

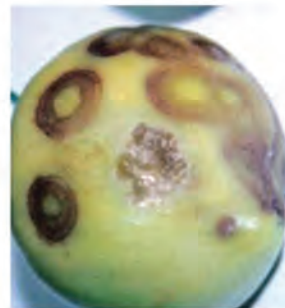
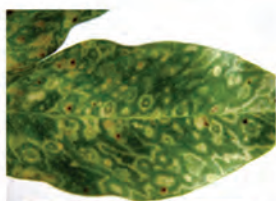


THE UNIVERSITY OF GEORGIA
COLLEGE OF AGRICULTURAL &
ENVIRONMENTAL SCIENCES

Tospoviruses

In Solanaceae and Other Crops in the Coastal Plain of Georgia

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TOSPOVIRUSES

IN SOLANACEAE AND OTHER CROPS

IN THE COASTAL PLAIN OF GEORGIA

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FOREWORD

I am pleased to announce the release of the research report *Tospoviruses in Solanaceae and Other Crops in the Coastal Plain of Georgia*. The study of tospoviruses and their transmission is a crucial need for agriculture because of the wide range of crops affected, the severity of damage caused by the viruses, the difficulty in preventing virus transmission and ultimately the economic impact resulting from infection. The challenges presented by tospoviruses are growing, and what began as a regional issue has developed national and international implications. This report is a compilation of the most recent research coming from the University of Georgia's Tifton Campus, located in the coastal plain region of the Southeast. The research presented here comes from teams of research and Extension scientists from a number of states who have collaborated to address this vexing issue. These scientists are meeting the challenge presented by this family of viruses and their combined efforts are yielding results to deal with this economically challenging issue. This body of work reflects the commitment by the University of Georgia's College of Agricultural and Environmental Sciences to address issues that pose a challenge to the agricultural industries and the citizens of Georgia and our nation. We hope that you find the contents of this publication useful.

Joe W. West
Assistant Dean
Tifton Campus

INTRODUCTION

JOHN SHERWOOD

The Tomato spotted wilt virus (TSWV) (genus Tospovirus; family Bunyaviridae) is transmitted by thrips (Thysanoptera: Thripidae) and replicates in both the thrips vectors and the plant hosts. Although worldwide in distribution, TSWV was of limited and sporadic significance until the mid-1980s. In peanut, for example, TSWV had not caused significant losses in the United States until 1982, but now the disease has become epidemic in peanut, tobacco, vegetables and many ornamentals. The emergence of *Frankliniella occidentalis*, the western flower thrips (WFT), as a worldwide resident, and the interstate and intercontinental shipping of plant materials likely contributed to TSWV becoming a global problem.

Although WFT is considered to be the most prominent vector species, *Frankliniella fusca* is considered the most important TSWV vector in Georgia. The epidemiology of the diseases caused by TSWV remains poorly understood. It was only confirmed in 1993 that TSWV multiplies in its vector, and it is not yet understood why some thrips species transmit TSWV and others do not. The wide plant host range of TSWV and the occurrence of at least 13 species of thrips that transmit TSWV and/or other tospoviruses make eliminating the primary inoculum sources of the virus impractical.

Understanding the relationship of TSWV with thrips requires a fundamental understanding of the structure of TSWV. TSWV virus particles (virions) are complex compared to many plant viruses. There are three RNAs in the virus genome that are individually encapsulated in many copies of the nucleocapsid protein. The three RNAs are collectively bound by a membrane envelope that is of host origin, much like three strings of spaghetti inside a tennis ball. Also inside the envelope are several copies of a virus-encoded “replicase” protein that is required to initiate virus replication in a new host. This complex virion structure is a characteristic that distinguishes TSWV from most other plant viruses.

TSWV virions are roughly spherical and are 80-110 nm in diameter. Two virus proteins processed during replication to contain sugars (i.e. glycoproteins (GPs)), are dispersed throughout the surface

of the viral envelope (much like the fuzz on a new tennis ball). These proteins are called glycoprotein 1 (GP1) and glycoprotein 2 (GP2) and differ slightly in size. The GPs in the envelope function in the maturation and assembly of virions, and in acquisition of virus in thrips leading to transmission.

Thrips deposit their eggs into plant tissue and the eggs hatch after 2-3 days, depending on the temperature and the plant host species. Two feeding larval stages are followed by two non-feeding pupal stages. The life cycle takes about 20-30 days from egg to adult, depending on the temperature. Thrips are dispersed over long distances by wind. TSWV must be acquired by thrips during the larval stage of their development to be transmitted; thus, only immature thrips that acquire TSWV, or adults derived from such immatures, transmit the virus.

Within 24 hours after larval thrips feed on virus-infected plants, the virus can be found in the cells of the midgut, the muscle cells around the midgut and the salivary glands. The virus must be in the salivary gland for transmission to the plant as virions are excreted with the saliva into host plants when the thrips feed. The capability of thrips to acquire TSWV decreases as the thrips age. Once acquired by the larvae, the virus is passed transtadially, i.e., TSWV persists through insect molts from larval to adult stages. The virus replicates in thrips, and thrips can transmit the virus after acquisition as larva and during their entire adult life. When adult thrips that have never been exposed to the virus feed on virus-infected plants, the virus can be found in the midgut and muscle cells around the midgut. However, the virus is not found in the salivary glands and these adults do not transmit it.

When the virus is excreted from the salivary glands during feeding, if the plant is a suitable host for the virus, the virus begins to replicate. If the plant is a suitable host for thrips, the thrips reproduce. As those thrips emerge, they feed on the virus-infected plant and the cycle begins again.

HISTORY

ALEX CSINOS, NATALIA MARTINEZ-OCHOA AND PAUL BERTRAND

Tospoviruses are the only plant-infecting members of the Bunyaviridae family. Most Bunyaviruses involve an insect vector, which spreads the virus between hosts. This is also the case with tospoviruses where several different species of thrips (Thysanoptera) serve as vectors. The main vectors in the United States are *Frankliniella occidentalis*, *F. fusca* and *Thrips tabaci*. Of these, *F. occidentalis*, the western flower thrips, has the broadest host range affecting a diverse variety of ornamental and vegetable crops. *F. fusca* has proven economically important as a vector in the peanut and tobacco industry, and *T. tabaci* vectors a strain of tospoviruses in onion and garlic crops (Campbell et al., 2005). There are new tospoviruses being reported all the time, but the current list includes seventeen tospovirus species. In the United States, only Tomato spotted wilt virus, *Impatiens necrotic spot virus* and *Iris yellow spot virus* have been reported. The host range varies for each tospovirus species, and the total number of reported susceptible hosts worldwide includes 848 named plant species within 106 identified families.

Tomato spotted wilt virus was first reported in Georgia in 1986, but was not considered a problem until 1989. The disease infects many of the commercial crops in Georgia and also many of the weeds found in the Coastal Plain in Georgia. Three of the reported TSWV vectors – *Frankliniella fusca* (Hinds) (Sakimura, 1963), *F. occidentalis* (Per-gande) (Sakimura, 1962), and *F. bispinosa* Morgan (Webb et al., 1997) – are present in Georgia. The wide host range of both the virus and thrips vectors makes managing the disease very difficult. The most effective way to minimize the impact of spotted wilt is by use of resistant cultivars. Significant progress has been made in developing such cultivars for peanuts, tomato and peppers; however, in the case of flue-cured tobacco, no resistance has yet been identified in commercial cultivars. Efforts to deploy other control options such as insecticide sprays, adjusting planting dates, rouging of infected plants, and replanting have had limited and unpredictable results on reducing TSWV infections.

Impatiens necrotic spot virus (INSV) was first reported in *Impatiens* sp. (Law and Moyer, 1990). INSV is closely related to TSWV, and was initially designated as TSWV-I, but later named as a separate species in the genus *Tospovirus*. INSV is an emerging virus found mostly in ornamentals under greenhouse production (Daughtrey et al., 1997). Field crops are also susceptible to INSV, but fewer reports have been made. INSV was first detected in 1998 in peanut growing in Georgia (Mitchell and Tift Counties) and Texas

(Frio County) (Pappu et al., 1999). Tomato and pepper are also hosts for INSV, although symptoms are less severe than with TSWV. Little is known about INSV distribution and impact on outdoor vegetable and field crops, but recent field surveys suggest that incidence of INSV in peanut might be increasing in mixed infections with TSWV or alone (Wells et al., 2001; Martinez-Ochoa et al., 2002). INSV has also been found in flue-cured tobacco (Martinez-Ochoa et al., 2003), yellow and purple nutsedge (Martinez-Ochoa et al., 2004), and several other weeds such as wandering cudweed, bristly starbur, smallflower morningglory, common ragweed, spiny sowthistle, dogfennel, etc. (Mullis et al., unpublished). For several years it was believed that *Frankliniella occidentalis* was the only confirmed vector of INSV (Peters et al., 1996), but more recently *F. fusca* has been shown to serve as a vector as well (Naidu et al., 2001).

SPOTTED WILT LOSSES

VEGETABLES: There has not been any systematic survey of losses caused by Tomato spotted wilt virus in vegetables in Georgia. Observations noted increasing losses in tomato and pepper through the mid-1980s and the mid-1990s. A rapid switch from farming on bare ground to plastic culture was effective in reducing losses in pepper and tomato through the mid-1990s. Losses as high as 85% in spring-grown tomatoes were observed in 1999 and 2000. In 2001, resistant varieties of tomato and pepper were introduced and have seen constant improvement in horticultural quality. In each crop, resistance is single gene resistance. In 2008, observations suggested resistance-breaking strains of TSWV were developing.

PEANUT: Spotted wilt losses in peanut are based on a consensus estimate from each of the scientists (plant pathology, entomology and agronomy) involved in the annual evaluation of the spotted wilt risk index for peanut (Brown et al., 1999). Annual losses based on these estimates are shown in Figure 1. The sharp declines in losses after 1997 are due to the implementation of a highly effective management program based principally on resistant varieties and planting date. Resistant varieties can reduce disease loss by 30%-75% for any planting date. Resistance-breaking strains of TSWV have not been found in peanut. The loss cycle between 2001 and 2008, when the variety Georgia Green made up ~95% of the peanut acreage in Georgia, suggests

a high level of partial resistance and loss fluctuating with disease pressure. Planting in May as opposed to April can result in a 40%-60% reduction of loss in susceptible to moderately resistant varieties. Other cultural practices offer additional additive disease reduction, particularly when April planting cannot be avoided (Brown et al., 1999).

TOBACCO: Spotted wilt losses in tobacco are shown in Figure 1. The estimates for disease incidence (% plant loss) are based on disease evaluations in 10-60 on-farm trials per year, plus estimates from county agents not in the farm trial program, plus a final general estimate of plant loss from tobacco crop specialists. The yield loss is estimated based on disease incidence figures. Tobacco shows a pattern of continued high loss after peanut losses declined sharply in 1998 (Figure 1.). Imidacloprid was labeled for

managing spotted wilt in 2000. An imidacloprid treatment can reduce losses by 25%-30% and was used on 75%-80% of the tobacco by 2005. Actigard treatment of seedlings prior to transplanting was approved in 2003. A combination of Actigard followed by imidacloprid treatment of seedlings can reduce spotted wilt incidence by about 50%. This program gained acceptance slowly so that by 2006 ~85% of the Georgia crop was treated in this manner. Between 2000 and 2008, disease loss curves for treated (imidacloprid only or Actigard + imidacloprid) plants parallel, at a lower level, losses for untreated plants. Loss estimates in Figure 1 take into account the relative portions of the crop untreated, treated with imidacloprid only, and treated with Actigard + imidacloprid. These portions are based on formal and informal surveys.

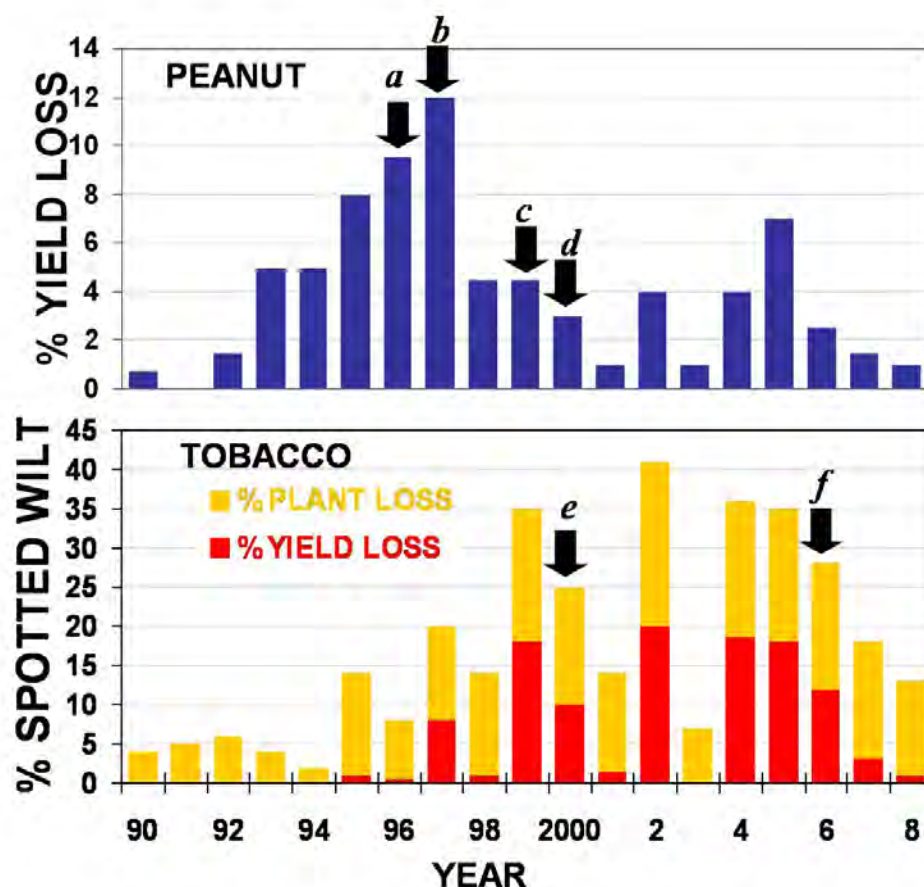


FIGURE 1. ANNUAL LOSSES FOR PEANUT AND TOBACCO.

- PEANUT:** a: Introduction of Georgia Green;
b: Publication of "Peanut risk Index";
c: Crop >95% Georgia Green;
d: >95% of crop planted after 1st of May
- TOBACCO:** e: imidacloprid labeled;
f: >85% use of Actigard + imidacloprid

TOSPOVIRUS DETECTION

STEPHEN MULLIS AND CLAUDIA NISCHWITZ

Symptoms by Tomato spotted wilt virus and other tospoviruses are highly variable and of little diagnostic value. Necrosis on all plant parts, chlorosis, ring patterns, mottling, silverying, and local lesions are the most characteristic symptoms. It has been shown that the same strains of the same tospovirus may vary widely in its symptom expression within the same plant. It is not clearly understood why many plants infected with the virus may not show any symptoms. During the last three tobacco growing seasons, half of the infected tobacco exhibited no outward signs of disease. The vast majority of the known plant hosts also showed no symptoms. Some of the symptoms that were exhibited may be mimics of some other pathogen or environmental stressor. This attenuates the need for testing protocols that will give a more definitive determination of tospovirus presence. Three current commonly-used techniques are double antibody sandwich-enzyme linked immunosorbent assay, reverse transcriptase-polymerase chain reaction, and mechanical sap inoculation. Additional serological and molecular methods are employed when thrips screening is needed.

Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) is commonly used in tospovirus detection. Antibodies specific to the virus are used to screen whether a particular virus is present in the tissue that is sampled. DAS-ELISA is used on a wide scale due to its reliability and the ease of screening a vast number of samples over short periods of time. Antibodies specific for a particular pathogen are coated into the wells of a polystyrene assay plate. Plant saps extracted from tissues of interest are loaded into the individual wells on the assay plate (Figure 2). After a series of incubations and washes, an enzyme conjugated antibody is added to the plate, to which a colorless substrate is added after incubating and washing. The plate is observed for color changes in the assay wells after an hour using a micro titer plate reader (Figure 3). The level of color change is given by the absorbance readings of the reader, and these readings indicate if the tested sample had the pathogen of interest within the extracted sap. Although it is commonly used on a day-to-day basis, other verification methods are used to back up the results of the serological testing. A related serological protocol employs

immunostrips. This tool gives a rapid result and is convenient because immunostrips can be used directly in the field. Immunostrips are lateral flow devices that have antibodies for a particular pathogen impregnated within an absorbent pad. Plant tissue is ground up in a small plastic bag, and then the immunostrip is put in the sap and observed for a color reaction. The immunostrips will only show a positive reaction when virus levels in the tissue are very high.

