CHATCHAWAN CHAISUEKUL

Tomato plant age effects on the transmission of *Tomato spotted wilt virus* and insecticide inhibition of the thrips-vector feeding.

(Under the direction of DAVID G RILEY)

Thrips (Thysanoptera: Thripidae) transmission of *Tomato spotted wilted virus* (TSWV) to tomato, *Lycopersicon esculentum* Mill, and feeding behavior were investigated relative to tomato plant age and a systemic insecticide, respectively. Tomato plants at selected ages were inoculated with virus using mechanical and thrips transmission. Both transmission methods were compared in terms of percent infection, symptom development and yield. Percent infection was assessed from Enzyme-Linked Immunosorbent Assay (ELISA) and TSWV symptoms. Plants exhibited symptoms from three weeks up to six weeks after infection. ELISA positive samples correlated with TSWV symptoms, but not in all samples. Tomato yield reduction correlated with the age at which infection of TSWV occurred. Thrips feeding response to tomato plants treated with a systemic insecticide, imidacloprid, was quantified by the number of feeding scars on tomato leaves. Feeding scars decreased as concentration of imidacloprid in tomato leaves increased.

INDEX WORDS: ELISA, Imidacloprid, Inoculation, Insecticide, *Lycopersicon esculentum*, Mechanical transmission, Plant age, Systemic insecticide Thripidae, Thrips, Thrips feeding, Thysanoptera, Tomato plant, *Tomato spotted wilt virus*, *Tospovirus*, Transmission, TSWV.

TOMATO PLANT AGE EFFECTS ON THE TRANSMISSION OF *TOMATO SPOTTED WILT VIRUS* AND INSECTICIDE INHIBITION OF THE THRIPS-VECTOR FEEDING

by

CHATCHAWAN CHAISUEKUL

B.A., University of Delaware, 1998

A Thesis Submitted to the Graduate Faculty

of The University of Georgia in Partial Fulfilment

of the

Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2000

TOMATO PLANT AGE EFFECTS ON THE TRANSMISSION OF *TOMATO SPOTTED WILT VIRUS* AND INSECTICIDE INHIBITION OF THE THRIPS-VECTOR FEEDING.

by

CHATCHAWAN CHAISUEKUL

Approved:

Major Professor: David G. Riley

Committee: Hannu R. Pappu
Darold P. Batzer

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
August 2000

ACKNOWLEDGEMENTS

I would like to thank my major professor Dr. David Riley for his excellent mentoring and advising. Dr. Riley provided me an opportunity to develop my professional skills. My research and thesis could not be accomplished without his guidance. The comments and suggestions from Dr. Pappu and Dr. Batzer as committee members had been invaluable. I thank the staff from Dr. Riley's lab: Dr. Tan, Jackie Davis, Donnie Cook, Tracey Stevens, and others, who helped me to set up and complete my experiments. My ELISA tests on thrips and tomato leaves could not be accomplished without the help and advice from Kippy Lewis and others from Dr. Pappu's lab.

I thank Coastal Plain Experiment Station, and the College of Agricultural and Environmental Sciences for supporting this research. Also, research was financed in part by USDA Southern Region IPM grant # 9804385.

Finally, I owe my success to my parents who have always supported me and encouraged me in my decisions since I can remember.

DEDICATION

To Prince Kritsada, a hard working farmer and reformist, who inspired me.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Tomato spotted wilt virus (TSWV) vectored by thrips (Thysanoptera: Thripidae) causes reduction in both quantity and quality of yield in infected plants. TSWV is a member of the genus *Tospovirus* of family *Bunyaviridae*. All other genera in this family contain virus pathogenic to humans and animals (Wijkamp et al., 1995). TSWV caused major economic damage to agricultural crops worldwide (German et al. 1992). Crops affected by TSWV include tobacco (McPherson et al. 1999), peanut (Stewart et al. 1989), petunia, geranium, chrysanthemum, aster and poinsettia (Gofflot and Verhoyen 1990, Marchoux et al. 1991), eggplant, melon, lettuce, pepper, and tomato (Marchoux et al. 1991, Bautista et al. 1995, Gitiatis et al. 1998). Economic losses due to TSWV in Georgia have been high as $100 million in a single year (Bertrand 1997).

TSWV has been found in at least 16 families of plants, mostly in family Solanaceae and Leguminaceae, after its first detection in tomato in the 1920s' (Brunt et al. 1996). Other families include Amaranthaceae, Amaryllidaceae, Apocynaceae, Asteraceae, Brassicaceae, Bromeliaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cucurbitaceae, Iridaceae, Malvaceae Papaveraceae, Polemoniaceae, and Tropaeolaceae (Brunt et al. 1996).

Symptom expression of plants infected with TSWV varies with plant species and cultivars (Kumar et al., 1993, Roca et al. 1997) and is affected by plant age (Moriones et al. 1998). Symptoms are generally similar to those caused by other plant viruses. Symptoms include stunting, leaf distortion, necrosis, wilting, mosaic, mottling and vein

clearing (Moriones et al. 1998). Black necrotic spots and severe wilting are uniquely distinct to TSWV which makes it easier to distinguish from other plant viruses in tomato. Irregular color patterns in flowers and fruits of tomato are generally found in TSWV-infected plants. Most flowers of infected tomato plants are pale yellow instead of normal bright yellow. Infected tomato fruits usually have mosaic or concentric rings after ripening (Figure 1.1). Some plants can have no symptom (asymptomatic) even though parts of the plant are infected with the virus (H.R. Pappu, unpublished data).

Figure 1.1. Photographs of symptoms of tomato plants infected with *Tomato spotted wilt virus.*

The severity of TSWV on tomato yield depends on time of infection of the plants. For example, when TSWV symptoms start to develop in young tomato plants, pre-blossom period indicate early infection, these infected plants usually develop severe symptoms such as stunting and severe wilting leading to death. However, the plants that

express symptoms when they are older in the blossoming and fruiting stages appear to tolerate the disease, and produce more fruits even though there still may be irregular ripening. Moriones et al. (1998) found that early-infected plants (less than 30 days old) produced less amount of fruits in both quantity and weight compared to late-infected plants. Based on their data, it appears that the effect of plant age at the time of infection plays an important role on plant growth and yield. This information may be critical to identify the period which insecticides or other control tactics can be used effectively. The method used in Moriones et al. (1998) was based on symptomatic plants, and assumptions were made that the plant would exhibit symptoms about 1 to 2 weeks of latent period after infection. Symptoms might not reflect the correct time of infection due to extenuating circumstances, such as level of plant resistance to TSWV (Pang et al. 1992) and temperature (Roggero and Pennazio 1997). Incubation times of TSWV between infection and symptom development have not been fully investigated for most crops.

The use of insecticides to control thrips vectors has been a popular practice although its effectiveness has been questioned (Ullman et al. 1997). In theory, lowering overall thrips populations with insecticides should effectively reduce the spread of TSWV, but insecticides needed to be highly effective when thrips reach the host plant population or they will be ineffective at suppressing primary infection. Moreover, insecticide resistance has been found in many thrips population to the commonly used insecticides. For example, some populations of western flower thrips were found to have resistance against several insecticides, methiocarb (Jensen, 1998), diazinon, methomyl, bendiocarb (Zhao et al., 1995), and cypermethrin (Kontsedalov et al. 1998).

Based on recent developments in TSWV management that certain insecticide treatments were highly effective for TSWV and thrips reduction in tomato (Riley and Pappu 2000), two important factors were selected for investigation. First, experiments were conducted to better define the role of the insecticide, imidacloprid (Admire®, Bayer Corp., Kansas City, MO) in thrips and TSWV management. Secondly, the effects of tomato plant age on transmission and symptom development of TSWV were investigated.

Protection of tomato from TSWV targeted at the thrips vectors is often commercially attempted with insecticides. A systemic insecticide, imidacloprid, has had some effectiveness against incidence of TSWV in field grown peppers when applied as soil drench at early age (D. Rogers, personal communication). This chemical may interact with TSWV, kill thrips, or deter thrips feeding. Changes in thrips feeding behavior in response to imidacloprid was suspected to provide an explanation for lower incidence of TSWV in certain crops since there is a lack of efficacy in terms of thrips mortality (D. Riley, personal communication).

My study used two methods of TSWV transmission, mechanical and thrips mediated, to tomato plants over different plant ages to determine the effects of time of inoculation on the yield. Kumar et al. (1993) used mechanical inoculation and thrips transmission to screen for resistance in multiple cultivars of tomato. They reported that these two methods produced indistinct percentage of TSWV-infected plants, but they concluded that resistance to thrips feeding or transmission behavior can only be screened by directly evaluating thrips transmission.

Purpose of the Study

Tomato spotted wilt could be potentially managed by developing a better management plan for the thrips vectors. Controlling thrips with insecticides is currently practiced in commercial tomato fields, but the effect of imidacloprid is poorly understood and the best time during the crop growth cycle for treatment is also not known. In order to better understand these two constraints, the following two main objectives were developed:

1. To test the effect of imidacloprid on thrips feeding behavior, and
2. To evaluate the effects of plant age at time of TSWV infection on TSWV symptoms and yield.

Hypotheses and Experiments

First, imidacloprid may reduce the transmission of TSWV by deterring thrips feeding, but feeding would have to be arrested completely on the leaf surface. The simplest hypothesis to test here is whether or not imidacloprid affects thrips feeding behavior at all.

$H\text{-}1_o$: Imidaclprid does not reduce thrips feeding on tomato leaves.

$H\text{-}1_A$: Imidacloprid reduces thrips feeding on tomato leaves.

Thrips were caged on tomato plants treated with imidacloprid at various concentrations during August 1999. Feeding response by number of feeding scars were recorded and compared to applied rates and leaf-tissue residues of imidacloprid.

Secondly, greater yield reduction is expected from plants infected with TSWV at an earlier age. The simplest hypothesis to test is whether or not plant age (focus on early

season) at the time of inoculation had any effect on tomato yield and virus symptomology.

$H\text{-}2_o$: Early and late TSWV infected plants will not be significantly different in terms of symptom expression and tomato yield.

$H\text{-}2_A$: Early infection of TSWV in tomato plants will reduce yield more and have earlier symptom expression than later TSWV infected plants.

Two methods of TSWV transmission, mechanical transmission and thrips transmission, were compared on 1,2 and 4 week-old tomato plants in the same experiment during May-August, 1999. The two techniques were conducted separately during March-June, 2000 to determine the effects of time of inoculation relative to the yield of tomato fruits using 1,2,3,4,5, and 6 week-old tomato plants for thrips transmission and 2,3,4,5,6, and 7 week-old tomato plants for mechanical transmission. Also, within the thrips transmission technique, two methods of inoculation of different age tomato plants were used: 1) inoculating plants over time with different viruliferous thrips populations with all plants planted in the field at the same time and 2) inoculating different age plants grown in pots in the greenhouse from a single viruliferous thrips population and then transplanting the pots to the field. The growth and TSWV symptoms of tomato were recorded and analyzed with the fruit yields, which was measured in terms of quantity by total weight and marketable quality of individual fruits.

Literature Cited

Bautista, R.C., Mau, R.F.L., Cho, J.J., and Custer, D.M. 1995. Potential of *Tomato spotted wilt virus* plant hosts in Hawaii as virus reservoirs for transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae). Phytopathology 85: 953-958.

Bertrand, P.F. 1997. 1996 Georgia plant disease losses. University of Georgia Cooperative Extension Service Publication 1-97: 1.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) 1996. `Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' URL http://biology.anu.edu.au/Groups/MES/vide/.

German, T. L., Ullman, D. E., and Moyer, J. W. 1992. Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. Annu. Rev. Phytopathol. 30: 315-348.

Gofflot, A. and Verhoyen, M. 1990. Heavy progression of *Tomato spotted wilt virus* infections transmitted by the thrips *Frankliniella occidentalis* in ornamental greenhouse crops in Belgium. Parasitica 46: 85-88.

Jensen, S.E. 1998. Acetylcholinesterase Activity Associated with Methiocarb Resistance in a Strain of Western Flower Thrips, *Frankliniella occidentalis* (Pergande). Pesticide Biochem. Physiol. 61: 191-200.

Kontsedalov, S., Weintraub, P.G., Horowitz, A.R., and Ishaaya, I. 1998. Effects of Insecticides on Immature and Adult Western Flower Thrips (Thysanoptera: Thripidae) in Israel. J. Econ. Entomol. 91: 1067-1071.

Kumar, N.K.K., Ullman, D.E., and Cho, J.J. 1993. Evaluation of Lycopersicon germ plasm for *Tomato spotted wilt virus* resistance by mechanical and thrips transmission. Plant Dis. 77: 938-94.

McPherson, R.M., Pappu, H.R., and Jones, D.C. 1999. Occurrence of five thrips species on flue cured tobacco and impact on spotted wilt disease incidence in Georgia. Plant Dis. 83: 765-767.

Moriones, E., Aramburu, J., Riudavets, J., Arno, J., and Lavina, A. 1998. Effect of plant age at time of infection by *Tomato spotted wilt virus* on the yield of field-grown tomato. Eur. J. Plant Pathol. 104: 295-300.

Pang, S.Z., Nagpala, P., Wang, M., Slightom, J.L., and Gonsalves, D. 1992. Resistance to heterologous isolates of *Tomato spotted wilt virus* in transgenic tobacco expressing its nucleocapsid protein gene. Phytopathology 82: 1223-1229.

Riley, D.G. and Pappu, H.R. 2000. Evaluation of tactics for management of thrips-vectored *Tomato spotted wilt tospovirus* in tomato. Plant Dis. 84:847-852.

Roca, E., Aramburu, J., and Moriones, E. 1997. Comparative host reactions and *Frankliniella occidentalis* transmission of different isolates of *Tomato spotted wilt virus* from Spain. Plant Pathol. 46: 407-415.

Roggero, P. and Pennazio, S. 1997.Thermal inactivation of *Tomato spotted wilt virus* in vivo. Physiol. Mol. Plant Pathol. 51: 35-40.

Terry, L.I. 1997. Host Selection, Communication and Reproductive Behaviour. pp. 65-118, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Ullman, D.E., Sherwood, J.L., and T.G. German. 1997. Thrips as Vectors of Plant Pathogens, pp. 539-565, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Wijkamp, I., Almarza, N., Goldbach, R., and Peters, D. 1995. Distinct levels of specificity in thrips transmission of Tospoviruses. Phytopathology 85: 1069-1074.

Zhao, G., Liu, W., Brown, J.M., and Knowles, C.O. 1995. Insecticide resistance in field and laboratory strains of western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1164-1170.

CHAPTER 2

REVIEW OF LITERATURE

Transmission of *Tomato spotted wilt virus*.

Brittlebank (1919) first reported *Tomato spotted wilt virus* (TSWV) from Australia in 1916 in tomato, *Lycopersicon esculentum* Mill. TSWV used to be known by other names, *Groundnut ring spot virus*, *Tomato chlorotic spot virus*, *Dahlia oak leaf virus*, *Dahlia ring spot virus*, *Dahlia yellow ring spot virus*, *Mung bean leaf curl virus*, *Pineapple yellow spot virus*, and *Watermelon silver mottle virus* (Brunt et al. 1996). However, these names were actually different species of viruses in genus *Tospovirus* (Wijkamp et al. 1995). *Impatiens necrotic spot virus*, a TSWV-like virus first found in *Impatiens species*, used to be considered to be a strain of TSWV, is now recognized as a distinct member of the genus *Tospovirus* (de Avila et al., 1993). The serological detection of TSWV by enzyme-linked immunosorbent assay (ELISA) has been accurate in both plants (Marchoux et al. 1991) and thrips (Cho et al. 1988).

Virions of TSWV are 80-100 nm in diameter and contain 5 % nucleic acid, 70 % protein, 20 % lipid, and 5% carbohydrate (Elliot 1990). The genome of TSWV contains three segments of circular single stranded RNA: RNA-L (negativesense), RNA-M (ambisense), and RNA-S (ambisense), and these three segments are shown to undergo reasortment (Qiu and Moyer 1999).

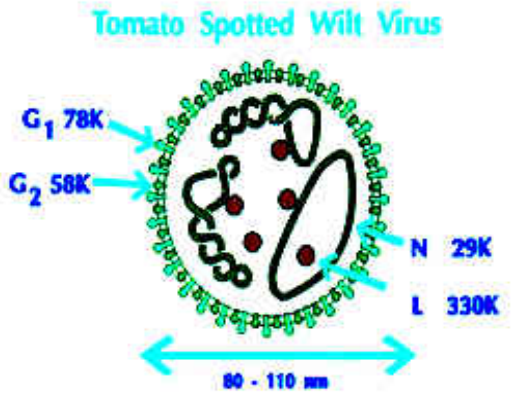


Figure 2.1. Structure of *Tomato spotted wilt virus* from Moyer et al. (1999). G_1 and G_2 are envelope proteins. N is the nucleocapsid and L is RNA-dependent RNA polymerase.

Many insect herbivores, especially those with sucking mouthparts, are highly efficient in the transmission of plant viruses. However, TSWV is transmitted by only a particular group of thrips (Thysanoptera: Thripidae), mainly *Thrips spp*. and *Frankliniella spp.* (Wijkamp et al. 1995, Ullman et al. 1997). TSWV is transmitted in a persistent manner, which requires a period of incubation in the vector host before being transmitted to the plant host (Ullman et al. 1997). Thrips only acquire the virus in their immature stages, and immature of *F. occidentalis* can acquire the virus as early as 30 minutes and transmit the virus 24 hours after feeding on infected plant tissue (Wijkamp et al. 1996[c]). A study in the Netherlands by Van De Wetering et al. (1996) reported that only the first instar of *F. occidentalis* could acquire the virus while other studies of *F. occidentalis* populations from other regions of the world reported both first and second instars could acquire the virus (Ullman et al. 1997). TSWV was found to be associated with salivary glands of thrips (Ullman et al. 1996). Immature thrips retain virus and infectability through out their life (Wijkamp et al. 1995). However, TSWV can not be

transmitted longitudinally (from infected parents to their gametes or their progenies) in the thrips vectors (Wijkamp 1996[b]). Since TSWV can not persist in thrips populations longer than 1 generation, alternative plant hosts (especially weeds) act as reservoirs for virus acquisition by subsequent thrips generations (Stewart et al. 1989, Johnson et al. 1996). Besides thrips transmission, TSWV could also be transmitted by mechanical inoculation, vegetative propagation, and grafting, but does not transmit through pollen and seed (Moyer et al. 1999).

Thrips as Viral Vectors.

Thrips, a group of tiny insects in order Thysanoptera, have been found to be vectors of at least four plant virus groups (families), bunyaviruses, ilarviruses, sobemoviruses, and caroviruses (Ullman et al. 1997). As many as 8 species of thrips have been reported to transmit TSWV (Wijkamp et al. 1995). *Thrips tabaci* Lindeman, *T. setosus* Moulton, *T. palmi* Karny, *Frankliniella schultzei* Trybom, *F. occidentalis* (Pergande), *F. fusca* (Hinds), and *F. intonsa* Trybom were reported to be vector of TSWV (Wijkamp et al. 1995, Ullman et al. 1997). Webb et al. (1997) also reported *F. bispinosa* (Morgan) as a vector of TSWV. *Frankliniella tenuicornis* (Uzel) *and Scirtothrips dorsalis* Hood had been previously reported to be vector of TSWV, but experimental verification has not been strongly proved (Ullman et al. 1997). *F. occidentalis* and *T. tabaci* are common vectors of multiple plant viruses (Ullman et al. 1997). In the field, practically all TSWV-infected plants are infested by thrips (Kumar et al. 1993).

Most thrips are herbaceous while some are predacious especially on the other species of thrips. TSWV-vectors are all herbaceous and polyphagous in the family

Thripidae (Wijkamp et al. 1995). Western flower thrips, *F. occidentalis*, is a major vector of TSWV, and high population densities of this thrips have been associated with the incidence of TSWV (Aramburu et al. 1997). *F. fusca* is also a major vector in peanut (Chamberlin et al. 1992), and is reported to be associated with weed hosts during winter (Johnson et al. 1995).

Figure 2.2. Photograph of western flower thrips, *Frankliniella occidentalis*, image by David G. Riley.

The life cycle of most TSWV thrips vectors is approximately 12-16 days. Female adult thrips lays her eggs in plant tissue, mostly floral tissue (Terry 1997). The larvae (wingless) hatch in about three days, survive in winter as diapaused first instars, and enter two or more instars before entering the prepupal and pupal stages in the soil (Moritz 1997). The pupae emerge as winged adults (some *F. fusca* are brachypterous) and migrate to plants by following visual and chemical cues (Terry 1997). Brachypterous

adults of *F. fusca* were reported to migrate to adjacent peanut fields during late spring after overwintering in old peanut fields (Chamberlin et al. 1992).

TSWV is transmitted by thrips in a persistent manner (requires a latent period after ingestion of virus before transmission) (Ullman et al. 1997). Several species of thrips could transmit TSWV, but Wijkamp et al. (1995) demonstrated that western flower thrips, *F. occidentalis,* was the most effective vector for TSWV when compared to *F. intonsa, F. shcultzei* and *T. tabaci.* Besides TSWV, *F. occidentalis, F. intonsa* and *F. shcultzei* have also been reported to transmit other *Tospoviruses* including: *Impatiens necrotic spotted virus* (INSV), *Groundnut ring spot virus* (GRSV), and *Tomato chlorotic spot virus* (TCSV) (Wijkamp et al. 1995). *T. tabaci* has been reported to transmit TSWV, and a decline in TSWV transmission efficiency has been noted in *T. tabaci* over the past 30 years (Ullman et al. 1997).

Female western flower thrips have been shown to be less efficient in transmitting TSWV, even though female thrips feed more than male thrips (Van de Wetering et al., 1998). Although TSWV replicates in infected thrips, it does not affect thrips fecundity or survivorship (Wijkamp et al. 1996[b]). Thrips acquire virus when they are first and second instars and maintain the infectability through out their life, but they cannot pass virus to offsprings (Ullman et al. 1996). Adult thrips which did not acquire TSWV during their larval stages cannot transmit virus because of a midgut barrier (Ullman et al. 1992).

At certain times of the year such as summer, thrips populations can increase as well as the number of infected individual thrips as temperature increased (Boissot et al. 1998). Bautista et al. (1995) reported that adult thrips preferred to land and feed on TSWV-infected plants, and diseased plants had higher larval yields compared to non-

Table 2.1. Plant viruses transmitted by thrips and their acquisition/transmission time.

Thrips species	Virus transmitted (Ullman et al 1997)	Acquisition/ Transmission time	Reference
Frankliniella occidentalis	Tospovirus		
	TSWV[1]	30 minutes/ 24 hours	Wijkamp et al. 1996[c]
	INSV[1], GRSV[1], TCSV[1]	12 hours/ 72 hours	Wijkamp et al. 1995
	Pollen borne viruses	-	
	PNRSV[2], PDV[2]		
F. intonsa	TSWV, TCSV	12 hours/ 72 hours	Wijkamp et al. 1995
	GRSV	-	
F. shcultzei	TSWV, TCSV, GRSV	12 hours/ 72 hours	Wijkamp et al. 1995
F. bispinosa	TSWV	-	
F. fusca	TSWV	-	
Thrips tabaci	TSWV, PNRSV, TSV[2], SoMV[3]	-	
T. palmi	WSMV[1],GBNV[1]	-	
T. setosus	TSWV	2 hours/ 24-96 hours	Tsuda et al. 1996

[1]Bunyaviridae: (TSWV) *Tomato spotted wilt virus*, (TCSV) *Tomato chlorotic spot virus*, (GRSV) *Groundnut ringspot virus*, (INSV) *Impatiens necrotic spot virus*, (GBNV) *Groundnut bud necrosis virus*, (WSMV) *Watermelon silver mottle virus*
[2]Ilarviridae: (PNRSV) *Prunus necrotic ringspot virus*, (PDV) *Prune dwarf virus*, (TSV) *Tobacco streak virus*
[3]Sobemoviridae: (SoMV) *Sowbane mosaic virus*

infected plants. Thrips feed on plant tissue by probing their mouthparts into sub-epidermal cells and draw cell contents from the breakage into their mouth (Kirk, 1997). Their feeding habit causes physical damage to plant tissue as well as facilitating transmission of plant viruses. The damage from thrips feeding alone may not be significant to plant growth and yield. However, with the combination of thrips and

TSWV, infected plants can be drastically decreased in growth and yield in both quantity and quality (Fajardo et al. 1997, Moriones et al. 1998, Riley and Pappu 2000).

