburu et al., 1997; Moriones et al., 1998; Nault et al., 2002; Chaisuekul et al., 2003). It is generally assumed that curative treatments will not adequately reduce thrips populations to control the threat of virus transmission (Murphy et al., 2004). However, it remains to be determined whether, or under what scenarios, releasing *A. cucumeris* to control WFT might adequately control TSWV.

My research focused on managing TSWV through thrips pest management, comparing industry-recommended release/application rates of the biological control agent *Amblyseius cucumeris* to spinosad (Conserve[®]). I compared these tactics using realistic scenarios of virus introduction and grower response under an IPM strategy; that is, to wait until a pest is detected before taking action. I measured the success of each thrips management tactic under two scenarios of virus introduction at the level of a whole greenhouse. Success was assessed in relation to the management of TSWV in the tomato crop using crop yield and percentage of infected plants at the end of each greenhouse season as measures of efficacy.

Materials and Methods

Cultures and colonies

Plant propagation

Seeds of the beefsteak tomato variety 'Geronimo' (*Lycopersicon esculentum* Mill.) were obtained through a donation from De Ruiter Seeds, Inc., Hybrid Seeds (Lakewood, CO). Plants were propagated in large thrips-proof screened cages inside greenhouses. These cages prevented plants from becoming infested by various arthropods, including thrips which might vector tospoviruses. Planting was done at least five weeks before each round of the experiment was scheduled to begin. At each planting, 500 seeds were sown. This number was sufficient to offset losses due to germination and poor seedling quality; also, because not all plants scheduled for virus treatments became infected, it provided enough plants to obtain the required number of experimental plants. Seeds were planted in 10.5 x 21 x 2.25-inch (26.7 x 53.3 x 5.7-cm) flats at a rate of 40 seeds per flat using a standard soilless aggregate mix (360 Metro Mix, Hummert International, Earth City, MO). Flats were misted with water one to two times each day, and plants were allowed to grow until they had two true leaves, which occurred on average around 21 days. Subsequently, tomatoes were individually transplanted into 3-gallon (11.35-liter) polyethelene bags filled with the 360 Metro Mix and placed on an automated hydroponic watering system.

Thrips colony

The western flower thrips (WFT) used in experiments were from a laboratory colony maintained at Kansas State University. This colony was initiated in 2005 and derived from an

established colony at the University of California, Davis which was originally derived from Hawaii. We reared our WFT on pods of green beans (*Phaseolus vulgaris* L.) in 6 x 6-inch (15.2 x 15.2-cm) Ziploc® containers with snap-on lids (S. C. Johnson & Son, Inc., Racine, WI). Each lid had a 2 x 3-inch (5.1 x 7.6-cm) hole that was covered by a piece of 132-mesh screening (Hummert International, Earth City, MO). The fine-mesh screening prevented thrips from leaving the container while allowing adequate ventilation. Rearing containers were kept in a room with an average temperature of 25°C and 25% RH, and under a 12:12 (L:D) photoperiod.

Virus propagation

We obtained TSWV via infected WFT from the laboratory of Dr. Diane Ullman, University of California at Davis, under USDA-APHIS-PPQ Permit Number 71401 issued to Kansas State University. This virus strain had been isolated originally from severely-infected plants in Hawaii and was propagated on plants (*Datura stramonium* or *Emilia sonchifolia*) using a colony of WFT as vectors. At Kansas State University, we maintained the TSWV isolate on the tomato cultivar 'Geronimo' by transferring uninfected first instar WFT to infected plants and allowing them to develop to adults. After emergence, infective thrips were transferred to new plants for culture or experiments. Occasionally, we used as a source of virus-infected plants (*Emilia sonchifolia* (L.) and *Datura stramonium* L.) obtained from Dr. Anna Whitfield, Department of Plant Pathology, Kansas State University.

Experiments

Greenhouses, irrigation system, and temperature monitoring

Experiments were conducted in four 25 x 25-ft (7.62 x 7.62-m) greenhouses at Kansas State University, Manhattan, Kansas. A shading compound (whitewash) was applied during

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early spring to the glass roof and side panes to aid in temperature reduction in the greenhouses during summer months. In addition, an evaporative cooling system was used to maintain lower temperatures. Cooling pads were outfitted with thrips-proof screening.

Fertigation of tomato plants was controlled automatically with an electronic control box attached to the hydroponic watering system. Water and fertilizer were delivered to each plant through spaghetti tubing, and drainage was allowed to occur through openings in the polyethylene bags. The hydroponic system used 4-18-38 tomato fertilizer at a rate of 0.5 lbs/gal (0.06 kg/l) water, Epsom salt at a rate of 0.25 lbs/gal (0.03 kg/l) water, and calcium nitrate at a rate of 0.5 lbs/gal (0.06 kg/l) water. Dositron injectors diluted the fertilizer at a rate of 100:1. Fertilizer concentration was reduced by 50% two weeks after transplanting. Drip lines delivered approximately 0.5 gallons (1.89 liters) of fertilizer per plant per day throughout the growing season.

Temperature and relative humidity were monitored in all greenhouses every 30 min using HOBO H8 data loggers (Onset Computer, Bourne, MA). Data were uploaded to a computer using BoxCar Pro4 software (Onset Computer, Bourne, MA). Subsequently, daily averages were computed using Microsoft Office Excel 2003 (Microsoft, Inc., Redmond, WA).

