## ABSTRACT

**SUTLIFF-SHIPLEY, SUSAN G.** Population Dynamics and Feeding Preferences of Thrips (Thysanoptera: Thripidae) as Tospovirus Vectors in North Carolina Cut Flower Production. (Under the direction of Dr. Christine A. Casey).

Many cut flower crops are susceptible to both of impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV) which are commonly vectored by *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergande) (Thysanoptera: Thripidae) in North Carolina. INSV and TSWV are among the most damaging plant diseases affecting the floriculture industry. Thrips were surveyed in field cut flower production in eight field locations across North Carolina during the 2005 growing season to determine regional and production differences in thrips species distribution and abundance. Thrips populations were monitored via yellow sticky traps and by directly sampling the blooms of 20 common floral crop species and 11 surrounding weed species. *F. tritici* (Fitch) was the most common thrips species overall and the most common virus vector was F. fusca. Relatively low numbers of *F. occidentalis* and *Thrips tabaci* (Lindeman), another reported vector, were also collected. The spatial and temporal occurrence of vector species varied regionally, but there were no significant differences in vector populations between conventional and organic production systems. There was a positive correlation between thrips sampled on sticky traps and thrips sampled directly on floral blooms. Weed species *Triodanis perfoliata*, *Ranunculus spp., Oxalis spp., Taraxacum officinale*, and *Trifolium incarnatum* and crop species *Centaurea cyanus, Chysanthemum spp., Antirrhinum majus, Achillea millefolium, Matricaria recutita*, and Lilium - Asiatic hybrid consistently supported the largest populations of adult TSWV vector species. *F. fusca*, was the most abundant TSWV vector species collected, comprising over 95% of vector species in the Piedmont region and 98% in the Coastal Plain region. The results of this study demonstrate the relative potential of cut flower crops and weed plants to act as important reproductive sites for *F. fusca* and *F. occidentalis* as innoculum sources of TSWV and INSV. Differences in seasonal patterns and within plant distribution should be considered in developing sampling protocols and management plans for tospovirus vectors. *F. occidentalis* is one of the most efficient

vectors of INSV and TSWV in greenhouse production. Indicator plants, which develop virus symptoms faster than crop plants, are a potential tool for detection of viruliferous thrips. If thrips do not prefer to feed on these relative to crop plants, their utility as indicators may be limited. Two accepted indicator plants, petunia and fava bean, were evaluated in a F. occidentalis feeding preference choice tests. Petunia leaves had significantly more feeding scars per $cm^2$ than fava bean leaves, suggesting that thrips will feed preferentially on petunia. The two indicator plants were also compared with important greenhouse crops in a thrips feeding preference choice test. Crop plants included: garden impatiens, New Guinea impatiens, gloxinia, begonia, kalanchoe, and chrysanthemum. Petunia leaves had significantly more feeding scars per $cm^2$ than the other plant species tested, suggesting that thrips will feed preferentially on petunia relative to other crop plants and thus, will be useful as a virus indicator. Six petunia varieties, 'Purple Wave', 'White Bedder', 'White Swan', 'Red Halo', 'Dreams–Midnight Blue', and 'Mix' (heirloom) petunia were also evaluated in a thrips feeding preference choice test. Feeding preference tests with non-viruliferous thrips showed that the varieties 'Mix' and 'Red Halo' had significantly more feeding scars than any other variety. Choice feeding tests using thrips infected with TSWV using the same petunia varieties were inconclusive. However, only the varieties 'Purple Wave', 'Mix' and 'White Bedder' showed characteristic necrotic lesions indicative of TSWV infection and presence of viral lesions suggest that these varieties may be more suitable as tospovirus indicator plants.

# POPULATION DYNAMICS AND FEEDING PREFERENCES OF THRIPS (THYSANOPTERA: THRIPIDAE) AS TOSPOVIRUS VECTORS IN NORTH CAROLINA CUT FLOWER PRODUCTION

By

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# DEDICATION

I dedicate this work to my husband Jason, whose unconditional love and support made this dream possible. I could not have done it without you! I also dedicate this work to my parents, Richard and Gail Sutliff for their encouragement throughout my research and coursework. I thank you all so very much!