Management of *Tomato spotted wilt virus*

One approach for the management of tomato spotted wilt consists of first identifying and classifying the virus and vector species, then selecting effective integrated pest management (IPM) control tactics, and developing a set of decision criteria for implementing the use of these tactics (Riley 1997). For example TSWV can readily be identified by ELISA (Marchoux et al. 1991), but identifying the most critical thrips vector species in the tomato crop system is important for selecting the appropriate control tactic. The control tactics could include host plant resistance (Culbreath et al. 1997, Diez et al. 1999, Sherman et al. 1996), insecticides (Chamberlin et al. 1992), cultural controls (Chamberlin et al. 1992), screens or other physical barriers (Diez et al. 1999), etc., but ranking these tactics for effectiveness can be complicated. The decision criteria, for example in peanut, include timing of insecticide treatments for the maximum benefit at the minimum cost, selecting planting dates and selecting resistant cultivars (Brown et al. 1998). Of all of the control tactics, plant resistance to virus or insect vectors has been reported to be highly effective strategy against TSWV (Cho et al. 1989, Sherman et al. 1996). The resistance could be selected from suitable cultivars (De Jager et al. 1995, Kumar et al. 1993), species (Kumar et al. 1995), or developed with genetic engineering (Sherman et al. 1996, Wijkamp et al. 1996[a]). Combination of genes, such as green fluorescent protein (GFP) and nucleocapsid (N), provided resistance to TSWV infection (Jan et al. 2000). Even so, it is possible for TSWV to overcome resistance, especially

when a single resistant gene is involved, possibly by genome reassortment (Qiu and Moyer 1999).

Insecticide use can be an effective tactic to control thrips. For example, imidacloprid: (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine), a systemic insecticide, has been observed to reduce incidence of TSWV in pepper and tomato but not in all cases (D. Rogers, personal communication). Riley and Pappu (2000) found that soil applied imidacloprid in combination with foliar appied insecticides could increase tomato yield by as much as 50% compared to a control.

Imidacloprid is also commercially known as 1.6 F Gaucho®, Admire® 2F, Confidor®, and Provado® (Bayer Corp. Kansas City, KS) (Thomson 1994). It has been approved in several crops as a systemic and contact insecticide (table 2.1). Imidacloprid is most effective against plant sucking insects, such as aphids, leafhoppers, planthoppers, whiteflies, and thrips, moderately effective against some Coleopteran insects such as Colorado potato beetles, Leaf beetles, Wireworms, and Ricewater beetles, and no activity against nematodes or mites (Thomson 1994). Imidacloprid has been reported to have negative impact to beneficial insects such as coccinellid predator, *Coleomegilla maculata* (Smith and Krischik 1999). Application rate of imidacloprid depends on the crop system and other management methods of insect pests (Thompson 1994).

Imidacloprid has low lethal dose to insect vectors of various viral diseases. It works by interfering with the transmission of stimuli in the insect nervous system, (Kidd, H. and D. James (eds.). 1994). Imidacloprid is selectively more toxic to insects than warm-blooded animals because it targets nicotinergic pathway that is more abundant in insects than in warm-blooded animals. The blockage of nicotinergic pathway leads to the

Table 2.2. Labeling and application information for imidacloprid (Admire 2F®). (Thomson 1994, Guillibeau 1999).

Treatment	Crops
Seed treatment: dressing, pelleting	Sugar beet, cereals, maize, sunflower, cotton
Soil treatment: granules, liquid application, tablets	Rice, vegetables, potatoes, lawns, ornamental plants, tobacco
Leaf treatment: sprays	Pomaceous and stone fruit, cotton, vines
Stem treatment: brush application (painting)	Citrus fruit, hops, pomaceous fruit

accumulation of acetylcholine resulting in the insect's paralysis, and eventually death. Imidacloprid effects on reduction of feeding behavior and reproduction rate have been reported on aphids (Boiteau and Osborn 1997).

Imidacloprid could be applied to plants in several methods (Table 2.1) but tomato and other vegetables are generally treated with soil application in the form of Admire 2F (Guillibeau 1999). Young tomato plants, 3 to 4 weeks old, are usually treated with imidacloprid as soil drench (Guillibeau 1999). Plants then take up imidacloprid through the roots and deposit the chemical in their tissues that will last up to 70 days, and imidacloprid in leaf tissue will dilute through time as plants grow (Thompson 1994). However, the diluted amounts of imidacloprid present in the leaf could sufficiently protect plants from sucking insects, depending on factors such as the initial amount applied and the critical time in the plant growth cycle for vector control (Kidd and James 1994). One unknown factor with imidacloprid treatment is how the antifeedant behavior induced by imidacloprid in aphids (Boiteau and Osborn 1997) is affected by starvation in the vector species. There is limited information on the effects of imidacloprid on thrips behavior. The effect of imidacloprid on thrips feeding is reported in the following chapter.

Literature Cited

Aramburu, J., Riudavets, J., Arno, J., Lavina, A., and Moriones, E. 1997. The proportion of viruliferous individuals in field populations of *Frankliniella occidentalis*: Implications for *Tomato spotted wilt virus* epidemics in tomato. Eur. J. Plant Pathol. 103: 623-629.

Bautista, R.C., Mau, R.F.L., Cho, J.J., and Custer, D.M. 1995. Potential of *Tomato spotted wilt virus* plant hosts in Hawaii as virus reservoirs for transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae). Phytopathology 85: 953-958.

Boissot, N., Reynaud, B., Letourmy, P. 1998. Temporal Analysis of Western Flower Thrips (Thysanoptera: Thripidae) Population Dynamics on Reunion Island. Environ. Entomol. 27: 1437-1443.

Boiteau, G. and Osborn, W.P.L. 1997. Behavioural effects of imidacloprid, a new nicotinyl insecticide, on the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera, Aphididae). Can. Entomol. 129: 241-249.

Brittlebank, C.C. 1919. Tomato diseases. J. Agric. Victoria. Aust. 17: 231-235.

Brown, S.L., Todd, J.W, Culbreath, A.K. Baldwin, J.A., Beaslet, J.P., and Pappu, H.R. 1998. Tomato spotted wilt of peanut: Identification and avoiding high risk situation. University of Georgia Cooperative Extension Service, report#B1165 pp. 1-3.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) 1996. `Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' URL http://biology.anu.edu.au/Groups/MES/vide/.

Chamberlin, J.R., Todd, J.W., Beshear, R.J., Culbreath, A.K., and Demski,, J.W. 1992. Overwintering hosts and wingform of thrips, *Frankliniella spp*., in Georgia (Thysanoptera: Thripidae): Implications for management of spotted wilt disease. Environ. Entomol. 21: 121-128.

Cho, J.J., Mau, R.F.L., Hamasaki, R.T., German, T.L., Hartman, R.W., Yudin, L.S., Gonsalves, D., and Provvidenti, R. 1989. A multidisciplinary approach to *Tomato spotted wilt virus* management of in Hawaii. Plant Dis. 73:375-383.

Cho, J.J., Mau, R.F.L., Hamasaki, R.T., and Gonsalves, D. 1988. Detection of *Tomato spotted wilt virus* in individual thrips by enzyme-linked immunosorbent assay. Phytopathology 78: 1348-1352.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Shokes, F.M., and Pappu, H.R. 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis. 81:1410-1415.

De Avila, A. C., de Haan, P., Kormelink, R., Resende, de O., Goldbach, R.W., and Peters, D. 1993. Classification of Tospoviruses based on phylogeny of nucleoprotein gene sequences. J. Gen. Virol. 74: 153-159.

De Jager, C.M., Butot, R.P.T., Klinkhamer, P.G.L., De Jong, T.J., Wolff, K., Van der Meijden, E. 1995. Genetic variation in chrysanthemum for resistance to *Frankliniella occidentalis*. Entomol. Exp. Appl. 77: 277-287.

Diez, M.J., Rosello, S., Nuez, F., Costa, J., Lacasa, A., Catala, M.S. 1999. Tomato production under mesh reduces crop loss to tomato spotted wilt virus in some cultivars. Hort. Science. 34: 634-637.

Fajardo, T.V.M., Lopes, C.A., Silva, W.L.C.E., and Avila, A.C.D. 1997. Dispersal of disease and yield reduction in processing tomato infected with *Tospovirus* in the Federal District, Brazil. Fitopatol. Bras. 22: 413-418.

Guillibeau, P. (ed.) 1999. Georgia Pest Control Handbook. Cooperative Extension Service, University of Georgia, pp. 137-213.

Jan, F.J., Fagoaga, C., Pang, S.Z., and Gonsalves, D. 2000. A minimum length of N gene sequence in transgenic plants is required for RNA-mediated *Tospovirus* resistance. J. Gen. Virol. 81: 235-242.

Johnson, R.R., Black, L.L., Hobbs, H.A., Valverde, R.A., Story, R.N., and Bond, W.P. 1995. Association of *Frankliniella fusca* and three winter weeds with *Tomato spotted wilt virus* in Louisiana. Plant Dis. 79: 572-576.

Johnson, W.C. III, Todd, J.W., Culbreath, A.K., and Mullinixx, B.G. Jr. 1996. Role of warm-season weeds in spotted wilt epidemiology in the southeastern Coastal Plain. Agron. J. 88: 928-933.

Kidd, H. and D. James (eds.). 1994. Agrochemicals Handbook. Third Edition. Royal Society of Chemistry. Cambridge, UK.

Kirk, W.D.J. 1997. Feeding, pp. 119-174, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Kumar, N.K.K., Ullman, D.E., and Cho, J.J. 1993. Evaluation of *Lycopersicon* germ plasm for *Tomato spotted wilt virus* resistance by mechanical and thrips transmission. Plant Dis. 77: 938-94.1

Kumar, N.K.K., Ullman, D.E., Cho, J.J. 1995. Resistance among *Lycopersicon species* to *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1057-1065.

Marchoux, G., Gebre-Selassie, K., and Villevieille, M. 1991. Detection of *Tomato spotted wilt virus* and transmission by *Frankliniella occidentalis* in France. Plant Pathol. 40: 347-351.

Moriones, E., Aramburu, J., Riudavets, J., Arno, J., and Lavina, A. 1998. Effect of plant age at time of infection by *Tomato spotted wilt virus* on the yield of field-grown tomato. Eur. J. Plant Pathol. 104: 295-300.

Moritz, G. 1997. Structure, Growth and Development, pp. 15-63, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Moyer, J.W., German, T.G., Sherwood, J.L., and Ullman, D.E. 1999. 'An Update on *Tomato spotted wilt virus* and Related Tospoviruses Version: 7th August 1999.' URL http://www.scisoc.org/feature/tospovirus/Top.html.

Qiu, W. and Moyer, J.W. 1999. *Tomato spotted wilt virus* adapts to the TSWV N gene-derived Resistance by genome reassortment. Phytopathology 89: 1-10.

Riley, D.G. and Pappu, H.R. 2000. Evaluation of tactics for management of thrips-vectored *Tomato spotted wilt tospovirus* in tomato. Plant Dis. 84: 847-852.

Sherman, J.M. Moyer, J.W., and Daub, M.E. 1996. Genetically engineered resistance to *Tomato spotted wilt virus* in chrysanthemum (*Dendranthema grandiflora*): a model system for virus protection in ornamental crops. Acta Hort. 431:432-441.

Smith, S.F. and Krischik, V.A. 1999. Effects of Systemic Imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae) Environ. Entomol. 28: 1189-1195.

Stewart, J.W., Cole, C., and Lummus, P. 1989. Winter survey of thrips (Thysanoptera: Thripidae) from certain suspected and confirmed hosts of *Tomato spotted wilt virus* in south Texas. J. Entomol. Sci. 24: 392-401.

Terry, L.I. 1997. Host Selection, Communication and Reproductive Behaviour. pp. 65-118, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Thomson, W.T. (ed) 1994. Agricultural Chemicals: Book I. Thomson Publication, Fresno, CA. p. 158.

Tsuda, S., Fujisawa, I., Ohnishi, J., and Hosokawa, D. Tomaru, 1996. Localization of tomato spotted wilt tospovirus in larvae and pupae of the insect vector *Thrips setosus* Phytopathology 86: 1199-1203.

Ullman, D.E., Cho, J.J., Mau, R.F.L., Hunter, W.B., Westcot, D.M., and Custer, D.M. 1992. Midgut epithelial cells act as a barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. Phytopathology 82: 1333-1342.

Ullman, D.E., Westcot, D.M., Chenault, K.D., Sherwood, J.L., German, T.L., Bandla, M.D., Cantone, F.A., and Duer, H.L. 1996. Compartmentalization, intracellular transport, and autophagy of *Tomato spotted wilt virus* proteins in infected thrips cells. Phytopathology 85: 644-654.

Ullman, D.E., Sherwood, J.L., and T.G. German. 1997. Thrips as Vectors of Plant Pathogens, pp. 539-565, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Van de Wetering, F. Goldbach, R., and Peters, D. 1996. *Tomato spotted wilt virus* ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900-905.

Van de Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., and Peters, D. 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower *Tospovirus* transmission by females. Entomol. Exp. Appl. 88: 9-15.

Webb, S.E., Kok-Yohomi, M.L., and Tsai, J.H. 1997. Evaluation of *Frankliniella bispinosa* as a potential vector of *Tomato spotted wilt virus*. (Abstr.) Phytopathology 87(suppl.):S102.

Wijkamp, I., Almarza, N., Goldbach, R., and Peters, D. 1995. Distinct levels of specificity in thrips transmission of *Tospoviruses*. Phytopathology 85: 1069-1074.

Wijkamp, I., Goldbach, R., and Peters, D. 1996[a]. Differential susceptibilities between leaf disks and plants in the transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis* to TSWV hosts and transgenic plants. Phytopathology 144: 355-362.

Wijkamp, I., Goldbach, and Peters, D. 1996[b]. Propagation of *Tomato spotted wilt virus* in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. Entomol. Exp. Appl. 81: 285-292.

Wijkamp, I., Van De Wetering, F., Goldbach, R., and Peters, D. 1996[c]. Transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis*; median acquisition and inoculation access period. Ann. App. Biol. 129: 303-313.

CHAPTER 3

THRIPS (THYSANOPTERA: THRIPIDAE) FEEDING RESPONSE TO CONCENTRATION OF IMIDACLOPRID IN TOMATO LEAF TISSUE[1]

[1] Chaisuekul, C. and D. G. Riley. 2000. Submitted to Journal of Entomological Science, 3/16/00.

Introduction

Thrips have become primary pests in many horticultural crops, particularly as vectors of tospoviruses (Ullman et al. 1997). They penetrate their stylets through upper plant cells and feed on materials from fractured cells. Thrips feeding alone can cause reduction in maturity and yield when plants are infested with high populations of thrips. When a plant virus is present in the crop system, thrips can transmit plant viruses both propagatively, requiring incubation inside the vector's body, as well as non-propagatively. *Tomato spotted wilt virus* (TSWV) is a propagative virus transmitted by several thrips species. TSWV has been particularly devastating in tomato and pepper in Georgia (Gitaitis et al. 1998, Riley and Pappu 2000). In tomato plants in the southeastern United States, the vector species are mainly *Frankliniella occidentalis* and *F. fusca* (Salguero Navas et al. 1991). Thrips acquire TSWV during their first and second instars by feeding on TSWV infected tissue and they remain infective through out their lives (Van de Wetering et al. 1996.). Adult *F. occidentalis* do not acquire TSWV because of a midgut barrier (Ullman et al. 1992). Viruses are retained in saliva tissue and can be transmitted to healthy plant tissue during feeding.

Foliar insecticides are effective for the control of thrips in certain vegetable crops (Sparks, et al. 1998). However, to prevent TSWV transmission by thrips vectors, insecticides have to be applied frequently and have to possess rapid efficacy to be able to kill the viruliferous thrips before inoculation can occur. A systemic insecticide, imidacloprid, (1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2- imidazolidinimine) under trade names Admire® and Provado® (Bayer Corp., Kansas City, KS) has been reported to be effective in reducing incidence of TSWV in some crops such as tomato and pepper

(D. Rogers, Bayer Corp., personal communication), but can increase incidence in peanut (J. Todd, Univ. of Georgia, personal communication) when used as a soil drench. The chemical interferes with the transmission of stimuli in the insect nervous system. Specifically, it causes a blockage in a type of neuronal pathway (nicotinergic) that is more abundant in insects than in warm-blooded animals (making the chemical selectively more toxic to insects than warm-blooded animals). This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis, and eventually death. It is effective on contact and via stomach action (Kidd and James, 1994). This chemical could prevent TSWV infection by suppressing viral expression in plant cells or inhibiting the transmission of TSWV by killing thrips or by deterring thrips from feeding on plant tissue. Since plants treated with imidacloprid have been observed to be infected with TSWV by mechanical inoculation (Chaisuekul, unpublished data), and mortality of thrips with imidacloprid is low (D. Riley, unpublished data), we suspected that imidacloprid is affecting thrips feeding behavior rather than affecting the virus or thrips mortality. Imidacloprid targeted against sucking insects such as leafhoppers, planthoppers, thrips, and whiteflies and it has been shown to affect feeding in aphids (Boiteau and Osborn, 1997) and corn flea beetles (Munkvold et al. 1996).

Recently, soil application of imidacloprid plus various foliar insecticide treatments have been used to reduce infection of TSWV in tomato (Riley and Pappu 2000), presumably affecting the thrips vector population. Viruliferous thrips were either reduced in numbers, deterred from feeding, or were unsuccessful in the transmission of virus. Our study investigated the effect of imidacloprid on feeding behavior of thrips in

tomato plants by comparing feeding response (number of feeding scars) to concentration of imidacloprid in the leaf tissue. The null hypothesis was that feeding response was not affected by concentration of imidacloprid.

Materials and Methods

In an experiment conducted in the summer of 1999 at Tifton, Georgia, various concentrations of imidacloprid (Admire 2F®, Bayer Corp., Kansas City, KS) were applied to 4-week old potted tomato plants, cv. `Sunny Hybrid' (Asgrow Seed Co., Kalamazoo, MI). One control and five rates of Admire were applied to the top of the soil in 6-inch pots as a soil drench. The applied rates of Admire 2F were 0, 0.5, 0.8, 1.1, 2.1, and 4.3 fl oz per 0.4046 hectare (based on 7,260 plants per 0.4046 hectare (1 acre)) or 0.0, 2.17, 3.26, 4.35, 8.69, and 17.39 µl per plant (100ml water). Formulated Admire 2F, according to the treatment rate, was first measured in µl for all 6 pots using a micropipette in 600 ml water. Then the mixtures were stirred and 100 ml was poured around the base of each tomato plant. These tomato plants later were transferred to a screened, thrips-exclusion cage in greenhouse. These plants were watered by drip tube irrigation, programmed for 20 minutes every other day.

Two weeks after the imidacloprid treatment, leaf samples taken from the fourth branch from the terminal bud were collected and sent to a pesticide analysis lab (Pesticide and Hazardous Waste Laboratory, University of Georgia, Athens, GA) to measure imidacloprid residue in the leaf tissue. Thrips, primarily *F. occidentalis*, were collected from cotton blossoms and caged for 72 hours in microcages clipped on the upper side of lowest leaves from the branch above the leaf taken for residual analysis. The microcage was made from a hair clip attached with hot glue to a plastic cap cut from 2.0 cm head of

a plastic transfer pipette (Samco® transfer pipets #202, Samco Scientific Corporation, San Fernando, CA). This microcage produces a circular feeding area of 1.5 cm in diameter on a leaf. After 72 h, thrips were removed from microcages and placed into 50% ethyl alcohol for identification. The thrips condition was categorized as either not present in the microcage after 72 h, present in the microcage and alive, or present in the microcage but dead. The circular areas on the tomato leaves were examined for feeding scars and recorded by digital camera for image analysis. After the first 72 h, new thrips were placed in the microcages on the next lowest leaves on the third branch, and the previous procedure was repeated. Five feeding tests were performed during 7/13/1999 to 8/2/1999.

Results and Discussion

The feeding scars were the areas on leaves showing feeding damage from thrips, usually 1 mm wide by 1-3 mm long section of damaged leaf cells (Appendix A, figure 1A). The damaged tissue could be categorized into white feeding scar areas (dry leaf tissue resulting from older thrips feeding) and black or dark feeding scar areas (wet leaf tissue from recent thrips feeding, less than 24 hours). In either category, thrips feeding scars were eliminated at the highest concentration of imidacloprid used in this test (Appendix A, figure 1B). The null hypothesis of no effect on feeding response by imidacloprid was disproved with the data collected in this test.

The results presented in Figure. 1 show that increased Admire soil drench concentration (µl per 100 ml water per pot) increased the amount of Admire in the leaf tissue (ppm), $R^2 = 0.97$ and $P=0.0003$ (ANOVA). High variation in the highest rate of Admire in our study could be caused by greater variability in leaching of applied Admire.

In Fig. 1, the results also show that the number of thrips feeding scars on tomato leaves negatively corresponded to the applied rates of Admire® and the amount of imidacloprid leaf residue. These data clearly demonstrate a reduction of thrips feeding, R^2= 0.98 (natural logarithm transformation of applied Admire rate plus 0.1µl) and *P*= 0.0002 (ANOVA), with increasing levels of imidacloprid, which increases in the leaf tissue as greater amounts were applied to the soil. It was clear from these observations that imidacloprid has an anti-feeding effect on thrips, even at concentrations lower than label recommendations. The critical rate of imidacloprid that provides anti-feeding activity on thrips will be studied further. Imidacloprid could interfere with thrips transmission of TSWV by means of inhibiting thrips feeding. We suspect that with the right insecticide program in addition to applications of imidacloprid to the soil, the incidence of TSWV infection could be reduced in tomato.

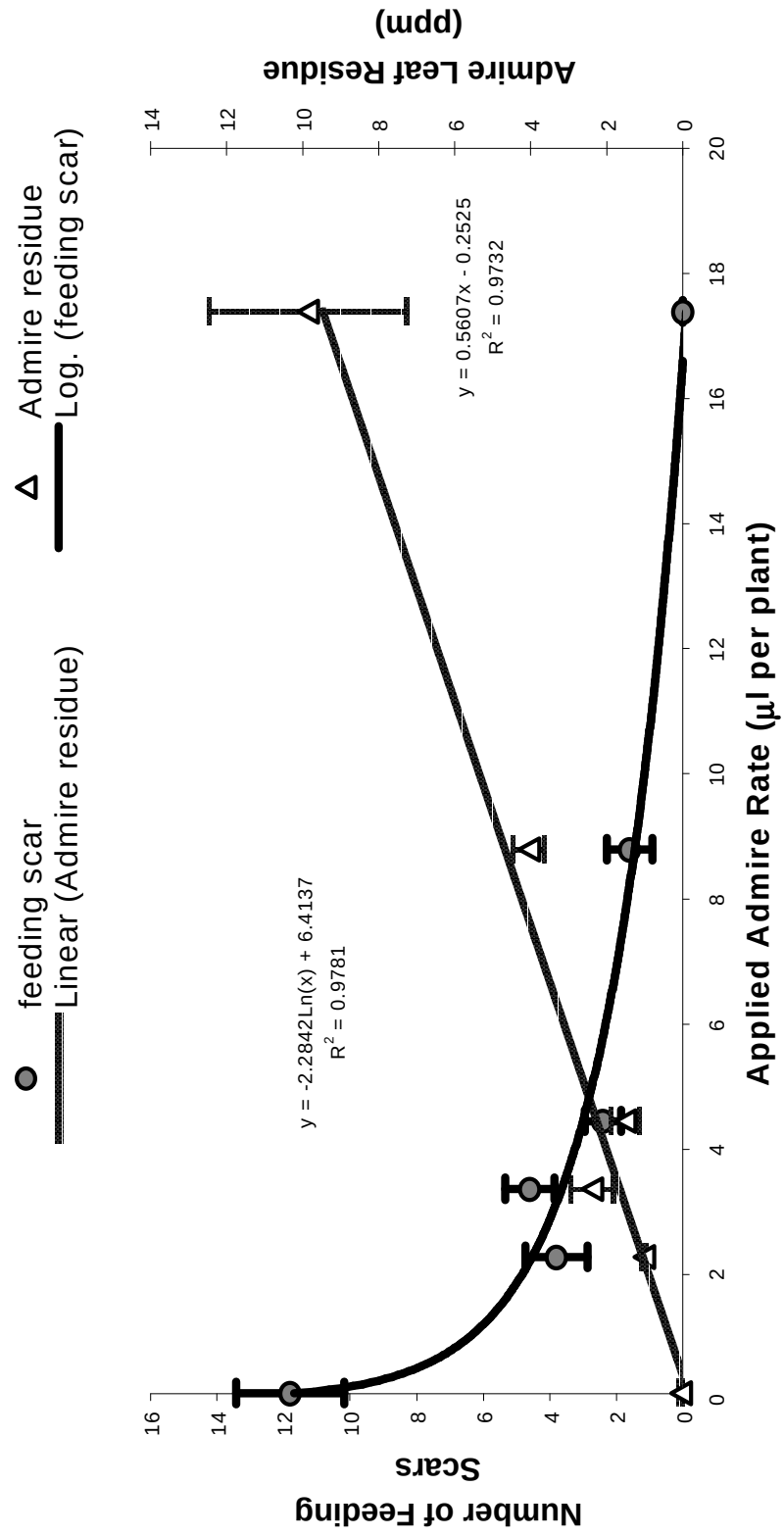


Figure 3.1 Average feeding scars per 6 thrips per 10.6 cm^2 to applied Admire rate (µl per plant) and Admire leaf residue (ppm).