Thrips

The western flower thrips in the experiment were approximately the same age (all had emerged within a few hours of one another). To synchronize the age of the thrips, first instars were transferred from bean pods to a separate container using a fine-haired brush within the first few hours of emergence. The virus-free colony was reared in a laboratory separate from the infected thrips used for virus propagation or experiments. No viruliferous colony was

continuously maintained. Therefore, subpopulations of 1,500-3,000 first instars were removed periodically from the virus-free colony for each virus acquisition event.

Plant inoculation and infection

Plants selected for virus transmission were relocated to a separate insect-free greenhouse when they had reached the two true leaf stage and were still in flats. Small fine camel hair brushes were used to transfer three to five viruliferous adult thrips to each selected plant. Thrips were allowed to feed for 24-48 h after which plants were fumigated using No-Pest Strips (Hot Shot® Spectrum, Atlanta, GA) to kill the thrips. Infected tomatoes were then transplanted to polybags and kept in an insect-free greenhouse for at least 2 to 3 weeks for symptoms to develop. All plants selected for the experiment showed distinctive visual symptoms of TSWV infection over a one- to two-week period after thrips inoculation and tested positive using the Immuno-strip on-site enzyme-linked immunosorbent assay ELISA kits (Agdia, Elkhart, IN) for TSWV.

Spatial and experimental design and schedule

We conducted a two-year experiment, consisting of four consecutive trials (turns) beginning winter-spring 2006 and ending in late fall 2007, to compare biological and chemical control of western flower thrips as a means of mitigating the spread of Tomato spotted wilt virus on greenhouse tomatoes. The start and end dates for the four turns were as follows: turn 1: April 15-June 12, 2006; turn 2: December 12, 2006-February 9, 2007; turn 3: April 21-July 3, 2007; turn 4: October 21-December 19, 2007. Turn 1 had an average temperature of 26.67 ± 0.85°C with an average relative humidity of $39.54 \pm 6.55\%$, turn 2 had an average temperature of 20.08 ± 0.78°C with an average relative humidity of $31.95 \pm 4.79\%$, turn 3 had an average temperature of 23.52 ± 1.11°C with an average relative humidity of $38.87 \pm 3.03\%$, turn 4 had an average temperature of 23.27 ± 4.63°C with an average relative humidity of $32.85 \pm 2.21\%$. Within each

turn, the experiment was conducted simultaneously in four 625 ft^2 (58 m^2) greenhouses as

described above. Each greenhouse had 80 tomato plants which were distributed into eight rows

of 10 plants each. Plants were further arranged into four double rows such that each plant in a

given row was paired, resulting in a 2 x 10 grid pattern. Within rows and between adjacent

double-rowed plants, the interplant spacing averaged 12 inches (30.5 cm) between stems. The

double rows were separated from adjacent double rows by approximately four feet. Each tomato

plant was connected to a trellis rope by a small clip and plants were trained to follow the line

vertically. Lateral shoots were trimmed on a regular basis to promote growth of the single main

stem. An electric vibrating probe was used to buzz each flower cluster several times a week to

increase pollination and fruit production. The spatial arrangement of the crop and horticultural

procedures used in the experiment followed typical commercial greenhouse practices.

There were two main treatment factors: thrips management method (3 levels) and virus

introduction strategy (2 levels). Thrips management consisted of biological control, chemical

control, or no control. In the biological control treatment, the predatory mite *Amblyseius*

cucumeris was released at the commercially-recommended rate of 250 mites/m^2/week (Koppert

Biological Systems, Romulus, MI), which resulted in eight weekly applications of 20,000 mites

per treated greenhouse. The chemical control treatment consisted of a single application of

spinosad at the labeled rate of 1 tsp/gal (1.3 ml/l) per greenhouse. In the 'no control' treatment,

thrips were left unmanaged.

We compared thrips management and related TSWV under two virus introduction

strategies which simulated ways in which a producer's crop might become infected with TSWV.

TSWV can enter a greenhouse either in infected plant material or in infected thrips. To simulate

the first scenario we made a uniform hand release of 500 uninfected adult thrips into greenhouses

in which 20% (16 of 80) of the tomato plants were already infected and expressing TSWV symptoms. Thrips were divided evenly into separate containers to insure an even release rate for each individual crop row. Infected plants were randomly spaced throughout the greenhouse using a random number generator for each turn. Under the second scenario, we released 500 viruliferous adult WFT evenly over the tomato crop. In both treatments WFT had molted to the adult stage approximately 72 h prior to release, and releases were done 3 days before any thrips management was applied. The delay of 3 days between thrips introduction and the initiation of action against thrips simulated what we would expect in a commercial greenhouse; that is, a grower would have some lag time between detection and action.

The three thrips management methods, combined with the two virus introduction strategies, resulted in 6 treatment combinations. However, because only four greenhouses were available at any given time, a complete block experimental design was not possible. Instead, at the outset of the study (first turn) we randomly assigned 4 of the 6 treatment combinations to the greenhouses. Then treatments were assigned for the remaining three turns such that the four treatment combinations comprised of biological and chemical control for each of the 2 virus introduction strategies would have three replications, while the no-thrips management treatment applied under each virus introduction strategy would have two replications. Treatments were assigned so that comparisons of greatest interest (biological vs. chemical control) would appear in the same greenhouse and/or turn. This resulted in a 2 x 3 randomized incomplete doubly-blocked design with 16 total greenhouse treatments over the 2-year study period.