Reverse transcriptase-polymerase chain reaction (RT-PCR) is a molecular technique used to amplify a specific region of the viral genome. This technique, although time-consuming and expensive, is valuable in verifying DAS-ELISA results (Figure 4). It can also be used to identify individual viral strains when the RT-PCR products are sequenced. Analyzing the sequenced products of RT-PCR is useful in understanding some of the epidemiological properties of the Tospoviruses. Phylogenetic analysis of some of the regional strains of TSWV and IYSV has offered some intriguing insights into the background and dynamics of the disease. In addition, due to the nature of analyzing the virus genome, a positive response using this method is the most definitive screen for tospoviruses.

PCR of thrips has been problematic, but a technique using universal primers for the ribosomal DNA gene was developed that can identify the stomach contents of thrips. Preliminary results indicate that the plant species that the thrips last fed on could be determined up to 15 hours after feeding, but not after 24 hours. This technique is dependent on the genetic material being on file in GenBank, and also presents a number of intriguing studies that can be conducted, including identifying a potential weed reservoir host that serves as the primary source of inoculum for tospovirus epidemics.

Mechanical sap inoculation is another helpful tool in detection and virus culture. Tissue from a suspected infected plant is used to inoculate a known indicator plant. These indicator plants include *Nicotiana tabacum* L., *Nicotiana glutinosa* L., *Nicotiana benthamiana* and *Emilia sonchifolia* (L.) DC. Successful viral transmission to one of the indicator plants is useful in two ways: it aids in the diagnosis of a plant virus and serves as a continuous culture of a virus over time.



FIGURE 2. Plant sap being extracted by a leaf press (left) and the use of mesh bags (right).

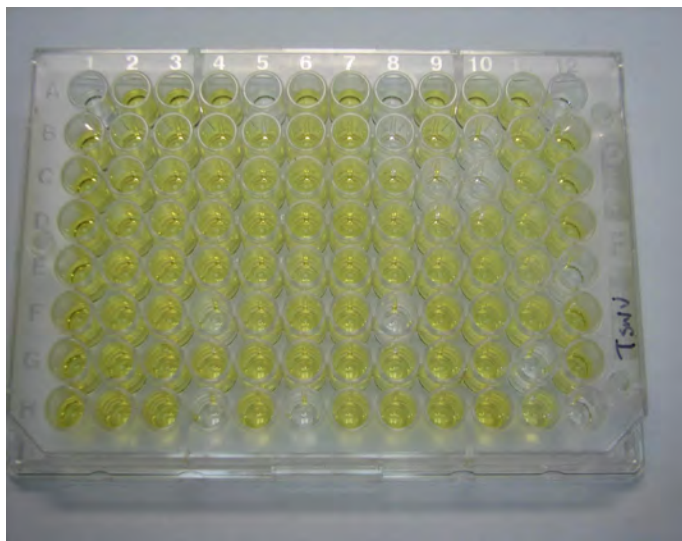


FIGURE 3. An ELISA plate after the color change. The yellow color is indicative of a positive reaction.

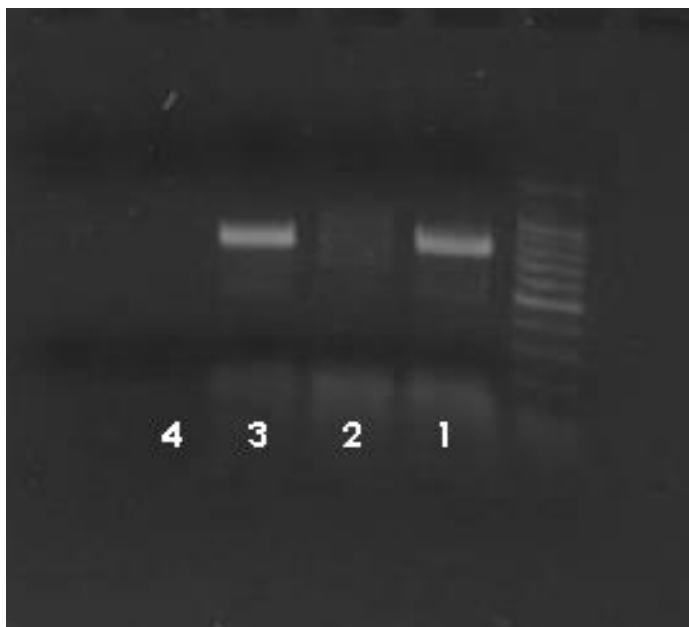


Figure 4. Image of a UV-illuminated agarose gel after electrophoresis. Lanes 1 and 2 are RT-PCRs of suspected TSWV nucleocapsid genes. Lane 3 is a positive control and lane 4 is a negative blank. In this image, Lane 1 is a positive reaction.

SYMPTOMATOLOGY OF TSWV IN TOBACCO

ALEX CSINOS

TSWV can cause symptoms at all stages of growth, from transplant to maturity. On tobacco, however, younger, actively growing plants appear to be more susceptible to infection than slow-growing, hardened-off plants.

The actual symptoms vary greatly on individual plant species and among virus hosts. Symptoms on tobacco vary from discrete individual spots to systemic necrosis. Often, individual discrete spots are local infections, while infections that become systemic move from the point of initial infection to the root systems, where the virus reproduces. Systemic infection often occurs when an environmental

factor such as a temperature change, irrigation or rainfall triggers sudden plant growth. Systemic infection results in specific leaves and the apical bud becoming necrotic. This systemic infection is often unilateral, indicating that it may be moving up in the xylem. Plants having ring spot symptoms on stems in absence of any foliar systems may occur late in the season. Very young, freshly transplanted tobacco may collapse and die without distinct symptoms.



HIGH DEGREE OF VARIABILITY



LARGE FOLIAR SPOTS OBSERVED EARLY IN THE SEASON



**MOST PLANTS WITH SYMPTOMS BY
2ND-4TH WEEK DIE OFF QUICKLY**

**FLUE-CURED TOBACCO FIELD WITH >90% STAND
LOSS DUE TO TSWV (BOWEN FARM, MAY 2002)**



2ND-4TH WEEK: VARIABLE SYMPTOMS



6TH-10TH WEEK: SYMPTOMS APPEAR ON TOP, AT TIMES ON ONE SIDE, BUD TIP 45°



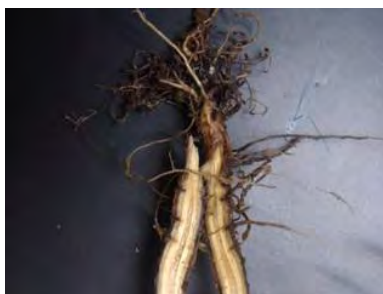
TYPICAL SYSTEMIC VEIN NECROSIS



NECROTIC SPOTS IN FLOWERS



2ND-4TH WEEK VARIABLE SYMPTOMS; NECROTIC LESIONS ON STEMS



CROWN AND ROOT STEM NECROSIS



TSWV MILD ISOLATES

THRIPS VECTORS OF TSWV

DAVID RILEY, ROBERT MCPHERSON AND LENNY WELLS

INTRODUCTION

Thrips are minute, slender-bodied insects (around 1/16-inch long when fully grown, Figure 5) that have become an important agricultural pest because of their ability to transmit plant viruses. Thrips-vectored Tomato spotted wilt virus (TSWV), of the genus *Tospovirus* (Family *Bunyaviridae*), is a serious disease of solanaceous crops (Sherwood et al., 2005). In Georgia, TSWV has reduced tomato yields by an estimated \$8.8 million in a single year (2000) (Riley, 2000). Also, this virus has become a major yield-limiting factor to production of many other crops in Georgia, including pepper, peanut and tobacco, with total losses estimated at \$100 million in 1996 (Bertrand, 1997; Pappu, 1997). TSWV is one of the most economically important members of the genus *Tospovirus* (family *Bunyaviridae*) (Moyer, 2000; Mumford et al., 1996; Peters and Goldbach, 1995). TSWV is

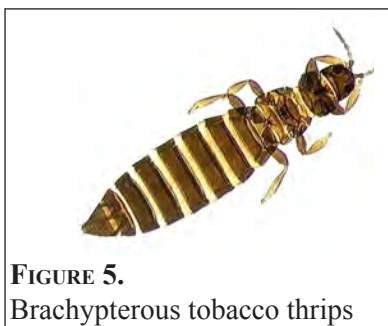


FIGURE 5.
Brachypterous tobacco thrips

transmitted by multiple species of thrips (Ullman, 1997). Thrips responsible for spotted wilt epidemics in Georgia and Florida can be largely attributed to western flower thrips, *Frankliniella occidentalis*, tobacco thrips, *F. fusca*, and possibly *F. bispinosa* (Salguero et al., 1991; Riley and Pappu, 2000, 2004; and Webb et al., 1997, respectively). Other species could also be involved in vectoring *Tospoviruses*, but the aforementioned species are thought to be the most important. In general, immature thrips in the first and second instars acquire TSWV from infected host plants, the virus replicates in the vector as it matures, and subsequently viruliferous adults spread the virus when they move to other plants (Figure 6). Virus multiplication in the thrips vector makes managing TSWV difficult because, once infected, adult thrips can migrate long distances to new host plants and quickly transmit the virus, often before thrips can be controlled. It is important to note that each generation of thrips must re-acquire the virus since the virus in adult females is not passed on to her eggs (Figure 7). Thus, the frequency of viruliferous adults can vary greatly over time. However, even

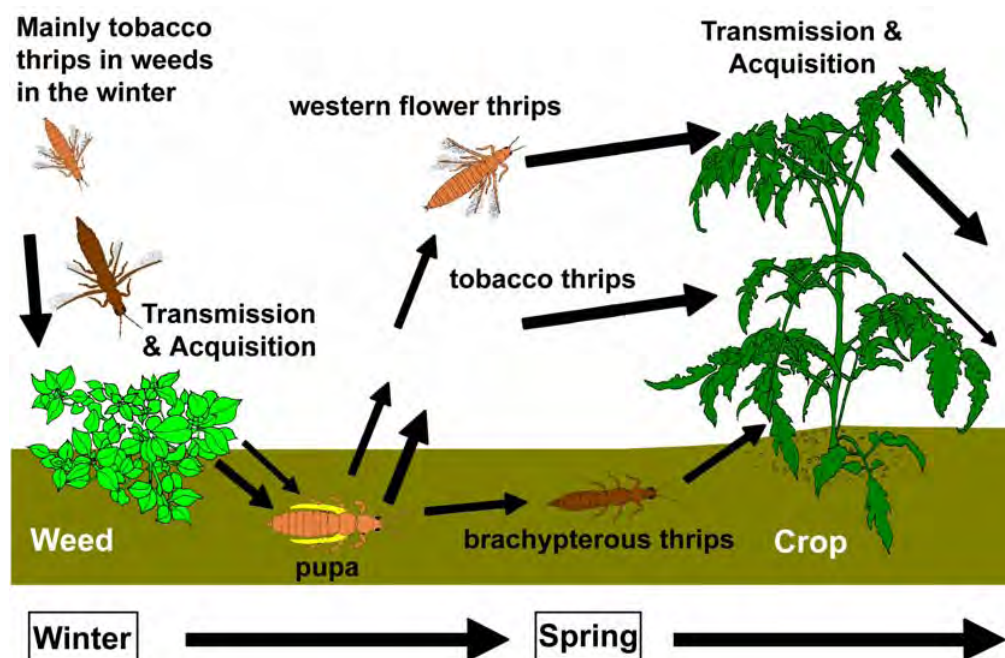


FIGURE 6. Illustration of thrips' movement of TSWV in the spring. The nymphs acquire the virus from weeds before the fully developed adults transmit the virus to a springtime crop, like tomato. (Drawing by Angelika Pia Schmid-Riley)

low percentages of viruliferous thrips can cause significant yield loss. Viruliferous thrips are usually <10% of the field population even in a severe TSWV year. The percentage tends to increase over time in the spring.

General Biology

Thrips belong to the insect order Thysanoptera (fringe-winged insects, their wings consisting of fine hairs rather than a membrane) and the main TSWV vector species all belong to the family Thripidae. In the Georgia solanaceous crops, TSWV is primarily transmitted by western flower thrips, *Frankliniella occidentalis* (Pergande), and tobacco thrips, *F. fusca* (Hinds) (Riley and Pappu, 2000, 2004; Salguero et al., 1991). Immatures are a cream to yellowish color and adults are either dark brown or yellow/orange, depending on the species. Adults usually have wings, although wings can be absent in tobacco thrips (brachypterous form, Figure 8). When wings are present, they are narrow and fringed with long hairs (Figure 7). Eggs are laid singly and are usually inserted in plant tissue (usually flower parts or young leaves). The eggs hatch in 3-4 days. Two larval stages last about 10-14 days, prepupa and pupa stages last around 7 days, in which the thrips are inactive and do not feed, and then they become adults. The only stages that can acquire TSWV are the two larval stages, and this only occurs when larvae feed on an infected plant host. It is not passed from adults to eggs, so each generation of thrips must re-acquire the virus for the disease epidemic to continue. This is fortunate because thrips can reproduce parthenogenically. That is, if female thrips remain unmated then all male offspring are produced asexually. If thrips mate, they produce mostly female offspring.

It is important to note that once thrips acquire the virus, they remain viruliferous (infected with virus) through-



FIGURE 8. Western flower thrips

out their life span. Several generations occur per year in Georgia, but only one or two generations occur in a single tobacco or tomato growing season. Thrips overwinter on host plants as adults or larvae (nymphs), and can be observed on winter weeds during warm periods throughout the winter months. Thrips extracted from weeds in Georgia have predominantly been in the larval stages. It has been observed that tobacco thrips can reproduce year-round in south Georgia in the winter onion crop and in weed hosts, even though winter populations are very low and the life cycle is greatly extended with the lower average temperatures. Infected thrips are capable of transmitting TSWV to a wide range of host plants spanning several hundred plant species from more than 70 plant families. Thus, these winter thrips populations are important for maintaining TSWV in the resident weed plant population. This sets the stage for TSWV epidemics in the following spring season.

FEEDING

Thrips use piercing and sucking mouthparts to feed on plant tissue. They produce an initial opening by penetrating the plant cuticle with their single mandible after rocking the head capsule downward and upward numerous times. Once an opening or food channel is punched through the outer cell wall, then a pair of maxillary stylets is extended into the plant tissue. Saliva is injected into the plant and cellular contents are drawn up through a pumping action. The adults transmit TSWV as saliva is injected into the plant tissue. Thrips feeding can damage plant tissue directly; however, the greatest loss to agriculture is caused by the species that vector (transmit) Tospoviruses like TSWV. Since TSWV transmission occurs through thrips feeding, understanding factors that affect thrips feeding is critical for managing this pest. For example, thrips vary greatly in their host preference and, given a choice, they will lay eggs more in one species of plant than another (Chaisuekul and Riley, 2005). Also, many factors can affect thrips feeding and thus transmission efficiency. For example, older plant age and treatment with the insecticide imidacloprid has been shown to reduce feeding by tobacco thrips (Joost, 2003). Unfortunately, imidacloprid

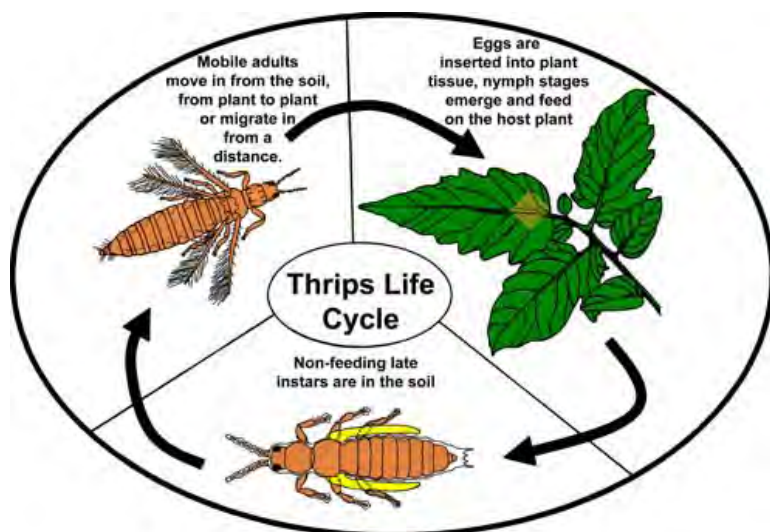


FIGURE 7. Generalized life cycle of thrips vectors of TSWV (Drawing by Angelika Pia Schmid-Riley)

has been shown to increase western flower thrips settling in peanut and feeding in tomato, so it is important to note that these effects can be specific for the thrips species.

THRIPS VECTOR SPECIES

Thrips have been found to be vectors of at least four plant virus groups (families): bunyaviruses, ilarviruses, sobemoviruses and caroviruses (Ullman et al., 1997). Previously, eight thrips species were 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 vectors 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 vectors of TSWV, but experimental verification had not been done for all species as has been done for *F. occidentalis* and *T. tabaci* (Ullman et al., 1997). Clearly, *F. occidentalis* and *T. tabaci* are common and important vectors of multiple plant viruses in many regions of the world (Ullman et al., 1997). In Georgia, western flower thrips, *Frankliniella occidentalis*, and tobacco thrips, *F. fusca*, appear to be the most important species (Riley and Pappu, 2000, 2004).