# BIOGRAPHY

Susan Sutliff-Shipley was born in Portsmouth, VA February 13, 1974. She grew up in Chesapeake, VA and graduated from Great Bridge High School in 1992. After high school, she attended Auburn University in Auburn, Alabama where she pursued a B.S. degree in Secondary Education with concentrations in Biology and English. After receiving her degree, she taught high school biology for six years in the North Carolina public school system. However, her interest in research and insects lead her to pursue a Master's degree in entomology

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LITERATURE REVIEW

## Description and Biology of the Thysanoptera in North Carolina

Knowledge of the natural history and diversity within the Thysanoptera order is beneficial in understanding thrips population dynamics. Insects of the order Thysanoptera, collectively called thrips, are between 1-2 mm in length with slender, spindle-shaped bodies. Thrips are worldwide in distribution. There are over 5,000 known species of Thysanoptera that are classified into two suborders, Tuberlifera and Terebrantia. Members of the suborder Tuberlifera possess an internal ovipositor while members of the suborder Terebrantia possess a saw-like ovipositor (Mound *et al.,* 1980). The family Thripidae (suborder Terebrantia) are primarily pest species and include two genera, *Thrips* and *Frankliniella*, which are serious pests of agricultural crops (Mound, 1997). The genus *Thrips* is the largest genus of Thysanoptera, consisting of 275 species worldwide. There are over 180 species in the genus *Frankliniella*, with the majority found in the neotropics (Mound & Kirby, 1998).

Eckle *et al*. (1996) identified 23 Terebrantia thrips species belonging to 14 different genera as well as 7 Tuberlifera species belonging to 4 different genera in tomato, pepper, and tobacco cropping systems across North Carolina. Of these, five thrips species have been identified as significant thrips pests in North Carolina. These species include *Frankliniella fusca* (Hinds), *Frankliniella occidentalis* (Pergande), *Frankliniella tritici* (Fitch), *Thrips tabaci* (Lindeman) and *Neohydrothrips variabilis* (Beach) (Eckel *et al.,* 1996). *F. fusca* and *F. tritici* are native to the southeastern United States (Mound & Kirby, 1998). *F. occidentalis*, native to the western U.S., was first recorded in the southeast in the early

1980's (Beshear, 1983). *T. tabaci* and *N. variabilis* are not indigenous to the U.S., but have become established pest species throughout the U.S. (Mound & Kirby, 1998).

Thrips are a serious concern to agricultural commodities because of the amount of crop damage they are able to inflict (Lewis, 1997). Thrips have asymmetrical mouthparts comprised of a pair of maxillary stylets and a single mandibular stylet (Chisholm & Lewis, 1984). Thrips feed by using the modified left mandible mouthpart to pierce plant cells and their stylets to suck out their contents (Lewis, 1973). Feeding activities result in deformed leaf and flower growth, leaf, petal, and fruit scarring, all of which can cause crop loss. Leaf and petal feeding damage usually appears as silvered patches however, feeding symptoms vary among flower crop species. Feeding damage symptoms in floral crops include petal discoloration, distortion, and streaking (van Driesche, 1998). In addition to feeding damage, some plants develop a local dead or discolored spot where thrips eggs have been inserted into plant tissue (van Driesche, 1998). Several thrips species also transmit the tospoviruses impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV) (German *et al.* 1992, Ullman *et al*. 1997).

## Tospoviruses in Relationship to their Thrips Vectors

Because thrips are the only known vectors of tospoviruses, it is important to understand the biology of these viruses in relation to the virus vector. Tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV) are closely related tospoviruses (family Bunyaviridae) that have been associated with a variety of North American crops (Daughtrey *et al.*, 1997). The tospovirus genus is the only genus in the family Bunyaviridae to infect plants (Francki *et al*., 1991). TSWV and INSV are among the most damaging of the

North American plant viruses (Sherwood *et al*., 2002). The disease that came to be known as TSWV was first observed in Australia in 1906 (Brittlebank, 1919) and was first detected in the southeast during the 1970's (Jones & Baker, 1991). The first detection of TSWV in North Carolina occurred in 1988 (Groves *et al.,* 2002). By 1997, TSWV was reported in nearly every county in North Carolina (Groves *et al.,* 2002). Prior to 1990, TSWV was classified as the single representative of a monotopic plant virus group, the "tomato spotted wilt virus group" (Matthews, 1982). de Avila *et al.* (1992) proposed classifying tospoviruses based on the percentage of amino acid sequence similarity between the nucleoprotein genes of members of the tospovirus genus. A new serotype was discovered in 1989 in impatiens and became known as TSWV-I and in 1990, TSWV-I became formalized as INSV (Law & Moyer 1990). INSV was first detected in peanut crops in Georgia and Texas in 1998 (Pappu *et al.*, 1999). Mixed infections of TSWV and INSV in tobacco have also been recently observed in North Carolina and Kentucky (Martinez-Ochoa *et al.*, 2003).