Data collection

Individual plants were monitored for visual evidence of TSWV infection in all greenhouse treatments on a weekly basis for 8 weeks. Data was not collected on non-

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experimental, infected plants for the infected plant virus introduction method. Virus symptoms expressed by tomato plants typically include leaf curling, bronzing, concentric ring spots, necrotic streaks, mottling, chlorosis, or lesions. Virus symptoms on tomato fruit may include pale or yellowed skin color, concentric ring spots, uneven blotchy skin color, or even fruit deformity. Mild symptoms in green unripe fruit may consist of pale green or white spots and blotches, which may remain white after ripening or may become pale red or yellow (Allen and Broadbent, 1986). We focused on necrosis, chlorosis, ring pattern (including concentric ring spots), mottling, and stunting as evidence of putative virus infection. Each time a plant was observed, it was assigned a numerical value from 1 to 3. A "1" indicated definite virus symptoms, a "2" denoted that symptoms were ambiguous, and a "3" was assigned if a plant did not exhibit visual symptoms. In cases where a "2" was assigned for a given week, it was converted to either a "1" or a "3" at the end of the experiment depending on whether or not the plant became infected or was uninfected. Infection status was confirmed with Immuno-strips (Agdia, Elkhart, IN). In addition to the terminal assays, a small sample of plants was randomly selected for ELISA testing periodically throughout each turn.

Tomato production was recorded on a weekly basis using the number of mature tomato fruits that were harvested from each plant and the total number of pounds harvested per greenhouse. Tomatoes were evaluated visually, counted, and classified as either marketable or non-marketable. Fruit that expressed TSWV symptoms on the surface such as abnormal coloration, necrotic spots, thrips feeding or oviposition blemishes, were separated and deemed unmarketable. Production and fruit quality data were tracked to the specific location of individual plants in the greenhouse using plant codes and maps. Yield comparisons were based only on marketable fruit.

Statistical analysis

The response data were analyzed according to mixed-effects models, where there were fixed effects for the two treatment factors (thrips management method and virus introduction strategy), and random effects for the two block factors (greenhouse location and turn (time)). Blocking by time was done because environmental conditions, primarily temperature, differed between the fall-winter and spring turns. We blocked by location in case factors associated with specific greenhouses had an effect on responses. We measured incidence of virus and tomato yields. Degree of tospovirus infection was assessed in two ways: by the cumulative number of weeks a plant showed TSWV symptoms, and by the proportion of plants with visual symptoms of TSWV each week. Data collection began week 3, when first symptoms occurred, until week 8 which represented the common ending period for the four turns.

For tomato yields and proportions of weekly virus infection, mixed-model analysis of variance was used to test for main effects of thrips management methods and virus introduction strategies and interactions between them. Models included random blocks, and both blocking factors, turn and greenhouse, had an incomplete assignment of treatments because of the unequal number of treatment combinations (thrips management methods x virus introduction strategies) that could be assigned to any given greenhouse or turn, and because logistical problems in one turn precluded running an intended treatment combination, which resulted in replacing it with an extra replication of an unplanned treatment combination. The cumulative number of weeks plants were infected was analyzed using generalized linear mixed models (mixed-effects logistic regression).

All data were analyzed using SAS software (SAS Institute Inc., 2005). Analysis of variance via PROC MIXED was used for analyzing yield data, and logistic regression via PROC GLIMMIX was used for the analysis of weekly virus incidence data. Pairwise comparisons of

means were performed using the LS MEANS procedure. Pairwise comparisons were performed using ordinary t-tests and parametric-bootstrap adjustments to control the type I error rate for multiple testing (Westfall and Young 1993). Because a high degree of variability was expected in the data and sample sizes were small, the alpha level we used was 0.10 as opposed to the standard level of 0.05. This means that chances of type I errors (false discoveries) are greater than usual in these results, but the use of the adjusted p-values does limit the potential for these to occur.

Results

Symptoms

Tomato spotted wilt virus causes a variety of observable symptoms, including severe deformity in fruit, fruit and leaf necrotic rings, non-uniform ripening of fruit, leaf mottling, leaf chlorosis, concentric ring spots, and stunted plant growth. Tomato plants that expressed TSWV symptoms at an early growth stage showed an increase in stunting of plant growth. One or more virus symptoms were observed in some plants during each eight-week greenhouse turn.   All plants that displayed one or more symptoms tested positive for the virus in the on-site ELISA test.

Length of virus infection

The length of time plants were infected was analyzed as the number of weeks symptoms were observed (Table 3.1). There was no significant interaction between virus introduction method and thrips management tactic in terms of number of weeks plants were infected ($F_{2,4} = 0.89$, $p = 0.4785$). There was a significant main effect for virus introduction method ($F_{1,4} = 8.68$, $p = 0.0405$). In the greenhouses in which virus was introduced through infected plant material, plants showed symptoms for a shorter time (1.6 ± 0.6 weeks) than in greenhouses into which viruliferous thrips were released (2.7 ± 0.6 weeks).   Likewise, there was a significant main effect for thrips management tactics ($F_{2,4} = 11.78$, $p = 0.0211$).   There was no difference in the number of weeks plants showed symptoms of infection between the biological and chemical controls ($t_4 = 1.78$, $p_{Adjusted} = 0.28$). However, plants under both biological and chemical treatments were infected less time than those for which no thrips treatment was applied ($t_4 = -$

3.42, $p_{Adjusted} = 0.06$ for biological control versus no treatment, and $t_4 = -4.85$, $p_{Adjusted} = 0.02$ for chemical control versus no treatment).