Literature Cited

Boiteau, G. and Osborn, W.P.L. 1997. Behavioural effects of imidacloprid, a new nicotinyl insecticide, on the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera, Aphididae). Can. Entomol. 129:241-249.

Gitaitis, R.D., Dowler, C.C., and Chalfant, R.B. 1998. Epidemiology of tomato spotted wilt in pepper and tomato in southern Georgia. Plant. Dis. 82:752-756.

Kidd, H. and D. James (eds.). 1994. Agrochemicals Handbook, Third Edition. Royal Society of Chemistry. Cambridge, England.

Munkvold, G.P., McGee, D.C., and Iles, A. 1996. Effects of imidacloprid seed treatment of corn on foliar feeding and *Erwinia stewartii* transmission by the corn flea beetle. Plant disease. 80: 747-749.

Riley, D.G. and Pappu, H.R. 2000. Evaluation of tactics for management of thrips-vectored *Tomato spotted wilt tospovirus* in tomato. Plant Dis. 84: 847-852.

Salguero Navas, V.E., Funderburk, J.E., Beshear, R.J., Olson, S.M., and Mack, T.P. 1991. Seasonal patterns of Frankliniella spp. (Thysanoptera: Thripidae) in tomato flowers. J. Econ. Entomol. 84: 1818-1822.

Sparks, A.N. Jr., Anciso, J.R., Riley, D.G., and Chambers, C. 1998. Insecticidal control of thrips on onions in South Texas: insecticide selection and application methodology. Subtropl. Pl. Sci. 50: 58-62.

Van de Wetering, F. Goldbach, R., and Peters, D. 1996. *Tomato spotted wilt virus* ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900-905.

Ullman, D.E., Sherwood, J.L., and T.G. German. 1997. Thrips as Vectors of Plant Pathogens, pp. 539-565, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Ullman, D.E., Cho, J.J., Mau, R.F.L., Westcot, D.M., and Custer, D.M. 1992. A midgut barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. Phytopathology 82: 1333-1342.

CHAPTER 4

MECHANICAL AND THRIPS TRANSMISSION OF *TOMATO SPOTTED WILT VIRUS* TO YOUNG TOMATO PLANTS. [2]

[2] C. Chaisuekul and D. G. Riley to be submitted to Plant Disease.

Introduction

Tomato spotted wilt virus (TSWV) is a plant virus of genus *Tospovirus* in family Bunyaviridae and (Wijkamp et al., 1995). TSWV has caused major damage to agricultural crops worldwide (German et al. 1992). It has become serious disease to several economically important crops such as tobacco (McPherson et al. 1999) and peanut (Stewart et al. 1989), petunia, geranium, chrysanthemum, aster and poinsettia (Gofflot and Verhoyen 1990, Marchoux et al. 1991), eggplant, melon, lettuce, pepper, and tomato (Marchoux et al. 1991, Bautista et al. 1995, Gitiatis et al. 1998). Symptom expression of plants infected with TSWV varies on plant species and cultivars (Kumar et al., 1993, Roca et al. 1997), plant age (Moriones et al. 1998), and environmental conditions. Some symptoms are generally similar to those caused by other plant virus such as stunting, leaf distortion, necrosis, wilting, mosaic, mottling and vein clearing (Moriones et al. 1998). Black spots and severe wilting are generally characteristic of TSWV infection of tomato. Some plants, though is infected, may not show symptom (asymptomatic plants). These asymptomatic plants could be identified by serological assays. Enzyme-linked immunosorbent assay (ELISA) of TSWV have been accurate in both plants (Marchoux et al. 1991) and thrips (Cho et al. 1988).

Thrips (Thysanoptera: Thripidae) are the only group of insects found to be able to transmit TSWV. As many as 8 species of thrips were found to be able to transmit TSWV (Wijkamp et al. 1995). TSWV is transmitted by *Thrips tabaci*, *T. setosus*, *T. palmi*, *Frankliniella schultzei*, *F. occidentalis*, *F. fusca*, and *F. intonsa* (Wijkamp et al. 1995, Ullman et al. 1997), and *F. bispinosa* (Webb et al. 1997). Some species, *Frankliniella tenuicornis and Scirtothrips dorsalis* had been previously reported to transmit TSWV, but

experimental verification in these species has not been done in some cases (Ullman et al. 1997). *F. occidentalis* (western flower thrips) is an important agent for TSWV-transmission, and its high population densities have been associated with the incidence of TSWV (Aramburu et al. 1997).

TSWV is transmitted in a persistent manner, which requires a period of incubation in vector before being transmitted to plant (Ullman et al. 1997). Only thrips that acquire virus in their immature stages could transmit the virus, and immature thrips could transmit the virus as early as 30 minutes after feeding on infected plant tissue (Wijkamp et al. 1996[c]). A study by Van De Wetering et al. (1996) reported that only the first instar of *F. occidentalis* could acquire the virus while other studies of *F. occidentalis* population from other regions reported that both first and second instars could acquire the virus (Ullman et al. 1997). TSWV was found to be associated with salivary glands of thrips (Ullman et al. 1996). However, adult thrips which did not acquire TSWV during their larval stages cannot transmit the virus because of a midgut barrier (Ullman et al. 1992). Immature thrips retain virus and infectability through out their life (Wijkamp et al. 1995), but TSWV can not pass to the progeny of viruliferous thrips (Wijkamp 1996[b]).

The control of thrips populations may reduce the incidence of TSWV because the density of thrips, especially of *F. occidentalis,* correlates to the incidence of TSWV (Aramburu et al. 1997, Riley and Pappu 2000). A management plan for thrips vectors could play a crucial role in the control of tomato spotted wilt disease. The age of the tomato plant that is most susceptible to TSWV has not been directly identified relative to thrips inoculation. Tomato plants that developed tomato spotted wilt disease early in the

season had drastically reduced growth and yield compared to tomato plants with late symptom development (Fajardo et al. 1997, Moriones et al. 1998).

Our study used two methods of TSWV transmission, mechanical transmission and thrips transmission, to infect tomato plants over different plant ages to determine the effects of plant age at infection on yield. Besides thrips transmission, TSWV, as with most plant viruses, can be transmitted by mechanical transmission (Kumar et al. 1993). Kumar et al. (1993) suggest that mechanical transmission provides a rapid screening of viral resistant plants because it requires relatively less preparation compared to inoculation by insect vectors. Kumar et al. (1993) concluded that the two transmission methods produced indistinct percentages of TSWV-infected tomato plants, however, if the resistance to TSWV is due to resistance to the vector, it can only be screened by thrips transmission. We hypothesized that the infection of TSWV in tomato plants at an early age affects the yield of tomato greater than at an older plant age, and that both methods of inoculation, mechanical and thrips, should provide a similar result with respect to the relationship between time of inoculation and effects on yield.

Materials and Methods

Experiment 1: Comparison between mechanical and thrips transmission of TSWV to tomato plants in field exclusion cages.

The comparison between the methods of TSWV inoculation was conducted during May - August 1999 in a field plot treated with 98% methyl bromide 550 kg/ 0.4046 ha (250lb/ a) to beds, 180 cm wide and 20 cm raised, and covered with black plastic mulch. Tomato plants were direct seeded Sunny Hybrid cultivar (Asgrow Seed Co., Kalamazoo, MI) in individual exclusion cages. These exclusion cages were

45x45x120 cm^3 in dimension made from an aluminium conduit pipe (2 cm in diameter) frame covered with screen bags (Figure 4.1). The screens were made from white or ivory fine mesh chiffon (quality 3871, Shason Inc., Japan) with 40 cm strapped fasteners (Velcro®, Velcro USA Inc., Manchester, NH, USA) opening at one corner. The bottom of the screens were sealed by weighing down with plastic tube sandbags around the frames. Thirty-six plants were randomly assigned to six treatments of either mechanical inoculation or thrips transmission at 7, 14, or 28 days after direct seeding. 10 g of slow released fertilizer , formula 14-14-14 (Osmocote®, Scotts-Sierra Horticulture Products Co., Marysville, OH), was added to the base of each plant during transplant.

Figure 4.1 Field cages used for thrips exclusion in field grown tomato in Experiment 1 and Experiment 3.

Symptomatic (e.g., showing TSWV wilting and/or necrotic leaf spots) tomato plants, verified by ELISA test, were identified and used as an inoculum source for

mechanical inoculation and as host plants to produce viruliferous thrips. Plants were mechanically inoculated as described in Kumar et al. (1993) except that the inoculum was applied to half of the leaves in each plant. Leaves from one TSWV-infected plant were collected and macerated with pre-chilled TSWV-inoculation buffer, 0.1 M potassium phosphate and 0.01 M sodium sulfite (Kumar et al. 1993) in chilled mortar. Inoculating plants were covered with carborundum dust before TSWV solution was rubbed over and under leaf surface. Carborundum was washed off the leaf surface with a water spray the next day.

Thrips were collected from blossoms of TSWV infected tomato plants in a nearby field one day before inoculation, and placed in self-sealed plastic bag. Approximately 20 thrips were put in each 20 ml vial. Each vial was placed opened next to the randomly pre-selected tomato plants that were at the selected plant ages. Approximately 72 hours after initiating inoculation access, tomato plants with both mechanical and thrips inoculation were drenched with a mixture of the insecticides, lambda-cyhalothrin (Karate 1EC, Zeneca AG Products, Wilmington, Delaware) and methamidophos (Monitor 4L, Bayer Corp., Kansas City, Kansas) at rates of 53.86 g + 709.76 litre per 4046.86 m^2 (1.9 oz + 1.5 pt per acre), respectively, to eliminate thrips within the cages. A sub sample of thrips collected after first week and second week had 23.86% (n= 88) and 25.56% (n=90) of viruliferous individuals, respectively.

Each week after inoculation, one leaf sample from third branch from the terminal end of each plant was collected in self-sealing plastic bags (Walmart Stores Inc., Bentonville, AR). Leaf samples were tested for the presence of virus with double antibody sandwiched (DAS) ELISA (Agdia TSWV-ELISA kit, Agdia Corp., Elkhart,

IN). Harvested fruit were weighed and graded (USDA standard) by color and shape regularity and by size: extra large (diameter more than 7.0 cm), large (diameter between 6.0-7.0 cm), medium (diameter between 5.5-6.0 cm), and cull (diameter less than 5.5 cm).

Damaged fruits were characterized as fruits with cracking surface, uneven shape or uneven ripening. Unmarketable fruits were either cull-size fruits or damaged fruit. Market value was calculated with the average price ($8.28/ 11.4 kg) of tomato in Georgia from May-November of 1991-1995. Fruit yield and viral expression were compared across treatments, i.e. inoculation type and plant age at inoculation, using ANOVA (Proc ANOVA, SAS Institute, 1998) and yield was correlated with the presence of TSWV using ELISA and symptom expression (Pearson's correlation, Proc CORR, SAS Institute, 1998).

Experiment 2: Comparison between mechanical and thrips transmission of TSWV to tomato plants of different ages in six-inch pots.

This experiment was conducted during May - August 1999 in Tifton, GA. Sunny Hybrid cultivar (Asgrow Seed Co., Kalamazoo, MI) were direct seeded in six inch-pots (15 cm in diameter and 25 cm in height). Plants were seeded 56, 42, 28, and 14 days before viral transmission. Three plants from each age class were randomly assigned to be mechanically inoculated and the other three plants to be thrips inoculated. These plants were kept in dark at 20°C for 24 hours before the inoculation. The mechanical and thrips inoculations were conducted as in Experiment 1 to all plants under the cover of clear plastic bags (20x40 cm^2) and left approximately 72 hours before plants were sprayed with lambda-cyhalothrin and methamidophos at the rate used in Experiment 1 and moved to a

thrips exclusion cage in green house. Plants were watered by drip tube at the base of each plant, and 10 g of slow released fertilizer, formula 14-14-14 (Osmocote®, Scotts-Sierra Horticulture Products Co., Marysville, OH), was added to the base of each plant at 2 and 6 weeks after inoculation, and after transplanted in field.

Leaf samples, from the same position as in Experiment 1, for ELISA test were collected from each plant weekly after plants were inoculated until plants were transplanted in field. Transplants were set at 60 cm intervals, as in Experiment 1 after they were 70 days old. Plants were observed weekly and recorded for TSWV symptoms. Fruits were harvested and graded as previously described. Fruit number and fruit weight were statistically analyzed as in Experiment 1.

Experiment 3: Thrips transmission of TSWV to tomato plants in field exclusion cages.

The thrips transmission of TSWV to six plant ages, 7, 14, 21, 28, 35, and 42 days, was evaluated during March-April 2000. Thirty six tomato plants in field exclusion cages were set up as previously described in Experiment 1. Thrips used in this test were obtained from an onion field about 200 meters from the field exclusion cages. Only immature thrips (*Frankliniella occidentalis* and *F. fusca*) were collected by an aspirator from the onion leaves, and they were placed in a plastic vial with TSWV-infected tomato leaves. These infected tomato leaves were collected from mechanically inoculated tomato plants at four weeks old that were positive for TSWV by ELISA. The assumed percent viruliferous thrips was 35.0% based on the data of Wijkamp et al. (1995) on transmission to petunia. Twenty immature thrips were put into each plastic vial, and then each vial was placed next to an assigned tomato plant. After 72 hours from inoculation, tomato plants were sprayed

with a mixture of insecticides, lambda-cyhalothrin and methamidophos, at the rate used in Experiment 1 to kill thrips. The screens of the exclusion cages were removed after plants had reached 70 days old under warm conditions (Appendix C).

For ELISA test, leaf samples from lowest leaf of third highest branch and the lowest leaf from lowest branch were collected from each plant weekly after plants were four weeks old as described in Experiment 1. Plants were observed weekly and recorded for TSWV symptoms. Fruits were harvested and graded as previously described. Fruit number and fruit weight were statistically analyzed as described in Experiment 1, except that Proc GLM and contrast analysis were also used (SAS Institute, 1998).

Experiment 4: Mechanical transmission of *Tomato spotted wilt virus* to young tomato plants treated with insecticides to exclude thrips.

Tomato plants ('Sunny Hybrid') had been mechanically inoculated TSWV during March and April, 2000. Thirty six tomato plants were transplanted to a field plot seven days after being direct seeded. Six of these plants were randomly selected to be inoculated with TSWV at each of the following ages, 14, 21, 28, 35, 42, and 49 days. Mechanical inoculation was conducted as described in Experiment 1.

Tomato plants were protected from thrips by a combination of sprayed insecticides, lambda-cyhalothrin and methamidophos at the rates used in Experiment 1, and a systemic insecticide, imidacloprid (Admire 2F®, Bayer Corp, Kansas City, Kansas) as soil drench at rate 0.067 ml/ 104 ml water/ plant, after transplanting. Plants were individually sprayed once a week until they started bearing fruits (70 days old). Insecticide residue from the last spray was deemed to be effective for at least one week since only dead thrips and other dead insects were observed on plant.

A freeze-dried sample of TSWV infected tomato leaf tissue collected from the same location in summer 1999 and verified by ELISA was used as an inoculum source. Plants were inoculated at randomly assigned ages using the mechanical inoculation method described in Experiment 1, except that plants were covered with brown paper bags for one day, and carborundum dust was washed with sprayed water after paper bags were removed. Leaf samples, from the same position of the plant as described in Experiment 3, were collected from each plant weekly after plants were four weeks old for ELISA tests. Plants were observed weekly and recorded for TSWV symptoms. Fruits were harvested and graded as previously described. Fruit number and fruit weight were statistically analyzed as in Experiment 3.

Experiment 5: Mechanical transmission of TSWV to tomato plants of different ages in pots treated with insecticides to exclude thrips.

Tomato plants (‘Sunny Hybrid’) were direct seeded in six-inch pots at 7, 10, 14, 42, 49, and 56 days before they were mechanically inoculated with TSWV. All inoculated plants, six in each age class, were remained in green house 7 days before they were to be transplanted to a field plots. Mechanical inoculation was conducted as described in Experiment 4 except that it was conducted in the in laboratory, and plants were kept in dark for approximately 24 hours before they were moved to a green house for one week and then to field plot as described in Experiment 1. Tomato plants were protected from thrips by combination of sprayed insecticides, lambda-cyhalothrin and methamidophos, and a systemic insecticide, imidacloprid, as applied to plants in Experiment 4, after transplanting. Plants were individually sprayed once a week until they started bearing fruits (70 days old). Insecticide residue from the last spray was

deemed to be effective for at least one week since only dead thrips and other dead insects were observed on plant.

A freeze-dried sample of TSWV infected tomato leaf tissue collected from the same location in summer 1999 and verified by ELISA was used as an inoculum source as in Experiment 4. Plants were covered with clear plastic bags for approximately 72 hours, and carborundum dust was washed with sprayed water after 24 hours. For ELISA, leaf samples collected from same position of the plant as in Experiment 2 were collected from each plant weekly after plants were more than four weeks old. Plants were observed weekly and recorded for TSWV symptoms. Fruits were harvested and graded as previously described. Fruit number and fruit weight were statistically analyzed as in previous Experiment 3.

Results and Discussion

Experiment 1: Comparison between mechanical and thrips transmission of TSWV to tomato plants in field exclusion cages.

Most plants were successfully infected with TSWV by both inoculation methods, but none of 36 plants were severely stunted. TSWV infection was verified by DAS-ELISA. With thrips transmission, earlier inoculation resulted in greater yield loss. Mechanical inoculation differed from thrips transmission in that the earliest inoculation date was not significantly different than later dates in terms of success of inoculation of TSWV and consequently, there was less effect on yield (Table 4.1). However, symptoms on the foliage corresponded more with the removal of screen cage netting (Figure 4.2) than time of transmission. Even so, plants which developed symptoms early in the season showed a greater reduction in yield than plants that expressed symptoms later in the

season (Figure 4.3). Mechanical and thrips inoculations differed the most at the earliest plant age in terms of effect on yield with thrips transmission resulting in a greater incidence of TSWV infection and less yield (Table 4.1). Overall weight of marketable fruit decreased in plants that developed symptom early and tested positive for ELISA early (Pearson correlation = -0.36, P=0.032 and -0.35, P= 0.035, respectively) across both inoculation methods. Also, weight of marketable fruit of inoculated plants decreased as plants exhibited earlier symptoms (F=5.03, P=0.032, Proc REG, SAS version 7, SAS Institute) and a higher average ELISA values (F=4.73, P=0.036, Proc REG, SAS version 7, SAS Institute). The results from this experiment supported the hypothesis that earlier transmission of TSWV by either mechanical or thrips transmission had a greater negative impact on yield than later transmission.

Experiment 2: Comparison between mechanical and thrips transmission of TSWV to tomato plants at different age in six-inch pots.

Although plant heights at 12 weeks were not significantly different between each age class, plants inoculated at 14 days in this experiment did not produce any fruit while plants from other age classes produced some fruits (Figure 4.4). No marketable fruit was produced by plants inoculated at age 14 and 28 days old and plants mechanically inoculated at age 42 days old (Table 4.2). The weight of TSWV-damaged fruits were as much as twice the weight of normal fruits in plants inoculated at 56 days old by both mechanical and thrips transmissions (Table 4.2). Total number of fruits per plant (y_1) and plant age (x weeks) at inoculation can be described as the following regression equation y_1= -7.17x+2.58, P<0.0001 (Proc REG, SAS version 7, SAS institute). Total fruit weight per plant (y_2 grams) and plant age (x weeks) at inoculation can be described as the

following regression equation y_2= -575.71x+192.62, *P*=0.0003 (Proc REG, SAS version 7, SAS institute). The result from this experiment was similar to the result from Experiment 1 in that there was a greater reduction of yield from plants with earlier inoculation than from plants with later inoculation. The effectiveness of mechanical and thrips inoculation in this experiment conducted in the lab appeared to be similar (Table 4.2) unlike what was observed in Experiment 1. Mechanical inoculation in this experiment was more effective in the than field inoculation to plants in Experiment 1 which had less than 12 hours of darkness and a temperature more than 20°C before the inoculation. Also, the plants in this experiment had a greater chance to be fed by viruliferous thrips since the covered plastic bags (approximately 4,000 cm^3) used in laboratory inoculation had less volume than the volume of field exclusion cages (approximately 243,000 cm^3) of Experiment 1. Also, the plants in this experiment grew in the limited space of 6" pots and in greenhouse-exclusion cage until they are 70 days old, so their growth may be impeded or more stressed which might have increased impact of TSWV on yield.

Experiment 3. Thrips transmission of TSWV to tomato plants in field exclusion cages.

Most plants (30 of 36) developed symptoms when they were 63-99 days old. Three plants, one each from inoculation at day 21, 28, and 42, were not infected with TSWV based on ELISA of the leaf tissue and lack of symptoms before removing of screens from cage frames. These three plants later showed symptoms 14 days after removing of screens, suggesting they were inoculated by field thrips population as soon as the exclusion cages were removed. Five plants, two from inoculation at day 7 and one

each from inoculation at day 14, 21, and 35, showed severe stunting. Results from ELISA test of leaf samples showed most plants, 33 of 36, were positive for TSWV when plants were 49 days old. Five plants were positive for TSWV when plants were 42 days old. None of the plants were TSWV-positive when they were 35 days old. Most plants were positive for ELISA before they exhibited symptoms (Figure 4.5).

The fruits with blossom end rot prematurely ripened and were variable in size. They were not distinguishable as TSWV-damaged or not, and so were analyzed separately. The tissue samples from the blossom end rot fruits were not found to be positive for TSWV based on ELISA. Concentric rings, typical of TSWV symptoms on ripened fruits, were observed from most plants that produced fruits. The total yield, both number and weight of fruits per plant, of plants inoculated in the first four age classes increased, but slightly reduced in the last two age classes (Figure 4.6). However, the proportion of number (y_1) and weight (y_2 grams) of normal fruits to TSWV-damaged fruits decreased in case of plants that were inoculated at younger ages (x days), $y_1=0.03x+2.01$, $P=0.0295$ and $y_2=0.02x+1.49$, $P=0.0287$, respectively (Proc REG, SAS version 7, SAS Institute). Two earlier inoculations, age 7 and 14 days, were significantly different from the four later inoculations, age 21, 28, 35, and 42 days) in normal fruit weight, F= 4.95, $P=0.036$ (Contrast analysis, Proc GLM, SAS version 7, SAS Institute) The yield of tomato in term of TSWV-damaged to normal fruits supported the hypothesis of greater yield reduction being associated with earlier transmission of TSWV. The yields of plant inoculated at 35 and 42 days did not continue to increase like the treatments between 7 days to 28 days suggesting that there was a leveling of yield response at the older plant ages (Table 4.3).

Experiment 4: Mechanical transmission of TSWV to tomato plants treated with insecticides to exclude thrips.