A total of 43 thrips species have been collected on flue-cured tobacco foliage and blooms in Georgia, and many of these same species are present on vegetables and other crops as well. However, only five species are commonly encountered during the growing season and include the tobacco thrips, *Frankliniella fusca* (Hinds), the western flower thrips, *F. occidentalis* (Pergande), the flower thrips, *F. tritici* (Fitch), *F. bispinosa* (Morgan) and the grain thrips, *Limothrips cerealium* (Haliday). *F. fusca* is the predominant species on the foliage and usually accounts for more than 90% of all the foliage thrips present, although *L. cerealium* can also be abundant on some sampling dates. Thrips are present at low densities (less than one per plant) on tobacco foliage soon after transplanting, then steadily increase during the next 3-4 weeks after transplanting. They usually peak on foliage in early to mid-May, around six weeks after transplanting, before the tobacco flowering process begins. Peak populations of 50+ adult thrips per plant are common in Georgia in most growing seasons. Thrips movement within the field occurs between 9:00 a.m. and 4:00 p.m., with almost no activity (captures on sticky cards) outside of this time range. Immature thrips (*F. Fusca*) can be quite high (more than 100 per plant) on some sampling dates in May.

In Georgia, the most common thrips in the tobacco blooms are *F. tritici* and *F. bispinosa*. Thrips can be collected from the blooms before the flowers fully open and

continue to be present until the flowers dry up. Thrips peak in tobacco blooms in late May at more than 100 adults per terminal floral branch (all blooms on a single plant). Immatures are very abundant. *F. occidentalis* are also present at relatively low populations in tobacco blooms every year.

The thrips complex in tobacco flowers is probably of minor importance regarding TSWV symptomatic plants in the crop because they occur so late in the growing season. However, these same thrips species are also found on numerous flowering weeds in the tobacco farmscape throughout the summer, fall and winter. Many of the thrips in tobacco blooms do not complete one generation on the crop because the blooms are removed during the topping process. Many of the eggs and young larvae that are on the blooms that are removed from the plant do not survive to adulthood because the blooms dry up prematurely. Two other thrips species on weeds in the tobacco farmscape have also tested positive for non-structural TSWV protein, indicating that these thrips also replicate the virus in their bodies and are potentially capable of transmitting TSWV. These other thrips include *Haplothrips graminis* (Hood) and *Chirothrips* spp. Both of these species are also present at low population densities on tobacco foliage.

In the Georgia tomato crop, TSWV is primarily transmitted by western flower thrips, *Frankliniella occidentalis* (Pergande), and tobacco thrips, *F. fusca* (Hinds) (Riley and Pappu, 2000, 2004; Salguero et al., 1991). Of the individual species counted in tomato blossoms in 1997 and 1998 trials, *F. occidentalis* represented $25 \pm 2\%$ and $31 \pm 2\%$, respectively, and *F. fusca* represented $2 \pm 0.7\%$ and $7 \pm 1\%$, respectively. The remainder consisted of other flower thrips (mainly *F. tritici* and *F. bispinosa*) and immature thrips (not identified to species). *Frankliniella occidentalis* was the predominant TSWV-vector species counted in the blossom sample in both years.

Tobacco thrips have been found to be more prevalent than western flower thrips on pre-flowering tomato in Georgia (Joost and Riley, 2004) representing 74% of the adults collected. As in tobacco, thrips numbers on young tomato plants are low (less than one per plant), but are sufficient to result in high incidence of TSWV. Once flowering occurs in the crop, thrips collected in blossoms are comprised mostly of flower thrips species and occur in much higher numbers. Relative to thrips species, two interesting correlations have been observed. *Frankliniella occidentalis* numbers in blossoms correlated better with % TSWV as detected by ELISA than *F. fusca* and correlated well with % TSWV based on plant symptoms. However, only *F. fusca* had a significant negative correlation with marketable tomato yield and positive correlation with TSWV-affected fruit. The way we interpret these data is that although both thrips species are good TSWV vectors, *F. fusca* occurs on tomato foliage in greater numbers earlier in the

growing season during the pre-blossom growth stages.

TSWV inoculation of young vegetative tomato plants has been associated with significant negative effects on tomato yield (Chaisuekul et al., 2003) and so *F. fusca* tends to have more of an impact on yield than *F. occidentalis*. Even so, *F. occidentalis* is an excellent vector and occurs in greater numbers than *F. fusca* later in the season when tomato blossoms occur. Because *F. occidentalis* is more prevalent than *F. fusca* in tomatoes grown at Tifton, Ga., during the course of recent studies, overall transmission of TSWV has been better correlated with *F. occidentalis*. The later the development of TSWV symptoms in the tomato plant, the lower the effect on the quality of tomato yield (Moriones et al., 1998). Thus, a pre-blossom thrips population early in the growing season could be important for correlating vector numbers to incidence of TSWV-related damage to the crop.

THRIPS VECTOR STUDIES RELATIVE TO PLANT AGE

Recent thrips exclusion studies, using field cages to protect tobacco transplants, have concluded that the most critical time for thrips to transmit TSWV infection in flue-cured tobacco is during the first six weeks after transplanting. Plants protected from thrips feeding for the first two weeks following transplanting had 29.2% TSWV symptomatic plants compared to uncaged plants (36.3%). When plants were protected from thrips for four weeks and six weeks after transplanting, the seasonal incidence of TSWV symptomatic plants was lowered to 23.0% and 12.5%, respectively. None of the tobacco plants protected with thrips exclusion cages showed any TSWV symptoms until 2-4 weeks after the cages were removed, indicating that TSWV infection was occurring in the field after the plants were exposed to thrips infestation and not being brought into the field by TSWV-infected plants obtained from the plant bed or greenhouse. Thus, thrips management practices need to focus on early-season control to be most beneficial in suppressing TSWV infection. Also, TSWV-symptomatic plants that appear later in the season, 10-12 weeks after transplanting, usually have only minor reductions in plant height, leaves per plant and yield per plant compared to the non-symptomatic plants. When TSWV symptoms appear early in the season, 4-8 weeks after transplanting, severe plant growth reductions and premature plant death usually occur.

In tomato cage and greenhouse vector studies at Tifton, tomato yield was lower as TSWV inoculation and TSWV symptoms occurred earlier (Chaisuekul et al., 2003). This effect was very dramatic in greenhouse studies where plants inoculated in the first few weeks after germination produced no marketable fruit. These results agree

with that of Moriones et al. (1998) in that earlier symptomatic plants produce less yield than later symptomatic plants. A notable effect was that symptoms developed rapidly across all treatments after the screen cages were removed regardless of when thrips vectors were introduced into the field cages. We suspect that the cages often used in thrips vector studies are also a source of shading or temperature differences, which could potentially affect TSWV symptom expression (Diez et al., 1999; Diaz et al., 2003).

Only around 1%-6% of all the thrips collected on tobacco throughout the growing season test positive for TSWV non-structural protein (using ELISA procedures) in any given year. Collections from a specific sampling date can have 10% or more thrips testing positive for TSWV, while other sampling dates have 0%-1% testing positive. Groves et al. (2001) reported no significant change in % viruliferous *F. fusca* and *F. occidentalis* in the spring in North Carolina (possibly because of too little sample replication), but the highest percentage for both species from January to May occurred in April (14.7% and 11.6%, respectively) and ranged from 1.1% to 14.6% throughout this period. In conclusion, it appears that thrips species present, population density, time of infestation, and percent TSWV positive (vectors capable of transmitting TSWV) all influence the cumulative infection rate of TSWV in flue-cured tobacco and other Solanaceous crops. Most of these effects all relate directly to plant age.

Recently, the effect of tomato, *Lycopersicon esculentum* (L.), plant and leaf age on the probing and settling behavior of *Frankliniella fusca* and *F. occidentalis* was studied using electrical penetration graph technique (EPG) and whole-plant bioassays. Male and female *F. fusca* probed and ingested more and for longer periods of time on three- and four-week-old plants compared to six- and eight-week-old plants. Female *F. fusca* probed and ingested more frequently than males in the plant age experiment but not in the leaf age experiment. *Frankliniella fusca* probed and ingested more frequently on two- and four-week-old leaves compared to one-week-old leaves. Males did probe and ingest longer than females in the plant age experiment and on the oldest leaf in the leaf age experiment. Both thrips species preferred to settle on three-week-old plants. *Frankliniella fusca* preferred to settle on four-week-old leaves after settling randomly for an hour. Thus, plant age does have an effect on *F. fusca* host selection and actual feeding, with younger plants preferred. We have observed in several studies that suppressing thrips early in the spring season on young plants (one to six weeks in age) with pre-plant, transplant and foliar insecticides or other tactics that reduce thrips present on young host crop plants can effectively reduce the incidence of TSWV.

Non-Crop Hosts of TSWV

STEPHEN MULLIS AND NATALIA MARTINEZ-OCHOA

Non-crop plant hosts are likely to contribute to pathogen spread by serving as reservoirs for the virus and reproductive hosts for thrips that transmit the virus. Several studies worldwide have reported findings of diverse plant hosts for tospoviruses. A survey of weeds around agricultural fields in Georgia from 2002-2009 have revealed a large number of plant species naturally infected with TSWV. The type of plants that did not test positive for TSWV during the entire survey was very small and included species such as field pansy (*Viola rafinesquii*), fumitory (*Fumaria officinalis*), hairy bittercress (*Cardamine hirsuta*), poorjoe (*Diodia teres*), common pokeweed (*Phytolacca americana*), American burnweed (*Erechtites hieracifolia*), alyceclover (*Alysicarpus vaginalis*), castorbean (*Ricinus communis*) and corn speedwell (*Veronica arvensis*). The weeds most commonly found infected with TSWV are outlined in Tables 1 and 2, some of which are new host recordings.

Most weeds testing positive for TSWV were non-symptomatic. Many of these weeds occur abundantly in the Southeastern U.S. and are cause of major losses in ag-

ricultural crops such as peanut, cotton, tobacco and vegetables. The incidence of TSWV in weeds varied from year-to-year (Figures 9, 10) and among seasons. Where the year-to-year and season-to-season TSWV incidence varied, there is a clear distinction of TSWV infection levels spiking during the late spring and early fall. This has been a constant observation throughout the weed surveys. The proportion of TSWV found in weeds relates positively with spotted wilt levels recorded in the crops affected those years. We believe the role of winter weeds is a key factor in understanding the epidemiology of TSWV, and perhaps virus levels detected prior to the cropping season might be an indicator of the spotted wilt to be expected. Other tospoviruses such as INSV and IYSV have been found in weeds in Georgia, and many overlapping hosts indicate that mixed infections are very likely to occur, increasing the complexity of the damage they can cause. The potential for survival and dispersal of tospoviruses is significant because the composition and amount of weed hosts found in Georgia year-round is very large.

TABLE 1. Survey of winter and spring weed species most commonly infected with *Tomato spotted wilt virus* (TSWV) in the surveyed area of Georgia from 2002 through 2009.

PLANT SPECIES (COMMON NAME)	TOTAL TSWV INCIDENCE
<i>Gamochaeta falcata</i> Lam. (narrowleaf cudweed)	31.5%
<i>Eupatorium capillifolium</i> (Lam.) Small (dogfennel)	21.6%
<i>Stellaria media</i> (L.) Cyrillo (chickweed)	21.1%
<i>G. purpurea</i> L. (purple cudweed)	15.2%
<i>Lepidium virginicum</i> L. (Virginia pepperweed)	8.9%
<i>Geranium carolinianum</i> L. (Carolina geranium)	8.2%
<i>Spergula arvensis</i> L. (corn spurry)	8.0%
<i>Raphanus raphanistrum</i> L. (wild radish)	6.9%
<i>Gamochaeta pennsylvanica</i> Wild (wandering cudweed)	6.6%
<i>Verbena rigida</i> Spreng. (stiff verbena)	6.3%
<i>Solidago canadensis</i> L. (Canada goldenrod)	6.1%
<i>Oenothera laciniata</i> Hill (cutleaf eveningprimrose)	4.2%

TABLE 2. Survey of summer and fall weed species most commonly infected with *Tomato spotted wilt virus* (TSWV) at nine farms in Georgia from 2002 through 2009.

PLANT SPECIES (COMMON NAME)	TOTAL TSWV INCIDENCE
<i>Jacquemontia tamnifolia</i> (L.) Griseb. (small-flower morningglory)	21.5%
<i>Amaranthus retroflexus</i> L. (redroot pigweed)	14.1%
<i>Ipomoea hederacea</i> Jacq. var. <i>integriuscula</i> Gray (entireleaf morningglory)	13.4%
<i>Eclipta prostrata</i> L. (eclipta)	13.1%
<i>Desmodium tortuosum</i> (Sw.) DC. (Florida beggarweed)	10.2%
<i>Wahlenbergia marginata</i> (Thunb.) A. DC. (southern rockbell)	8.2%
<i>Richardia scabra</i> L. (Florida pusley)	7.2%
<i>Acanthospermum hispidum</i> DC. (bristly starbur)	6.9%
<i>Croton glandulosus</i> var. <i>septentrionalis</i> Muell (tropic croton)	6.8%
<i>Senna obtusifolia</i> (L.) Irwin and Barneby (sicklepod)	5.3%
<i>Sida rhombifolia</i> L. (arrowleaf sida)	4.5%
<i>Portulaca pilosa</i> L. (broadleaf pink purslane)	3.7%