It has been hypothesized that each vector thrips species arose independently as a tospovirus vector and that a coevolutionary relationship between thrips and tospoviruses is unlikely, lacking a systematic relationship between vector species (Mound, 2002). Mound (1996) proposed that a thrips-tospovirus relationship arose from either a plant pathogen that optimized its dispersal by acquiring a vector, a thrips pathogen that adapted to infect plants, or a vertebrate pathogen whose ability to infect plants was mediated by thrips. Thrips vector species are commonly highly polyphagous and reproduce and feed on a wide range of hosts, increasing the probability of feeding on an infected plant.

Tospoviruses are spherical in shape (80-110 nm diameter) and the viron has a lipid envelope. The membranous envelope contains two proteins that may be involved in

recognition of receptors in the vector. Within the viral envelope, there are three single-stranded RNA molecules of differing sizes (S RNA, M RNA, and L RNA) (de Haan *et al.*, 1990, 1991, Pappu *et al.*, 2000).

The thrips life cycle consists of an egg stage followed by two larval feeding stages (1st and 2nd instars). Development continues with two non-feeding pupal stages (pupa and propupa) and pupation typically occurs in the soil. After development, adults are able to mate within a few days. The life cycle takes 20-30 days from egg to adult depending on temperature, day length, and the plant species it is feeding on (Soria & Mollema 1992, Gaum *et al.* 1994, Brødsgaard 1994, Katayama 1997, Lewis, 1973). Both TSVV and INSV can only be acquired by first instar larvae, but can be transmitted by second instars and adults (Sakimura, 1963, Wijkamp & Peters, 1993). TSVV and INSV replicate in thrips (Ullman *et al.*, 1993) and after a latent period, pass the virus to uninfected plants through plant feeding (van de Wetering *et al*., 1996). Latent periods of *F. occidentalis*, *F. fusca*, and *T. tabaci* have been documented to range from 4-7 days, 4-12 days, and 4-18 days, respectively (Sakimura, 1963, Wijkamp & Peters, 1993). The latent period is temperature dependent such that at low temperatures, the ability to transmit viruses increases significantly (Wijkamp & Peters, 1993 Wijkamp *et al.*, 1995, Chatzivassiliou *et al.*, 2002). Viruliferous thrips do not pass the virus to their progeny (Sakimura, 1962, 1963).

Thrips are able to diapause in the soil during the winter, so viruliferous thrips can be an innoculum source between cropping seasons (Lewis, 1973). However, more thrips have been observed overwintering on plants rather than diapausing in the soil, indicating that active populations overwintering and reproducing on winter host plants may play a more significant role in TSWV spread than do diapausing thrips (Cho *et al.,* 1995, Groves *et al.*,

2001). Thrips' minute size enables access to recessed areas of the plant which provide microclimates that inhibit desiccation of thrips freezing (Kirk, 1997). Winter host plants, such as natural vegetation near agricultural areas, are of great importance for thrips vectors that overwinter. Thrips vector species are highly phytophagous and reproduce and feed on a wide range of hosts, increasing the probability of feeding on an infected plant. Perennial weed species might also allow the virus to persist between plantings of short-lived crops (Bos, 1981) such as seen in field cut flower production. In a study of weed reservoir sources for TSWV in Hawaii, 44 species of weeds were found to have high incidence of TSWV (Cho *et al.*, 1986). Researchers in Canada found that 113 of the native plant species in southern Ontario were susceptible to TSWV and that of those species, 86% were ovipository hosts for *F. occidentalis* (Stobbs *et al.*, 1992). Populations of *F. fusca* and *F. occidentalis* have been found to overwinter on host plants in North Carolina (Cho *et al.,* 1995, Groves *et al.,* 2002). Groves *et al.* (2002) also suggested that summer annual plant species may provide a bridge between seasons of overwintering weed hosts harboring tospovirus.