Most plants, 34 of 36, developed symptoms when they were 63-99 days old. Two plants, one each from inoculation at age 14 and 28 days, were not infected with TSWV based on ELISA. Two plants, one each from inoculation at age 21 and 42, developed severe stunting, however these two plants resumed growth and later produced flowers and fruits. The result from ELISA showed that most plants, 33 of 36, were positive for TSWV when plants were 49 days old, mostly before the appearance of symptoms. Five plants were positive when plants were 42 days old. None of the plants showed positive ELISA when they were 35 days old (Figure 4.7).

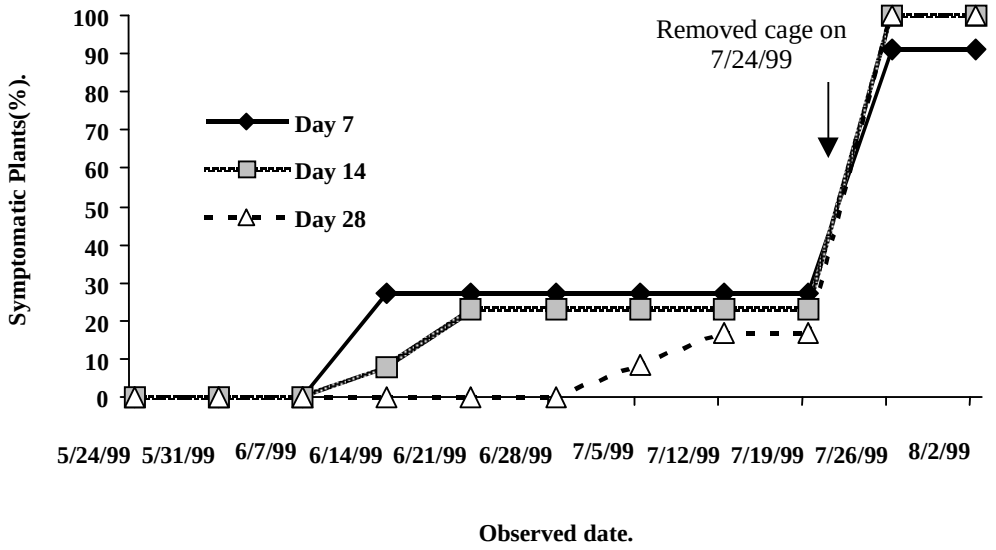
Tomato plants inoculated at an earlier age produced less fruits in terms of number and weight except the plants inoculated at 14 days which produced slightly higher fruit weight than plants inoculated at 21 and 28 days (Figure 4.8). Normal fruit weight and height of plants inoculated at 14, 21, and 28 days were significantly lower than at 35, 42, and 49 days, F=6.53, P=0.016 and F=5.04, P=0.032, respectively (Contrast analysis, Proc GLM, SAS version 7, SAS Institute). The result from this experiment also supported the hypothesis that there is a greater yield reduction in plants inoculated with TSWV at an earlier plant age compared to the two groups of inoculated ages, the first three vs. the last three dates. Regression analysis on the data presented in Figure 4.8 provided a clear trend of later dates of inoculation resulting in increased yield when the first date was excluded ($y_{number} = 0.757x_{day} - 1.733$, P=0.012, $y_{weight} = 55.58x_{day} - 410.58$, P= 0.006). The difficulty of mechanically inoculating the youngest seedling (7 and 14 days old) was discussed in Experiment 1.

Experiment 5: Mechanical transmission of TSWV to tomato plants of different ages in pots treated with insecticides to exclude thrips.

Plants inoculated at young ages, 7, 14, and 21 days, developed wilting and necrotic spots two weeks after inoculation, but most of them survived after new normal looking leaves appeared. Three plants inoculated at 7 days consequently died after 35 days. Plants inoculated at 42, 49, and 56 days developed symptoms 14-42 days after inoculation (Figure 4.9). One plant from age class 42 days was not infected. Most plants were detected as positive for TSWV by ELISA about 35-42 days after inoculation (Figure 4.9).

Plants inoculated at 7 and 14 days had severely stunted growth, no branching and no production of flowers. Plants inoculated at 21 days had only terminal growth but still produced flowers and fruits as well as some plants inoculated at age 7 days (Figure 4.10). Normal fruit weight (Figure 4.10), and plant height at 80 days (Table 4.4) decreased as plant were inoculated at earlier ages as evidenced by significant treatment (age) effects, $F=8.44$, $P<0.001$ and $F=29.52$, $P<0.001$, respectively (Proc GLM, SAS version 7, SAS Institute). As in Experiment 2, there was a stark difference in the first three dates compared to he last three dates in terms of marketable weight, % TSWV positive plants and TSWV-damage (Table 4.4). Again the potential stress associated with growing in pots and transplanting appear to exaggerate the differences between early and late inoculations.

A. Percent TSWV-symptomatic plants.



B. Percent TSWV-ELISA positive plants.

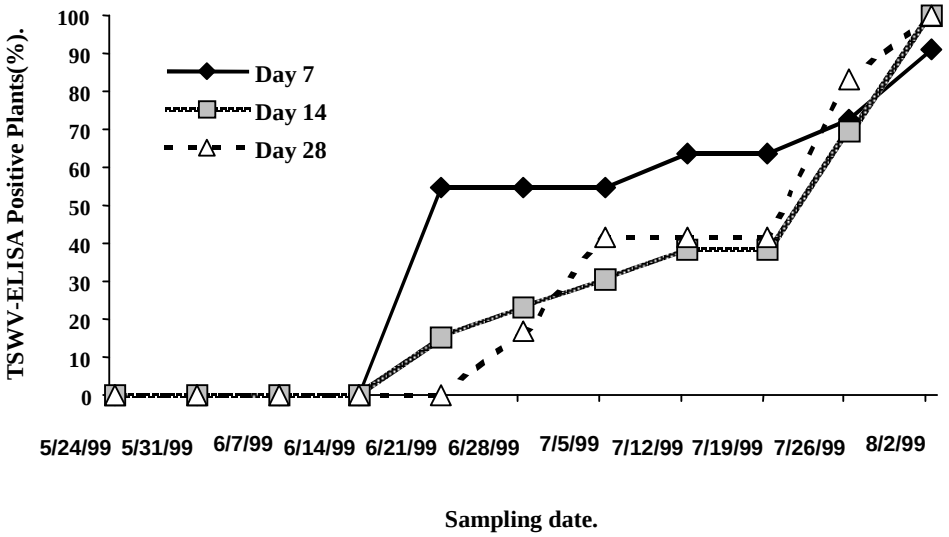
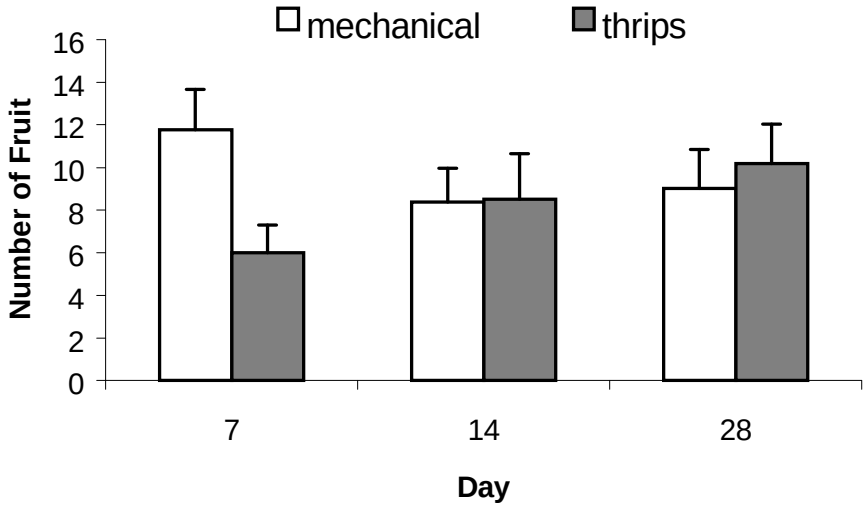


Figure 4.2 Percent observed symptomatic plants (A) and percent TSWV positive (DAS-ELISA) (B) plants over time by age of inoculation (7, 14, and 28 days old). Screens were removed on 7/24/99.

A. Total Fruit Number



B. Total Fruit Weight

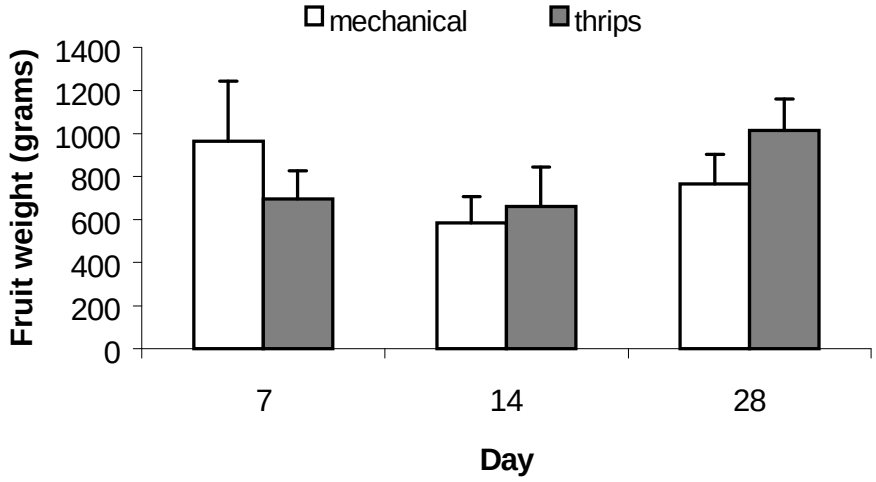


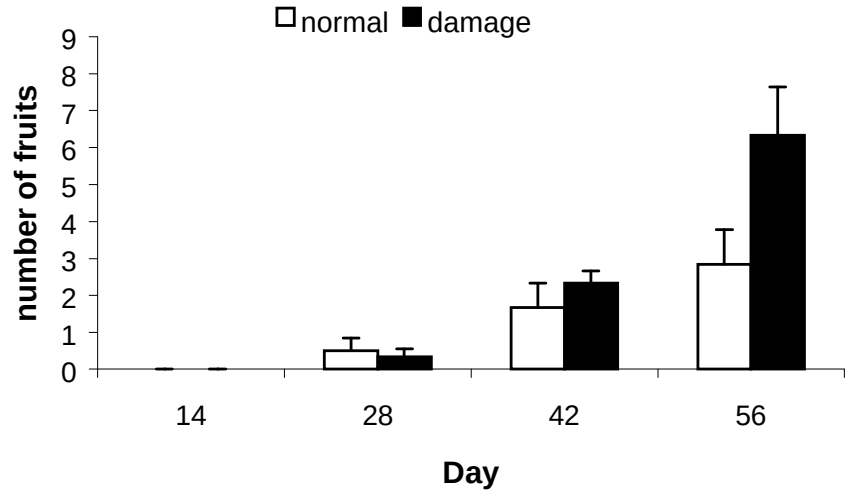
Figure 4.3 Average number (A) and weight (B) of fruits per plant from different ages (7, 14, and 28 days old) of inoculated tomato plants by mechanical and thrips transmission.

Table 4.1. Yield data and estimated market value of harvested tomato fruits per plant from each inoculation by plant age (7, 14, and 28 days).

Inoculation method	Plant age (days)	n	Average percent TSWV positive plants	Marketable fruit weight (grams)	Fruit market value (in US $)[1]	TSWV-damaged fruit weight (grams)	Loss (in US $) to TSWV damages	Plant height at age 63 days (cm)
Thrips								
	7	6	0.5365 a	51.5 a	0.038	615.5 a	0.448	51.3±2.9 a
	14	6	0.2911 b	192.6 a	0.140	384.5 a	0.280	50.2±6.0 a
	28	6	0.2640 b	222.9 a	0.162	696.1 a	0.507	53.2±3.2 a
Mechanical								
	7	5	0.3556 a	355.4 a	0.259	377.9 a	0.275	59.0±2.7 a
	14	7	0.3381 a	97.5 b	0.071	379.0 a	0.276	51.0±3.0 a
	28	6	0.1600 a	172.2 a,b	0.126	434.2 a	0.316	53.2±4.0 a

[1]Market value was calculated from weight of normal fruits in three sizes, extra large, large, and medium, with the average price ($8.28/ 11.4 kg) of tomato in Georgia from May-November of 1991-1995.
[2]Means followed by the same letter within inoculation method not significantly different (LSD, $P<0.05$).

A. Number of Fruits



B. Fruit weight

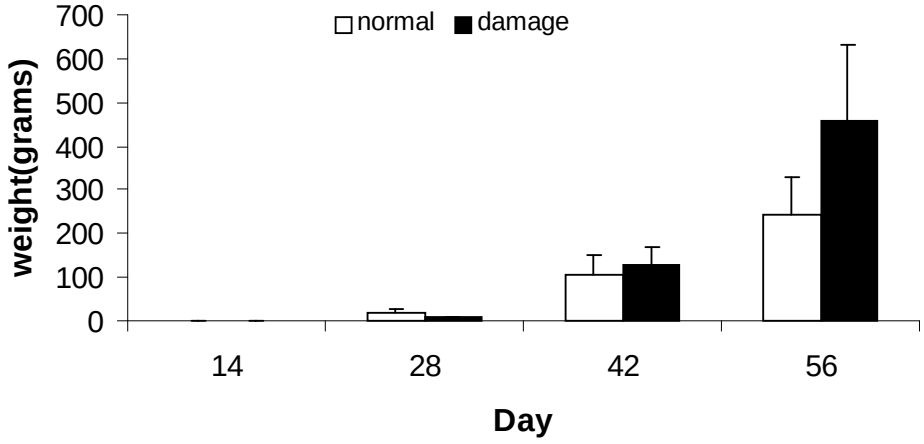


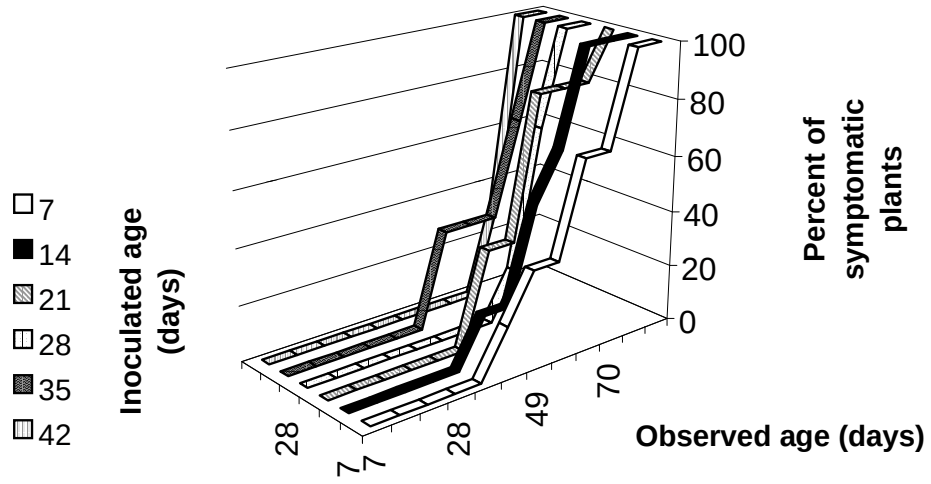
Figure 4.4 Average number (A) and weight (B) of fruits per plant from different ages (14, 28, 42, and 56 days old) of inoculated tomato plants by mechanical and thrips transmission.

Table 4.2. Yield data and estimated market value of harvested tomato fruits per plant from each inoculation by plant age (14, 28, 42, and 56 days old).

Inoculation method	Plant age (days)	n	Average percent TSWV positive plants	Marketable fruit weight (grams)	Fruit market value (in US $)	TSWV-damaged fruit weight (grams)	Loss (in US $) to TSWV damages	Plant height at age 84 days
Thrips								
	14	3	0.740 a	0 a	0.000	0 a	0.000	30.7 ± 7.8 a
	28	3	0.653 b	0 a	0.000	0 a	0.000	42.3 ± 12.9 a
	42	3	0.587 b,c	89.7 a	0.065	132.3 a,b	0.096	56.0 ± 14.6 a
	56	3	0.560 c	148.0 a	0.108	539.7 b	0.393	52.3 ±1.9 a
Mechanical								
	14	3	0.740 a	0 a	0.000	0 a	0.000	44.3 ± 2.3 a
	28	3	0.650 b	0 a	0.000	0 a	0.000	63.3 ± 2.8 a
	42	3	0.617 b	0 a	0.000	119.8 a,b	0.087	49.3 ± 16.2 a
	56	3	0.553 c	159.0 b	0.116	378.5 b	0.276	54.7 ± 4.8 a

[1]Market value was calculated from weight of normal fruits in three sizes, extra large, large, and medium, with the average price ($8.28/ 11.4 kg) of tomato in Georgia from May-November of 1991-1995.
[2]Means followed by the same letter within inoculation method not significantly different (LSD, $P<0.05$).

A. Percent TSWV-symptomatic plants.



B. Percent TSWV-ELISA positive plants.

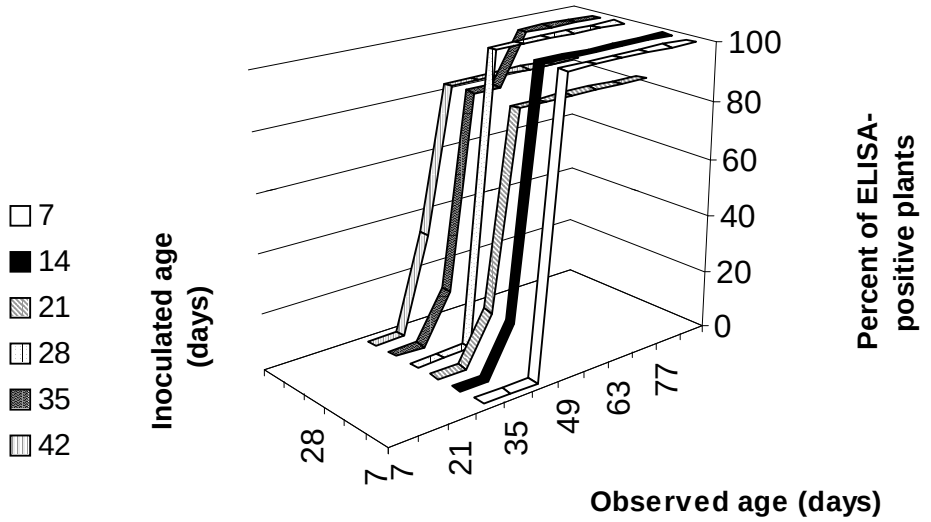
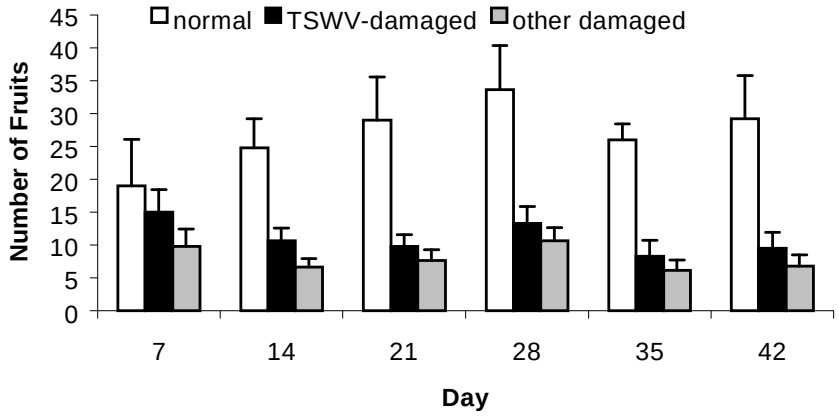


Figure 4.5 Percent observed symptomatic plants (A) and percent TSWV positive (DAS-ELISA) (B) plants over time by age of inoculation (7, 14, 21, 28, 35 and 42 days old). Screens were removed when plants were 70 days old.

A. Fruit number.



B. Fruit weight.

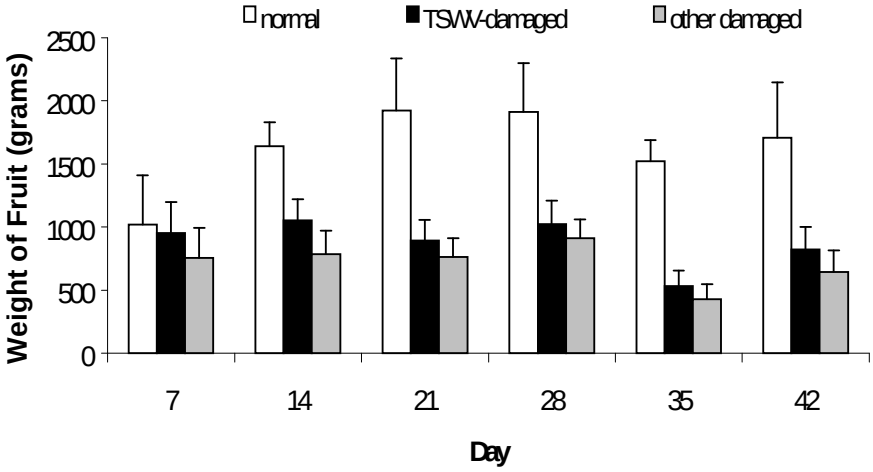


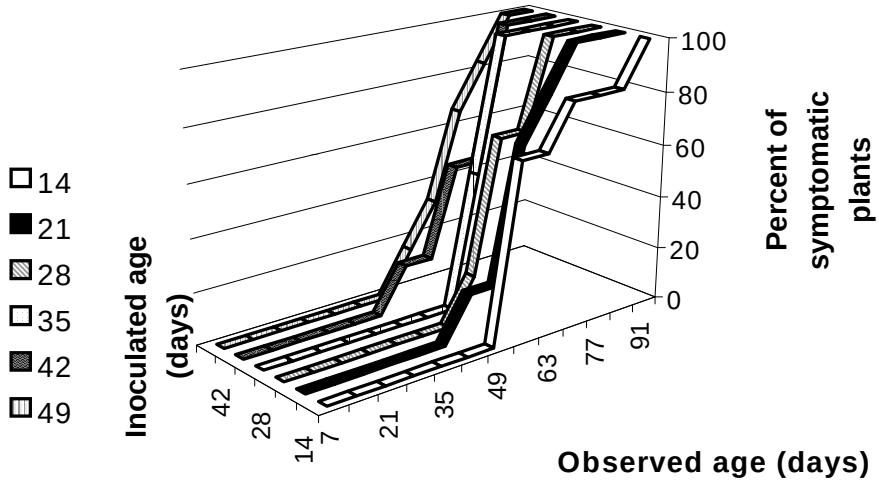
Figure 4.6 Average number (A) and weight (B) of fruits per plant from thrips inoculation to tomato plants of different ages (7, 14, 21, 28, 35, and 42 days old). Other damaged fruits, usually with blossom end rot, were unidentifiable for TSWV-damages.

Table 4.3 Yield data and estimated market value of harvested tomato fruits per plant from thrips inoculation by plant age (7, 14, 21, 28, 35, and 42 days).

Plant age (days)	n	Average percent TSWV positive plants	Marketable fruit weight (grams)	Fruit market value (in US $)[1]	TSWV-damaged fruit weight (grams)	Loss (in US $) to TSWV damages	Plant height at age 80 days (cm)
7	6	0.312 a	378.5 a	0.276	51.7 a	0.038	85.0 ± 3.4 a,b
14	6	0.229 a	927.5 a,b	0.676	187.5 a	0.136	69.8 ± 11.4 a
21	6	0.229 a	1075.8 b	0.784	82.5 a	0.060	95.2 ± 4.4 a,b
28	6	0.208 a	768.3 a,b	0.560	42.5 a	0.031	84.3 ± 13.8 a,b
35	6	0.354 a	615.8 a,b	0.449	34.2 a	0.025	96.8 ± 9.3 b
42	6	0.333 a	786.7 a,b	0.573	88.3 a	0.064	85.3 ± 5.8 a,b

[1]Market value was calculated from weight of normal fruits in three sizes, extra large, large, and medium, with the average price ($8.28/ 11.4 kg) of tomato in Georgia from May-November of 1991-1995.
[2]Means followed by the same letter within inoculation method not significantly different (LSD, $P<0.05$).