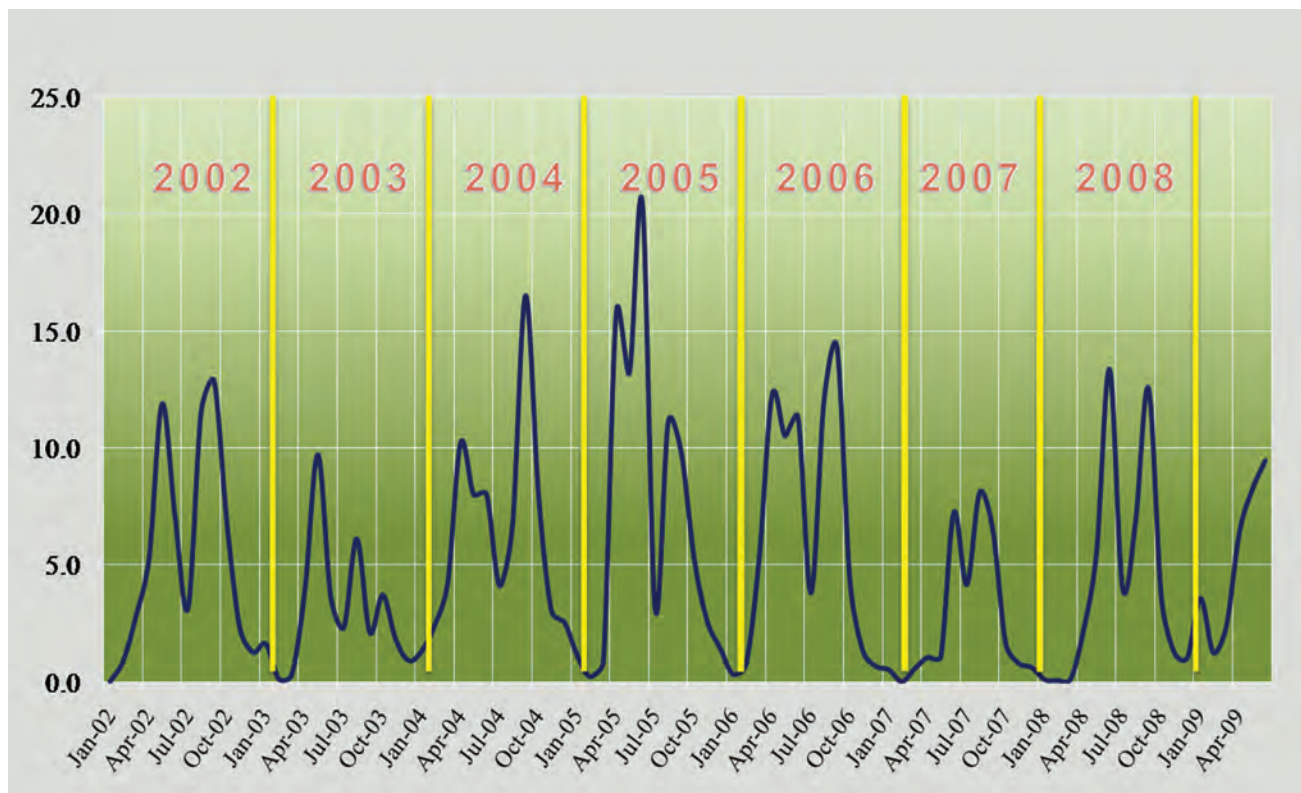


FIGURE 9. Total percentage TSWV in weeds screened 2002-2009

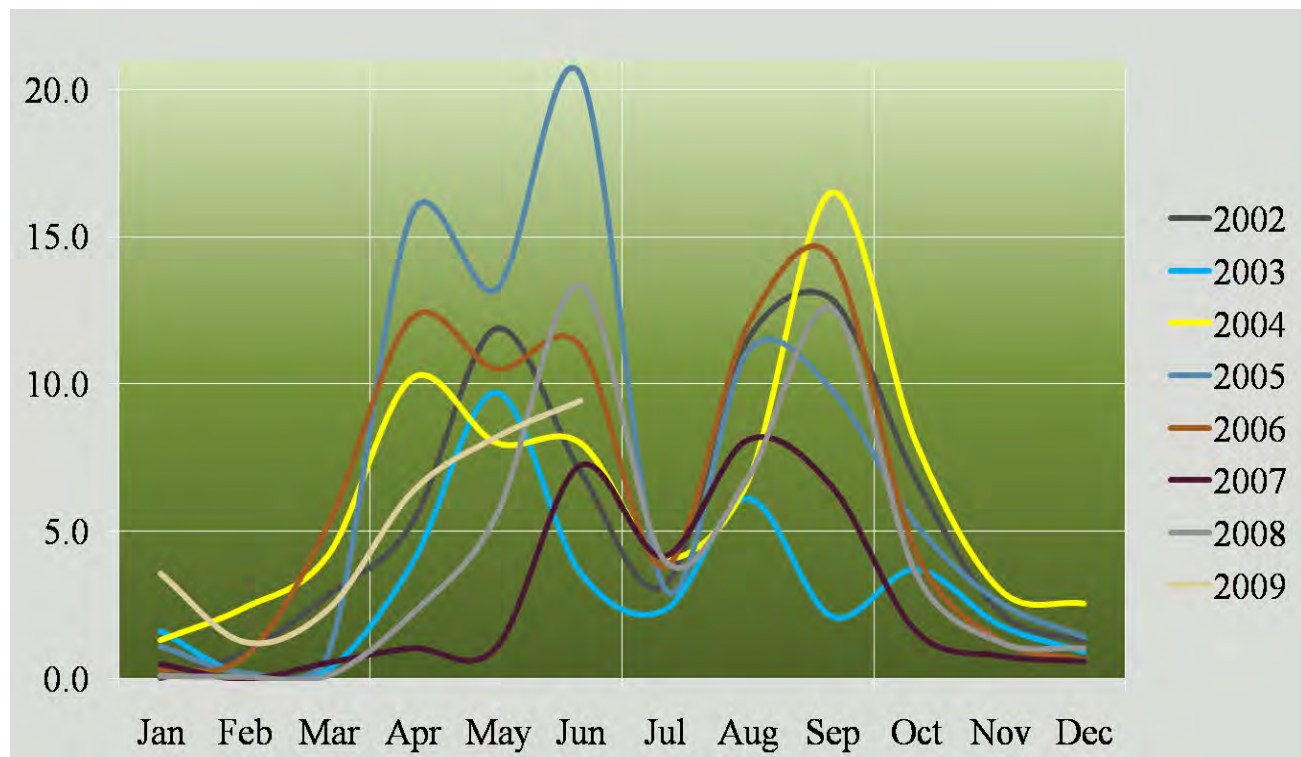


Figure 10. Total percentage TSWV in weeds by month. Note the increase in spring and fall.

ENVIRONMENT

ALEX CSINOS AND STEPHEN MULLIS

In Brazil, where growers have dealt with TSWV on tobacco for more than 25 years, they have made observations suggesting that rainfall may have an effect on TSWV incidence. Periods of rainfall at or just before transplanting have tended to reduce the incidence of TSWV on tobacco. In Georgia, in years where rainfall amounts were greater than 5 inches in the month of March, TSWV was low, when compared to those years where rainfall was less than 5 inches (Figure 11). The incidence is relative to location only. If the same area is examined over several years, those years with heavy rainfall resulted in lower TSWV; particularly in early season. Heavy rainfall early in the year may have a harmful effect on thrips reproduction, and thus reduces the number of potential vectors visiting the crop. In addition, heavy rainfall during March may keep weeds in surrounding fields green longer. Thrips may be reluctant to leave a succulent viable host for another crop. Thus the crop plants may be out of phase with moving viruliferous thrips and highest susceptibility of the crop to infection. More recent data has indicated that heavy spring rains may

not have a lasting effect on reducing TSWV infection, and thus total incidence and severity of TSWV at the end of the season may still be high. In 2005, heavy rains in the last week of March and cool temperatures in April resulted in no significant infection occurring until mid-May. However, with increased temperatures, both thrips populations and TSWV infection increased dramatically. Thus, infection of the host may be limited at or during rainfall events, but subsequent environmental conditions may be favorable for thrips development and subsequent TSWV infection. There is a pronounced temperature effect on TSWV losses when the running average temperature from the four months preceding transplanting are regressed against the disease loss and incidence. The increase in temperature has a positive significant effect on TSWV disease incidence (Figure 12). In the event that the window of infection for TSWV is early in the season (transplanting time), the heavy rain at that time would be expected to reduce TSWV infection. However, if the infection window is later (May) then March, rains may not have an effect on the level of infection.

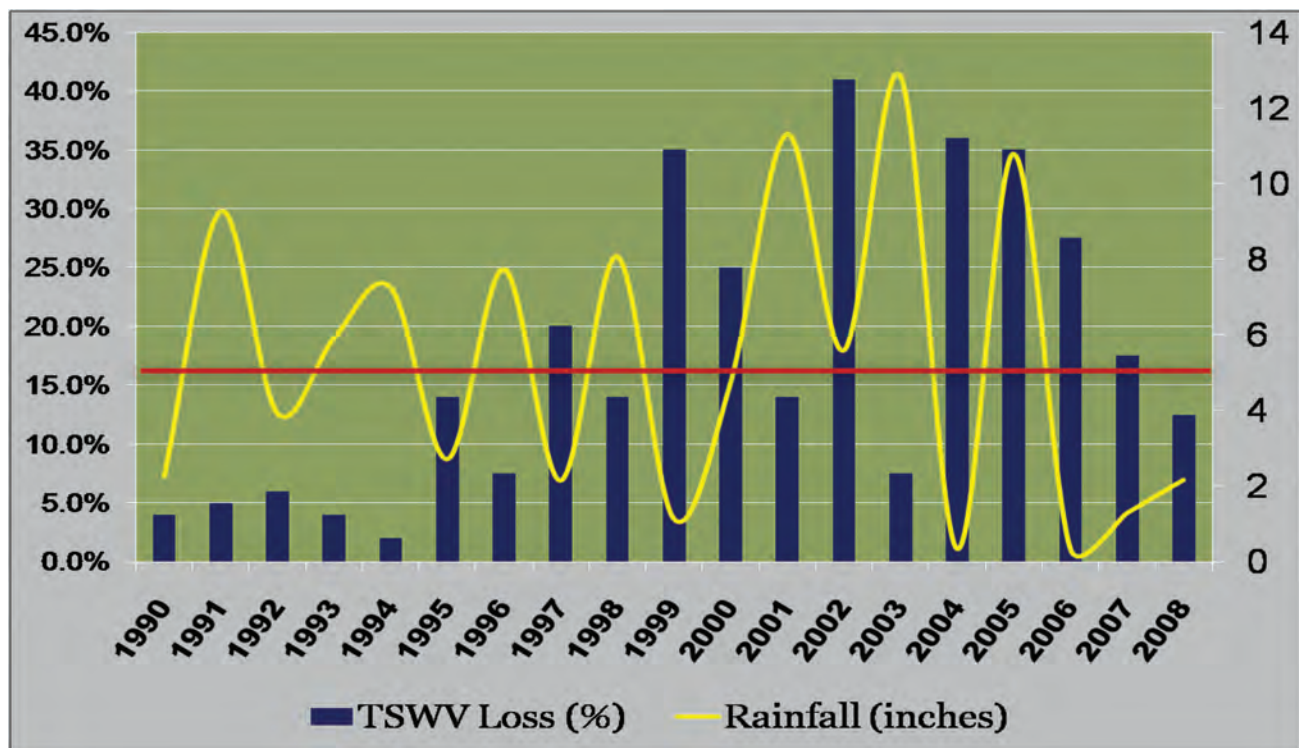


Figure 11. Early spring rainfall and TSWV losses

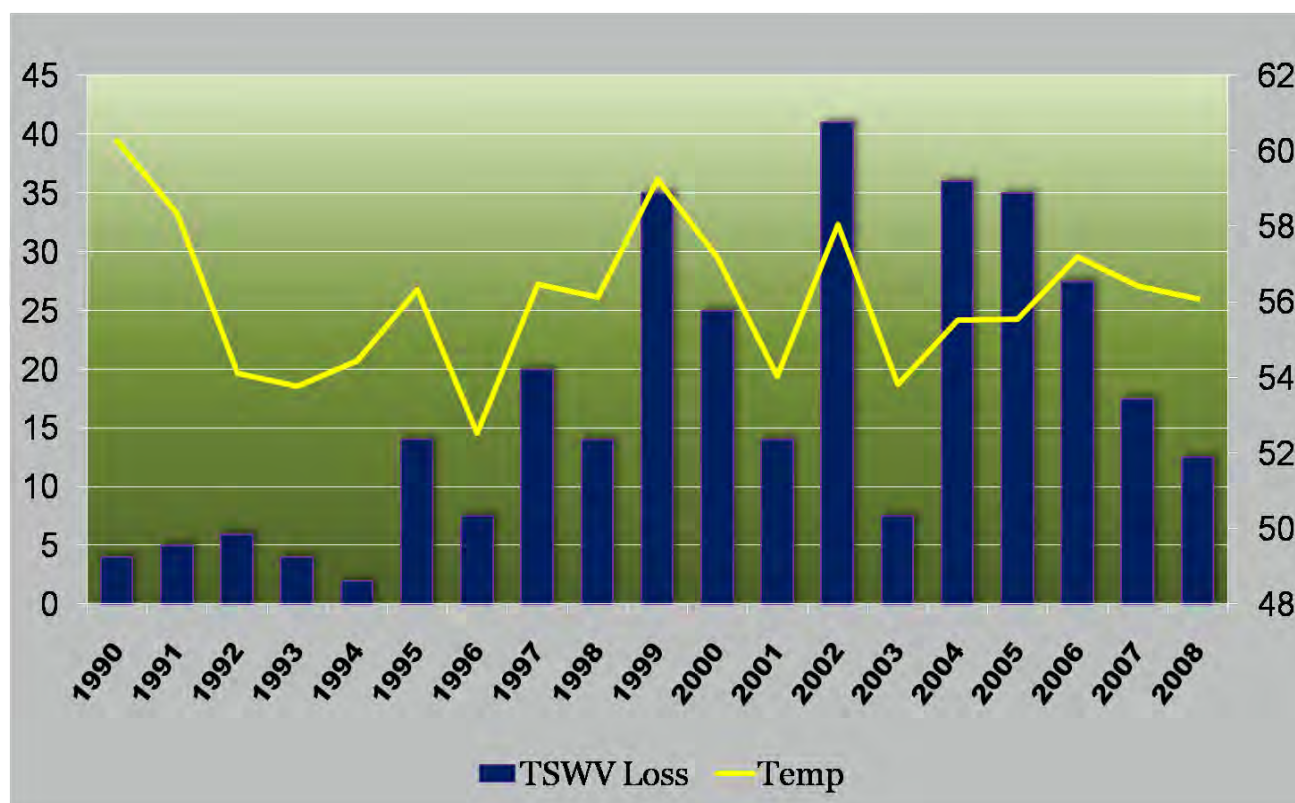


Figure 12. Running averages of air temperatures in the four months preceding transplanting and TSWV losses

TOSPOVIRUSES IN GEORGIA VEGETABLES

RON GITAITIS

Incidence of TSWV in Georgia has increased on pepper and tomato since it was first observed on tomatoes in the state during the late 1980s. Depending on the season, TSWV can be responsible for up to 100% loss in individual pepper and tomato fields and was a contributing factor to the decline of the field-grown transplant industry. Infected transplants grown in southern Georgia were implicated in TSWV losses in the northern United States and Canada.

Symptoms on tomato vary considerably, with lesions developing on stems, reddish-brown ring spots on leaves (Figure T1A) that can coalesce and give the appearance of bronzing (Figure T1B), or larger interveinal areas of necrosis and spotting on leaves (Figure T2). Depending on the timing and degree of infection, plants can be severely stunted (Figure T3) leaves can turn a purple hue with cupping, or plants can show severe wilt stress and die prematurely (Figure T4). Tomato fruit generally display a ring spot symptom (Figure T5A) with uneven maturation and development (Figure T5B). In pepper, symptoms again range from a coalescing of ring spots (Figure P1) to interveinal necrosis. Frequently, pepper leaves display a fan or V-shaped area of chlorosis at the base of the leaf (Figure P2). As in tomato, systemic symptoms include severe stunting, wilt and eventual death of the plant (Figure P3). Fruit symptoms in pepper also can vary from irregular chlorotic blotching to necrotic pits (Figure P4).

The epidemiology of TSWV in tomato and peppers is not fully understood, but it appears that plant position in the field is correlated with the risk of becoming infected with the virus. It has been documented that the disease distribution frequently is in a pattern of a gradient, with a higher degree of disease incidence along the edge. The presence of a disease gradient may imply that the source of the inoculum is in close proximity to the edge of the field containing the highest incidence of diseased plants. The lack of a gradient generally means that the source of inoculum is of some great distance from the field. Alternatively, the presence of disease gradients may be a result of thrips vectors “preferring” to alight on plants at the edge of the field. It is not known if thrips actively select plants along the edge because of an attraction to the contrasting colors of plants and soil or if it is because of the movement of air currents. Whatever the reason, more disease occurring along the edge because of a greater likelihood of thrips landing on those plants was supported by data in which an “edge effect” was observed in the middle of

tomato fields that had bare-ground alleys separating blocks of tomatoes. There were higher levels of disease along each new edge despite being in the middle of the field.

The disease gradients from the edge of tomato and pepper fields were observed to not flatten over time. This generally is interpreted to mean that there is little secondary spread within the field and that new infections are a result of either a continual influx of adult thrips carrying primary inoculum or of a staggered incubation period with some plants taking longer to show symptoms if all plants were inoculated at one time. Additional statistical analysis using methods such as ordinary runs analysis and doublet analysis have confirmed that in pepper and tomato there is little to no spread from plant to plant within a field. Lack of secondary spread and lack of mechanical transmission by farm equipment also was observed in beds of tomato transplants that were clipped with a rotary mower on several occasions over the course of their development. Clipping was a common practice in field-grown transplants and was necessary to promote uniform growth and harvest, to maintain plant size for packaging and shipment, to harden plants for greater survival after transplanting and to de-blossom plants if they developed too fast or had to be held extended periods of time because of inclement weather at their final destination. Although clipping was implicated in the transmission of other diseases, there was no evidence of mechanical transmission of TSWV by clipping tomato transplants.

Onion is a relatively new member to the list of hosts being damaged by tospoviruses in Georgia. In 2003, TSWV and Iris Yellow Spot Virus (IYSV) were found in onion seedbeds. In other areas of the world only IYSV has been reported on onion, however, in Georgia approximately 50% of the infections in onion are caused by TSWV. As with other crops, onions display a range of symptoms when infected with either of these viruses. In some varieties, such as the Japanese bunching types, there are definitive ring spot symptoms and lesions with a “green island” (Figure O1). In the more traditional Vidalia Sweet onion varieties, symptoms include irregular whitish to gray spots (Figure O2), sunken bleached areas (Figure O3), gray, cracked, leathery lesions (Figure O4), tip burn, leaf dieback and an overall appearance of stress and poor growth. Symptoms on bulbs are rare but can occur (Figure O5). There are a lot of uncertainties about the long-term impact of IYSV and why TSWV has only recently begun to be a problem in onion even though it has been affecting other crops such as peanut, pepper, tobacco and

tomato for close to 20 years. Nonetheless, onions have had either TSWV, IYSV or both viruses annually since 2003.

Since IYSV was first observed in Georgia in 2003, it was speculated that the virus may have been introduced from onions imported from Peru. Some commercial onion growers import onions during the off-season to maintain a constant flow of onions and maximize the use of their equipment and labor. In the course of importing and re-packing imported onions, onions are graded and those that fail to make the grade are dumped into cull piles in the field. Both the IYSV symptoms and thrips were observed on sprouting onions in the cull piles. Since genetic variation in the N gene of TSWV was found to be linked to the geographic origin of the virus, we amplified and sequenced the N gene of IYSV collected from onions both in Georgia and in Peru. The sequences were subjected to maximum parsimony and bootstrap analysis. When compared with all known strains in GenBank, the results indicated that all known virus strains in the world grouped in to eight distinct clades, three of which consisted of single isolates. The phylogenetic analysis placed the IYSV isolates from Peru and Georgia into a distinct and well-supported clade. The phylogenetic results were further supported by calculation of the divergence in N-gene nucleotide sequences of Georgia and Peru strains compared with strains from other parts of the world. Isolates from Georgia and Peru had the lowest percent divergence, with an average of 1.2%. The average divergence between Georgia strains and strains from other South and Central American countries, Europe, Australia, Japan and the western U.S. was higher (2.1%, 11.5%, 11.4%, 6.8% and 3.4%, respectively). The average divergence of Peruvian isolates compared with the other South and Central American, European, Australian and Japanese isolates and isolates from the western U.S. (2.2%, 11.4%, 11.1%, 6.7% and 3.4%, respectively) was similar to the divergence rate of the Georgian isolates, supporting the hypothesis that IYSV gene flow occurred from Peru to Georgia.