Weekly virus incidence

No virus symptoms were observed until 3 weeks after the experiment started. The proportion of infected plants and the statistical analysis of main effects and their interaction for each week from weeks 3-8 are presented in Tables 3.2 and 3.3. There were no significant interactions between virus introduction method and thrips management strategy. From weeks 3-5 the incidence of virus-infected plants was significantly greater in greenhouses in which virus was introduced by infected thrips than in those in which infected plants were the means of introduction, while in week 6 the effect of virus introduction was marginally significant. In the last two weeks there were no significant differences between the different virus introduction treatments in terms of virus incidence. Greenhouses into which virus was introduced in infected plants ended up with 49% of the originally uninfected plants showing virus symptoms, and those in which the virus was introduced in infected thrips ended up with 56% of the plants infected.

Management tactics had a significant effect on weekly virus incidence throughout the length of the experiment (Tables 3.2 and 3.3). The significance of all pairwise comparisons among management treatments are presented in Table 3.3. Plants in greenhouses under biological control showed marginally higher virus incidence than those under chemical control in weeks 4 and 5, but in other weeks were not significantly different. There was no significant difference in virus incidence between biological control and no thrips management in week 4, but in all other weeks greenhouses under biological control had marginally less infection than those with no thrips management. Plants in greenhouses under chemical control consistently

had less infection than those without thrips control. The final percentage of infected plants was 44% under biological control, 37% under chemical control, and 79% with no thrips management.

Effect of TSWV on fruit yield

There was no interaction between virus introduction and thrips management in terms of total fruit yield ($F_{2,4} = 0.36$, $p = 0.7212$).   The method by which virus was introduced into a greenhouse had a significant effect on total fruit yield ($F_{1,4} = 11.69$, $p = 0.0268$), but thrips management tactic did not ($F_{1,4} = 0.53$, $p = 0.6246$). Plants in greenhouses in which virus was introduced using infected plants yielded 8.14 ± 3.84 (mean $\pm$ SEM) fruit per plant, while plants in greenhouses in which virus was introduced using viruliferous thrips yielded 5.38 ± 3.86 (mean $\pm$ SEM) tomatoes per plant.

Figures and Tables

Figure 3.1 Weekly comparison of virus introduction method averaged across all control methods for percentage of plants infected with TSWV.