A. **Percent TSWV-symptomatic plants.**



B. Percent TSWV-ELISA positive plants.

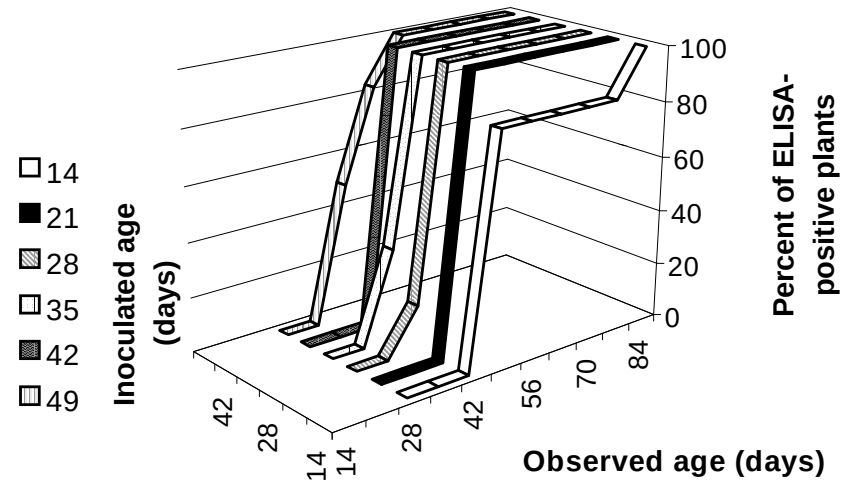
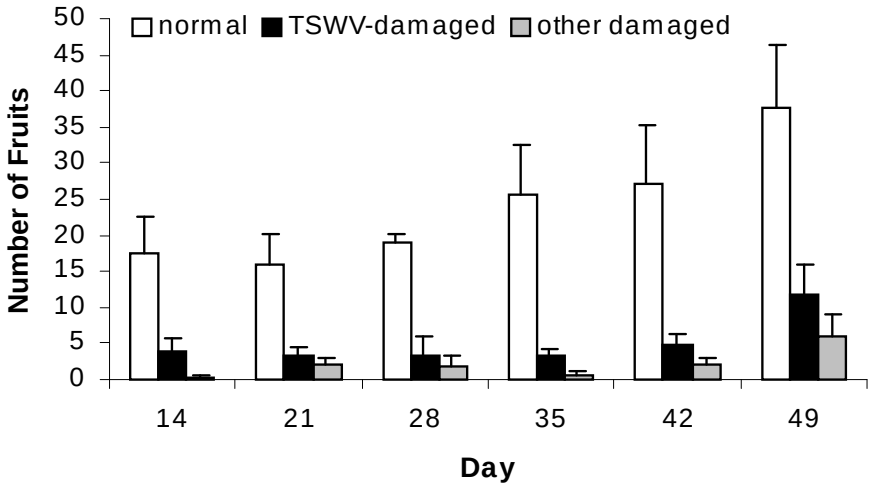


Figure 4.7 Percent observed symptomatic plants (A) and percent TSWV positive (DAS-ELISA) (B) plants over time by age of inoculation (14, 21, 28, 35, 42 and 49 days old).

A. Number of Fruits



B. Weight of Fruits

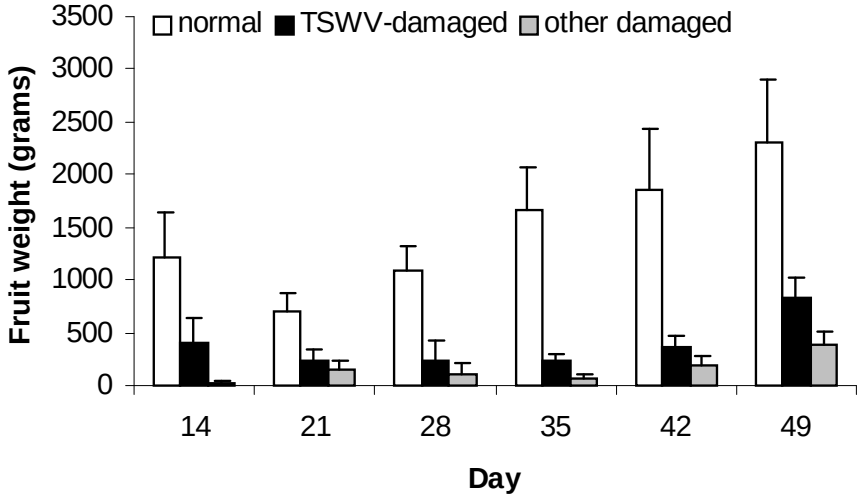


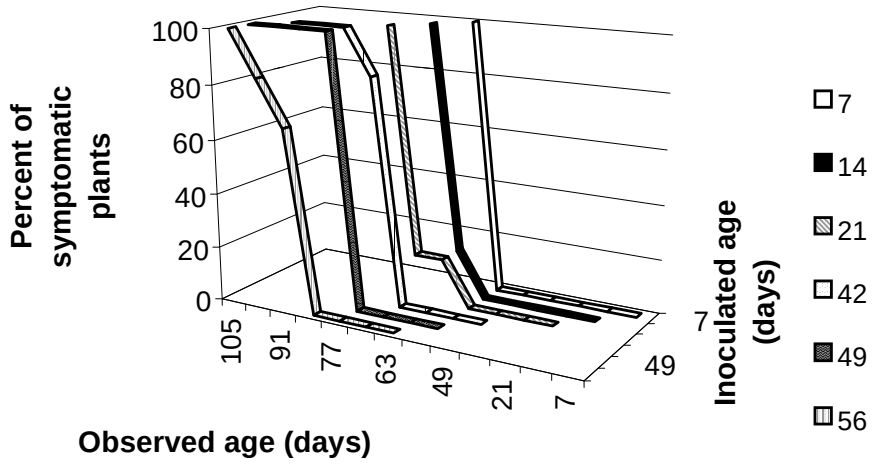
Figure 4.8 Average number (A) and weight (B) of normal and TSWV-damaged fruits per plant from mechanically inoculated tomato plants of different ages (14, 21, 28, 35, 42, and 49 days old). Other damaged fruits, usually with blossom end rot, were unidentifiable for TSWV-damages.

Table 4.4 Yield data and estimated market value of harvested tomato fruits per plant from mechanical inoculation by plant age (14, 21, 28, 35, 42, and 49 days).

Plant age (days)	n	Average percent TSWV positive plants	Marketable fruit weight (grams)	Fruit market value (in US $)[1]	TSWV-damaged fruit weight (grams)	Loss (in US $) to TSWV damages	Plant height at age 80 days (cm)
14	6	0.347 a	741.7 a,b	0.540	315.8 a,b	0.230	62.7 ± 3.7 a,b
21	6	0.417 a,b	225.8 b	0.165	50.0 a,b	0.036	57.0 ± 5.7 b
28	6	0.430 a,b	455.0 a,b	0.332	39.2 b	0.028	60.3 ± 2.0 b
35	6	0.430 a,b	925.0 a ,b	0.674	44.2 a,b	0.032	64.3 ± 3.1 a,b
42	6	0.444 b	1209.2 a	0.881	75.0 a,b	0.055	65.0 ± 5.7 a,b
49	6	0.458 b	1413.3 a	1.030	328.3 a	0.239	72.8 ± 2.9 a

[1]Market value was calculated from weight of normal fruits in three sizes, extra large, large, and medium, with the average price ($8.28/ 11.4 kg) of tomato in Georgia from May-November of 1991-1995.
[2]Means followed by the same letter within inoculation method not significantly different (LSD, $P<0.05$).

A. Percent TSWV-symptomatic plants.



B. Percent TSWV-ELISA positive plants.

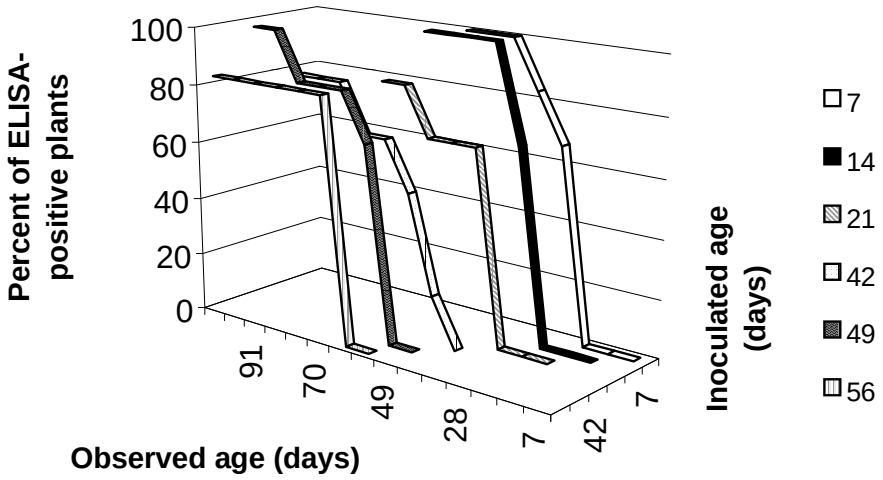
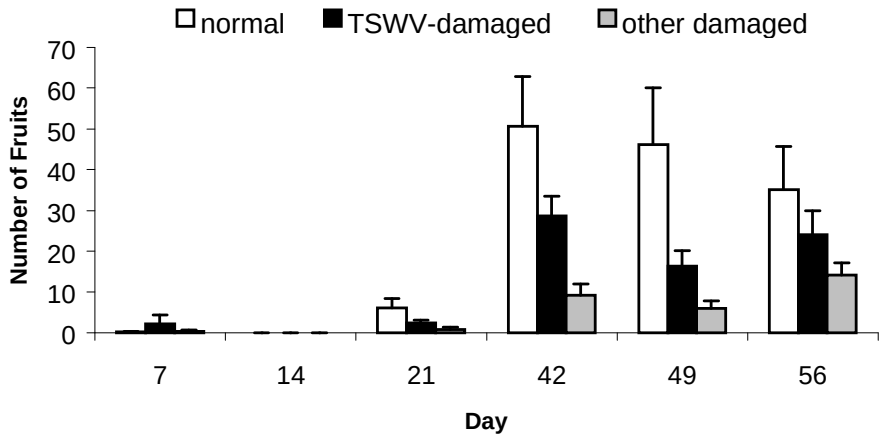


Figure 4.9 Percent observed symptomatic plants (A) and percent TSWV positive (DAS-ELISA) (B) plants over time by age of inoculation (7, 14, 21, 42, 49 and 56 days old).

A. Fruit number.



B. Fruit weight.

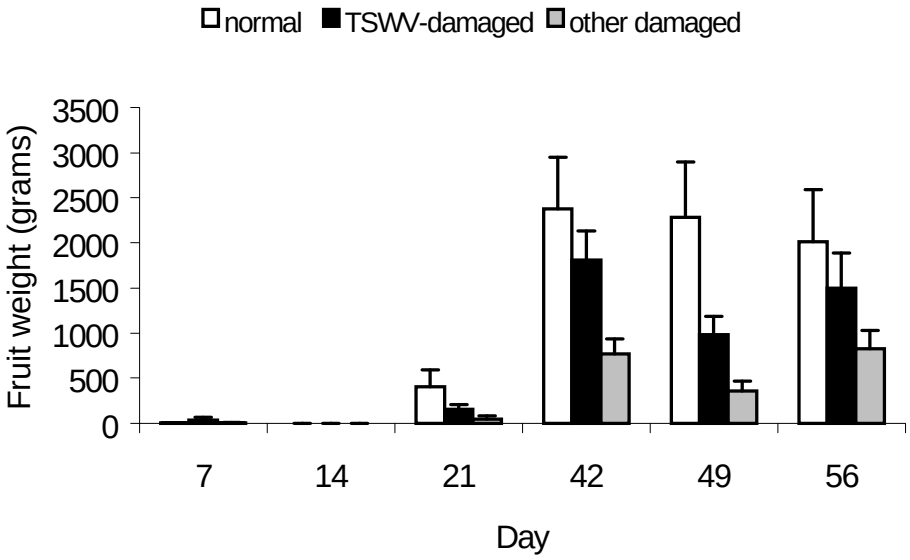


Figure 4.10 Average number (A) and weight (B) of fruits per plant of mechanically inoculated tomato plants at different ages (7, 14, 21, 42, 49, 56 days). Other damaged fruits, usually with blossom end rot, were unidentifiable for TSWV-damages

Table 4.5 Yield data and estimated market value of harvested tomato fruits per plant from mechanical inoculation by plant age (7, 14, 21, 42, 49, and 56 days).

Plant age (days)	n	Average percent TSWV positive plants	Marketable fruit weight (grams)	Fruit market value (in US $)[1]	TSWV-damaged fruit weight (grams)	Loss (in US $) to TSWV damages	Plant height at age 80 days (cm)
7	6	0.810 a	0 a	0.000	0 a	0.000	30.8 ± 7.9 a
14	6	0.757 a	0 a	0.060	0 a	0.000	24.2 ± 5.2 a
21	6	0.563 b	240.0 a	0.175	43.3 a	0.032	46.5 ± 5.3 b
42	6	0.500 b	1467.5 b	1.069	664.2 b	0.484	90.5 ± 8.0 c
49	6	0.437 b	1230.8 b	0.897	352.5 a,b	0.257	92.3 ± 4.2 c
56	6	0.397 b	1017.8 b	0.742	491.4 b	0.358	83.6 ± 11.1 c

[1]Market value was calculated from weight of normal fruits in three sizes, extra large, large, and medium, with the average price ($8.28/ 11.4 kg) of tomato in Georgia from May-November of 1991-1995.
[2]Means followed by the same letter within inoculation method not significantly different (LSD, $P<0.05$).

Table 4.6 Correlations of inoculation age, plant height, number of tomato fruits, and fruit weight with ELISA positives and symptoms of TSWV from each experiment.

Treatment	Inoculation age		Plant height		Number of fruit		Fruit weight	
	R[1]	*P*>95%	R	*P*>95%	R	*P*>95%	R	*P*>95%
Experiment 1								
Inoculation age	1.000	-	0.189	0.268	0.129	0.45	0.133	0.438
ELISA	-0.030	0.861	0.051	0.767	-0.314	0.062	-0.333	0.047
Symptom	-0.158	0.357	-0.233	0.172	-0.419	0.011	-0.388	0.019
Mean ELISA	-0.217	0.203	-0.167	0.330	-0.346	0.038	-0.328	0.050
Experiment 2								
Inoculation age	1.000	-	0.336	0.1081	0.799	<0.0001	0.675	0.0003
ELISA	-0.465	0.022	-0.371	0.0742	-0.195	0.360	-0.115	0.593
Symptom	-0.270	0.203	0.350	0.094	0.002	0.994	0.007	0.974
Mean ELISA	0.269	0.203	-0.431	0.035	-0.165	0.440	-0.147	0.494
Experiment 3								
Inoculation age	1.000	-	0.371	0.028	0.125	0.475	0.239	0.166
ELISA	0.009	0.961	-0.173	0.319	0.082	0.638	0.210	0.225
Symptom	0.007	0.969	-0.177	0.309	0.080	0.645	0.210	0.225
Mean ELISA	0.370	0.833	-0.0585	0.739	-0.416	0.813	-0.064	0.716
Experiment 4								
Inoculation age	1.000	-	0.372	0.026	0.465	0.004	0.488	0.003
ELISA	-0.383	0.046	-0.139	0.420	0.037	0.831	0.088	0.601
Symptom	-0.383	0.046	-0.139	0.419	0.037	0.831	0.088	0.601
Mean ELISA	0.179	0.297	-0.473	0.004	-0.345	0.039	-0.251	0.139
Experiment 5								
Inoculation age	1.000	-	0.860	<0.0001	0.765	<0.0001	0.754	<0.0001
ELISA	-0.706	<0.0001	-0.595	0.0001	-0.542	0.0006	-0.527	0.0010
Symptom	-0.787	<0.0001	-0.558	0.0004	-0.443	0.0069	-0.413	0.0124
Mean ELISA	0.063	0.715	0.019	0.9116	-0.039	0.821	-0.070	0.683

[1]Pearson Correlation coefficients, n= 36, 24, 36, 36, and 36, for Experiment 1,2,3,4, and 5, respectively.

The correlation between inoculation ages, plant height, and number and weight of fruit were significant in all experiments except Experiment 1 and 3 (Table 4.6). Although the inoculation age in Experiment 1 was not significantly correlated to yield, proportion of days that plants exhibited symptoms (number of days that plant exhibited symptoms divided by number of total observed days) significantly correlated to yield in Experiment 1. The higher yield in the first inoculated age of Experiment 3 was partially responsible for reducing the correlation between inoculated age and other responses. The proportion of leaf samples that were positive by ELISA and total leaf samples tested for TSWV by ELISA of plants appears to be the most consistent correlation with response to

inoculation age in the three mechanical inoculation experiments. The lack of correlation in the two thrips inoculation studies between ELISA and inoculation age is a surprising result and may require further investigation (Table 4.6)

Conclusion

The hypothesis that early infection results in greater yield loss than by later infection was supported by the data from each of the five experiments. However, the pattern of response was slightly different depending on which inoculation technique was used to test the hypothesis. For example, mechanical inoculation of TSWV to the youngest plants was apparently less effective than thrips transmission at the same age. It was observed that mechanical inoculation to tomato plants at one week caused severe plant stress and variable inoculation efficiency. Mechanical inoculation may have inflicted serious damage to plant tissue and obstructed growth of the seedling. Inoculated plants with only one pair of leaves, especially less than 4-weeks old, had lagging growth compared to non-inoculated plants. The inoculated leaves usually developed premature wilting from either carborundum abrasion or at infection. The rapid death of leaf tissue could have cause the unsuccessful inoculation in some plants.

One interesting observation from these experiments was that symptoms may not always correspond closely to the presence of virus based on ELISA possibly due to factors such as nutrient deficiency, solar intensity, and temperature. What is obvious from these tests is that some plants may test positive for TSWV using ELISA without symptom expression. In general, these results agree with Moriones et al. (1998) that earlier symptomatic plants produce less yield than later symptomatic plants. What was most notable was that symptoms developed rapidly across all treatments after the screen

cages were removed, which was a source of shading. The infection of TSWV after removal of screens and termination of insecticide application occurred when fruits were mature and ready to harvest, so the later infection from natural thrips population probably did not significantly affect the yield of each plant. However, removing of screen could have influenced the symptom expression or the possibility of infection from natural thrips population. Symptoms were also observed to vary from one plant to another plant, possibly because of the variable level of TSWV tolerance from plant to plant. Interestingly, ELISA measurements were less correlated with yield loss than symptoms, which suggest that plant response to the virus is variable.

None of the 36 tomato plants in Experiment 1 showed severe stunting while 15-30% were expected to be severely affected, especially in young plants, based on the assumptions of Moriones et al (1998). However, TSWV-inoculated plants in six-inch pots of similar age class in Experiments 2 and 5 had developed severe stunting symptoms, and most of the early inoculated plants did not produce any fruit, (Figure 4.4 and Figure 4.10). Uninfected tomato plants in six-inch pots were able to produce small fruits. Possibly, limited root space in pots might have resulted in the expression of severe symptoms by stressing the plant through limiting water intake and consequently growth. Although there was variable symptom intensity across the different experiments, the trend of greater yield loss occurring with the earliest inoculations was evident in all experiments. Thus, the prevention of thrips inoculation early in the tomato growing season needs to be emphasized in TSWV management programs in order to reduce the impact of TSWV.

Literature Cited

Aramburu, J., Riudavets, J., Arno, J., Lavina, A., and Moriones, E. 1997. The proportion of viruliferous individuals in field populations of *Frankliniella occidentalis*: Implications for *Tomato spotted wilt virus* epidemics in tomato. Eur. J. Plant Pathol. 103: 623-629.

Bautista, R.C., Mau, R.F.L., Cho, J.J., and Custer, D.M. 1995. Potential of *Tomato spotted wilt virus* plant hosts in Hawaii as virus reservoirs for transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae). Phytopathology 85: 953-958.

Cho, J.J., Mau, R.F.L., Hamasaki, R.T., and Gonsalves, D. 1988. Detection of *Tomato spotted wilt virus* in individual thrips by enzyme-linked immunosorbent assay. Phytopathology 78: 1348-1352.

Fajardo, T.V.M., Lopes, C.A., Silva, W.L.C.E., and Avila, A.C.D. 1997. Dispersal of disease and yield reduction in processing tomato infected with *Tospovirus* in the Federal District, Brazil. Fitopatol. Bras. 22: 413-418.

German, T. L., Ullman, D. E., and Moyer, J. W. 1992. Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. Annu. Rev. Phytopathol. 30: 315-348.

Gitaitis, R.D., Dowler, C.C., and Chalfant, R.B. 1998. Epidemiology of tomato spotted wilt in pepper and tomato in southern Georgia. Plant Dis. 82: 752-756.

Gofflot, A. and Verhoyen, M. 1990. Heavy progression of *Tomato spotted wilt virus* infections transmitted by the thrips *Frankliniella occidentalis* in ornamental greenhouse crops in Belgium. Parasitica. 46: 85-88.

Kumar, N.K.K., Ullman, D.E., and Cho, J.J. 1993. Evaluation of *Lycopersicon* germ plasm for *Tomato spotted wilt virus* resistance by mechanical and thrips transmission. Plant Dis. 77: 938-94.

Marchoux, G., Gebre-Selassie, K., and Villevieille, M. 1991. Detection of *Tomato spotted wilt virus* and transmission by *Frankliniella occidentalis* in France. Plant Pathol. 40: 347-351.

McPherson, R.M., Pappu, H.R., and Jones, D.C. 1999. Occurrence of five thrips species on flue cured tobacco and impact on spotted wilt disease incidence in Georgia. Plant Dis. 83: 765-767.

Mizelle, W.O. 1995. Vegetable economics: A planning guide for 1996, 1991-1995 prices, Georgia vegetable prices. Cooperative Extension Service, the University of Georgia College of Agricultural and Environmental Sciences p. 16.

Moriones, E., Aramburu, J., Riudavets, J., Arno, J., and Lavina, A. 1998. Effect of plant age at time of infection by *Tomato spotted wilt virus* on the yield of field-grown tomato. Eur. J. Plant Pathol. 104: 295-300.

Riley, D.G. and Pappu, H.R. 2000. Evaluation of tactics for management of thrips-vectored *Tomato spotted wilt virus* in tomato. Plant Dis. 84:847-852.

Roca, E., Aramburu, J., and Moriones, E. 1997. Comparative host reactions and *Frankliniella occidentalis* transmission of different isolates of *Tomato spotted wilt virus* from Spain. Plant Pathol. 46: 407-415.

SAS Institute. 1998. User's manual, version 7th ed. SAS Institute, Cary, NC.

Stewart, J.W., Cole, C., and Lummus, P. 1989. Winter survey of thrips (Thysanoptera: Thripidae) from certain suspected and confirmed hosts of *Tomato spotted wilt virus* in south Texas. J. Entomol. Sci. 24: 392-401.

Ullman, D.E., Cho, J.J., Mau, R.F.L., Hunter, W.B., Westcot, D.M., and Custer, D.M. 1992. Midgut epithelial cells act as a barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. Phytopathology 82: 1333-1342.

Ullman, D.E., Westcot, D.M., Chenault, K.D., Sherwood, J.L., German, T.L., Bandla, M.D., Cantone, F.A., and Duer, H.L. 1996. Compartmentalization, intracellular transport, and autophagy of *Tomato spotted wilt virus* proteins in infected thrips cells. Phytopathology 85: 644-654.

Ullman, D.E., Sherwood, J.L., and T.G. German. 1997. Thrips as Vectors of Plant Pathogens, pp. 539-565, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Van de Wetering, F. Goldbach, R., and Peters, D. 1996. *Tomato spotted wilt virus* ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900-905.

Van de Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., and Peters, D. 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower *Tospovirus* transmission by females. Entomol. Exp. Appl. 88: 9-15.

Webb, S.E., Kok-Yohomi, M.L., and Tsai, J.H. 1997. Evaluation of *Frankliniella bispinosa* as a potential vector of *Tomato spotted wilt virus*. (Abstr.) Phytopathology 87(suppl.):S102.

Wijkamp, I., Almarza, N., Goldbach, R., and Peters, D. 1995. Distinct levels of specificity in thrips transmission of *Tospoviruses*. Phytopathology 85: 1069-1074.

Wijkamp, I., Goldbach, R., and Peters, D. 1996[a]. Differential susceptibilities between leaf disks and plants in the transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis* to TSWV hosts and transgenic plants. Phytopathology 144: 355-362.