Three of the five most numerous thrips species in North Carolina are capable of transmitting tospoviruses. *F. fusca* and *F. occidentalis* have been determined as the primary thrips vector species of both TSWV and INSV in North Carolina (Eckle *et al.*, 1996, Daughtrey *et al.*, 1997, Naidu *et al.*, 2001). *T. tabaci* is another TSWV vector common in North Carolina (Eckle *et al.*, 1996), but has only been found to transmit virus at low efficiencies (Wijcamp *et al.*, 1995).

There are over 900 plants reported to be susceptible to TSWV, most belonging to the Solanaceae and Compositae families (Peters, 1998) and over 600 ornamental plant species susceptible to both TSWV and INSV (Daughtrey *et al.,* 1997). The symptoms of

TSWV and INSV vary widely and differ greatly between time of year, age of plant, plant species, and virus strain (Jones and Baker, 1991). Variation in symptom may be attributed to the mixing of viral strains in the plant (Ie, 1970). Identification of both INSV and TSWV in plants is accomplished in the laboratory with enzyme-linked immunosorbent Assay (ELISA) or polymerase chain reaction (PCR). Under field conditions, ELISA tests for rapid detection of INSV and TSWV in plants can be used.

Not all tospovirus host plants are sources of the disease. Many plants are susceptible to tospoviruses but do not support thrips reproduction and are considered a "dead end" for virus spread (Duffus, 1971). In North Carolina, Groves *et al.* (2002) found that the plant species *Scleranthus annuus* (L.), *Sonchus asper* (L.) Hill, *Stellaria media* (L.) Vill., and *Taraxacum officinale* (Weber ex F. H. Wiggers.) consistently supported both *F. fusca* reproduction and TSWV infection while species such as *Rumex crispus* (L.) and *Plantago lanceolata* (L.), although susceptible to TSWV, supported below average *F. fusca* reproduction and are effectively dead-end hosts. Two factors, our lack of an immature key and the difficulty in rearing thrips larvae to the adult stage, limits our knowledge of the plant species that serve as both reproductive hosts and are susceptible to TSWV or INSV.

## Thrips Host Plant Selection

Many thrips species of economic importance are highly phytophagous, thus, an understanding of factors influencing host plant selection is beneficial in determining feeding preferences among thrips species. Responses to wind, color, UV reflectance, and volatiles are all involved in thrips host plant selection. Although thrips are considered to be weak flyers, they are able to disperse via wind currents. It is widely assumed that once thrips are

carried in a wind current they have minimal control over their flight path and destination, however, there is abundant circumstantial evidence that some thrips species exhibit a level of control to land on host crops or even specific host plants (Lewis, 1997). Irwin & Yeargan (1980) found differences in different thrips species ability to control landings when carried in wind currents. *N. variabilis*, for example, are blown more readily and are less able to control their direction and landing response compared to *F. tritici*, which are intermittent flyers that attempt to orientate towards the wind where they better can direct their flight. However, long-distance dispersal is limited because flying thrips are unable to feed and their ability to resist dehydration determines how long they can remain airborne (Lewis, 1997). Thus, most thrips disperse over a series of short flights (Funderburk and Stavisky, 2004). Flower thrips such as *F. tritici* are highly dispersing and move rapidly between flowers (Ramachandran *et al.* 2001).

The primary characteristic in thrips locating a host plant is color (Terry, 1997). Thrips are able to locate hosts via visual clues such as the colors blue, white, and yellow (Frey *et al.,* 1994*,* Cho *et al.*, 1995, Childers & Brecht, 1996, de Kogal & Koschier, 2003). Yudin *et al.* (1988) found that all hosts of *F. occidentalis* contained twice as many thrips when flowers were present than those plants of the same age with the flowers removed. They also found that the least preferred pre-flowering host (*Verbesina encelioides* (Cav.) Benth. & Hook. f. ex Gray) supported 60 times more thrips when flowering. Similar studies involving *F. occidentalis*, were found in chrysanthemum (de Jager *et al*.,1993) and between flowering and non-flowering weed species (Bautista & Mau, 1994). UV reflectance also plays a role in thrips host selection. Matteson & Terry (1992) found two peaks in photo reception occurring at 365 nm in the UV region and 540 nm in the green-yellow region.