Since viruses are obligate parasites and cannot survive in the absence of a host, an important question arose regarding the IYSV infestation in Georgia. If onion were the only known host, then the disease cycle could easily be broken using crop rotation. However, if IYSV could infect other hosts, it had the potential to overseason in Georgia and become endemic. Consequently, a survey of weeds adjacent to cull piles where IYSV was identified was conducted. Results from the survey indicated that spiny sowthistle (*Sonchus asper*) served as a host for IYSV. As such, another survey was conducted to determine how widespread the virus

had become using spiny sowthistle as an indicator plant.

Spiny sowthistles were collected from most counties within the Vidalia onion-growing zone. In addition, several counties outside the onion-growing zone and fell on four different transect lines were surveyed. Three of the transect lines more or less radiated outward from the original cull pile (one to the north, one to the northwest and one to the west). The fourth transect line ran south along the Atlantic coast and covered counties that were along the eastern border of the Vidalia onion-growing zone. Samples were collected for three years (2007-2009). Results indicated that in 3 years, IYSV infected sowthistles were found as far as 164 km to the north, 110 km to the northwest and 147 km to the west of the approximate center of the Vidalia onion-growing zone. However, no infected sowthistles were detected during the three-year span in any county lying adjacent to the zone to the east.

These results demonstrate that the thrips-vectored virus moved great distances over a relatively short time, but the movement appeared directional (e.g., to the north and west). The results also indicated that the virus appears to have become endemic in Georgia as spiny sowthistles at the far end of the northern transect line as well as sowthistles inside the onion growing-zone were positive in all three years of the survey. A BLAST search of sequences obtained from selected sowthistle samples indicated the same strain as previously associated with Peru.

Other research of IYSV in onion in Georgia indicated that the virus is extremely localized in onion leaves. To determine the distribution of IYSV in mature onion leaves, 90 onion plants were tested for IYSV. Leaf areas near symptomatic lesions or signs of heavy thrips feeding were selected for processing. From these 90 plants, 51 leaves tested positive and were used for further analysis. Individual leaves were cut into 2.54 cm segments from tip to base and each segment was tested with DAS-ELISA. The results indicate that IYSV distribution within the leaf was highly variable, as only 27.5% of individual 2.54 cm segments (n=527) tested positive and the range ran from a low of only 5% of segments testing positive to a high of 91% of the leaf segments testing positive. There was no leaf in which 100% of the segments tested positive. These results show that distribution of detectable virus in onion leaves is highly variable and that more segments per leaf sample may be required to determine the presence or absence of IYSV in mature onion samples.

TOMATO



T1. Tomato infected with *Tomato spotted wilt virus*. A) Close-up image of multiple dark ringspots. B) From a distance, ringspots coalesce to create a “bronzing” appearance.



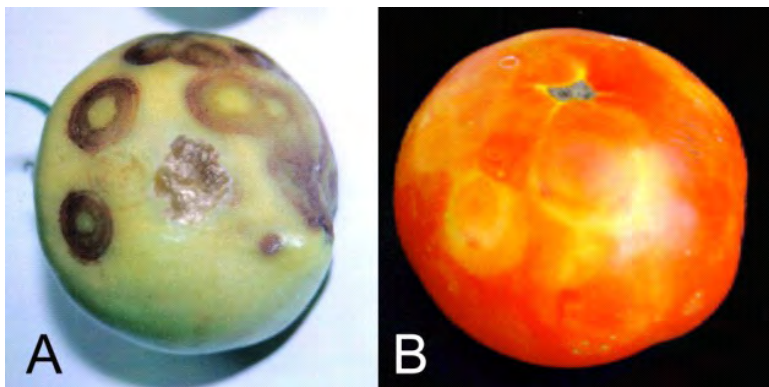
T2. Tomato foliage with *Tomato spotted wilt virus*.



T3. Early infection with *Tomato spotted wilt virus* will result in severely stunted and wilted tomato plants.



T4. Tomato plant with systemic wilt caused by *Tomato spotted wilt virus*.



T5. *Tomato spotted wilt virus* symptoms on A) green fruit; B) ripe fruit.

PEPPER



P1. Ringspot type symptoms caused by *Tomato spotted wilt virus* on upper surface of bell pepper leaves.



P2. Close-up view of ringspot type symptoms caused by *Tomato spotted wilt* on upper surface of bell pepper leaf.



P3. Early infections with *Tomato spotted wilt virus* will result in severely stunted pepper plants.



P4. Symptoms caused by *Tomato spotted wilt* on mature pepper fruit.

ONION



O1. Ringspots with prevalent "green islands" on Japanese bunching type onion leaf caused by *Iris yellow spot virus*.



O2. Irregular, bleached lesions on onion caused by *Iris yellow spot virus*.



O3. Sunken, bleached lesion on leaf caused by *Tomato spotted wilt virus*.



O4. Dieback of onion leaf infected with *Tomato spotted wilt virus*.



O5. Rare symptoms on onion bulb that had a mixed infection of both *Tomato spotted wilt virus* and *Iris yellow spot virus*.

MANAGEMENT OF THRIPS VECTORS OF TSWV

DAVID RILEY, ROBERT MCPHERSON, LENNY WELLS AND STEVE BROWN

The rationale for a thrips-TSWV risk management program is based on two main assumptions: (1) certain pre-season field measurements can be consistently associated with high incidence of tomato spotted wilt during the following season and (2) certain control tactics can consistently reduce the impact of TSWV in the target crop.

The pre-season field measurements are currently based on TSWV host weeds or crops and their relative importance in producing viruliferous (virus-carrying) thrips. In the tomato crop, early-season virus transmission has a much greater impact on yield than if the virus is transmitted to the plant later in the growing season (based on several studies conducted at Tifton, Ga.). The research on control tactics is being updated each year so that the amount of risk reduction associated with each tactic can change somewhat. If a new tactic is discovered, then the relative tactic ranking can also change, so it will be important to use the most current risk-management program available. Current information will be posted yearly at <http://www.tswv.org>.

Thrips-TSWV control tactics should be targeted for early in the crop-growing season to have the greatest impact on yield. Virus transmission that occurs later in the season may not be as important in terms of yield, but in tomato can be very important in terms of TSWV-caused irregularly ripened fruit. Irregular ripening caused by TSWV can show up after commercial fruit have been treated with ethylene for ripening and can result in reduced tomato quality after harvest. Data from 2001 suggested that the tomato plants with slight to moderate symptoms during early to mid-season resulted in the greatest amount of TSWV irregular-ripened fruit in the harvest, not plants with symptoms appearing late-season. Thus, the critical control period for reducing TSWV-related yield loss is early to mid-season in tomatoes. Late-season control of thrips for thrips-related yield quality loss, such as dimpling of the fruit, is likely to be much less important than the TSWV damage.

Managing the thrips vector can be difficult because immature thrips can acquire TSWV from infected weed host plants surrounding vegetable fields (Groves et al., 2001b). The year-to-year differences in timing and intensity of TSWV are related to the thrips and virus in our weed populations. In early spring, prior measurements suggest that TSWV incidence in winter weeds in January-February > 2%-8 % is associated with a high-incidence

year and < 2 %, a low incidence year. This information will be used to establish an assessment of TSWV risk for vegetable crops, which will determine (in part) the level of within-season vector control tactics that should be used. Because within-season TSWV and vector management procedures (e.g., reflective mulches, intensive chemical control of thrips and resistant cultivars) are costly and management intensive, the availability of an assessment of TSWV risk before the season will enable growers to limit their use to those situations in which they are truly needed.

Because the virus replicates in the vector as it matures, viruliferous adults can quickly spread the virus when they move into the field before thrips can be controlled (Ullman et al., 1997). However, some success in control tactics for thrips vectors has been documented (Riley, 2001; Riley and Pappu, 2000). Some of the primary control tactics available in tomato include the use of reflective plastic mulch (Greenough et al., 1990), host plant resistance to the virus (Kumar et al., 1993, 1995), and insecticides combined with other tactics (Brown and Brown, 1992; Riley and Pappu, 2000). In tomato and pepper, simultaneous evaluation of multiple control tactics for thrips and tomato spotted wilt management in tomato has been done (tomato: Riley and Pappu, 2000, 2004; pepper: Reitz, et al., 2003). Improvements in commercially available materials, specifically the release of TSWV-resistant tomato cultivars “BHN-444,” “BHN-555” and “BHN-640 (BHN Research Inc., Bonita Springs, Fla.), the TSWV-tolerant pepper “Heritage” (Harris Moran-Harris Seeds, Rochester, N.Y.), and the metallic reflective plastic mulch “RepelGro” (ReflecTec Foils Inc., Lake Zurich, Il.), have improved management options in tomato and pepper in recent years.

In tobacco, conventional insecticides labeled for beetle and aphid control generally provide effective short-term suppression of thrips infestations. Although these materials reduce thrips populations for several days after the foliar application is made, the seasonal incidence of TSWV that is vectored by thrips is not suppressed. Research data suggests that repeated applications of certain insecticides, once or twice a week for up to six weeks, can reduce the incidence of TSWV-symptomatic plants. However, repeated foliar applications in the early tobacco growing season do not always suppress TSWV. Also, this practice of repeated early-season insecticide applications is not recommended for tobacco growers because it can

lead to insecticide resistance and is not very economical.

A beneficial insecticide treatment for TSWV suppression is a greenhouse tray drench application or a transplant water application of the neonicotinyl insecticides imidacloprid (Admire) or thiamethoxan (Platinum). Growers are advised to treat their crop with one of these materials prior to transplanting because this thrips management practice consistently reduces the seasonal incidence of TSWV. In tomato, a combination of imidacloprid (Admire) soil drench, plus weekly foliar applications of methamidophos (Monitor - SLN label) and lambda-cyhalothrin (Warrior) in the first four weeks after transplant significantly decreased TSWV and increased yields. Since tomato is a high-value crop, this expense can be justified. In pepper, imidacloprid (Admire) at planting can help reduce the incidence of TSWV in the spring crop.

The currently labeled insecticides/nematicides that are applied in tobacco fields in PPI applications are not effective in reducing TSWV infection. In fact, some of these materials, when no other tray drench, transplant water or foliar applications are made, actually have higher incidence of TSWV-symptomatic plants than the plots where no PPI insecticides/nematicides are used. Many of the insecticides applied for budworm and hornworm control are effective in reducing thrips populations; however, these applications are made later in the season (usually 6-8 weeks after transplanting) and have no impact in suppressing TSWV infection.

In tomato studies at Tifton, three tactics (early-season insecticides, the TSWV-resistant tomato and reflective mulch), reduced the incidence of TSWV and improved tomato yields when the level of TSWV exceeded 17%. Averaged over three years, there was considerable economic incentive for using all three tactics in a preventative manner. Obviously, when TSWV incidence is low (e.g., 2%, as in 2000), none of the tactics show a yield response. Unfortunately, a pre-season prediction of TSWV severity is not currently available, although some overwintering host plant surveys show promise in this area (Groves et al., 2001b). Based on these studies, control tactics should be implemented in regions where TSWV is a problem in order to mitigate long term risks to production. We recommend a cautious approach when using insecticides because of the threat of insecticide resistance in thrips (Kontsedalov et al., 1998). This is an additional reason to focus treatments on early season tomato and not treat all season long.

The reflective metallic mulch is an effective tactic that appears to be cost effective as long as possible harvest delays from soil cooling are not a concern. Also, the commercial acceptability of the fruit of the BHN444 globe type is not as high as standard deep globe, blocky-type tomato hybrids and may not be acceptable to some growers if the TSWV risk to production does not warrant sacrificing fruit

marketability. If a risk index can be developed for TSWV in tomato as has been done in peanut (Brown et al., 1999), then the selection of tactics can be based on the severity of the predicted TSWV incidence, with high risk involving all available tactics and moderate risk using some selection of one or two tactics. This would help to preserve the efficacy of the existing tactics for thrips vector control and mitigation of the negative economic impact of TSWV on tomato.

Several treatments (control tactics) evaluated from 1998 to 2002 resulted in less irregular-ripened fruit and high tomato yield relative to an untreated standard. Several of these tactics have been commercially validated as well, but in high-TSWV risk situations, multiple tactics with additive effects will be required to economically reduce the impact of the virus. Table 1 lists the current effective treatments for TSWV/thrips management in tomato, but it should be noted that multiple tactics are often needed together to achieve commercially acceptable control levels.

The best insecticide treatment, imidacloprid soil drench plus lambda-cyhalothrin plus methamidophos foliar treatments beginning as soon as the tomatoes are transplanted, needs to be targeted at early season if it is to be effective. Late-season insecticide treatments are completely ineffective for reducing thrips-vectored TSWV. The only purpose of controlling thrips late-season would be to reduce direct thrips feeding damage to the fruit. Likewise, the metallic silver plastic mulch is most effective during early season when the reflective surface is greatest. Certainly, host plant resistance in the tomato cultivar BHN444 and the metallic silver reflective mulch have greatly improved yields where they are used in areas of high TSWV incidence.

It is important to remember that, since transmission of the virus occurs through thrips feeding, a program that prevents thrips feeding, kills thrips before they can feed, and/or reduces the attractiveness of the crop so that fewer thrips occur on the plant will be the most effective. Feeding consists of slight to deep probing and which type of feeding activity results in the most virus transmission is currently being investigated. This is extremely important because the anti-feeding effect of imidacloprid seems to work well in pepper and tomato, but not in peanut. It is also important to note that even though a product like imidacloprid can reduce thrips feeding initially, this alone may not be enough to reduce virus transmission since behavioral effects can be overcome with starvation. It is important to not only repel thrips feeding, but also to remove the thrips from the host plant as quickly as possible before repellence is overcome. This can be done either through mortality of the thrips (e.g., lambda-cyhalothrin plus methamidophos foliar treatments) or overall repellence off of the crop through the use of metallic silver mulch.

TABLE 3. Thrips-vectored TSWV control tactics or treatments that have been found effective in experimental trials.

CONTROL TACTIC	DESCRIPTION	COMMENTS
1. Metallic reflective mulch	Aluminum-plastic mulch such as ReflecTek's "RepelGro" reduces early-season thrips/TSWV on tomato and reduces soil temperature, affecting symptom development. The silver mulch is more effective on tomato than pepper, where black mulch is just as effective.	Excellent tactic with good control, but some additional production and disposal costs.
2. TSWV-resistance	Resistance tomato cultivars such as BHN Research's "BHN-444," "BHN-555" and "BHN-640." Note that this is single gene resistance only. (See Tables 4 & 5)	Excellent tactic with good control, but tomato type can have market limitations.
3. Early-season insecticide (thrips feeding repellent + contact thripicide)	Transplant treatment (drench/drip) with a neonicotinoid such as Bayer's "Admire" to inhibit thrips feeding <i>plus</i> 4 weekly sprays with effective contact thripicide material such as methamidophos+lambda cyhalothrin beginning at transplant.	Control of thrips with insecticides has been very controversial because early timing is so critical and thrip feeding must be stopped before killing.
4. Chemical-induced plant resistance	Use of a plant activator, such as Syngenta's "Actigard" in the greenhouse and at transplanting can provide some TSWV resistance.	High doses of plant activator can cause phytotoxicity, so care needs to be taken.
5. TSWV weed-risk index	It is proposed that early movement of thrips off of spring weeds (such as chickweed) that have high incidence of TSWV and thrips can indicate that the following tomato crop can expect greater TSWV incidence (unless weather reduces migration from weeds), especially early season.	A model is being developed that will be available at the TSWV management website http://www.tswv.org
6. TSWV weed-management	It is proposed that managing weeds and TSWV host crops can effectively reduce migration onto the crop in subsequent seasons.	Trials in weed management have only shown that mowing weeds around the field within two weeks of transplanting in the spring can actually increase the incidence of TSWV, presumably by forcing thrips vectors off of the weeds onto the adjacent crop.

MANAGEMENT OF TSWV IN TOBACCO

ALEX CSINOS, KATHERINE STEPHENSON, STEVAN LAHUE AND LARA LEE HICKMAN

Studies conducted in 1999 and 2000 at four locations each year established the use, rate and efficacy of imidacloprid and acibenzolar-S-methyl to effectively manage TSWV in tobacco (Csinos et al., 2001). Those results have been confirmed in Georgia, South Carolina and North Carolina.

Current label recommendations for use of imidacloprid are 1.0-2.8 oz. of formulation (Admire 2F) per 1,000 plants applied as a tray drench, washed into the root ball 3-7 days before transplanting (Cooperative Extension). Recommendations for the use of acibenzolar-S-methyl (Actigard 50 WP) are variable depending on whether plants are produced in field plant beds or in floats. In general, 0.5 oz. of Actigard 50 WP will treat 32,000-50,000 plants. Treatment should be made 5-7 days prior to transplanting.