Weekly comparison of virus introduction method averaged across all control methods
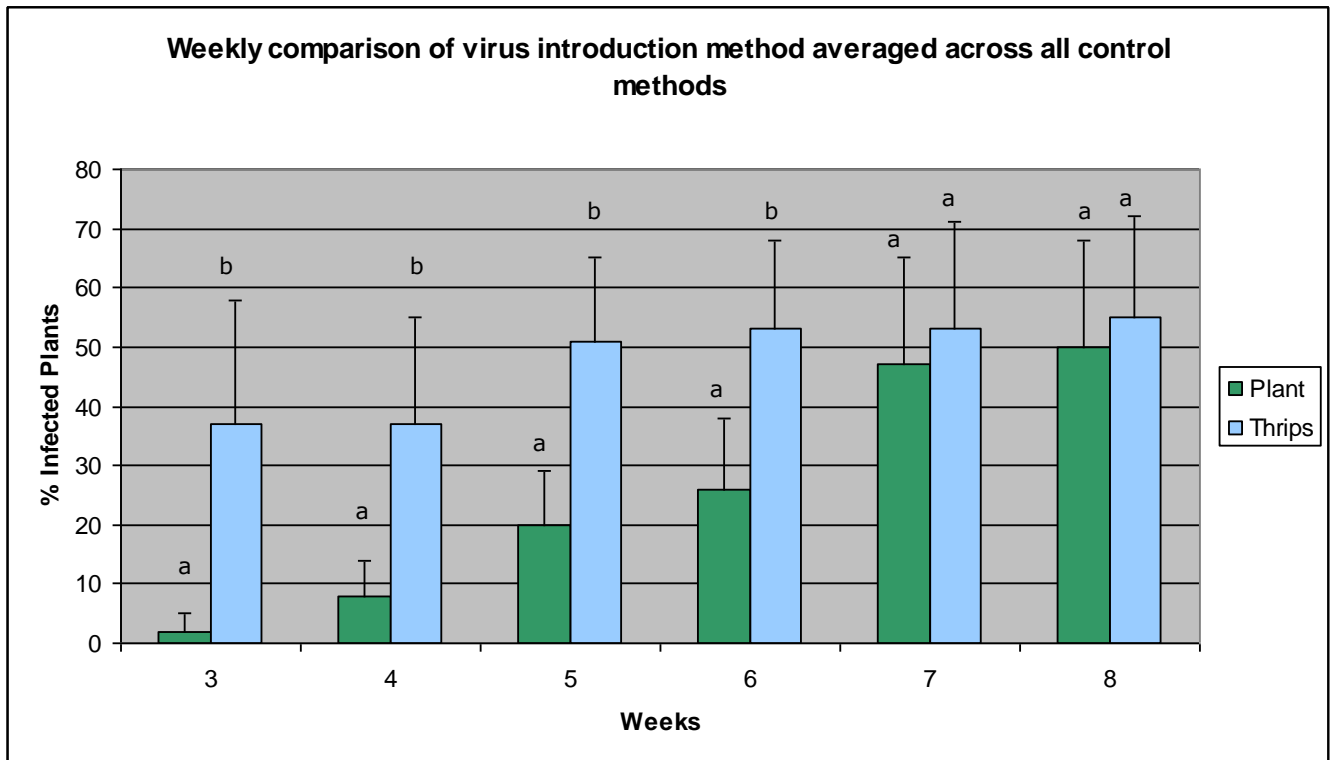
Figure 3.2 Weekly comparison of control methods averaged across both introduction methods for percentage of plants infected with TSWV.
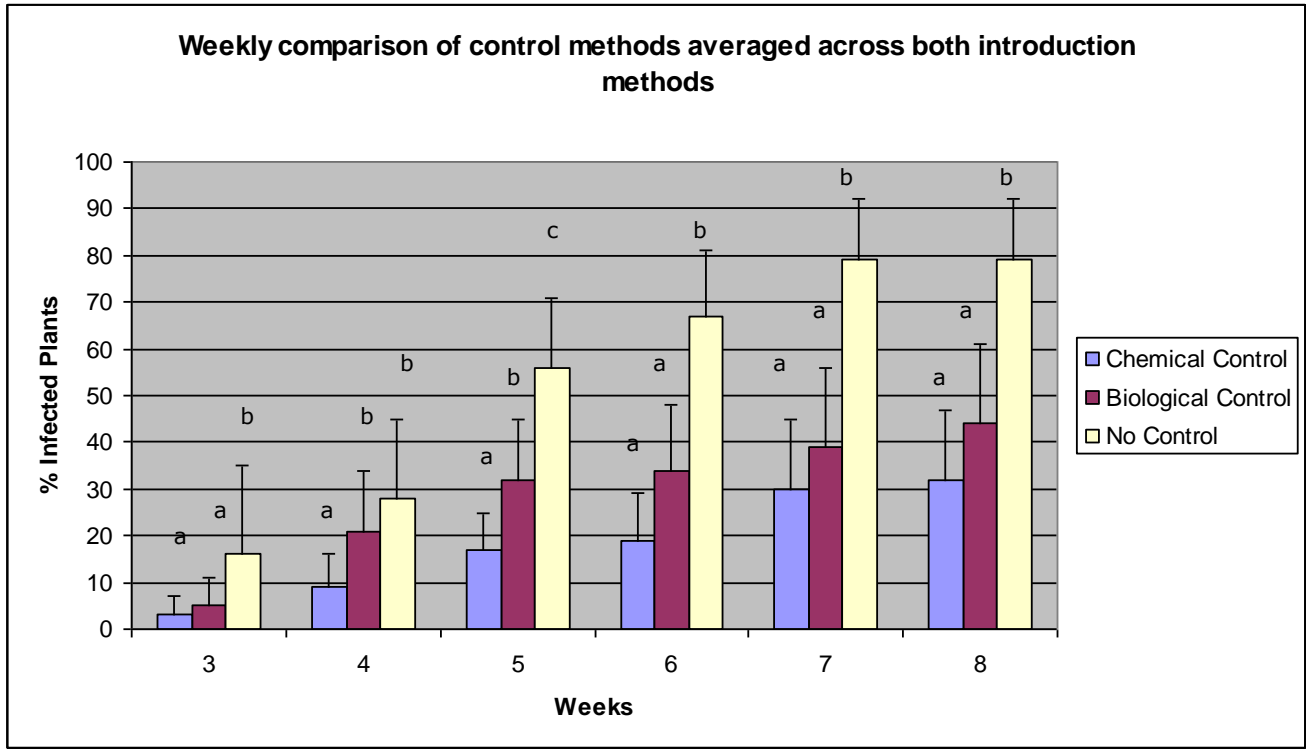
Table 3.1 Number of weeks (Mean ± SEM) tomato plants showed symptoms of infection by Tomato Spotted Wilt Virus as a function of method of virus introduction and thrips management tactic.

Thrips management tactic	Method of virus introduction	
	Infected plants	Infected thrips
Biological	1.07 ± 0.64 a	2.70 ± 0.66 x
Chemical	0.91 ± 0.64 a	1.60 ± 0.64 x
None	2.81 ± 0.71 b	3.97 ± 0.71 y

* There were no significant interactions between virus introduction method and thrips management strategy.

Table 3.2 Weekly incidence (Mean ± SEM proportion) of tomato plants showing symptoms of Tomato Spotted Wilt Virus as a function of the method of virus introduction and the thrips management tactic.

Method of virus introduction	Thrips management tactic	Weeks after virus introduction					
		3	4	5	6	7	8
Infected plants	Biological	0.02 ± 0.02a	0.08 ± 0.06a	0.17 ± 0.09a	0.18 ± 0.10a	0.33 ± 0.18a	0.39 ± 0.19a
	Chemical	0.01 ± 0.01a	0.05 ± 0.04a	0.13 ± 0.07a	0.15 ± 0.09a	0.29 ± 0.17a	0.32 ± 0.17a
	None	0.03 ± 0.04a	0.10 ± 0.09a	0.33 ± 0.15a	0.55 ± 0.18a	0.78 ± 0.17a	0.78 ± 0.17a
Infected thrips	Biological	0.11 ± 0.15xy	0.42 ± 0.21x	0.46 ± 0.21xy	0.50 ± 0.20x	0.55 ± 0.15x	0.57 ± 0.17x
	Chemical	0.09 ± 0.11x	0.17 ± 0.12x	0.22 ± 0.11x	0.25 ± 0.13x	0.31 ± 0.17x	0.32 ± 0.17x
	None	0.57 ± 0.34y	0.56 ± 0.21x	0.76 ± 0.12y	0.77 ± 0.13x	0.80 ± 0.14x	0.80± 0.14x

* There were no significant interactions between virus introduction method and thrips management strategy.