They also suggested that thrips use this peak sensitivity to yellow-green in long-range orientation to plants and then use contrasts within plants to find flowers; thrips are able to locate blue spectral hues when both UV and yellow-green receptors are initiated. Floral petal configuration is also thought to affect host plant preferences. Chrysanthemum flowers with disc florets are more attractive to *F. occidentalis* than flowers with spider-type florets (Broadbent & Allen, 1995, de Jager *et al.*, 1995).

Secondarily, olfactory cues in conjunction with visual cues are used to locate host plants. For example, *F. occidentalis* were attracted to extracted chrysanthemum volatile chemicals in choice tests, but were unable to locate the flowers without visible clues (Koschier *et al.*, 2000, de Kogal & Koschier, 2003). In laboratory experiments, Frey *et al.* (1994) found that blue sticky traps combined with the odor geraniol created an additive effect, capturing 1.9 times as many *F. occidentalis* than color alone. However, these odor-infused traps were found to be ineffective in greenhouse situations due to possible competition with other floral plants. Similar results were found by Teulon *et al.* (1993) with anisaldehyde-baited yellow sticky traps in greenhouse peppers.

Once thrips locate a potential feeding host, specific plant selection may be determined by nutritional requirements (de Jager *et al*., 1995, Mollema & Cole, 1996). However, little is known about thrips nutritional requirements (Brodbeck *et al*., 2002). Healthier plants are more attractive to thrips. Ananthakrishnan and Gopichandran (1993) suggested that thrips may prefer hosts with more amino acids because the short larval feeding stage and thrips require proteins necessary for rapid growth. Thrips crop damage was correlated to concentration of amino acids, particularly phenyalanine, a component of cuticle production and hardening, necessary to prevent desiccation or fungal infection (Mollema & Cole, 1996).

Also, Anderson *et al.,* (1992) found that glutamine, a component of amino acids, may also stimulate thrips feeding. It has been also found that plant carbohydrate concentration increases *F. occidentalis* feeding rates, but not as strongly as plant protein concentration (Brown *et al.,* 2002). Additionally, sugars added to pesticides have been shown to increase thrips insecticide consumption (Parrella, 1995).

## Conclusions

Several aspects influence the relationship between the thrips vector and tospoviruses. Dispersal and vectoral capacity of thrips populations, sources of virus reservoirs, the association of the thrips vectors with its host plant, and the replicative nature of the virus are all factors that contribute to the success of certain thrips species to vector TSWV/INSV. Thus, the objectives of this study were to: evaluate temporal and spatial dynamics of thrips and tospovirus vectors on field grown floral crops in geographically distinct areas; examine possible differences in thrips species distribution in conventional and organic production practices; determine which sampling methods are appropriate to identify tospovirus vectors in cut flower production; and to determine the population dynamics of thrips species on cut flower cultivars and surrounding weed species in field cut flower production. Additionally, feeding preferences of *F. occidentalis* for two tospovirus indicator plant species, crop plants species, and different petunia varieties were examined.

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CHAPTER 2

## Thrips species composition in North Carolina field cut flower production and implications for tomato spotted wilt and impatiens necrotic spot epidemiology

Field cut flower production has increased over the past fifteen years as farmers seek to diversify and to increase profit margins (Kelly, 1991). North Carolina ranks 8$^{th}$ in the nation in floriculture production with cut flower profits over $3.17 million (wholesale) in 2003 (North Carolina Department of Agriculture & Consumer Services). Thrips are one of the most serious insect pests in cut flower production due to their feeding damage and their capacity to vector plant viruses. Thrips feeding damage may result in petal scarring and distortion, incomplete petal expansion, and crop leaf scarring. Because the value of cut flowers is in their unblemished appearance, tolerance for this type of damage is low. However, tospoviruses such as tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV) may pose a serious threat as these tospoviruses are among the most damaging of the North American plant viruses (Sherwood *et al.*, 2002). TSWV is commonly associated with field grown vegetable crops while INSV is typically associated with greenhouse floral crops (Daughtrey *et al.*, 1997). Recently, however, INSV was detected in peanut crops in Georgia and Texas in 1998 (Pappu *et al.*, 1999). Mixed infections of TSWV and INSV in tobacco have also been recently observed in North Carolina and Kentucky (Martinez-Ochoa *et al.*, 2003). As farms integrate and floral crops become field crops, it is important to evaluate the potential risk of tospoviruses such as TSWV and INSV in floriculture production.