Some growth delays and phytotoxicity may be observed with the application of Actigard. Young and tender plants tend to be more affected than older, hardened-off plants. Drought, very hot weather, wind damage, chemical injury or any other stresses to the plants may increase phytotoxicity. Plants should be transplanted as fresh as possible; however, plants stunted due to Actigard treatment generally recover. Admire or Actigard applied alone provides 20%-30% control; however, the use of both materials in the greenhouse prior to transplanting can provide 40%-60% control, the additive sum of the two treatments if they were used alone.

Tobacco cultivars do not have appreciable resistance to TSWV and cultivar selection is not recommended in disease management. However, some studies have demonstrated that NC71 tends to be less susceptible to TSWV than K-326 (Figures 13 and 14).

RESEARCH ON TSWV MANAGEMENT IN THE FIELD

Field trials have also been initiated to evaluate the use of Actigard (acibenzolar-S-methyl or ASM) in the field to further reduce TSWV incidence. Actigard has an activity of 10-14 days, with 2-3 days required for activation of the plant. The tobacco field season in Georgia typically starts the last week of March and is usually complete by the end of August. Float house applications of ASM and imidacloprid (Admire) are made 3-5 days prior to transplanting and are expected to provide a few weeks of protection in the field. However, tobacco is typically in the field for 18-20 weeks, leaving tobacco unprotected most of the growing season. Data collected over several years have indicated that rate of infection is reduced dramatically after plants are topped (decapitated). It is not known whether

the cause is the physical process of removing flowers, the plant chemical changes associated with topping, the application of herbicides (suckercides) to manage auxiliary growth or the high temperatures usually occurring in mid-June. Based on these facts, the first 11 weeks of growth are the most important for TSWV management in the field, and since float house applications of both ASM and imidacloprid provide 2-3 weeks protection, the 8-week period from mid-April to mid-June would be the period targeted for field applications of Actigard for TSWV management.

Trials were initiated to evaluate the application of Actigard 50 WP at 0.5 oz./A in addition to float house treatment with Actigard and Admire Pro. Field applications were made at 7-day intervals starting at 7 days with applications as late as 49 days post-plant, depending on the year (Csinos, et al., 2004, 2005, 2006, 2007, 2008). Evaluation of the data indicates that applications of Actigard post-transplant can significantly decrease TSWV and increase yield in most years.

Application timing is critical because application must be made prior to infection and must allow three days for plant activation to occur. Modeling analysis has shown that non-treated control plants become infected 1-2 weeks sooner than plants treated with ASM and imidacloprid prior to transplanting. Data collected from plots receiving ASM field sprays indicate that the best application coincides with the first symptoms observed on non-treated control plots. Thus, the first symptom observed on non-treated control plots can be used as the trigger to treat plants that have been treated in the float house with ASM and imidacloprid (Figures 15, 16 and 17). Subsequent years of study have revealed that the infection window may be variable and may occur at any point in the 12-week post-transplant period. Thus, a single application of ASM may be insufficient to reduce TSWV infection, and multiple applications 7-10 days apart may be required to provide protection (Figures 18, 19 and 20). The initiation of symptoms and rapid increase in disease correlates with a spike in thrips numbers found in the field (see thrips numbers). Thrips are fair weather insects and are active during sunny, warm periods. They are less active during cool, cloudy, rainy and windy conditions (McPherson, 2004).

Based on data indicating that infection windows are variable and likely dependent on weather conditions, further research will be needed to develop a full-season management program for TSWV in flue-cured tobacco. The incorporation of a resistant/tolerant tobacco cultivar will be key to developing a durable management program.

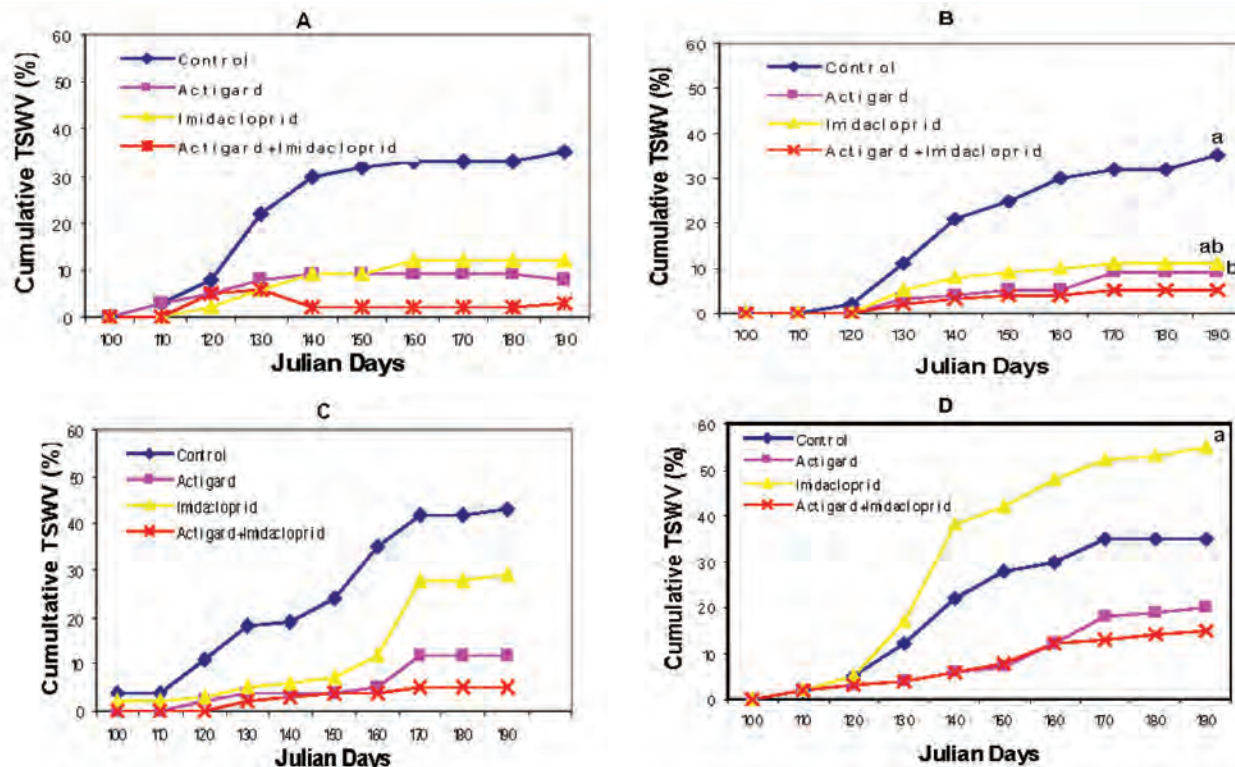


FIGURE 12. Area under the disease curve (AUDPC) for cumulative symptomatic TSWV tobacco treated with acibenzolar-S-methyl, imidacloprid, and combinations A: Blackshank Farm; B: Blackshank nursery; C: Entomology site; D: Bowen Farm. Curves (areas) followed by the same letter(s) are not significantly different from each other, $P=0.05$.

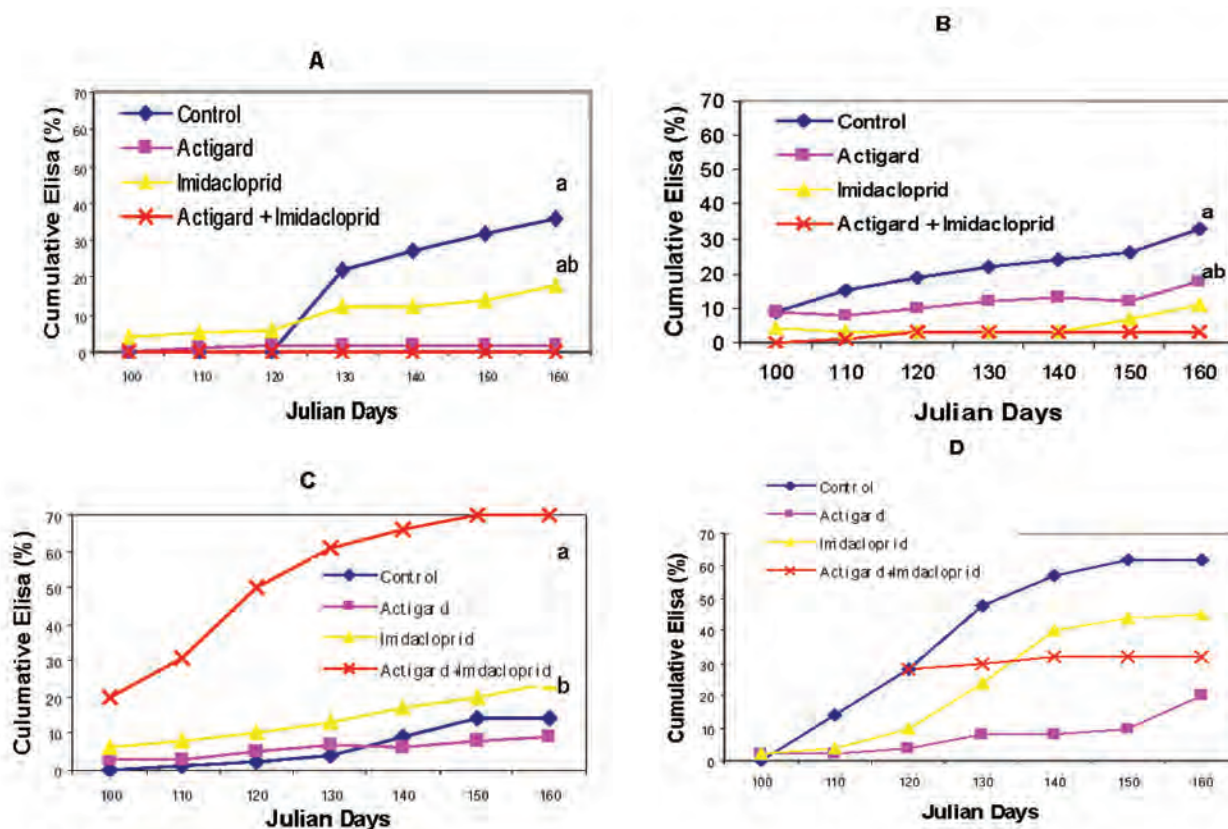


FIGURE 13. AUDPC for cumulative enzyme-linked immunosorbent assay (ELISA) positive samples. A: Blackshank Farm; B: Blackshank nursery; C: Entomology site; D: Bowen Farm. Curves (areas) followed by the same letter(s) are not significantly different from each other, $P=0.05$.

2003 Actigard Timing % TSWV Symptomatic

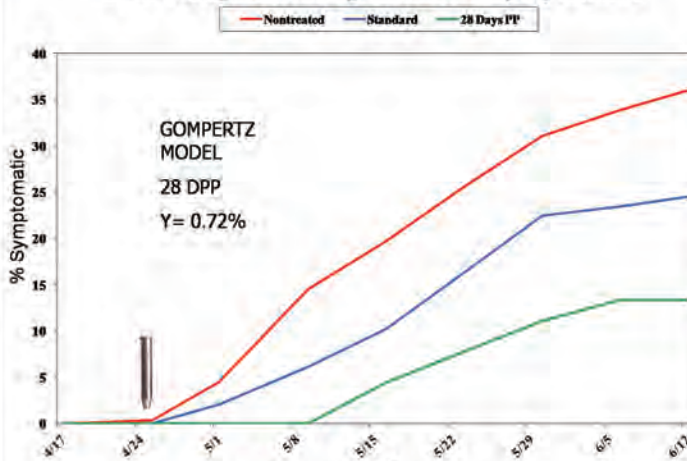


FIGURE 15

2004 Actigard Timing % TSWV Symptomatic

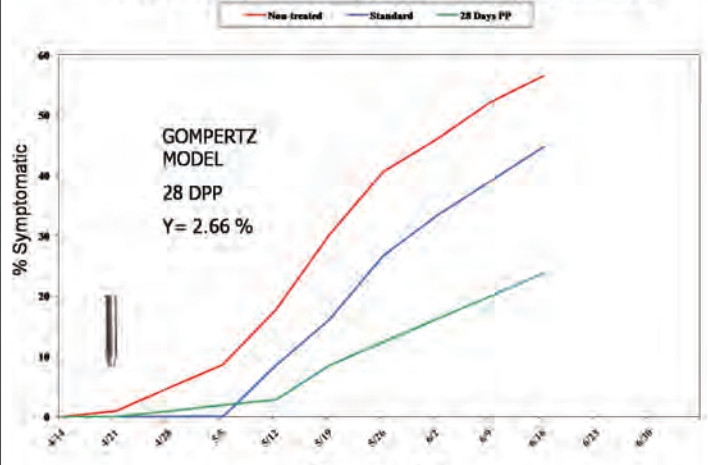


FIGURE 16

2005 Actigard Timing % TSWV Symptomatic

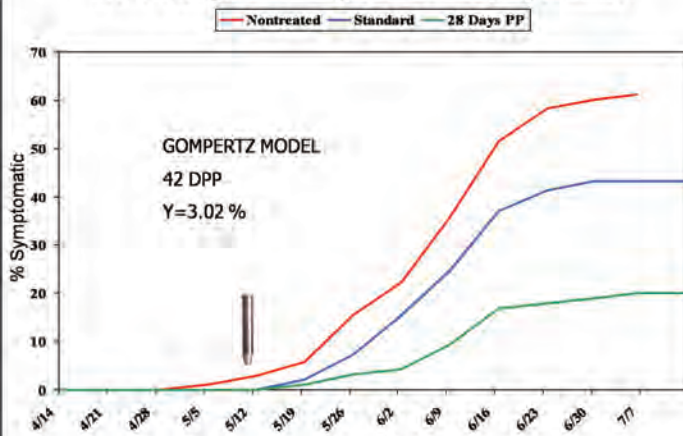


FIGURE 17

2006 Actigard Timing % TSWV Symptomatic

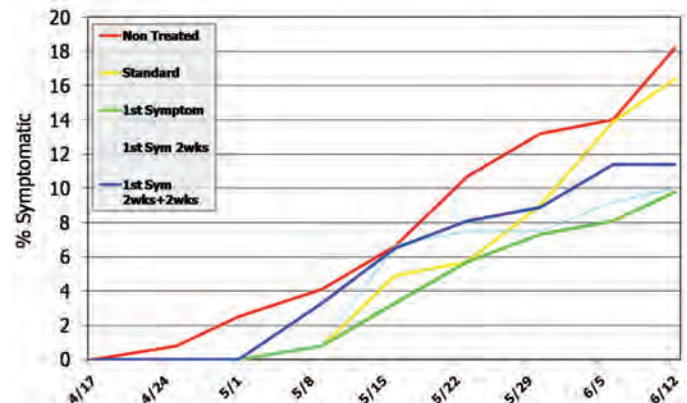


FIGURE 18

2007 Actigard Timing % TSWV Symptomatic

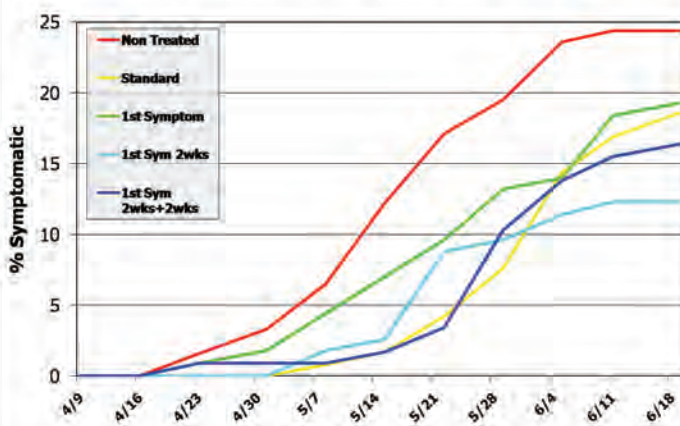


FIGURE 19

2008 Actigard Timing % TSWV Symptomatic

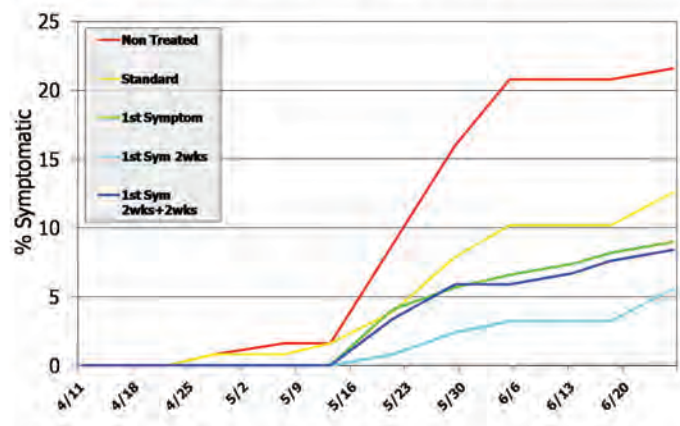


FIGURE 20

EVALUATION OF FLUE-CURED TOBACCO ENTRIES FOR RESISTANCE TO TSWV

ALEX CSINOS, ALBERT JOHNSON, FRANCIS REAY-JONES, STEVAN LAHUE, LARA LEE HICKMAN AND STEPHEN MULLIS

Breeding for resistance to TSWV was initiated at the Clemson University Pee Dee REC in Florence, S.C., in 2001 by Dr. Albert Johnson. Crosses were conducted on station and evaluations were made on station and in several counties in S.C., often where high TSWV pressure occurs. From 2001 to 2006, 444 breeding lines were evaluated for resistance to TSWV. During the first two years, one line with good resistance to TSWV was identified. In 2003, 12 lines expressed high levels of TSWV resistance; two of these lines resulted from crosses in the F1 generation, indicating that the resistance factor may be a dominant characteristic. In 2004, 12 advanced breeding lines had less than 10% of plants expressing TSWV symptoms (compared to NC 2326 [44%] and K 346 [35%]). Four resistant breeding lines had less than 5% infected plants. In 2005, two advanced breeding lines had less than 20% of plants with TSWV symptoms (compared to NC 2326 [78%] and K 346 [77%]). Plant selections were made from earlier crosses from F3-F5 segregating populations to enhance tobacco quality and TSWV resistance. Three stable lines had less than 10% of the plants expressing symptoms of TSWV (compared to NC 2326 [50%]

and K 346 [50%]). Hybrid crosses were made in 2006 and 2007 in an effort to speed up the process of developing resistant cultivars with good agronomic qualities.