Table 3.3 Significance of effects from mixed logistic regression model analysis of weekly virus incidence.

Effect	Weeks after virus introduction					
	3	4	5	6	7	8
Introduction	$F_{1,4} = 24.48$	$F_{1,4} = 17.24$	$F_{1,4} = 17.51$	$F_{1,4} = 6.44$	$F_{1,4} = 0.16$	$F_{1,4} = 0.11$
	$p = 0.0078$	$p = 0.0142$	$p = 0.0139$	$p = 0.0641$	$p = 0.7057$	$p = 0.7566$
Management	$F_{2,4} = 13.16$	$F_{2,4} = 5.16$	$F_{2,4} = 10.20$	$F_{2,4} = 8.37$	$F_{2,4} = 5.92$	$F_{2,4} = 6.41$
	$p = 0.0174$	$p = 0.0780$	$p = 0.0269$	$p = 0.0372$	$p = 0.0638$	$p = 0.0565$
Interaction	$F_{2,4} = 3.00$	$F_{2,4} = 0.83$	$F_{2,4} = 2.09$	$F_{2,4} = 0.99$	$F_{2,4} = 0.12$	$F_{2,4} = 0.10$
	$p = 0.1602$	$p = 0.4988$	$p = 0.2389$	$p = 0.4462$	$p = 0.8938$	$p = 0.9033$

* There were no significant interactions between virus introduction method and thrips management strategy.

Table 3.4 Significance of differences in weekly virus incidence between thrips management tactics (pairwise comparisons). Adjusted p-values (p_{ADJ}) are corrected to control error rate for multiple testing.

Pairwise comparisons	Weeks after virus introduction					
	3	4	5	6	7	8
Biological v Chemical	$t_4 = 0.96$	$t_4 = 2.21$	$t_4 = 2.74$	$t_4 = 1.95$	$t_4 = 0.81$	$t_4 = 1.13$
	$p = 0.3902$	$p = 0.0920$	$p = 0.0519$	$p = 0.1235$	$p = 0.4615$	$p = 0.3204$
	$p_{ADJ} = 0.6209$	$p_{ADJ} = 0.1783$	$p_{ADJ} = 0.1085$	$p_{ADJ} = 0.2374$	$p_{ADJ} = 0.7196$	$p_{ADJ} = 0.5455$
Biological v None	$t_4 = -2.38$	$t_4 = -0.74$	$t_4 = -2.50$	$t_4 = -2.76$	$t_4 = -2.75$	$t_4 = -2.63$
	$p = 0.0764$	$p = 0.5000$	$p = 0.0668$	$p = 0.0510$	$p = 0.0512$	$p = 0.0584$
	$p_{ADJ} = 0.1400$	$p_{ADJ} = 0.7480$	$p_{ADJ} = 0.1392$	$p_{ADJ} = 0.1019$	$p_{ADJ} = 0.1060$	$p_{ADJ} = 0.1129$
Chemical v None	$t_4 = -4.88$	$t_4 = -2.87$	$t_4 = -4.44$	$t_4 = -4.09$	$t_4 = -3.39$	$t_4 = -3.57$
	$p = 0.0082$	$p = 0.0457$	$p = 0.0114$	$p = 0.0150$	$p = 0.0276$	$p = 0.0234$
	$p_{ADJ} = 0.0152$	$p_{ADJ} = 0.0895$	$p_{ADJ} = 0.0226$	$p_{ADJ} = 0.0321$	$p_{ADJ} = 0.0604$	$p_{ADJ} = 0.0462$

Discussion

In most cases where biological control is used commercially for thrips and other greenhouse pests, a preventative strategy is employed whereby natural enemies are released periodically from the beginning of the cropping cycle without regard to pest presence. However, this approach is costly compared to most chemical controls, thereby contributing to the unwillingness of growers to adopt biological control. A better alternative is to time releases of biological control agents to coincide with early pest appearance, thus conforming to an IPM strategy. However, for biological control to be successful as a reactive treatment against virus vectors like the WFT on greenhouse crops, both the vector population and the plant pathogen must be maintained at low, non-threatening levels. Previous investigators have shown that release of the predatory mite, *Amblyseius cucumeris*, at moderate to high levels can substantially reduce WFT populations on greenhouse-grown cyclamen (de Courcy Williams, 2001) and tomatoes (Shipp and Wang, 2003). However, these studies did not consider the role of biological control in mitigating the spread of tospoviruses. Moreover, neither study compared the efficacy of biological control in relation to chemical control. My experiment evaluated both virus incidence and marketable crop yields of tomatoes, comparing biological control to chemical control under two sets of conditions in which greenhouse crops might become infected by Tomato spotted wilt virus.