TSWV was first observed in the southern U.S. during the 1970's (Jones & Baker, 1991) with its first detection in North Carolina in 1988 (Groves *et al.,* 2002). By 1997, TSWV was reported in nearly every county in North Carolina (Groves *et al.,* 2002). TSWV and INSV were initially thought to be two strains of the same virus, however, in 1989, INSV (formerly TSWV-I) was determined to be a distinct virus (Law and Moyer, 1990, Daughtrey *et al.*, 1997). Both TSWV and INSV are acquired by first instar thrips feeding on infected plants (Wijcamp & Peters, 1993). These viruses replicate in thrips (Ullman *et al.*, 1993) and after a latent period, pass the virus to uninfected plants through plant feeding (van de Wetering *et al*., 1996). Viruliferous thrips do not pass the virus to their progeny (Sakimura, 1962, 1963). *Frankliniella fusca* (Hinds) and *Frankliniella occidentalis* (Pergande) have been determined as the primary thrips vector species of both TSWV and INSV in North Carolina (Eckle *et al.*, 1996, Daughtrey *et al.*, 1997, Naidu *et al.*, 2001). *Thrips tabaci* (Lindeman) is another TSWV vector common in North Carolina (Eckle *et al.*, 1996), but has only been found to transmit virus at low efficiencies (Wijcamp *et al.*, 1995). Plants are most susceptible to these viruses early in their development and less susceptible as they mature (Daughtrey *et al.*, 1997). This potentially poses a more serious problem for floral crops compared to other crops in North Carolina because in floral production, planting times are staggered due to the seasonality of flower blooming whereas with crops such as tobacco or tomato, crops are planted at one time and remain in the field throughout the growing season. Thus, tospovirus susceptible crop stages may be longer in duration than in other cropping systems.

Symptoms of TSWV and INSV vary greatly among hosts, but typical symptoms include necrotic spots, stunting, or wilting of the host plant. Although specific TSWV losses

in floral crops have not been reported, crop losses in individual fields of flue-cured tobacco, tomato, and pepper have approached 50% in North Carolina (Groves *et al.* 2002). Floral losses due to INSV were estimated at $675,000 (retail value) during 1989-1990 in Pennsylvania (Daughtrey *et al.*, 1997). In Georgia, tospovirus ornamental crop losses have averaged over $500,000 annually during the 2000-2003 growing seasons (Williams-Woodard, 2001, 2002, 2003, 2004).

Natural infections of both INSV and TSWV have been found in field crops such as peanut and tobacco in several southeastern states including North Carolina (Martinez-Ochoa *et al.*, 2003). Groves *et al*., (2002) suggested that summer annual weed plant species may provide a bridge between seasons of overwintering weed hosts harboring tospovirus. For example, dandelion (*Taraxacum officinale* Weber ex F. H. Wiggers) was identified as a ubiquitous summer weed that served as both a reproductive host for *F. fusca* and a consistent TSWV innoculum source. Because *T. officinale* flowers throughout the spring and summer months in North Carolina as well as cut flower crops, cut flower crops might also serve as summer tospovirus bridge plants. Efforts to manage thrips and tospoviruses in cut flower production are hampered because the species composition and seasonal abundances of thrips that inhabit field cut flowers are unknown. The objective of this study was to identify thrips species and evaluate temporal and spatial abundance of thrips species and tospovirus vectors on cut flower crops and surrounding weed species in North Carolina field cut flower production. This information is needed to better understand factors influencing the timing and abundance of thrips infesting cultivated cut flower fields and the development of TSWV and INSV epidemics. Another objective was to examine possible differences in thrips

species distribution in cut flower production across two distinct geographical areas because environmental factors such as climate or soil type may affect the overwintering ecology of tospovirus vector species. These factors were studied because Piedmont soils are mainly loam and clay-loam while Coastal Plain soils contain more sand and sandy soils release more moisture and hold more heat than loam or clay-loam soils. A third objective was to examine possible differences in thrips species distribution in conventional and organic production practices. The final objective was to determine which sampling methods are appropriate to identify tospovirus vectors in cut flower production.