In 2008, 20 tobacco cultivars or lines were evaluated for resistance to TSWV at the University of Georgia Bowen Farm in Tifton, Ga. Entries were randomized and replicated five to 10 times in a split plot design. Each entry was either not treated or treated in the float house with Admire Pro and Actigard as recommended by Cooperative Extension. Two of the entries had lower TSWV than the NC 71 standard in the non-treated plots and 17 entries were lower than the standard when treated with Admire Pro and Actigard. ELISA was performed on several entries but no significant differences were detected. Yields for both the treated and non-treated entries were not significantly different from the NC 71 standard. Yield was increased by the float house treatments in six of the 12 entries evaluated.

These entries have demonstrated resistance in South Carolina and have showed some promise in trials in Georgia. The 2008 year was a relatively light year for TSWV and further evaluations will be required.

TABLE 4. Johnson variety trial % TSWV, % ELISA TSWV results and Dry Weight Yield

VARIETY ¹	% TSWV SYMPTOMATIC ²			% ELISA TSWV ³	DRY WEIGHT YIELD ⁴ (LBS/A)		
	A NON-TREATED	B TREATED		A NON-TREATED	A NON-TREATED	B TREATED	
CU 109	10.0 abc	7.7 bcd	xx	---	---	---	---
CU94	12.6 abc	4.5 cd	xy	10.0 ab	2154.8 b	2608.6 a	xy
CU61	12.0 abc	4.3 cd	xy	12.0 ab	2283.5 ab	2526.7 a	xy
CU75	8.7 bc	4.7 cd	xy	13.3 ab	2141.1 b	2377.6 a	xy
CU90	7.6 bc	5.2 cd	xy	18.0 ab	2237.8 ab	2349.6 a	xx
CU100	7.8 bc	7.1 bcd	xx	4.0 b	2381.4 ab	2431.5 a	xx
CU108	13.3 abc	7.8 bcd	xy	---	---	---	---
CU95	9.5 abc	6.6 bcd	xy	7.8 ab	2178.4 b	2440.4 a	xy
CU110	8.7 bc	2.7 d	xy	6.0 ab	2111.5 b	2187.6 a	xx
CU92	9.2 abc	6.3 bcd	xy	---	---	---	---
H9	17.3 ab	7.4 bcd	xy	10.0 ab	2204.5 b	2252.3 a	xx
H11	10.0 abc	4.8 cd	xy	---	---	---	---
H22	6.0 c	3.1 cd	xx	20.0a	2383.0 ab	2126.3 a	xy
H50	10.0 abc	13.2 ab	xx	---	---	---	---
H59	16.4 ab	7.7 bcd	xy	---	---	---	---
H103	18.9 a	10.4 bc	xy	---	---	---	---
H106	5.4 c	4.3 cd	xy	4.0 b	2071.8 b	2501.1 a	xy
H117	12.9 abc	6.2 bcd	xy	---	---	---	---
NC71	16.1 ab	18.7 a	xx	12.0 ab	2428.6 ab	2610.0 a	xy
K326-T	4.5 c	1.5 d	xy	4.0 b	2682.7 a	2593.5 a	xx

¹ DATA ARE MEANS OF FIVE REPLICATIONS. MEANS IN SAME COLUMN (VERTICALLY) FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (P=0.05) ACCORDING TO FISHER'S LSD TEST. MEANS IN THE SAME LINE (HORIZONTALLY-X,Y) FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (P=0.05) ACCORDING TO FISHER'S LSD. 21 TREATMENTS CONSISTED OF SELECTED VARIETIES OF TOBACCO. EACH PLOT WAS 2 ROWS, 1 ROW TREATED WITH ACTIGARD AND ADMIRE AND 1 ROW NON-TREATED.

² PERCENT TSWV WAS CALCULATED USING STAND COUNTS THAT WERE MADE FROM 17 APRIL THROUGH 26 JUNE WITH TSWV BEING RECORDED AND FLAGGED EVERY 7 DAYS.

³ DRY WEIGHT YIELD WAS CALCULATED BY MULTIPLYING GREEN WEIGHT TOTALS BY 0.15. POUNDS PER ACRE WAS CALCULATED BY MULTIPLYING DRY WEIGHT CONVERSION PER PLOT BY 6,491 DIVIDED BY THE BASE STAND COUNT. TOBACCO WAS PLANTED IN 44-INCH ROWS, WITH 22 INCHES BETWEEN PLANTS, WHICH EQUALS 6,491 PLANTS/A. FOURTEEN VARIETIES WERE SELECTED OUT OF THE TREATMENT LIST TO COLLECT YIELD ON. THESE ARE HIGHLIGHTED IN TABLE 1.

⁴ FINAL HARVEST TESTING WAS COMPLETED ON 24 JULY. TEN ROOT SAMPLES WERE COLLECTED PER PLOT. ELISA TESTING WAS PERFORMED IN THE LAB USING DOUBLE ANTIBODY SANDWICH-ENZYME LINKED IMMUNOSORBENT ASSAY (DAS-ELISA) ALKALINE PHOSPHATASE ANTISERA KITS. ELISA TEST RESULTS ARE PERCENT POSITIVE PLANTS.

TOMATO AND PEPPER PLANT GROWTH AND YIELD AS AFFECTED BY TSWV

JUAN CARLOS DÍAZ-PÉREZ

EFFECT OF TSW ON PLANT DEVELOPMENT, FRUIT YIELD AND FRUIT QUALITY

Tomato spotted wilt disease (TSW), caused by Tomato spotted wilt virus (TSWV), is an economically important disease of tomato and pepper (Cho et al., 1998). The disease is a major constraint to tomato and pepper production in the southern U.S. (Riley and Pappu, 2000; Brown et al., 2005) and in many other places in the world (Cho et al., 1998). Fruit yield losses are significant because the majority of currently available tomato and pepper cultivars are susceptible to TSWV.

Successful tomato and pepper production depends on a healthy plant growth throughout the growing season. Early plant infection with TSWV typically results in severe symptoms or plant death (Francki and Hatta, 1981; Gitaitis, 1998; Moriones et al., 1998). Diaz-Perez et al. (2003) found that there is an increasing reduction of vegetative top fresh weight, fruit number and fruit yield (total and marketable) with increasingly earlier expression of TSWV symptoms during tomato plant development. Compared to symptomless plants, total fruit yield of symptomatic tomato plants are reduced by about 2% for each day before harvest that plants first exhibited TSWV symptoms (Diaz-Perez et al., 2003). Similar results were obtained in an additional study using artificially inoculated plants as well as in plants under natural TSW infection (Díaz-Pérez et al., 2007).

In order to obtain high tomato and pepper yields, appearance of TSWV symptoms should be delayed as much as possible during the entire season, but particularly during early stages of plant development. Use of TSWV-resistant cultivars and utilization of colored mulches may be helpful in TSWV management.

The mechanisms through which TSWV affects tomato plant growth and yield are not fully understood; however, TSWV infections in tobacco have been associated with reductions in leaf chlorophyll content and diminished rates of CO₂ assimilation (Goodman et al., 1986). Wilting associated with TSWV is probably an indication of plant water deficits, which may also affect gas exchange and shoot growth. Unpublished data (Diaz-Perez, et al.) show that TSWV-infected tomato plants exhibit reduced rates of photosynthesis and transpiration.

EFFECT OF CULTIVAR, PLASTIC FILM MULCH AND ENVIRONMENTAL FACTORS ON TSW CULTIVAR

The number of resistant tomato and pepper cultivars has significantly increased during the last five years. A list of commercially available tomato and pepper cultivars resistant to TSW is shown in Tables 5 and 6. Fruit quality of the first TSW-resistant tomato cultivars was often lower compared to that of the susceptible cultivars. However, the most recently released tomato cultivars seem to have improved fruit quality attributes compared to the first TSW-resistant cultivars. In addition to resistance to TSW, future tomato cultivars will benefit from having resistance to Tomato yellow leaf curl, a viral disease transmitted by whiteflies. Tomato yellow leaf curl causes significant reductions in tomato yield in Florida and has been increasing in importance in Georgia in the last decade.

PLASTIC MULCH AND ENVIRONMENTAL MODIFICATION

Integrated management is probably the most effective method to manage TSWV and delay the expression of symptoms (Cho et al., 1998). The use of pesticides alone to control thrips is usually ineffective for management of TSWV (Brown, 1989; Cho et al., 1998), although imidacloprid applications to tomato transplants may offer some protection (Riley and Pappu, 2000). As a complement to chemical control, resistant cultivars and cultural control measures such as the use of silver reflective mulches may be considered important components of a TSW management strategy (Brown et al., 1989; Olson et al., 2000; Schalk and Robbins, 1987).

Plastic film mulches, particularly silver (metallic reflective) mulches, have also been found to reduce the populations of thrips and other insect vectors (Csizinszky et al., 1995; Farias-Larios and Orozco-Santos, 1997; Olson et al., 2000; Schalk and Robbins, 1987). In a five-year study in Florida, tomato plants grown on silver reflective mulch had lower thrips numbers and TSWV incidence compared to plants grown on black plastic mulch (Olson et al., 2000). Similarly, Riley and Pappu (2000) in Georgia found that silver reflective mulch in combination with

intensive insecticide applications and resistant cultivars resulted in reduced thrips populations and TSWV incidence. Reduction in thrips population has been attributed to the mulch color effect on the vector, due to a modification of the light environment around the plant (Csizinszky et al., 1997). However, appearance of TSWV symptoms may depend on factors other than the number of thrips.

Colored plastic film mulches modify root zone temperature (RZT) under the mulch in addition to modifying the light environment around the crop. RZT directly affects plant growth and yield in tomato, tomatillo, pepper, potato, cucumber and watermelon (Díaz-Pérez and Batal, 2002; Díaz-Pérez et al., 2005; Díaz-Pérez et al., 2008; Ibarra et al., 2004; Ibarra et al., 2006; Ibarra et al., 2008). These effects on plant growth have been associated with effects on plant responses to TSWV in tomato (Díaz-Pérez et al., 2007).

The RZT under the mulch may modify the appearance of TSW symptoms. In a study in the spring season (Tifton, Ga.) that included eight plastic film mulches and three cultivars, appearance of first TSWV symptoms was delayed on gray mulch, followed by black-on-silver, silver-on-black and silver-painted mulches (Díaz-Pérez et al., 2003). The TSW-susceptible cultivars “Florida-91” and “Sun Chaser” showed first symptoms of TSW 3-4 days earlier than the TSW-resistant “BHN-444.” Among mulches, TSW incidence was highest for tomato plants grown on white mulch, with incidences on the other mulches and bare soil ranging from 14% to 35%. Among cultivars, “BHN-444” had the lowest incidence of TSW (12%) and the highest fruit yield, while “Florida-91” and “Sun Chaser” had incidences that were about three times greater. Symptoms of TSWV were delayed in tomato plants grown on gray,

silver-on-black, black-on-silver and silver-painted mulches, where the mean RZT for the season approached the optimal RZT (26.1° C) for tomato plant growth and yield. These results suggested that RZT, as modified by plastic mulches, may influence the appearance of TSWV symptoms. Environmental factors other than RZT may also determine the impact of TSWV on tomato plants, as suggested by the study of Díez, et al. (1999), who found that growth and yield of tomato plants grown under mesh were higher compared to plants grown in open-air conditions.

In another study on colored mulches during the fall season (Tifton, Ga.), tomato plants on black mulch showed the earliest appearance of TSW symptoms, and had significantly reduced vegetative growth and fruit yields compared to plants on white, gray and silver mulches. These results suggested that utilization of plastic mulches that created conditions of high RZT stress resulted in reduced plant growth and yield and predisposed the plants to earlier expression of TSW symptoms compared to plants grown at RZTs more favorable to tomato plant growth [optimal RZT = 26.1° C (Díaz-Pérez, et al., 2003)]. Since these plant responses to TSW under heat stress occurred in artificially inoculated plants as well as in plants under natural TSW infection, high RZTs probably affected the plants directly, independently of any possible effects on the thrips vectors.

In conclusion, environmental modification with plastic mulches may contribute to the management of TSW in tomato and pepper by creating more optimal root zone temperature conditions for plant growth. Minimizing plant heat stress seems to reduce the severity of symptoms of TSW in tomato (Díaz-Pérez et al., 2007) and possibly other crops.

TABLE 5. List of commercial tomato cultivars resistant to *Tomato spotted wilt virus*.

CULTIVAR	SOURCE	TSWV ^z RESPONSE	CHARACTERISTICS
Amelia	Harris Moran	R *	Determinate plant; main season; round fruit; open field
Bella Rosa	Sakata	R *	Determinate plant; large, round fruit; heat tolerant; open field
BHN 444	BHN Research	R *	Determinate plant; early; large fruit; fruit shape may vary; open field
BHN 602	BHN Research	R *	Determinate plant, midseason maturity; fruit are globe; open field
BHN 640	BHN Research	R *	Determinate plant; early-midseason maturity; fruit are globe-shaped but tend to be slightly elongated and green shouldered; open field
BHN 685*	BHN Research	R *	Determinate, vigorous plant; midseason; large to extra-large, blocky globe fruit (roma); open field
Capaya	Seminis	R	Semi-determinate; vigorous plant; early maturity, large, roma fruit; open field
Crista	Harris Moran	T *	Determinate plant; medium earliness; large, round fruit; open field
FTM 2305	Sakata	T *	Determinate plant
Galilea	Hazera Genetics	T *	Determinate; vigorous plant; oval fruit (roma); open field
Hedvig	Hazera Genetics	T *	Determinate plant
Huichol	Seminis	R	Determinate; vigorous plant; oval fruit (roma); long shelf life; open field
Inbar	Hazera Genetics	R *	Determinate; early maturity; medium size plant; oblate, medium size fruit; open field
Lia	Hazera Genetics	R	Determinate; vigorous plant; medium size, oval fruit (Roma); open field
Muriel	Sakata	R *	Determinate plant; large, elongated fruit (roma); open field
Muriel	Sakata	R *	Determinate; medium maturity; large fruit (roma); open field
Nico	Harris Moran	T *	Determinate; medium maturity; large, flat to round fruit; open field
Panzer	Rogers	R	Indeterminate; medium maturity; vigorous plant; round, extra-large fruit; greenhouse
Picus	Seminis	R *	Determinate; main season, medium to large plant; large, elongated (roma) fruit; open field
Pilavy	Rogers	R	Indeterminate; medium maturity; vigorous plant; round, extra-large fruit; greenhouse and shade houses; used for grafting
PS 01522935	Seminis	R	Determinate; midseason; vigorous plant; round, large and extra-large fruit; open field
PS 01522942	Seminis	R	Determinate; midseason; vigorous plant; deep oblate to globe, large and extra-large fruit; open field
Quincy	Seminis	R *	Determinate and tall plant; full season maturity; the fruit is firm and has an oblate shape; open field.
Red Defender	Harris Moran	R *	Determinate; medium maturity; large, round fruit; open field
Redline	Syngenta	R *	Determinate; vigorous plant; large, round fruit; open field.
Shanty	Hazera Genetics	R *	Determinate; vigorous plant; large, oval fruit; open field.
Talladega	Syngenta	R *	Determinate; midseason; medium to large plant; developed primarily for the Southeast U.S. growing regions; round fruit; open field
Top Gun	Twilley	R *	Semi-determinate; medium-to-large size, round fruit; open field.
Tous 91	Hazera Genetics	R *	Determinate plant
Xaman	Seminis	R	Determinate plant; 70-75 days to maturity; medium size plant; elongated (roma), large fruit; open field

^z R = Resistance to TSWV (as reported by the seed company); T = Tolerance to TSWV (as reported by the seed company);

* = resistance or tolerance confirmed under Georgia conditions [Riley, D. and T. Kelley (unpublished data)].