Wijkamp, I., Goldbach, and Peters, D. 1996[b]. Propagation of *Tomato spotted wilt virus* in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. Entomol. Exp. Appl. 81: 285-292.

Wijkamp, I., Van De Wetering, F., Goldbach, R., and Peters, D. 1996[c]. Transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis*; median acquisition and inoculation access period. Ann. App. Biol. 129: 303-313.

CHAPTER 5

CONCLUSIONS

Thrips Feeding Response to Imidacloprid

Presently, combinations of certain control tactics for thrips vectors *of tomato spotted wilt virus* (TSWV) have been effective in certain crops. However, the role of insecticides such as imidacloprid in reducing TSWV has been poorly understood. The data from the experiment in Chapter 3 have revealed one important effect of imidacloprid on thrips feeding behavior. Imidacloprid has been shown to have an anti-feeding effect on thrips in Chapter 3, so the null hypothesis presented in Chapter 1 that imidaclprid does not reduce thrips feeding on tomato leaves has been rejected. The anti-feeding effect on thrips helps to explain the reduction of TSWV incidence in tomato plants treated with imidacloprid in certain situations. The data clearly demonstrated a reduction of thrips feeding with increasing levels of imidacloprid in leaf tissue. These results supported the hypothesis that imidacloprid has an anti-feeding effect on thrips, even at concentrations lower than label recommendations.

Transmission of *Tomato spotted wilt virus* to Young Tomato Plants

From the five experiments in Chapter 4, greater yield reduction was observed from plants infected with TSWV at an earlier plant age than at older ages. The null hypothesis proposed in Chapter 1 that early and late TSWV infected plants will not be significantly different in terms of symptom expression and yield is rejected. Early

transmission of TSWV to tomato produced yield reduction in both transmission methods, mechanical and thrips transmission. There was no significant difference between the two transmission methods in all but the earliest age classes as previously described in Experiments 1 and 2 of Chapter 4. TSWV-inoculated plants in six-inch pots of the similar age class, Experiments 2 and 5, had developed severe stunting, and most of the early-inoculated plants could not produce any fruit. Possibly, limited root space in pots could have caused the expression of symptoms to be more severe than that seen in the cage studies. Mechanical inoculation to tomato plants at one week caused severe plant stress and variable inoculation efficiency. Mechanical inoculation might have inflicted serious damage to leaf. The rapid death of leaf tissue could have caused the unsuccessful inoculation.

Based on these studies, symptoms may not always correspond closely to the presence of virus based on ELISA testing, possibly due to factors, such as nutrient deficiency, solar intensity, and temperature. What is obvious from these tests is that some asymptomatic plants may test positive for TSWV when tested by ELISA. Interestingly, the average ELISA measurements were less correlated with yield loss than symptoms, which suggest that plant response to the virus is variable. The percentage of time that plants were positive for ELISA appears to be the most consistent correlation with the inoculation age. Thus, the combination of ELISA and observation of symptoms are useful in the confirmation of infected plants for the purpose of predicting yield loss. Even though, there was variable symptom intensity across different experiments, the trend of greater yield loss occurring with the earliest inoculations was evident in all of the experiments. Thus the prevention of thrips inoculation early in the tomato growing season needs to be emphasized in TSWV management programs.

Future Research and Application to Tomato Spotted Wilt Management

One of the major implication from this research project is that early inoculations of TSWV to tomato plants can cause greater yield reduction than later inoculations. The activity of TSWV and physiological response in young plants compared to mature plants needs to be further examined because the variable response to TSWV in plants at different ages may provide some insight as to how this virus interacts with the host plant. The mechanism of symptom expression and its severity needs to be investigated since plants inoculated at the same age exhibit a variety of symptom expression levels from asymptomatic to severely stunted growth. When tomato plants were infected at an earlier age, their yields decreased drastically. Young tomato plants, especially those are less than 28 days old after seeded, should be protected from TSWV at a higher intensity of control tactics early in the season based on these studies. Usually, the decision to apply insecticide and other control methods are based on density of thrips vectors or symptomatic plants. Direct damage from thrips feeding scars and number of thrips on sticky traps have been used to assess the threshold density of thrips vectors that needs to be controlled. However, this study suggests early calendar treatment could be warranted. Only few species of thrips are able to transmit TSWV effectively, so identifying these species in the field in early plant age would help to provide a good assessment for calendar control of TSWV vectors during the critical period. Identifying infected plants through symptom expression still provides the best opportunity for estimating disease condition. TSWV-infected plants can be identified by serological testing, especially Enzyme-linked Immunosorbent Assay (ELISA), or symptoms. However, infected plants

may not exhibit symptom from two weeks after infection or even as late as near harvest time as presented in Chapter 4.

Protection of plants from feeding of thrips vectors could require systemic insecticides such as imidacloprid, which should be applied as early as possible in the tomato growing season. Although imidacloprid concentration in plant tissue reduces overtime as plant grows, the late-infection of TSWV may not affect plant yield as does early infection. From this research it appears that tomato seedlings should be protected from TSWV for at least the first four weeks. Application of foliar insecticides should be highly intensive during pre-blossomed period if the natural thrips population is high, and the application of insecticides could be discontinued after plants start bearing fruits.

The feeding preference of thrips for different plant ages and the health condition of tomato plants could be crucial for controlling the vectors of TSWV. Thrips assessment of plant chemical defense, either naturally occurring or artificially applying with insecticides, should be investigated in order to develop the alternative chemical control method. One critical question not addressed by this research is how does thrips starvation affect the anti-feeding behavior induced by imidacloprid. The residue of imidacloprid in each plant varied from plant to plant in this study, probably due to soil physical and chemical properties and plant's ability to uptake and deposit imidacloprid in the tissue. Thrips may prefer to feed on plants with lowest toxicity and take advantage of this variability. What is certain is that more research needs to be conducted on thrips feeding in relation to their ability to transmit Tospoviruses. This research is critical for developing an effective management program in the future.

REFERENCES

Aramburu, J., Riudavets, J., Arno, J., Lavina, A., and Moriones, E. 1997. The proportion of viruliferous individuals in field populations of *Frankliniella occidentalis*: Implications for *Tomato spotted wilt virus* epidemics in tomato. Eur. J. Plant Pathol. 103: 623-629.

Bautista, R.C., Mau, R.F.L., Cho, J.J., and Custer, D.M. 1995. Potential of *Tomato spotted wilt virus* plant hosts in Hawaii as virus reservoirs for transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae). Phytopathology 85: 953-958.

Bertrand, P.F. 1997. 1996 Georgia plant disease losses. University of Georgia, Cooperative Extension Service Publication 1-97: 1.

Boissot, N., Reynaud, B., Letourmy, P. 1998. Temporal Analysis of Western Flower Thrips (Thysanoptera: Thripidae) Population Dynamics on Reunion Island. Environ. Entomol. 27: 1437-1443.

Boiteau, G. and Osborn, W.P.L. 1997. Behavioural effects of imidacloprid, a new nicotinyl insecticide, on the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Homoptera, Aphididae). Can. Entomol. 129: 241-249.

Brittlebank, C.C. 1919. Tomato diseases. J. Agric. Victoria. Aust. 17: 231-235.

Brown, S.L., Todd, J.W, Culbreath, A.K. Baldwin, J.A., Beaslet, J.P., and Pappu, H.R. 1998. Tomato spotted wilt of peanut: Identification and avoiding high risk situation. University of Georgia Cooperative Extension Service, report#B1165 pp.1-3.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) 1996. `Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' URL http://biology.anu.edu.au/Groups/MES/vide/.

Chamberlin, J.R., Todd, J.W., Beshear, R.J., Culbreath, A.K., and Demski,, J.W. 1992. Overwintering hosts and wingform of thrips, *Frankliniella spp*., in Georgia (Thysanoptera: Thripidae): Implications for management of spotted wilt disease. Environ. Entomol. 21: 121-128.

Cho, J.J., Mau, R.F.L., Hamasaki, R.T., German, T.L., Hartman, R.W., Yudin, L.S., Gonsalves, D., and Provvidenti, R. 1989. A multidisciplinary approach to *Tomato spotted wilt virus* management of in Hawaii. Plant Dis. 73:375-383.

Cho, J.J., Mau, R.F.L., Hamasaki, R.T., and Gonsalves, D. 1988. Detection of *Tomato spotted wilt virus* in individual thrips by enzyme-linked immunosorbent assay. Phytopathology 78: 1348-1352.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Shokes, F.M., and Pappu, H.R. 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis. 81:1410-1415.

De Avila, A. C., de Haan, P., Kormelink, R., Resende, de O., Goldbach, R.W., and Peters, D. 1993. Classification of Tospoviruses based on phylogeny of nucleoprotein gene sequences. J. Gen. Virol. 74: 153-159.

De Jager, C.M., Butot, R.P.T., Klinkhamer, P.G.L., De Jong, T.J., Wolff, K., Van der Meijden, E. 1995. Genetic variation in chrysanthemum for resistance to *Frankliniella occidentalis*. Entomol. Exp. Appl. 77: 277-287.

Diez, M.J., Rosello, S., Nuez, F., Costa, J., Lacasa, A., Catala, M.S. 1999. Tomato production under mesh reduces crop loss to tomato spotted wilt virus in some cultivars. Hort. Science. 34: 634-637.

Fajardo, T.V.M., Lopes, C.A., Silva, W.L.C.E., and Avila, A.C.D. 1997. Dispersal of disease and yield reduction in processing tomato infected with *Tospovirus* in the Federal District, Brazil. Fitopatol. Bras. 22: 413-418.

German, T. L., Ullman, D. E., and Moyer, J. W. 1992. Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. Annu. Rev. Phytopathol. 30: 315-348.

Gitaitis, R.D., Dowler, C.C., and Chalfant, R.B. 1998. Epidemiology of tomato spotted wilt in pepper and tomato in southern Georgia. Plant Dis. 82: 752-756.

Gofflot, A. and Verhoyen, M. 1990. Heavy progression of *Tomato spotted wilt virus* infections transmitted by the thrips *Frankliniella occidentalis* in ornamental greenhouse crops in Belgium. Parasitica. 46: 85-88.

Guillibeau, P. (ed.) 1999. Georgia Pest Control Handbook. Cooperative Extension Service, University of Georgia pp. 137-213.

Jan, F.J., Fagoaga, C., Pang, S.Z., and Gonsalves, D. 2000. A minimum length of N gene sequence in transgenic plants is required for RNA-mediated *Tospovirus* resistance. J. Gen. Virol. 81: 235-242.

Jensen, S.E. 1998. Acetylcholinesterase Activity Associated with Methiocarb Resistance in a Strain of Western Flower Thrips, *Frankliniella occidentalis* (Pergande). Pesticide Biochem. Physiol. 61: 191-200.

Johnson, R.R., Black, L.L., Hobbs, H.A., Valverde, R.A., Story, R.N., and Bond, W.P. 1995. Association of *Frankliniella fusca* and three winter weeds with *Tomato spotted wilt virus* in Louisiana. Plant Dis. 79: 572-576.

Johnson, W.C. III, Todd, J.W., Culbreath, A.K., and Mullinixx, B.G. Jr. 1996. Role of warm-season weeds in spotted wilt epidemiology in the southeastern Coastal Plain. Agron. J. 88: 928-933.

Kidd, H. and D. James (eds.). 1994. Agrochemicals Handbook. Third Edition. Royal Society of Chemistry. Cambridge, England.

Kirk, W.D.J. 1997. Feeding, pp. 119-174, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Kontsedalov, S., Weintraub, P.G., Horowitz, A.R., and Ishaaya, I. 1998. Effects of Insecticides on Immature and Adult Western Flower Thrips (Thysanoptera: Thripidae) in Israel. J. Econ. Entomol. 9: 1067-1071.

Kumar, N.K.K., Ullman, D.E., and Cho, J.J. 1993. Evaluation of *Lycopersicon* germ plasm for *Tomato spotted wilt virus* resistance by mechanical and thrips transmission. Plant Dis. 77: 938-94.

Kumar, N.K.K., Ullman, D.E., Cho, J.J. 1995. Resistance among *Lycopersicon species* to *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1057-1065.

Marchoux, G., Gebre-Selassie, K., and Villevieille, M. 1991. Detection of *Tomato spotted wilt virus* and transmission by *Frankliniella occidentalis* in France. Plant Pathol. 40: 347-351.

McPherson, R.M., Pappu, H.R., and Jones, D.C. 1999. Occurrence of five thrips species on flue cured tobacco and impact on spotted wilt disease incidence in Georgia. Plant Dis. 83: 765-767.

Mizelle, W.O. 1995. Vegetable economics: A planning guide for 1996, 1991-1995 prices, Georgia vegetable prices. Cooperative Extension Service, the University of Georgia College of Agricultural and Environmental Sciences p. 16.

Moriones, E., Aramburu, J., Riudavets, J., Arno, J., and Lavina, A. 1998. Effect of plant age at time of infection by *Tomato spotted wilt virus* on the yield of field-grown tomato. Eur. J. Plant Pathol. 104: 295-300.

Moyer, J.W., German, T.G., Sherwood, J.L., and Ullman, D.E. 1999. 'An Update on *Tomato spotted wilt virus* and Related Tospoviruses Version: 7th August 1999.' URL http://www.scisoc.org/feature/tospovirus/Top.html.

Munkvold, G.P., McGee, D.C., and Iles, A. 1996. Effects of imidacloprid seed treatment of corn on foliar feeding and *Erwinia stewartii* transmission by the corn flea beetle. Plant disease. 80: 747-749.

Pang, S.Z., Nagpala, P., Wang, M., Slightom, J.L., and Gonsalves, D. 1992. Resistance to heterologous isolates of *Tomato spotted wilt virus* in transgenic tobacco expressing its nucleocapsid protein gene. Phytopathology 82: 1223-1229.

Qiu, W. and Moyer, J.W. 1999. *Tomato spotted wilt virus* adapts to the TSWV N gene-derived Resistance by genome reassortment. Phytopathology 89: 1-10.

Riley, D.G. and Pappu, H.R. 2000. Evaluation of tactics for management of thrips-vectored *Tomato spotted wilt tospovirus* in tomato. Plant Dis. 84: 847-852.

Roca, E., Aramburu, J., and Moriones, E. 1997. Comparative host reactions and *Frankliniella occidentalis* transmission of different isolates of *Tomato spotted wilt virus* from Spain. Plant Pathol. 46: 407-415.

Roggero, P. and Pennazio, S. 1997.Thermal inactivation of *Tomato spotted wilt virus* in vivo. Physiological and molecular plant pathology. 51: 35-40.

SAS Institute. 1998. User's manual, version 7th ed. SAS Institute, Cary, NC.

Salguero Navas, V.E., Funderburk, J.E., Beshear, R.J., Olson, S.M., and Mack, T.P. 1991. Seasonal patterns of Frankliniella spp. (Thysanoptera: Thripidae) in tomato flowers. J. Econ. Entomol. 84: 1818-1822.

Smith, S.F. and Krischik, V.A. 1999. Effects of Systemic Imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae) Environ. Entomol. 28: 1189-1195.

Sparks, A.N. Jr., Anciso, J.R., Riley, D.G., and Chambers, C. 1998. Insecticidal control of thrips on onions in South Texas: insecticide selection and application methodology. Subtropl. Pl. Sci. 50: 58-62.

Stewart, J.W., Cole, C., and Lummus, P. 1989. Winter survey of thrips (Thysanoptera: Thripidae) from certain suspected and confirmed hosts of *Tomato spotted wilt virus* in south Texas. J. Entomol. Sci. 24: 392-401.

Terry, L.I. 1997. , Host Selection, Communication and Reproductive Behaviour. pp. 65-118, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Thomson, W.T. (ed) 1994. Agricultural Chemicals: Book I. Thomson Publication, Fresno, CA. p. 158.

Tsuda, S., Fujisawa, I., Ohnishi, J., and Hosokawa, D. Tomaru, 1996. Localization of tomato spotted wilt tospovirus in larvae and pupae of the insect vector *Thrips setosus* Phytopathology 86: 1199-1203.

Ullman, D.E., Cho, J.J., Mau, R.F.L., Hunter, W.B., Westcot, D.M., and Custer, D.M. 1992. Midgut epithelial cells act as a barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. Phytopathology 82: 1333-1342.

Ullman, D.E., Westcot, D.M., Chenault, K.D., Sherwood, J.L., German, T.L., Bandla, M.D., Cantone, F.A., and Duer, H.L. 1996. Compartmentalization, intracellular transport, and autophagy of *Tomato spotted wilt virus* proteins in infected thrips cells. Phytopathology 85: 644-654.

Ullman, D.E., Sherwood, J.L., and T.G. German. 1997. Thrips as Vectors of Plant Pathogens, pp. 539-565, In (ed.) T.L. Lewis, Thrips as Crop Pests, CAB International, London, UK.

Van de Wetering, F. Goldbach, R., and Peters, D. 1996. *Tomato spotted wilt virus* ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathology 86: 900-905.

Van de Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., and Peters, D. 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower *Tospovirus* transmission by females. Entomol. Exp. Appl. 88: 9-15.

Webb, S.E., Kok-Yohomi, M.L., and Tsai, J.H. 1997. Evaluation of *Frankliniella bispinosa* as a potential vector of *Tomato spotted wilt virus*. (Abstr.) Phytopathology 87(suppl.):S102.

Wijkamp, I., Almarza, N., Goldbach, R., and Peters, D. 1995. Distinct levels of specificity in thrips transmission of *Tospoviruses*. Phytopathology 85: 1069-1074.

Wijkamp, I., Goldbach, R., and Peters, D. 1996[a]. Differential susceptibilities between leaf disks and plants in the transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis* to TSWV hosts and transgenic plants. Phytopathology 144: 355-362.

Wijkamp, I., Goldbach, and Peters, D. 1996[b]. Propagation of *Tomato spotted wilt virus* in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. Entomol. Exp. Appl. 81: 285-292.

Wijkamp, I., Van De Wetering, F., Goldbach, R., and Peters, D. 1996[c]. Transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis*; median acquisition and inoculation access period. Ann. App. Biol. 129: 303-313.

Zhao, G., Liu, W., Brown, J.M., and Knowles, C.O. 1995. Insecticide resistance in field and laboratory strains of western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1164-1170.

APPENDIX A

THRIPS FEEDING RESPONSE DATA AND IMAGES OF FEEDING SCARS.

Table 1. Admire® rate for tomato plants in thrips feeding behavior study.

Level	Rate fl oz per acre	Rate8.69 µl per pot (100ml water)	Rate µl / 6 pots (600ml water)
R0	0	0.0	0.0
R1	16	2.17	13
R2	24	3.26	19.6
R3	32	4.35	26.1
R4	64	8.69	52.2
R5	128	17.39	104.3

Actually measurement of Admire 2F®: rate µl / 6 pots in 600ml water before measure the mixture 100ml applied to each plant.

Table 2. Feeding of thrips on tomato plants treated with various concentration of imidacloprid (rate as in table 1) .

Plant	Leaf residue	# of feeding scars[1], black(B[2])/ # of feeding scars, white(W[3])/thrips condition(T[4])														
		16 July 1999			19 July 1999			22 July 1999			26 July 1999			2 August 1999		
		# scars			# scars			# scars			# scars			# scars		
		B	W	T	B	W	T	B	W	T	B	W	T	B	W	T
R0-1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1
R0-2	0	0	0	1	2	0	1	1	0	0	0	1	1	1	0	1
R0-3	0	2	0	1	5	4	1	2	2	2	4	3	1	2	0	1
R0-4	0	1	1	1	0	0	1	0	0	2	1	0	1	0	0	1
R0-5	0	0	0	0	2	2	0	0	0	1	4	0	1	6	0	1
R0-6	0.33	4	0	1	3	0	1	0	3	1	0	0	1	0	1	1
R1-1	0.77	0	0	1	0	0	1	0	0	1	0	1	1	5	0	1
R1-2	1.2	1	0	1	0	0	1	0	0	1	2	0	0	0	0	1
R1-3	1.13	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
R1-4	1	1	0	1	0	0	0	1	0	1	0	0	1	0	0	1
R1-5	0.99	1	0	1	0	0	1	0	2	0	0	0	1	0	0	1
R1-6	0	0	0	0	1	0	1	0	0	0	4	0	1	0	0	1
R2-1	1.6	1	1	1	0	0	1	0	0	1	2	0	1	0	0	0
R2-2	1.27	0	0	1	1	1	0	1	0	1	1	1	1	0	0	1
R2-3	4.66	3	0	1	0	1	1	0	0	1	0	3	1	2	0	1
R2-4	1.04	0	0	1	0	2	0	0	0	1	0	0	1	0	0	1
R2-5	3	0	0	1	0	0	0	0	0	1	0	0	1	0	0	2
R2-6	2.74	0	0	1	0	0	0	1	2	1	0	0	1	0	0	1
R3-1	1.08	0	0	1	0	0	1	0	3	1	0	0	1	0	0	1
R3-2	2.62	0	0	1	0	1	1	0	0	0	0	0	1	0	0	2
R3-3	2.42	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1
R3-4	0.44	0	1	1	2	0	1	0	1	1	0	0	1	0	0	0
R3-5	0.67	0	0	1	0	0	0	0	0	2	2	0	1	0	0	1
R3-6	1.84	0	0	1	0	0	1	0	0	1	0	1	1	1	0	0
R3-1	4.95	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1
R3-2	5.11	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1
R3-3	0	0	1	1	0	0	0	0	0	2	0	0	1	0	0	1
R3-4	0	1	2	1	0	0	1	2	0	1	0	0	1	0	0	1
R3-5	2.08	0	0	1	0	0	1	0	0	1	0	0	0	1	0	1
R3-6	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0	1
R4-1	2.32	0	0	1	0	0	2	0	0	1	0	0	1	0	0	0
R4-2	12.49	0	0	1	0	0	2	0	0	2	0	0	2	0	0	1
R4-3	11.04	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
R4-4	20.27	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1
R5-5	4.87	0	0	2	0	0	0	0	0	2	0	0	1	0	0	1
R5-6	8.15	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1

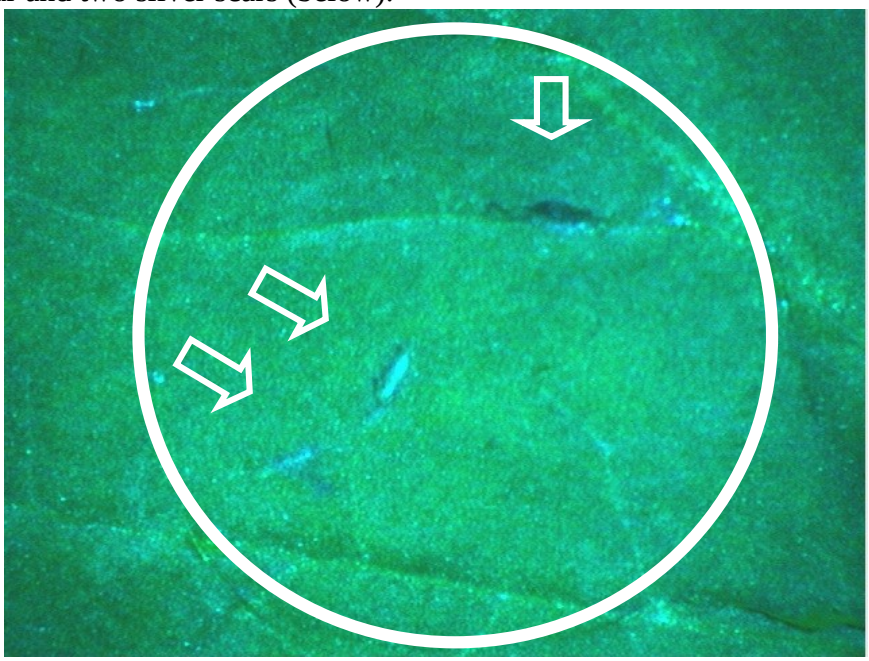
[1]Feeding scars are area on leaves showing feeding damage from thrips, usually 1 mm wide and 1-3 mm long.

[2] B: Black feeding scar -area of wet leaf tissue from thrips feeding.

[3]W: White feeding scar -area of dry leaf tissue from thrips feeding, sometimes called silver scar.

[4]T: Thrips condition: 0-not present in microcage after 72 hours.
1-present in microcage and alive.
2-present in microcage but dead.