The timing of first virus symptoms – at about three weeks -- was the same for greenhouses in which weekly releases of predatory mites were made and those treated with spinosad. However, the rate of infected tomato plants, as measured by the cumulative proportion

of plants expressing symptoms of TSWV, was greater during the middle weeks of the experiment in greenhouses where predators were released than in greenhouses where plants were treated with spinosad. This pattern was observed both when virus was introduced into the greenhouse on viruliferous thrips and when uninfected thrips were released with 20% of the tomato plants already infected with TSWV. Despite the differences in progression of infected plants, marketable tomato yields were comparable for biological and chemical control. The similarity in yields may reflect the fact that there were no statistical differences in the final percentage of plants expressing symptoms, or that tomatoes were not harvested for a longer period. It should also be noted that for this experiment fruit was not ready to be harvested until week 4 or 5 of an eight week long trial. Therefore, yield would have been greater per plant if data had been collected over a longer period as would be the case for a typical crop season. Alternatively, differences in virus rates between the thrips management methods may not have been sufficient to influence yields. However, Moriones et al. (1998) found that tomato plants showing symptoms of TSWV had very low yields of marketable fruit. They also noted that plants developing symptoms early in the growing season produced significantly fewer and smaller tomatoes than plants that were older at the first signs of infection. For many agricultural and horticultural crops, the age at which plants are infected is often one of the most important factors in the amount of economic loss experienced (Taylor et al., 2001, Tolman et al., 2004). For this study it can be concluded that the more time a plant spent uninfected with TSWV, the greater the probability it was able to produce marketable fruit.

Even though experimental yields were not different, producers are very sensitive to the presence of virus on greenhouse crops (Schumacher et al., 2006) and make decisions accordingly. For example, most growers will remove infected plants, or even an entire crop,

when virus is detected. Therefore, I expect that typical tomato yields would be lower in unmanaged compared to thrips-managed crops, and under biological control compared to chemical control, based on the schedule and rate of predators used in this study. However, the fact that experimental yields were similar has implications for grower education programs.

Tomato spotted wilt virus can enter greenhouses by two routes – through infected plants and by invasion in viruliferous thrips vectors. My data demonstrated that the mode of virus introduction had a profound effect on virus incidence and tomato production. Both the amount of time and total percentage of plants that were infected with TSWV were greater, and yields lower, when infected thrips was the source of virus as compared to infected plants. The correspondence between yield and time and proportion of plants infected agrees with the findings of Moriones et al. (1988). This difference is related to the transmission cycle of WFT. That is, when nonviruliferous adult thrips encounter an infected plant, oviposition must occur followed by hatching and acquisition of the virus by first instars as they feed. It is only after second generation thrips complete their life cycle and become adults that infection of new plants is possible as they disperse and feed on healthy plants. In contrast, when viruliferous thrips were released, infection could take place immediately (Wijkamp et al., 1996). Thrips are better transmitters as younger adults as transmission efficiency decreases by day five (Whitfield et al., 2008). Thrips are also able to transmit the virus throughout their entire adult life, which creates a greater probability for the spread of the virus within the first few weeks if viruliferous thrips are introduced into the greenhouse (Ullman et al., 1992). It should be noted that the single release of viruliferous thrips was an uncontrolled source of variability in this experiment as the level of virus acquisition was not estimated for each of the thrips populations before it was released in the greenhouse crop. The differences in results obtained between the two virus introduction methods

are important because the most common route by which TSWV and other tospoviruses likely enter greenhouses is by infected thrips that have visited diseased weeds or crop plants outside of greenhouses. This suggests that use of reactive versus preventative biological control will require efficient and effective thrips monitoring procedures combined with the release of sufficient numbers of predators upon detection of the pest. For a grower to adopt a biological control program it is first necessary to show that biological control will be as effective in managing thrips and tospoviruses as chemical control.

It is noteworthy that while the spread of TSWV occurred at a faster rate initially in greenhouses where viruliferous thrips were released, the first symptoms of virus were detected at about three weeks for both virus introduction methods. Based on the predicted time course of the virus in developing thrips and the transmission and incubation periods within tomato plants, I would have expected visual symptoms of virus to be slightly delayed in greenhouses where uninfected adult WFT were released in the presence of infected plants compared to those in which viruliferous thrips were released. That is, where nonviruliferous thrips were released onto infected plants, oviposition, hatching and development of first instars (which acquire virus) to infective adults capable of transmission would normally take about 10 to 14 days depending on greenhouse temperatures. My observations indicate that an additional 2 weeks is required for plants to express visual symptoms of TSWV, which would total about 3.5 to 4 weeks. Where viruliferous adults were released, transmission should have occurred immediately with only an expected 2-week delay in symptoms.

Because I compared biological to chemical control experimentally under very challenging conditions (high populations of WFT and large inoculum levels of TSWV) whereby a large amount of virus was present either in infected thrips or infected plants, it is difficult to

determine whether releasing *A. cucumeris* at commercially-recommended rates when low densities of WFT are observed would be less effective than spinosad in preventing the spread of TSWV. However, it is likely that in most commercial operations neither thrips infestations nor the amount of tospovirus present would be as high as those tested experimentally. It is also possible that even under conditions where virus is present, increasing the number of predatory mites released would delay the timing and number of plants infected relative to a program based on spinosad treatments. Jacobson (et al., 2001) stated that repeated inundative introductions of *A. cucumeris* may provide a reasonable management strategy for WFT, but he noted the limitation of doing so based on the high costs involved.