TABLE 6. List of commercial pepper cultivars resistant to *Tomato spotted wilt virus*.

CULTIVAR	SOURCE	TSWV^z RESPONSE	CHARACTERISTICS
Cyrus	Hazera Genetics	R	Hot pepper; medium upright plant; medium earliness; red when ripe; green-house, tunnel
Declaration	Harris Moran	R	Bell pepper; blocky fruit; early; vigorous plant; for bush or stake culture; large fruit; open field
HA-250	Hazera Genetics	R	Bell pepper; thick fruit wall; medium to tall plant; shade house; greenhouse
Heritage	Harris Moran	R *	Bell pepper; vigorous plant; large fruit; main season, open field
Hila	Hazera Genetics	R	Bell pepper; early maturity; medium size plant; large fruit; yellow when ripe; greenhouse and tunnel
Lord King	Hazera Genetics	R	Bell pepper; blocky fruit; medium earliness; medium size plant; medium to large fruit; greenhouse and shade house
Magico	Harris Moran	R *	Bell pepper; vigorous plant; large fruit; early; open field
Plato	Seminis	R *	Bell pepper; medium to large plant; large fruit; open field
Riata	Harris Moran	R	Bell pepper; blocky fruit; medium size plant; medium to large fruit; open field
Rioja	Hazera Genetics	R	Lamuyo pepper; compact to medium size plant; red when ripe; open field, shade house
Sargon	Hazera Genetics	R	Bell pepper; early maturity; compact plant; large fruit; open field
Sir John	Hazera Genetics	R	Bell pepper; late maturity; tall plant; large fruit; red when ripe; greenhouse and shade house
Stiletto	Rogers	R *	Bell pepper; blocky fruit; medium earliness; open field
Vargas	Hazera Genetics	R	Bell pepper; early maturity; medium size plant; medium to large fruit; red when ripe; greenhouse and shade house
Zin	Hazera Genetics	R	Bell pepper; early maturity; tall plant; medium to large fruit; red when ripe; greenhouse and shade house

^z R = RESISTANCE TO TSWV (AS REPORTED BY THE SEED COMPANY); T = TOLERANCE TO TSWV (AS REPORTED BY THE SEED COMPANY);

* = RESISTANCE OR TOLERANCE CONFIRMED UNDER GEORGIA CONDITIONS [RILEY, D. AND T. KELLEY (UNPUBLISHED DATA)].

MANAGEMENT OF THRIPS VECTORS AND TSWV IN PEANUT

STEVE BROWN

The only known means of virus transmission is via vectors belonging to a few species of thrips vectors (German et al., 1992; Peters et al., 1996). Only first instar larvae of *Frankliniella occidentalis* (Pergande), one vector species, can acquire the virus from an infected plant (van de Wetering et al., 1996). After acquisition, the virus replicates in the vector and the viruliferous thrips is capable of transmission for the duration of its life (Peters et al., 1996; Ullman et al., 1993; Wijkamp et al., 1993). In Georgia, the primary vectors are tobacco thrips, *F. fusca* (Hinds), and western flower thrips, *F. occidentalis* (Ullman et al., 1993). Most spotted wilt in peanut is thought to be the result of primary transmission, but some secondary transmission probably occurs as well (Camann et al., 1995), mostly by *F. fusca*, which readily reproduces on peanut (Todd et al., 1994). Peanut cultivars exhibit a wide range of susceptibility to spotted wilt (Figures 20 and 21). The mechanism of resistance is unknown, but since thrips populations on resistant cultivars do not appear to be significantly lower than those on susceptible cultivars, differences in cultivar susceptibility are not thought to be due to differential preference by vectors (Culbreath et al., 1994, 1992, 1996, 1999, 2000).

Prior to severe outbreaks of spotted wilt in Georgia, planting date was found to influence the incidence of the disease on peanuts grown in southern Texas (Mitchell et al., 1991), where peanuts planted early and late in the normal planting season tended to have more spotted wilt than peanuts planted in the middle of the planting season, and those planted within a recommended "window" expressed less severe symptoms. Although actual planting dates are slightly different, a similar trend was found in Georgia (Todd et al., unpublished data).

Optimum planting dates and the magnitude of the planting date effect vary slightly from year to year, but in general, avoiding early and late planting reduces incidence and severity of spotted wilt in Georgia. Again, the mechanism of the planting date effect is not totally understood, but mid-season planting dates may avoid thrips population peaks (Todd et al., 1996). Although primary infection may occur throughout the growing season, young peanut tissue has been shown to be more susceptible to infection by peanut bud necrosis virus (a tospovirus closely related to TSWV) than more mature tissue (Buiel and Parleviet, 1996). Since planting dates that avoid synchronization of young peanut plants with peak thrips populations appear to

significantly reduce TSWV infection levels, plant age may affect peanut susceptibility to TSWV in a similar manner.

An association between low plant populations and high levels of spotted wilt was noted soon after the disease began to impact peanut production in Georgia, and recent research has confirmed this observation (Brenneman and Walcott, 2001; Gorbett and Shokes, 1994). Brenneman and Walcott (2001) found that 92% of the effect of plant population on yield was due to its indirect effect on spotted wilt. They characterized that effect as $\text{yield (lbs./A)} = 3728 - 31.5 (\text{TSWV severity}) + 176.4 (\text{stand} - 2.9 \text{ plants/m})$. Individual peanut plants may have higher numbers of thrips feeding on them in low plant populations than in high plant populations (Todd, et al., unpublished data), and therefore may have a higher probability of infection. In many cases, the actual number of infected plants per hectare may be nearly the same in low and high plant populations, but higher plant populations result in more uninfected plants per hectare, which help compensate for yield losses on infected plants. Field survey data has also indicated that as populations drop below 13 plants per meter of row, spotted wilt severity progressively increases (Brown, et al., unpublished data).

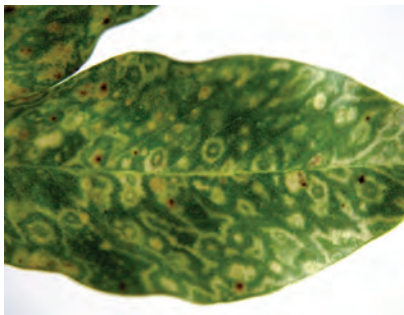
In past reports, the use of insecticides to control thrips vectors has been mostly ineffective in suppressing spotted wilt (Funderburk et al., 1990; Todd et al., 1996). More recent reports in tomato and tobacco suggest that certain intensive early season treatments might be effective (Riley and Pappu, 2004; McPherson et al., 2005). Lowering vector populations with insecticides probably reduces secondary spread, but most infection is thought to be the result of primary infection from overwintering and immigrating thrips (Todd et al., 1997). Despite the overall disappointing results with insecticides in peanut, in-furrow applications of phorate granules have provided consistent, low-level suppression of spotted wilt (Todd et al., 1996). The mechanism of this suppression is not known, but the level of thrips control obtained with phorate is not greater than that obtained with other insecticides. Gallo-Meagher et al. (2001) found oxidative stress in phorate-treated peanuts consistent with systemic acquired resistance mechanisms identified in other plant/pathogen associations (Friedrich et al., 1993). Their study (Gallo-Meagher et al., 2001) identified 22 genes that were turned on and 24 genes that were turned off in phorate-treated peanut plants compared to non-treated plants.

Eighteen- to 25-cm twin row spacing (utilizing the same

seeding rate per hectare as single row spacing) has become increasingly popular in Georgia. Research has shown a strong tendency for twin row patterns to have significantly higher yields, a one- to two-point increase in grade (percentage of total in-shell weight attributed to sound, mature kernels), significantly reduced spotted wilt severity and significantly increased net returns per hectare (Baldwin et al., 1999). The reason for reduced spotted wilt is not fully understood, but more rapid ground coverage in twin row patterns may affect the ability of thrips to locate a seedling host.

Peanut growers utilize a variety of tillage methods, each with its own merits and disadvantages for a given situation. Strip tillage (tillage of a narrow band for the seed furrow, leaving the remainder of the land undisturbed) has some distinct advantages, including reduced soil erosion and reduced time and labor required for planting, but in some situations, yields have been disappointing. Previous studies have shown that peanuts grown in reduced tillage systems have less thrips damage (Campbell et al. 1985, Minton et al. 1991). Our on-farm observations during the early 1990s indicated a reduction in spotted wilt as well,

and these observations have since been confirmed in replicated research plots (Baldwin et al. 2001, Johnson et al. 2001, Monfort et al. 2004). Wheat straw applied to the soil surface prior to peanut emergence at the rate of 1,217, 2,437 and 4,876 kg/ha to simulate different levels of crop residue resulted in 16%, 34% and 51% reductions in thrips damage, respectively, and 21%, 49% and 65% reductions in spotted wilt severity, respectively, compared to that of peanut grown on bare ground. The cause of this effect is unknown, but ground cover may interfere with thrips' ability to visually locate host plants. Slight reductions in spotted wilt do not always justify a change in tillage methods; however, tillage must be considered as a production practice that contributes to the overall variation in spotted wilt severity.



Varied expression of
TSWV
in Peanut



TOSPOVIRUS SUMMARY AND OUTLOOK

ALEX CSINOS AND STEPHEN MULLIS

Tospoviruses have become some of the greatest concerns in tobacco, peanut and vegetable production in the Southeast. Some headway has been made over the past 15 years in managing these diseases, in particular where disease resistance can be incorporated into the management plans. This is definitely the case for peanut, tomato and pepper where traditional breeding programs have developed cultivars that have at least partial resistance. These resistant cultivars have become crucial to the management programs and have been very effective in reducing TSWV losses.

Crops such as tobacco and onion do not have cultivars with resistance and thus management programs are dependent on cultural techniques and chemicals. There has been some limited success with some of these programs in tobacco, though disease losses due to TSWV continue to be high. Although transgenic tobacco lines, immune to TSWV, have been developed, GMO crops are not well accepted in the world market and thus are unacceptable to tobacco companies. The susceptibility and resultant crop damage on onion has just recently been brought to light and thus impact of TSWV on the onion industry in Georgia is yet uncertain. In other geographical areas of the world, IYSV has been a devastating disease in onion, but as of now, its effects have been minimal in this area.

Tospoviruses are a great concern worldwide, but little information is available on managing them. The Coastal Plain of Georgia has become the center of TSWV dam-

age on crops grown in the Southeast. We suspect that environment and hosts play an important role in the severity of the virus in Georgia. Many crops and weeds are susceptible to TSWV infection and the vector thrips also use those same plants as hosts. With the virus, hosts and the vector all present during the entire year, epidemics of TSWV will occur each year the environment is suitable.

Host resistance and critical biological aspects of the disease may be the only foreseeable management areas we can capitalize on. Insecticides such as imidacloprid (Admire) and phorate (Thimet) are used primarily to target the insect vectors of TSWV. Acibenzolar-S-methyl (Actigard) is a plant activator and induces systemic acquired resistance (SAR) in plants to protect against infections. These materials are currently recommended and are being used with limited success, but alone may not provide acceptable control of the disease.

Management of tospoviruses and other similar viruses vectored by insects will continue to be serious threats to Southeast agriculture since both the viruses and the thrips vectors are endemic. Both traditional breeding programs and transgenic breeding programs (if accepted) will provide the best input into management programs for tospoviruses. Understanding the biology of both the virus and its thrips vector, and their relationship to environmental conditions favorable for epidemics, continues to be a priority in research programs.

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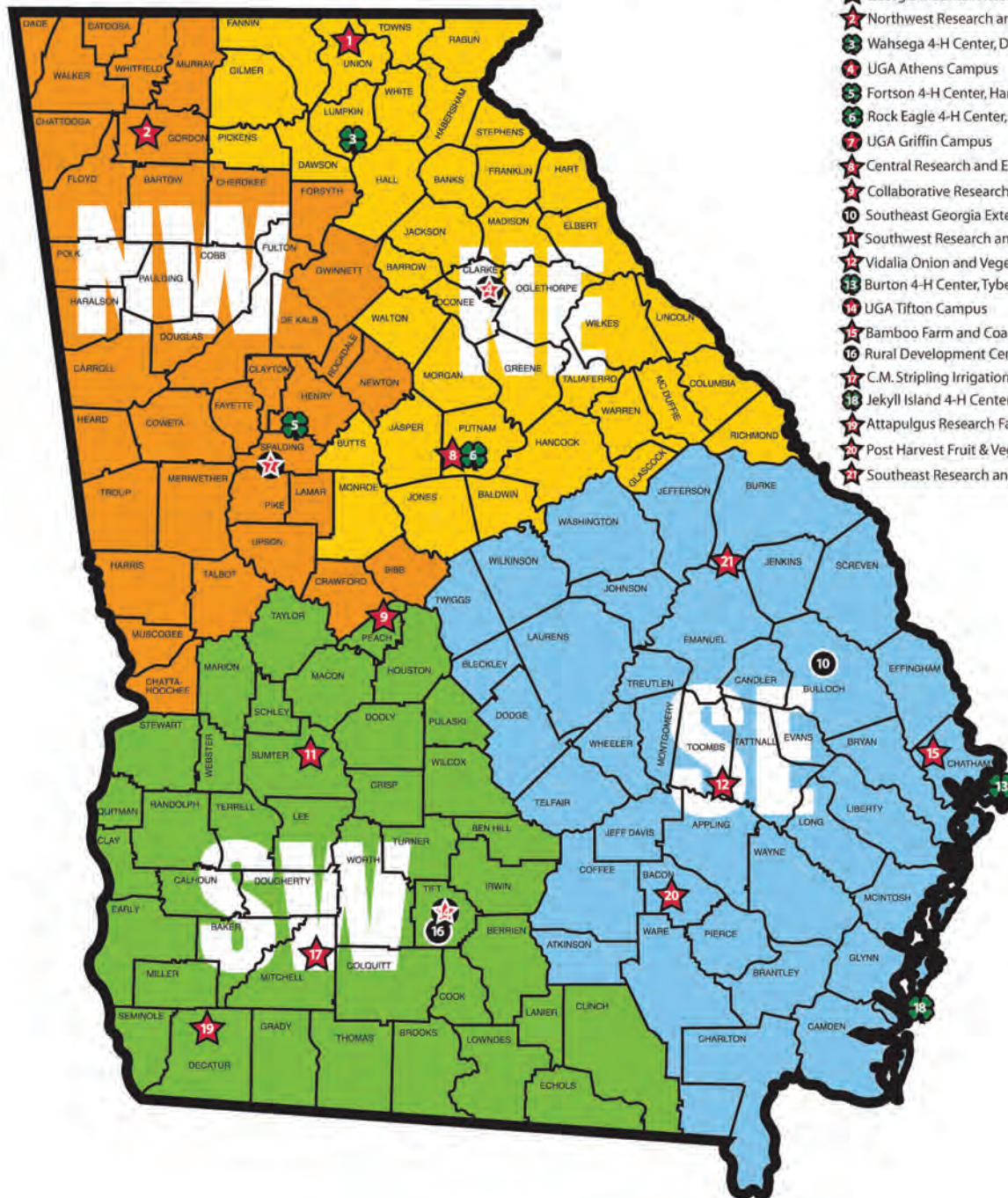
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Conversion Table

U.S. <i>Abbr.</i>	<i>Unit</i>	<i>Approximate Metric Equivalent</i>
Length		
mi	mile	1.609 kilometers
yd	yard	0.9144 meters
ft or '	foot	30.48 centimeters
in or "	inch	2.54 centimeters
Area		
sq mi or mi ²	sqare mile	2.59 square kilometers
acre	acre	0.405 hectares or 4047 square meters
sq ft or ft ²	square foot	0.093 square meters
Volume/Capacity		
gal	gallon	3.785 liters
qt	quart	0.946 liter
pt	pint	0.473 liter
fl oz	fluid ounce	29.573 milliliters or 28.416 cubic centimeters
bu	bushel	35.238 liters
cu ft or ft ³	cubic feet	0.028 cubic meter
Mass/Weight		
ton	ton	0.907 metric ton
lb	pound	0.453 kilogram
oz	ounce	28.349 grams
Metric <i>Abbr.</i>	<i>Unit</i>	<i>Approximate U.S. Equivalent</i>
Length		
km	kilometer	0.62 mile
m	meter	39.37 inches or 1.09 yards
cm	centimeter	0.39 inch
mm	millimeter	0.04 inch
Area		
ha	hectare	2.47 acres
Volume/Capacity		
liter	liter	61.02 cubic inches or 1.057 quarts
ml	milliliter	0.06 cubic inch or 0.034 fluid ounce
cc	cubic centimeter	0.061 cubic inch or 0.035 fluid ounce
Mass/Weight		
MT	metric ton	1.1 tons
kg	kilogram	2.205 pounds
g	gram	0.035 ounce
mg	milligram	3.5 x 10 ⁻⁵ ounce

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