## Materials and Methods

From 21 April to 20 September 2005 thrips populations were monitored in organic and conventional production and in two distinct geographical regions (Piedmont and Coastal Plain) (Fig. 1). The following Piedmont locations were sampled throughout the entire season: Farm A (Graham, Alamance Co., organic), farm B (Chapel Hill, Chatham Co., organic), farm C (Raleigh, Wake Co., conventional) and farm D (Clayton, Johnston Co., conventional). Farm E (Efland, Orange Co., conventional) was sampled in the latter half of the growing season and is included only for sampling method analysis. The following Coastal Plain locations were also sampled throughout the entire season: Farm F (Kinston, Lenoir Co., conventional), farm G (Jacksonville, Onslow Co., conventional). Farm H (LaGrange, Lenoir Co., organic) was sampled only during the first half of the growing season because the sampling site was destroyed and was replaced with farm J (Goldsboro, Wayne Co., organic), a farm which had a similar agroecosystem.

**Aerial trap collections.** Thrips were sampled using 17.8 x 10.2 cm, double-sided, yellow sticky cards with an imprinted grid pattern to facilitate counting (Seabright Laboratories, Emeryville, CA). Each trap contained approximately 76 sq. cm of adhesive area. The tops of the traps were reinforced with duct tape (3M, Minneapolis, USA) to prevent wind damage. Traps were fastened to ¾-inch (1.9 cm.) plastic spring clamps (Wolfcraft, USA) via 0.2 x 10.5 cm. cable ties (Thomas & Betts, Memphis, TN) which were attached to 60 cm tomato stakes. Traps were placed above the crop canopy to intercept dispersing thrips from broader areas (Gillespie &Vernon, 1990, Brødsgaard, 1989). At each field location, four traps were equally placed along the perimeter of a plot, with one in each cardinal direction. A fifth trap was placed randomly within an interior crop row. Plot sizes were approximately 1/5 of an acre in size (810 square meters). Traps were replaced at ~14-d intervals, and taken to the laboratory and stored at ~4.5°C until thrips could be counted and identified. As traps were collected in the field a sheet of polyethylene wrap (The Glad Products Company, Oakland, CA) was placed over each trap to protect it from debris. The total number of thrips on each trap were counted and recorded. A subset of 50 thrips was removed from each card (25 from each side) by using a solvent (HistoClear II, Great Lakes IPM, Vestaburg, MI) to loosen the thrips from the card. Thrips removed were chosen in a random pattern using the grid blocks of the card. The thrips were then mounted onto a glass microscope slide using euparal mounting medium (Bioquip, Rancho Dominguez, CA) and identified to species using a compound microscope. When fewer than 50 thrips were present on the card, as was the case in the early part of the sampling project, all thrips were removed from the card and were identified to species.

Vector species occurrence was compared among regions, production type, and date. Farms A, B, C, and D in the Piedmont region and farms F, G, H, and J in the Coastal Plain region were included in the species composition analysis. Temporal analysis of species composition ranged from May 20th –August 24th when all farms were in production. Overall means between production systems, regions, and species composition were analyzed via ANOVA and means were separated using Tukey-Kramer HSD (SAS JMP 6, Cary, North Carolina, 2005). Correlation analysis (PROC CORR) was used to examine the relationship between individual flower samples and mean trap counts of vector species. Correlation analyses were conducted using SAS, version 9.1.

**Individual weed and flower crop samples.** Individual plant samples were collected at ~14-d intervals, concurrent with aerial trap collections. At each farm visit, three different flower crop species and three different weed species were randomly sampled (Table 1 & 2). Because of seasonal bloom times for weed and floral species and individual farm cut flower selections, it was not possible to sample the same plant species across time and location. When possible, similar weed and flower crop species were sampled across all farms. Individual samples were collected via white beat trays (HDPE cutting trays, Gage industries, Lake Oswego, OR) using a single bloom. Flower blooms were tapped 3-5 times directly over a white beat tray and collected thrips were transferred into a 5-dram vial containing 70% ethanol using a fine-tipped paintbrush. The thrips were later mounted onto a glass microscope slide using euparal mounting medium and were identified to species using a compound microscope. Sampling dates used in the analysis ranged from April 22nd – September 20th and included all farms. Only plants that were sampled at least three times over the course of this study were included in this analysis. Data was analyzed via ANOVA

and means were separated using Tukey-Kramer HSD (SAS JMP 6, Cary, North Carolina, 2005).