A. Image of tomato leaf surface, R4-4 (Rate 8.69 µl per plant), date 16 July 1999, one black scar and two silver scars (below).



B. Image of a non-feed upon tomato leaf surface, rate 17.39 µl per plant, date 26 July 1999 (below).

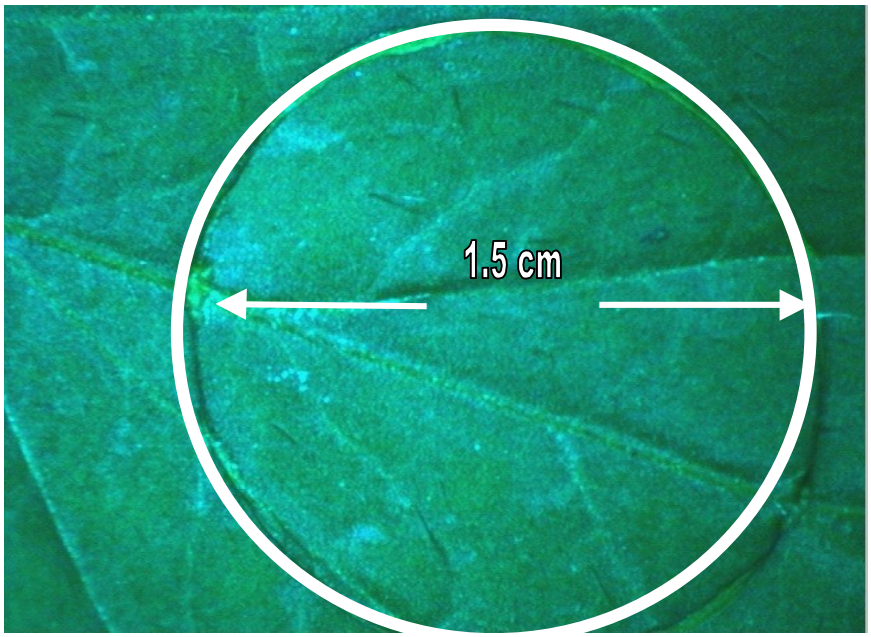


Figure 1. Feeding scars of thrips on tomato leaves treated with two different concentrations of imidacloprid (Admire®)

APPENDIX B

ELISA DATA AND TOMATO HARVEST DATA

Table 1. Percentage of ELISA positive of leaf samples from the same plant. Leaf samples from Experiment 3,4, and 5 of chapter 4.

	N	%ELISA positive	% Symptom
Leaf section			
Terminal	48	29.37	47.92
Low	48	64.75	85.42
Leaf symptom			
Not visible	62	61.29	-
Visible	62	75.81	-

Table 2. TSWV-ELISA result of leaf samples from tomato plants infected with TSWV by thrips transmission in Experiment 3 of chapter 4.

Plant Number	Treatment (days old)	Wk4	wk5	wk6	wk7	wk8	wk9	wk10	wk11	Mean
1	14	5E-04	0	0.511	0.008	0.039	0.008	0.004	0.059	0.079
2	35	0.002	0.238	0.543	0.045	0.008	0.045	0.022	0.006	0.114
3	35	0.002	0.048	0.457	0.03	0	0.03	0	0.014	0.073
4	21	0.002	0.011	0.511	0	0.043	0	0	0.032	0.075
5	42	0.005	0	0.436	0.015	0.029	0.015	0.68	1.386	0.321
6	42	0	0.011	0.489	0.97	0.069	0.97	0.422	0.088	0.377
7	7	0	0.016	0.532	0.045	0.065	0.045	0.009	0.009	0.09
8	14	0	0	0.372	2.598	0.033	2.598	0	0.003	0.701
9	7	0	0.026	0.266	1.523	0.038	1.523	0	0.004	0.422
10	21	0	0	0.33	0.189	0	0.189	0	0	0.089
11	7	0	0	0.298	0.205	0.004	0.205	0	0.004	0.089
12	21	0	0	0.383	0.053	0.046	0.053	0	0.004	0.067
13	28	0	0	0.415	0.129	0.054	0.129	0	0.002	0.091
14	42	0	0.042	0.457	0.106	0.034	0.106	0.031	0.002	0.097
15	42	0	0.423	1.277	0	0.073	0	0	0.011	0.223
16	28	0	0	0.16	0.03	0.046	0.03	0.013	8E-04	0.035
17	42	8E-04	0	0	0	0.005	0	0.013	0.011	0.004
18	28	0.007	0	0.223	0	0.031	2.715	0.627	1.116	0.59
19	14	0.002	1.862	0.138	0	0.001	0	0.204	0.111	0.29
20	7	0	0	0.181	0	1.02	1.073	0.4	0.007	0.335
21	35	0	0	0	0	1.753	0	0.84	0.918	0.439
22	14	0	0.016	0.128	0	0.146	0	0.138	0.088	0.064
23	42	0	0.735	0.117	0	0.028	0	0	0.002	0.11
24	28	0	0.042	1.309	0.023	0.071	0.023	0	0.006	0.184
25	35	0.002	0	1.755	0.136	0.06	0.136	0	0.002	0.262
26	28	0	0	1.649	0.045	0.001	0.045	0.391	1.195	0.416
27	7	0	0	0.713	0	0	0	0.009	0.08	0.1
28	14	0	0.032	1.84	0	0.045	0	0.009	0.003	0.241
29	21	0	0	1.532	3.712	0.034	3.712	0	0.004	1.124
30	21	3E-04	0.265	1.426	3.576	0.075	3.576	0.036	0.002	1.119
31	7	0.002	0.063	1.16	2.038	0.084	2.038	0	1.054	0.805
32	35	0.002	0	0.191	0.098	0.333	0.098	0	0.053	0.097
33	35	0.003	0.011	1.032	0.068	0.065	0.068	0	8E-04	0.156
34	28	0.002	0.032	0.904	0.023	0.015	0.023	0	8E-04	0.125
35	14	0.002	0.048	1.309	0	0	0	0	0	0.17
36	21	0.002	0	0.064	0.008	0.047	0.008	0.018	0	0.018
Positive Control		3.75	0.19	0.098	0.132	2.155	0.495	0.24	1.245	1.038
Negative Control		0.007	0.001	0.004	0	0.105	0	0.015	0.007	0.017

Table 3. TSWV-ELISA result of leaf samples from tomato plants infected with TSWV by mechanical inoculation in Experiment 4 of chapter 4.

Plant number	Treatment (days old)	Wk4	wk5	wk6	wk7	wk8	wk9	wk10	wk11	Mean
1	14	0.001	0	1.34	0.009	0	0.008	0.047	0.002	0.176
2	42	0	0.058	1.468	0	0.082	0.012	0.031	0.002	0.207
3	35	0	0.302	1.457	0.013	0.023	0.026	0.058	0	0.235
4	35	0	0	1.34	0	0.043	0	0.043	0	0.178
5	35	0	0.053	1.287	0	0	0.028	0.043	8E-04	0.177
6	14	0.002	0.085	0.362	0	0.226	0.026	0.054	0.002	0.095
7	14	0.002	0	1.426	0	0.043	0.004	0.035	0	0.189
8	42	0.053	0.037	1.16	0	0	0.012	0.039	0.005	0.163
9	28	0	0.026	1.149	0	0.061	0.01	0.039	0	0.161
10	49	8E-04	0.984	1.032	0	0.062	0.01	0.023	0	0.264
11	14	0	0	0.904	0	0.101	0.016	0.047	0	0.133
12	49	0	1.751	0.404	0	0.223	0.002	0.012	0	0.299
13	42	0	0.016	0.553	1.485	1.587	3.455	2.354	1.412	1.358
14	28	0	0.058	0.457	0	0.093	0.02	0.086	0.288	0.125
15	42	0	0	0.383	0.004	0.023	0.006	0.078	0.002	0.062
16	21	0	0.005	0.383	0.022	0	0.03	0.047	0	0.061
17	28	0	0.032	0.34	0	0.043	0	0.027	8E-04	0.055
18	14	0.039	0.053	0.394	0	0.03	0.004	0.027	0.008	0.069
19	21	0	0.016	0.521	0	0.098	0	0	0	0.079
20	21	0	0	0.394	0	0.061	0.016	0.008	0.002	0.06
21	42	0	0.053	0.468	0	0.076	0	0.016	0	0.077
22	28	0	0	0.468	0	0.017	0.004	0.039	0	0.066
23	21	0.005	0.037	0.532	0	0.054	0.012	0.031	8E-04	0.084
24	28	0.048	1.746	0.415	0	0.075	0	0.019	0.002	0.288
25	49	0.003	0.143	0.34	0	0.105	0	0.027	0.002	0.078
26	21	0	0	3.883	1.229	0.986	2.099	0.564	1.173	1.242
27	35	0	0.021	1.277	0.017	0.027	0.02	0.004	0.242	0.201
28	35	0	0.243	0.404	0	0.038	0.014	0.027	0.002	0.091
29	49	0	0.058	0.319	0.004	0.058	0.002	0.016	0	0.057
30	21	0	0	0.319	0	0.081	0.022	0.012	0.002	0.054
31	35	0	0.005	0.394	0	0.014	0	0.004	0	0.052
32	42	0	0.026	0.351	0.017	0.001	0.012	0.023	0.52	0.119
33	49	0	0.032	0.404	0	0.021	0.004	0.016	0.002	0.06
34	28	0	0	0.34	0	0.013	0.032	0.012	0	0.05
35	14	0	0.021	0	0	0	0.008	0	0.799	0.104
36	49	0	0	0.053	2.065	0.912	2.917	2.054	1.013	1.127
Positive Control		3.75	0.19	0.098	0.231	2.155	0.495	0.257	1.291	1.058
Negative Control		0.007	0.001	0.004	0	0.205	0	0	0.007	0.028

Table 4. TSWV-ELISA result of leaf samples from tomato plants infected with TSWV by mechanical inoculation in Experiment 5 of chapter 4.

Plant number	Treatment (days old)	Wk4	wk5	wk6	wk7	wk8	wk9	wk10	wk11	Mean
1	7	0	0	0	0	0.103	0.532	2.288	0.002	0.366
2	42	0.002	0	0.032	0.113	0.057	0.1	0.307	0	0.076
3	14	0	0	0	0.892	0.592	0	0	0	0.185
4	42	0	0.063	0.138	0	0	0.061	0.019	0	0.035
5	7	0	0	0	0.234	1.114	0.066	0.043	0	0.182
6	49	0.002	0.026	0.064	2.26	1.577	2.106	0.521	1.129	0.961
7	14	0	0	0	0	0.897	0	0	0	0.112
8	7	0	0	0	0.571	0.779	0	0	0	0.169
9	42	0.002	0.212	3.074	5.277	0.406	2.69	2.12	0.432	1.777
10	49	0.002	0.074	0.085	0.043	0	0.068	0.098	0.046	0.052
11	49	0.002	0.042	0.106	0.074	0	0.066	0.018	0	0.038
12	21	0	0	0	0.03	0	0.068	0.009	0.002	0.014
13	56	0.002	0.048	0.085	0.065	0	0.052	0	0	0.031
14	21	0	0	0	0.108	0	0.075	0.013	0	0.025
15	14	0	0	0	0.251	1.871	2.079	2.538	0.845	0.948
16	49	0	0.026	0.138	0.165	1.611	0.267	2.062	1.18	0.681
17	56	0.003	0.116	0.106	0.26	2.051	1.914	2.4	0.459	0.914
18	7	0	0	0	0	0	3.14	0.738	1.418	0.662
19	14	0	0	0	0.662	0.521	1.276	1.053	1.191	0.588
20	14	0	0	0	0.338	0.941	1.238	1.596	1.214	0.666
21	49	0	0.032	3.713	0.017	0.396	0.097	2.031	0.071	0.795
22	56	0	0	2.84	0.039	0	0.075	0.791	0.002	0.468
23	21	0	0	0	0.104	0.012	0.066	0.08	0.02	0.035
24	21	0	0	0	0.1	0	0.059	0.196	1.286	0.205
25	14	0	0	0	0.074	0.661	0.949	1.16	0.022	0.358
26	56	0	0.011	0.16	0.1	0.131	1.245	0.564	0	0.276
27	42	0.003	0.032	0	0	0.01	0.405	0.151	0	0.075
28	42	0	0.069	0.074	0.061	0.012	0.066	0.031	0	0.039
29	49	0.002	0.048	0.096	0.126	0	0.051	0.004	0	0.041
30	56	0	0.09	0.191	0.281	0.002	0.027	0.173	0	0.096
31	42	0	0.063	0.245	1.108	1.226	0.977	1.507	1.31	0.804
32	21	0	0	0	0	0.281	1.062	1.262	0.809	0.427
33	21	0	0	0	0.121	0.026	0.218	0.062	0.125	0.069
34	56	0	0.063	0.149	0.19	0.404	2.132	0.556	1.604	0.637
35	7	0	0	0	0.268	0.826	0.918	0.769	1.029	0.476
36	7	0	0	0	0.264	1.267	0.833	1.169	1.05	0.573
Positive Control		3.75	0.19	0.098	0.231	2.155	0.467	0.257	1.296	0.825
Negative Control		0.007	0.001	0.004	0	0.205	0.025	0	0.004	0.024

Table 5. Harvested tomato fruits number and weight from Experiment 1 of chapter 4.

Size		Extra Large				Large				Medium				Cull				Total			
Viral damage		Clean		damage		clean		damage		Clean		Damage		clean		damage		clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
1	m2	0	0	0	0	0	0	0	0	1	113	0	0	0	0	2	-	1	113	2	0
2	t2	0	0	0	0	1	135	0	0	0	0	0	0	4	308	1	-	5	443	1	0
3	m2	0	0	0	0	0	0	0	0	1	95	1	198	4	201	5	-	5	296	6	198
4	m2	0	0	1	397	0	0	0	0	0	0	3	361	3	227	2	-	3	227	6	758
5	t4	0	0	0	0	1	125	0	0	5	559	1	57	3	142	0	-	9	826	1	57
6	m2	0	0	0	0	0	0	0	0	0	0	1	113	2	128	3	-	2	128	4	113
7	m4	1	178	1	340	1	162	0	0	0	0	0	0	7	450	0	-	9	790	1	340
8	t4	0	0	0	0	1	147	2	367	0	0	1	85	1	65	2	-	2	212	5	452
9	t1	0	0	1	312	0	0	0	0	0	0	0	0	0	0	1	-	0	0	2	312
10	t1	0	0	1	284	0	0	0	0	0	0	2	263	0	0	1	-	0	0	4	547
11	t4	0	0	2	454	0	0	1	142	0	0	1	85	0	0	1	-	0	0	5	680
12	m4	0	0	1	255	0	0	0	0	2	198	0	0	2	142	1	-	4	340	2	255
13	t2	0	0	0	0	0	0	0	0	0	0	2	203	0	0	0	-	0	0	2	203
14	t1	0	0	1	284	0	0	4	624	0	0	2	244	1	57	3	-	1	57	10	1151
15	t2	0	0	0	0	0	0	1	137	2	243	2	240	1	57	3	-	3	300	6	377
16	m1	0	0	1	284	0	0	3	439	7	689	1	129	4	330	2	-	11	1019	7	852
17	m2	0	0	0	0	0	0	1	175	1	113	2	217	2	121	7	-	3	234	10	392
18	m4	0	0	0	0	0	0	0	0	2	229	4	404	4	252	6	-	6	481	10	404
19	m4	0	0	0	0	1	152	2	274	1	114	0	0	0	0	0	-	2	266	2	274
20	t2	0	0	0	0	0	0	0	0	2	217	0	0	1	57	2	-	3	274	2	0
21	m1	0	0	0	0	3	444	0	0	2	227	0	0	1	57	1	-	6	728	1	0

Size		Extra Large				Large				Medium				Cull				Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage		clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
22	t1	0	0	0	0	0	0	2	425	1	124	2	184	1	85	0	-	2	209	4	609
23	m4	0	0	0	0	0	0	1	227	0	0	1	85	0	0	4	-	0	0	6	312
24	m1	0	0	0	0	1	162	3	400	0	0	4	411	1	57	3	-	2	219	10	811
25	t2	0	0	1	284	1	135	3	340	0	0	2	198	1	81	5	-	2	216	11	822
26	t1	0	0	2	528	1	113	0	0	1	113	0	0	5	227	0	-	7	454	2	528
27	t4	0	0	3	684	0	0	4	454	0	0	3	284	5	142	3	-	5	142	13	1421
28	t2	0	0	2	419	0	0	4	287	4	426	2	198	0	0	4	-	4	426	12	905
29	m2	0	0	0	0	0	0	0	0	2	198	5	567	2	144	4	-	4	342	9	567
30	m2	0	0	1	340	0	0	0	0	0	0	3	284	0	0	0	-	0	0	4	624
31	t1	0	0	0	0	0	0	0	0	2	185	3	280	0	0	3	-	2	185	6	280
32	m1	0	0	0	0	1	142	0	0	0	0	0	0	1	85	8	-	2	227	8	0
33	m1	0	0	0	0	0	0	0	0	1	113	1	227	3	180	3	-	4	294	4	227
34	t4	0	0	0	0	0	0	5	754	2	280	3	285	0	0	2	-	2	280	10	1039
35	t1	0	0	1	340	0	0	0	255	0	0	2	198	1	28	1	-	1	28	4	794
36	m4	0	0	0	0	0	0	2	227	0	0	8	794	2	113	0	-	2	113	10	1021

Table 6. Harvested tomato fruits number and weight from Experiment 2 of chapter 4.

Size		Extra Large				Large				Medium				Cull				Total			
Viral damage		clean		damage		clean		damage		Clean		damage		clean		damage		clean		damage	
Plant	Treatment (weeks-replicate)	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
1	2-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
2	2-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
3	2-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
4	2-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
5	2-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
6	2-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
7	4-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
8	4-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-	0	0	1	0
9	4-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
10	4-4	0	0	0	0	0	0	0	0	0	0	0	0	1	28	1	-	1	28.4	1	0
11	4-5	0	0	0	0	0	0	0	0	0	0	0	0	2	74	0	-	2	74	0	0
12	4-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
13	6-1	0	0	0	0	0	0	0	0	3	269	2	170	0	0	1	-	3	269	3	170
14	6-2	0	0	0	0	0	0	0	0	0	0	0	0	1	57	2	-	1	56.7	2	0
15	6-3	0	0	0	0	0	0	0	0	0	0	2	161	0	0	1	-	0	0	3	161
16	6-4	0	0	0	0	0	0	0	0	0	0	2	198	2	101	0	-	2	101	2	198
17	6-5	0	0	0	0	0	0	0	0	0	0	2	227	4	210	1	-	4	210	3	227
18	6-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-	0	0	1	0

Size		Extra Large				Large				Medium				Cull				Total			
Viral damage		clean		damage		clean		damage		Clean		damage		clean		damage		clean		damage	
Plant	Treatment (weeks-replicate)	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
19	8-1	0	0	0	0	1	144	2	229	1	104	4	454	0	0	2	-	2	248	8	683
20	8-2	0	0	1	255	0	0	2	327	0	0	4	510	1	82	1	-	1	82	8	1092
21	8-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0
22	8-4	0	0	1	199	1	114	2	254	1	115	0	0	1	68	5	-	3	297	8	453
23	8-5	0	0	0	0	0	0	0	0	0	0	0	0	5	208	6	-	5	208	6	0
24	8-6	0	0	0	0	2	250	0	0	2	194	5	527	2	172	3	-	6	616	8	527

Table 7. Harvested tomato fruits number and weight from Experiment 3 of chapter 4.

Size		Extra Large				Large				Medium				Cull				Blossom End Rot		Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage				clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
1	14	2	360	0	0	5	620	2	305	6	555	1	100	18	610	3	20	5	445	31	2145	11	870
2	35	2	390	0	0	2	225	0	0	2	175	0	0	21	650	0	0	10	762	27	1440	10	762
3	35	0	0	0	0	3	345	0	0	5	470	0	0	22	1155	1	5	0	0	30	1970	1	5
4	21	3	475	0	0	2	245	0	0	7	655	0	0	26	920	1	20	2	365	38	2295	3	385
5	42	2	305	1	180	2	265	0	0	3	290	0	0	20	970	8	435	5	255	27	1830	14	870
6	42	1	160	0	0	1	115	0	0	5	465	0	0	38	1330	0	0	7	770	45	2070	7	770
7	7	0	0	0	0	1	105	1	120	5	450	2	190	31	1205	11	215	6	670	37	1760	20	1195
8	14	2	375	0	0	2	235	0	0	2	180	4	385	29	935	5	140	9	900	35	1725	18	1425
9	7	0	0	0	0	3	350	0	0	6	656	0	0	26	1270	4	160	12	1480	35	2276	16	1640
10	21	1	185	0	0	3	375	0	0	3	300	0	0	28	1445	2	90	14	1090	35	2305	16	1180
11	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	21	2	390	0	0	1	125	0	0	6	610	0	0	27	830	0	0	10	1175	36	1955	10	1175
13	28	0	0	0	0	2	235	0	0	8	755	0	0	31	1330	2	25	9	913.5	41	2320	11	938.5
14	42	0	0	1	165	0	0	0	0	2	200	2	185	25	940	0	0	10	817	27	1140	13	1167
15	42	6	1035	0	0	4	495	0	0	2	210	0	0	31	1520	4	110	12	1095	43	3260	16	1205
16	28	0	0	0	0	1	125	1	130	1	90	0	0	30	1310	3	60	9	920	32	1525	13	1110
17	42	2	395	0	0	3	390	0	0	4	395	0	0	24	755	0	0	7	920.1	33	1935	7	920.1
18	28	0	0	0	0	3	365	0	0	4	375	0	0	42	1235	4	185	18	1322	49	1975	22	1507
19	14	2	345	0	0	1	115	0	0	4	355	0	0	26	1135	2	45	12	1640	33	1950	14	1685
20	7	0	0	0	0	0	0	0	0	0	0	0	0	7	250	6	220	10	280.1	7	250	16	500.1
21	35	0	0	0	0	0	0	0	0	2	225	0	0	29	1365	9	380	9	230	31	1590	18	610

Size		Extra Large				Large				Medium				Cull				Blossom End Rot		Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage				clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
22	14	3	515	0	0	5	575	0	0	3	285	0	0	5	375	1	75	6	765	16	1750	7	840
23	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	28	1	155	0	0	5	590	1	125	10	1015	0	0	33	1730	4	125	14	1265	49	3490	19	1515
25	35	1	170	0	0	4	490	1	130	3	300	1	75	7	445	0	0	5	650	15	1405	7	855
26	28	1	165	0	0	0	0	0	0	4	390	0	0	2	130	0	0	10	715.1	7	685	10	715.1
27	7	0	0	0	0	2	215	0	0	4	375	0	0	26	965	1	5	12	1342	32	1555	13	1347
28	14	1	175	1	150	3	330	1	105	2	190	0	0	2	125	0	0	3	460	8	820	5	715
29	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	55	6	361.8	0	0	8	416.8
30	21	2	425	2	365	6	675	1	130	4	390	0	0	9	500	1	28	6	700	21	1990	10	1223
31	7	0	0	0	0	1	120	0	0	0	0	0	0	2	145	6	260	19	768.5	3	265	25	1028
32	35	0	0	0	0	2	255	0	0	6	555	0	0	21	1075	0	0	4	360	29	1885	4	360
33	35	0	0	0	0	0	0	0	0	1	95	0	0	23	745	1	30	9	570.1	24	840	10	600.1
34	28	0	0	0	0	0	0	0	0	4	350	0	0	20	1115	1	35	4	325	24	1465	5	360
35	14	0	0	0	0	0	0	0	0	4	355	1	80	22	1100	3	220	5	495	26	1455	9	795
36	21	3	535	0	0	3	365	0	0	7	705	0	0	31	1385	4	90	8	905	44	2990	12	995

Table 8. Harvested tomato fruits number and weight from Experiment 4 of chapter 4.