Summary and Conclusions

My research provides new evidence that biological control using the predatory mite, *Amblyseius cucumeris*, has potential not only for reducing direct damage from western flower thrips (de Courcy Williams, 2001; Shipp and Wang, 2003), but also for mitigating the spread of Tomato spotted wilt virus. I base this conclusion on a comparison of relative infection schedules, infection rates and crop yields between biological and chemical control. Although in the early stages of infection incidence of the virus differed under biological and chemical control, biological control using recommended rates of *A. cucumeris* was ultimately as successful at controlling the spread of the TSWV as one application of spinosad, Conserve®, at labeled rates. Thus, it may be reasonable to consider biological control as an alternative for reducing the risk of TSWV on greenhouse tomatoes. However, to be commercially successful, additional research is needed under whole greenhouse conditions to develop procedures whereby applications of predators are able to prevent plant infection and maintain yields of marketable tomatoes within acceptable standards.

To achieve this goal, the timing and number of predator releases, as well as release rates, need to be determined. Various suppliers of biological control agents, such as the predatory mite *A. cucumeris*, recommend a variety of release rates for general greenhouse crops. However, because each greenhouse structure and crop layout offers different conditions which may affect predator efficiency, these factors need to be considered and assessed experimentally. My study used a trellising system that allowed plants to reach heights up to 10 feet (~3 meters), but utilized a relatively small amount of floor space. Experimental data are also needed because current recommendations vary greatly among commercial insectaries and other suppliers of biological control. For example, for curative applications in response to heavy thrips infestations, Koppert Biological Systems (Romulus, MI; www.koppert.com), recommends a rate of 250 mites/m^2/week, BetterGrow Hydro (Bell, CA; www.bghydro.com) recommends 10,000 mites per 1,000 ft^2 /week and Greenfire, Inc. (Chico, CA; www.greenfire.net) recommends a rate of 25-250 mites per plant. For applications of beneficials to be effective, the biological control agents need to be introduced into the crop at the right time. When introduced preventatively or with relatively low pest numbers, most biological control agents have a higher potential for suppressing pest population numbers. For a successful pest management program to be implemented it is necessary to become acquainted with both the biology of the biological control agent as well as the pest. Eggs of *A. cucumeris* mature in 8-11 days depending on the relative humidity and temperature in the greenhouse. The adults as well as the first and second nymphal stages will consume approximately 1 thrips larva/day as they will only prey on the early larval stages of the thrips pest. The number of releases of beneficials in the greenhouse crop should be based on the level of the pest population at the time.

There may be limiting factors when implementing a biological control program for a greenhouse crop. In cases where high numbers of viruliferous thrips have already entered the greenhouse, it is essential to implement immediate thrips control tactics to minimize or, ideally, eliminate the possible transmission of the virus. The process of pest suppression in relation to the release of a natural enemy into a crop is a more gradual process when compared to a chemical treatment which has a more immediate suppressive effect on the pest population. Another limiting factor may be the cost of implementing an effective biological control program. To achieve the desired level of pest control adequate release rates and supplemental releases of natural enemies may result in varying weekly pest management costs. Currently, Koppert Biological Systems sells bottles of 50,000 *A. cucumeris* mites for $44.07 a bottle, not including the additional shipping costs. BetterGrow Hydro charges $92.95 and Greenfire charges $70.00 for the same amount not including the shipping costs. The shipping costs should be considered when making a cost comparison to other pest management treatments as other non-biological control products may be ordered in large amounts and have extended shelf lives. In contrast, biological agents require use within a relatively short time after they are packaged and shipped to the grower. As there are restrictions when implementing a biological control program, there are also limiting factors when implementing a strictly chemical based control program for a greenhouse crop. A quart of Conserve SC® costs approximately $140.00 without shipping. The maximum application rate for a tomato crop is 0.2 fl oz/1 gallon of water. There are restrictions that do not allow this product to be applied more than 6 times per growing season in a tomato crop, with no more than 3 consecutive applications made to a generation cycle, nor continuous use for more than 30 days. There is a minimum waiting period of 4 days before reapplying the chemical.

Thrips and viruses cannot be controlled with chemical or biological treatments alone, but require a more comprehensive integrated management plan. Detecting early signs of virus symptoms and populations of thrips before introducing new plants into a greenhouse crop is essential. Growers need to systematically inspect all new plant material as it enters the greenhouse. New plant material also should be isolated from the rest of the greenhouse crop until it is certain they are thrips- and virus-free. Plants that become infected with TSWV should be removed and destroyed immediately. Monitoring for TSWV can be done using an ELISA test or by distributing indicator plants that show TSWV symptoms earlier than other plants throughout the greenhouse. Regular scouting for populations of WFT and TSWV should be carried out on a regular basis. Thrips populations can be monitored with yellow or blue sticky cards. It is important to make sure that other biological control agents are not harmed by the application of natural products such as various plant extracts or minerals, which contain components with a controlling or protecting effect against pests and/or diseases. A chemical treatment such as a spinosad may prove an important element in a well-designed IPM program due to its compatibility with most predators and parasitoids (Williams et al., 2003). To help conserve a predatory mite such as *A. cucumeris*, applications of supplemental food sources can be made to help promote survivorship during times of low pest populations within the crop. IPM programs that incorporate applications of predatory mites, in combination with other natural enemies such as nematodes (Arthurs et al., 2003, Ebssa et al., 2006) or *O. insidiosus* (Shipp and Wang, 2003), may be able to further reduce thrips populations.

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