Size		Extra Large				Large				Medium				Cull				Blossom End Rot		Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage				Clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
1	14	0	0	0	0	0	0	0	0	1	100	0	0	1	45	1	60	0	0	2	145	1	60
2	42	0	0	0	0	0	0	0	0	7	645	0	0	9	450	2	125	1	385.6	16	1095	3	510.6
3	35	0	0	0	0	0	0	0	0	0	0	0	0	21	1175	4	170	0	0	21	1175	4	170
4	35	0	0	0	0	0	0	0	0	3	290	0	0	10	640	1	60	0	0	13	930	1	60
5	35	1	195	0	0	1	115	0	0	1	85	0	0	5	300	1	80	0	0	8	695	1	80
6	14	0	0	0	0	1	135	0	0	4	410	0	0	6	395	2	125	0	0	11	940	2	125
7	14	0	0	0	0	1	155	0	0	2	195	0	0	14	805	0	0	0	0	17	1155	0	0
8	42	0	0	0	0	0	0	0	0	2	180	0	0	9	485	1	75	0	0	11	665	1	75
9	28	0	0	0	0	4	495	0	0	0	0	0	0	10	650	2	130	0	0	14	1145	2	130
10	49	0	0	0	0	0	0	0	0	6	525	1	100	16	855	1	45	0	0	22	1380	2	145
11	14	0	0	0	0	0	0	0	0	0	0	0	0	12	360	1	65	0	0	12	360	1	65
12	49	1	145	1	190	3	390	0	0	9	855	0	0	41	995	3	160	3	250	54	2385	7	600
13	42	0	0	0	0	0	0	0	0	0	0	0	0	3	90	3	165	0	0	3	90	3	165
14	28	2	325	0	0	3	370	0	0	1	85	0	0	10	665	0	0	0	0	16	1445	0	0
15	42	2	330	1	155	5	570	0	0	8	775	1	100	19	1060	2	100	2	90	34	2735	6	445
16	21	0	0	0	0	1	110	0	0	4	330	0	0	13	630	0	0	0	0	18	1070	0	0
17	28	0	0	0	0	1	110	2	235	3	270	0	0	20	1165	5	375	10	620	24	1545	17	1230
18	14	0	0	1	170	7	805	0	0	7	650	2	180	14	845	1	60	0	0	28	2300	4	410
19	21	0	0	0	0	1	110	1	125	2	200	0	0	30	680	1	75	6	425	33	990	8	625
20	21	0	0	0	0	1	130	0	0	1	110	0	0	8	365	0	0	2	230	10	605	2	230
21	42	2	320	0	0	9	1060	1	105	8	745	1	90	27	905	3	100	7	525	46	3030	12	820

Size		Extra Large				Large				Medium				Cull				Blossom End Rot		Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage				clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
22	28	0	0	0	0	1	100	0	0	0	0	0	0	18	270	0	0	0	0	19	370	0	0
23	21	0	0	0	0	0	0	1	100	2	170	1	75	17	805	1	15	1	65	19	975	4	255
24	28	0	0	0	0	3	385	0	0	5	490	0	0	13	755	0	0	0	0	21	1630	0	0
25	49	1	160	0	0	13	1575	1	135	12	1125	1	95	34	1290	0	0	6	513.5	60	4150	8	743.5
26	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	35	2	320	0	0	8	980	0	0	8	710	0	0	25	700	2	140	3	295	43	2710	5	435
28	35	0	0	0	0	5	565	0	0	15	1400	2	170	30	1105	1	65	1	60	50	3070	4	295
29	49	3	485	0	0	5	570	3	370	7	660	1	85	18	670	1	70	4	415	33	2385	9	940
30	21	0	0	0	0	1	100	0	0	1	95	0	0	13	425	2	100	3	221.7	15	620	5	321.7
31	35	1	140	0	0	4	465	0	0	3	285	1	95	11	530	4	290	0	0	19	1420	5	385
32	42	1	150	0	0	9	1075	0	0	15	1405	0	0	27	925	2	20	2	145	52	3555	4	165
33	49	0	0	0	0	8	935	0	0	10	970	3	285	25	930	3	80	4	241.7	43	2835	10	606.7
34	28	0	0	1	195	1	105	1	105	3	255	0	0	8	350	2	125	1	80	12	710	5	505
35	14	4	670	3	590	7	755	4	495	9	825	2	210	17	490	2	45	2	140	37	2740	13	1480
36	49	0	0	0	0	0	0	1	115	0	0	2	185	8	185	9	315	20	890	8	185	32	1505

Table 9. Harvested tomato fruits number and weight from Experiment 5 of chapter 4.

Size		Extra Large				Large				Medium				Cull				Blossom End Rot		Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage				clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	42	0	0	2	325	14	1655	3	360	13	1225	6	580	40	1505	29	780	6	591.9	67	4385	46	2637
3	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	42	1	160	2	300	5	625	3	340	8	700	0	0	77	1730	18	335	7	965	91	3215	30	1940
5	7	0	0	0	0	0	0	0	0	0	0	0	0	1	20	11	165	2	35	1	20	13	200
6	49	0	0	1	170	0	0	1	115	0	0	1	80	3	210	16	700	12	640	3	210	31	1705
7	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	42	2	325	0	0	4	490	0	0	11	1040	4	365	38	970	8	185	22	1360	55	2825	34	1910
10	49	1	255	2	325	5	655	1	115	11	1025	1	115	39	1255	1	10	2	185	56	3190	7	750
11	49	1	160	0	0	4	515	1	135	7	690	3	290	76	1785	3	180	7	570	88	3150	14	1175
12	21	0	0	0	0	0	0	0	0	1	80	0	0	1	50	1	70	0	0	2	130	1	70
13	56	2	320	0	0	5	595	1	120	8	730	10	955	53	1520	16	530	15	990	68	3165	42	2595
14	21	0	0	0	0	0	0	0	0	0	0	0	0	3	70	1	70	0	0	3	70	1	70
15	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	49	2	325	0	0	0	0	0	0	2	160	5	390	2	95	13	415	3	70	6	580	21	875
17	56	1	240	0	0	4	490	0	0	3	345	6	515	34	780	6	345	18	1270	42	1855	30	2130
18	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	49	2	420	0	0	5	595	0	0	12	1145	4	380	43	1625	5	205	10	570	62	3785	19	1155

Size		Extra Large				Large				Medium				Cull				Blossom End Rot		Total			
Viral damage		clean		damage		clean		damage		clean		damage		clean		damage				clean		damage	
Plant	Treatment	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt	#	wt
22	56	3	590	0	0	4	465	0	0	1	85	0	0	14	680	2	35	19	930	22	1820	21	965
23	21	0	0	0	0	0	0	0	0	1	85	1	80	6	275	3	200	0	0	7	360	4	280
24	21	4	530	0	0	0	0	0	0	4	400	1	90	4	285	0	0	3	195	12	1215	4	285
25	14	1	150	0	0	2	240	0	0	1	105	0	0	5	365	0	0	0	0	9	860	0	0
26	56	0	0	2	355	4	470	2	240	14	1250	2	195	19	1595	10	235	10	570	37	3315	26	1595
27	42	1	170	2	295	4	455	5	570	8	755	7	635	43	678	5	60	11	1010	56	2058	30	2570
28	42	3	420	0	0	3	325	1	95	2	175	0	0	22	400	5	100	5	410	30	1320	11	605
29	49	1	140	0	0	9	1045	0	0	3	255	0	0	49	1335	4	150	2	105	62	2775	6	255
30	56	1	140	2	335	3	360	1	135	10	945	0	0	40	1545	3	15	2	220	54	2990	8	705
31	42	0	0	0	0	0	0	1	120	2	285	0	0	3	165	16	785	4	275	5	450	21	1180
32	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	21	0	0	0	0	3	345	0	0	0	0	1	90	10	290	1	45	2	85	13	635	4	220
34	56	0	0	0	0	0	0	0	0	0	0	0	0	1	10	2	75	9	140	1	10	11	215
35	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	56	0	0	3	480	1	100	1	110	0	0	0	0	21	860	0	0	26	1660	22	960	30	2250

APPENDIX C

WEATHER DATA

Coastal Plain Experiment Station
The University of Georgia
Tifton, Tift, GA
Historical Data Weather Data
(Temperature min, Temperature max and Precipitation amount)

Date,	Max. Temp. (° F)	Min. Temp. (° F)	Rain (in)
Mar 1, 1999	69.3	46.0	0.00
Mar 2, 1999	75.0	43.0	0.00
Mar 3, 1999	59.7	42.4	0.15
Mar 4, 1999	59.0	33.6	0.00
Mar 5, 1999	68.2	34.9	0.00
Mar 6, 1999	68.9	48.9	0.08
Mar 7, 1999	62.6	46.0	0.00
Mar 8, 1999	61.5	39.9	0.00
Mar 9, 1999	69.6	45.7	0.18
Mar 10, 1999	66.0	41.4	0.00
Mar 11, 1999	58.3	38.5	0.00
Mar 12, 1999	62.1	40.6	0.00
Mar 13, 1999	71.2	46.4	0.00
Mar 14, 1999	69.6	49.8	0.52
Mar 15, 1999	56.8	40.6	0.00
Mar 16, 1999	70.2	37.9	0.00
Mar 17, 1999	73.4	45.0	0.00
Mar 18, 1999	76.3	49.1	0.00
Mar 19, 1999	79.0	51.8	0.00
Mar 20, 1999	77.9	53.4	0.00
Mar 21, 1999	62.4	50.2	0.06
Mar 22, 1999	71.6	42.8	0.00
Mar 23, 1999	75.4	40.5	0.00
Mar 24, 1999	75.7	47.7	0.00
Mar 25, 1999	75.4	51.8	0.12
Mar 26, 1999	64.4	45.9	0.00
Mar 27, 1999	62.2	41.0	0.00
Mar 28, 1999	72.1	39.9	0.00
Mar 29, 1999	79.7	48.6	0.00
Mar 30, 1999	72.5	53.1	0.00
Mar 31, 1999	78.3	58.6	0.02
Apr 1, 1999	78.3	63.7	0.17
Apr 2, 1999	81.7	65.7	0.00
Apr 3, 1999	84.2	61.2	0.00
Apr 4, 1999	84.2	66.2	0.00
Apr 5, 1999	87.3	62.8	0.00
Apr 6, 1999	84.2	66.6	0.00
Apr 7, 1999	86.4	66.6	0.00
Apr 8, 1999	84.2	64.0	0.01
Apr 9, 1999	85.8	68.2	0.00
Apr 10, 1999	87.8	68.4	0.00
Apr 11, 1999	86.5	68.7	0.00
Apr 12, 1999	80.6	57.0	0.00
Apr 13, 1999	77.2	50.9	0.00
Apr 14, 1999	82.0	51.4	0.00
Apr 15, 1999	86.9	63.7	0.00
Apr 16, 1999	66.4	51.4	0.00
Apr 17, 1999	67.5	49.1	0.00
Apr 18, 1999	68.0	42.3	0.00
Apr 19, 1999	72.1	41.0	0.00
Apr 20, 1999	82.8	52.3	0.00
Apr 21, 1999	84.7	57.7	0.00
Apr 22, 1999	85.3	56.5	0.02
Apr 23, 1999	86.2	57.0	0.00
Apr 24, 1999	90.9	66.0	0.00
Apr 25, 1999	74.5	61.9	0.00
Apr 26, 1999	81.5	62.2	0.00
Apr 27, 1999	87.6	67.6	0.00
Apr 28, 1999	81.7	61.5	1.64
Apr 29, 1999	62.2	50.5	0.15
Apr 30, 1999	50.5	46.9	0.08
May 1, 1999	67.5	48.7	0.00
May 2, 1999	76.1	51.8	0.00
May 3, 1999	81.3	50.9	0.00
May 4, 1999	84.2	55.4	0.00
May 5, 1999	78.8	65.5	0.36
May 6, 1999	84.9	63.9	0.43
May 7, 1999	73.8	63.7	0.51
May 8, 1999	82.2	60.8	0.00
May 9, 1999	84.4	56.5	0.00
May 10, 1999	86.4	60.1	0.01
May 11, 1999	84.9	62.6	0.07
May 12, 1999	81.7	60.8	0.00
May 13, 1999	83.7	63.7	0.63
May 14, 1999	79.2	64.0	0.00
May 15, 1999	74.3	61.3	0.00
May 16, 1999	81.3	57.0	0.00
May 17, 1999	83.1	60.8	0.00
May 18, 1999	84.4	60.1	0.00
May 19, 1999	84.9	65.3	0.00
May 20, 1999	86.5	63.3	0.00
May 21, 1999	86.5	62.6	0.00
May 22, 1999	87.4	66.0	0.00
May 23, 1999	87.1	65.3	0.00
May 24, 1999	86.5	68.5	0.00
May 25, 1999	87.4	60.6	0.00
May 26, 1999	86.2	66.7	0.00
May 27, 1999	82.4	65.1	0.03
May 28, 1999	86.2	62.1	0.03
May 29, 1999	80.6	65.5	0.07
May 30, 1999	86.5	66.0	0.00
May 31, 1999	86.2	64.6	0.00
Jun 1, 1999	86.7	67.1	0.00
Jun 2, 1999	88.9	66.7	0.00
Jun 3, 1999	91.4	70.5	0.00
Jun 4, 1999	92.8	70.5	0.00
Jun 5, 1999	88.2	69.3	0.27
Jun 6, 1999	88.5	70.5	0.00
Jun 7, 1999	87.1	67.3	0.01
Jun 8, 1999	91.0	68.2	0.00
Jun 9, 1999	90.5	68.9	0.00
Jun 10, 1999	78.8	70.9	1.56
Jun 11, 1999	82.2	71.6	0.36
Jun 12, 1999	85.1	71.1	0.06
Jun 13, 1999	88.2	67.3	0.00
Jun 14, 1999	89.2	71.1	0.00
Jun 15, 1999	90.7	72.1	0.00
Jun 16, 1999	89.4	70.3	0.23
Jun 17, 1999	84.0	69.8	0.92
Jun 18, 1999	84.0	67.5	0.00
Jun 19, 1999	82.2	64.6	0.00
Jun 20, 1999	77.7	62.8	0.00
Jun 21, 1999	84.0	68.0	0.00
Jun 22, 1999	85.5	68.5	0.67
Jun 23, 1999	85.8	71.1	0.00
Jun 24, 1999	84.0	71.4	0.11
Jun 25, 1999	83.3	71.4	0.70
Jun 26, 1999	84.6	72.0	0.19
Jun 27, 1999	81.0	71.4	0.27
Jun 28, 1999	84.0	71.6	0.02
Jun 29, 1999	88.9	68.4	2.20
Jun 30, 1999	86.4	70.7	0.00
Jul 1, 1999	89.6	71.1	0.00
Jul 2, 1999	88.3	69.3	0.00
Jul 3, 1999	88.9	70.2	0.15
Jul 4, 1999	88.0	71.6	0.00
Jul 5, 1999	90.3	71.4	0.00
Jul 6, 1999	93.2	70.7	0.28
Jul 7, 1999	86.7	70.5	0.06
Jul 8, 1999	89.8	69.3	0.00
Jul 9, 1999	90.3	71.4	0.00
Jul 10, 1999	88.2	72.9	0.00
Jul 11, 1999	89.1	73.6	0.00
Jul 12, 1999	87.1	71.8	0.10
Jul 13, 1999	75.9	72.0	0.05
Jul 14, 1999	79.3	70.9	0.09
Jul 15, 1999	86.5	71.4	0.05
Jul 16, 1999	89.1	71.6	0.00
Jul 17, 1999	87.4	71.4	0.09
Jul 18, 1999	88.3	72.1	0.35
Jul 19, 1999	88.5	69.1	0.00
Jul 20, 1999	90.5	71.6	0.00
Jul 21, 1999	91.4	73.9	0.01
Jul 22, 1999	93.6	74.7	0.00
Jul 23, 1999	96.1	71.6	0.78
Jul 24, 1999	91.8	71.6	0.02
Jul 25, 1999	83.3	71.4	2.62
Jul 26, 1999	93.7	71.8	0.00
Jul 27, 1999	95.5	74.7	0.00
Jul 28, 1999	94.8	75.0	0.00
Jul 29, 1999	92.8	76.1	0.00
Jul 30, 1999	98.1	75.9	0.00
Jul 31, 1999	98.6	76.1	0.00
Aug 1, 1999	99.0	77.4	0.00
Aug 2, 1999	96.4	76.3	0.04
Aug 3, 1999	92.3	72.0	0.00

Aug 4, 1999 90.5 70.9 0.00
Aug 5, 1999 92.8 72.7 0.00
Aug 6, 1999 97.0 73.4 0.00
Aug 7, 1999 96.8 75.4 0.00
Aug 8, 1999 91.6 73.9 0.10
Aug 9, 1999 89.4 75.0 0.04
Aug 10, 1999 90.5 73.2 0.00
Aug 11, 1999 93.7 76.1 0.00
Aug 12, 1999 97.5 75.7 0.00
Aug 13, 1999 99.0 74.1 0.03
Aug 14, 1999 96.3 72.7 0.24
Aug 15, 1999 90.7 70.9 0.00
Aug 16, 1999 91.6 74.1 0.07
Aug 17, 1999 91.9 72.5 0.00
Aug 18, 1999 94.5 74.7 0.00
Aug 19, 1999 92.5 73.8 0.00
Aug 20, 1999 91.8 73.8 0.00
Aug 21, 1999 92.7 73.4 0.51
Aug 22, 1999 89.4 71.4 0.00
Aug 23, 1999 91.4 69.1 0.00
Aug 24, 1999 89.8 71.6 0.47
Aug 25, 1999 91.2 70.5 0.00
Aug 26, 1999 91.4 72.7 0.07
Aug 27, 1999 92.5 73.8 0.00
Aug 28, 1999 92.5 70.9 0.00
Aug 29, 1999 90.7 73.9 0.00
Aug 30, 1999 91.4 69.6 0.00
Aug 31, 1999 84.9 66.7 0.00

Mar 1, 2000 76.3 50.0 0.00
Mar 2, 2000 75.2 52.7 0.00
Mar 3, 2000 77.2 44.1 0.00
Mar 4, 2000 65.7 50.4 0.06
Mar 5, 2000 73.6 44.4 0.00
Mar 6, 2000 79.5 48.9 0.00
Mar 7, 2000 79.2 53.4 0.00
Mar 8, 2000 79.7 51.3 0.00
Mar 9, 2000 76.6 53.2 0.00
Mar 10, 2000 79.0 59.2 0.00
Mar 11, 2000 76.1 54.1 0.53
Mar 12, 2000 60.1 40.3 0.00
Mar 13, 2000 67.1 37.6 0.00
Mar 14, 2000 72.0 44.2 0.00
Mar 15, 2000 77.5 49.1 0.01
Mar 16, 2000 74.3 61.2 1.46
Mar 17, 2000 75.0 60.6 0.00
Mar 18, 2000 61.5 52.2 0.00
Mar 19, 2000 68.9 53.4 0.44
Mar 20, 2000 70.0 52.5 0.50
Mar 21, 2000 74.7 48.6 0.00
Mar 22, 2000 73.4 51.8 0.00
Mar 23, 2000 70.3 51.3 0.00
Mar 24, 2000 77.4 47.3 0.00
Mar 25, 2000 80.1 53.8 0.00
Mar 26, 2000 78.4 58.6 0.25
Mar 27, 2000 72.3 57.7 0.20
Mar 28, 2000 74.1 48.9 0.01
Mar 29, 2000 79.3 53.4 0.00
Mar 30, 2000 68.2 58.6 1.26
Mar 31, 2000 79.3 53.1 0.00
Apr 1, 2000 78.3 56.7 0.00
Apr 2, 2000 76.6 64.0 0.00
Apr 3, 2000 78.6 61.3 0.02
Apr 4, 2000 64.8 46.9 0.01
Apr 5, 2000 64.8 38.1 0.00
Apr 6, 2000 75.2 44.6 0.01
Apr 7, 2000 76.1 49.6 0.00
Apr 8, 2000 73.0 42.4 0.04
Apr 9, 2000 65.1 34.0 0.00
Apr 10, 2000 73.8 40.6 0.00
Apr 11, 2000 77.5 54.0 0.00
Apr 12, 2000 81.3 54.7 0.00
Apr 13, 2000 81.3 54.0 0.00
Apr 14, 2000 54.0 48.7 0.12
Apr 15, 2000 74.8 52.0 0.00
Apr 16, 2000 81.9 54.5 0.00
Apr 17, 2000 82.6 60.1 0.00
Apr 18, 2000 75.2 52.3 0.00
Apr 19, 2000 82.0 51.1 0.00
Apr 20, 2000 84.0 56.5 0.01
Apr 21, 2000 80.8 57.6 0.00
Apr 22, 2000 73.4 48.7 0.00
Apr 23, 2000 74.5 47.5 0.00
Apr 24, 2000 68.4 57.6 0.70
Apr 25, 2000 64.9 53.6 0.00
Apr 26, 2000 73.4 48.4 0.00
Apr 27, 2000 76.6 49.3 0.01
Apr 28, 2000 77.2 56.7 0.40
Apr 29, 2000 77.2 51.3 0.00
Apr 30, 2000 79.3 51.8 0.00
May 1, 2000 81.1 53.6 0.00
May 2, 2000 83.5 59.2 0.00
May 3, 2000 85.3 61.2 0.00
May 4, 2000 82.6 61.2 0.00
May 5, 2000 82.9 61.7 0.00
May 6, 2000 86.4 60.3 0.00
May 7, 2000 86.2 59.5 0.00
May 8, 2000 87.4 61.2 0.00
May 9, 2000 86.5 61.5 0.01
May 10, 2000 86.7 64.4 0.00
May 11, 2000 89.6 68.2 0.00
May 12, 2000 90.0 68.4 0.00
May 13, 2000 91.2 69.6 0.00
May 14, 2000 84.7 66.9 0.00
May 15, 2000 82.8 59.5 0.00
May 16, 2000 84.0 60.6 0.00
May 17, 2000 87.8 64.2 0.00
May 18, 2000 87.6 66.7 0.01
May 19, 2000 89.2 64.9 0.00
May 20, 2000 90.0 66.2 0.00
May 21, 2000 86.2 68.9 0.00
May 22, 2000 88.5 70.0 0.00
May 23, 2000 91.4 67.5 0.00
May 24, 2000 92.3 72.7 0.00
May 25, 2000 93.2 74.5 0.01
May 26, 2000 92.7 74.7 0.00
May 27, 2000 93.9 71.1 0.00
May 28, 2000 93.0 75.4 0.00
May 29, 2000 86.0 67.3 0.00
May 30, 2000 82.9 63.5 0.00
May 31, 2000 85.3 59.5 0.00
Jun 1, 2000 91.6 61.9 0.04
Jun 2, 2000 95.9 69.1 0.00
Jun 3, 2000 96.1 68.2 0.00
Jun 4, 2000 97.2 66.9 0.02
Jun 5, 2000 86.9 69.3 0.86
Jun 6, 2000 85.8 66.9 0.00
Jun 7, 2000 82.0 60.1 0.00
Jun 8, 2000 86.2 61.5 0.00
Jun 9, 2000 87.3 65.1 0.00
Jun 10, 2000 89.1 63.0 0.00
Jun 11, 2000 87.4 65.1 0.00
Jun 12, 2000 91.2 70.9 0.00
Jun 13, 2000 92.1 68.9 0.00
Jun 14, 2000 94.8 71.1 0.60
Jun 15, 2000 89.4 70.7 0.41
Jun 16, 2000 89.8 70.2 0.00
Jun 17, 2000 91.9 70.3 0.00
Jun 18, 2000 91.4 70.5 0.00
Jun 19, 2000 93.4 70.9 0.00
Jun 20, 2000 94.8 73.4 0.00
Jun 21, 2000 90.0 70.9 0.00
Jun 22, 2000 92.7 72.5 0.01
Jun 23, 2000 91.4 70.5 0.00
Jun 24, 2000 92.1 68.2 0.00
Jun 25, 2000 92.1 71.1 0.00

BIOGRAPHY

Chatchawan Chaisuekul was born on January, 30 1976 in Bangkok, Thailand. He is a youngest son of Boonchoo and Wandee Chaisuekul. He received elementary education from Phayathai and secondary education from Suankularb Wittayalai in Bangkok. After finished high school in 1993, he attended Chulalongkorn University at Faculty of Science where he was awarded a scholarship from the Development and Promotion of Science and Technology Talent Project, and later to continued his undergraduate program at University of Delaware in 1994.

After received Bachelor of Arts in biology from University of Delaware in 1998, he attended a graduate program in entomology at University of Georgia where he conducted his master's research under the guidance of Dr. David G. Riley.