## Results

**Aerial trap collections.** Across all farms, a significant number of thrips were captured during the first two weeks in June compared to any other sampling interval with a mean number of 1607.57 ± 436.27 ($F = 5.3$, $df = 7, 46$, $p<0.0002$) (Fig. 2) as well as when separated regionally with a mean number of 931 ± 171.26 captured in the Coastal Plain region ($F = 7.87$, $df = 7, 15$, $p<0.0004$) and 2115 ± 671.74 in the Piedmont region ($F = 4.31$, $df = 7, 23$, $p<0.0035$) (Fig. 3).

Lists of the mean number of each species captured by date and region are located in Tables 3 and 4. The predominant species captured in both locations was *F. tritici* (Fitch), eastern flower thrips, a non-vector species. *F. tritici* species comprised 84% (n = 5,808) of the species identified in the Piedmont region and 57% (n = 2,936) in the Coastal Plain region. There were significantly more *F. tritici* in the Piedmont region with a mean of 578.27 ± 110.97 compared to a mean of 253.56 ± 39.76 in the Coastal Plain region ($F = 6.13$, $df = 1, 53$, $p<0.0165$). The mean numbers of *F. tritici* differed temporally with significantly more captured during the first two weeks in June than in any other sampling interval with a mean of 1778 ± 570.25 ($F=5.23$, $df = 7, 23$, $p<0.0011$) in the Piedmont region and a mean of 649.49 ± 143.63 ($F = 8.94$, $df = 7, 15$, $p<0. 0002$) in the Coastal Plain region.

Approximately 2.5% (n = 176) of the adult thrips identified were capable of transmitting both TSWV and INSV in the Piedmont region compared to 5.3% (n = 277) in the Coastal Plain. Mean numbers of vector species differed temporally with significantly

more captured during the first two weeks in June than in any other sampling interval with a mean of 70.32 ± 16.01 ($F=10.26$, $df = 7, 46$, $p<0.0001$) in the Piedmont region and a mean of 99.76 ± 29.64 ($F = 7.71$, $df = 7, 15$, $p<0.0005$) in the Coastal Plain region. Adult *F. fusca* was the predominate vector species identified in both regions, comprising 90% (n = 159) of the total vectors in the Piedmont region and 92% (n = 254) in the Coastal Plain region. The mean numbers of *F. fusca* differed temporally with significantly more captured during the first two weeks in June than in any other sampling interval with a mean of 66.74 ± 12.795 ($F=11.48$, $df = 7, 23$, $p<0.0001$) in the Piedmont region and a mean of 99.76 ± 29.64 ($F = 8.29$, $df = 7, 15$, $p<0.0003$) in the Coastal Plain region.

A substantial number of onion thrips, *T. tabaci*, were also collected, particularly in the Coastal Plain region with a mean of 125.06 ± 27.8 compared to a mean of 21.76 ± 7.64 in the Piedmont region ($F = 16.45$, $df = 1, 52$, $p<0.0002$). *T. tabaci* comprised 23% (n = 1,178) of the total population in the Coastal Plains region, compared to 3.8% (n = 260) in the Piedmont region. TSWV transmission efficiencies for *T. tabaci* are unknown in North Carolina and were not included as vector species in the analysis of vector numbers.

Soybean thrips, *Neohydrothrips variabilis* (Beach) accounted for 8% of the species identified in the Piedmont region (n = 571) and 10% of the total population in the Coastal Plain region (n = 539). The mean numbers of *N. variabilis* fluctuated temporally in the Coastal Plain region with significantly more captured during the late August through early September compared to those captured from mid-June through early July ($F = 4.1$, $df = 7, 15$, $p<0.0005$). However, in the Piedmont region, the mean numbers of *N. variabilis* differed temporally with significantly more captured during the first two weeks in June than in any other sampling interval with a mean of 200.78 ± 67.92 ($F=4.36$, $df = 7, 23$, $p<0.0033$).

Grain thrips, *Limnothrips cerealium* (Fitch) accounted for 0.8% of the species identified in the Piedmont region (n = 54) and 3.7% of the total population in the Coastal Plain region (n = 193). Species belonging to the Phlaeothripidae family accounted for 0.9% of the species identified in the Piedmont region (n = 59) and 1.4% of the total population in the Coastal Plain region (n = 70). Mean numbers of Phlaeothripidae species fluctuated temporally in the Piedmont region ($F = 3.32$, $df = 7, 23$, $p<0.0136$).

Overall, more thrips were captured on sticky traps in organic production than in conventional production with an overall mean number of thrips per card of 751.574 ± 183.4 in organic production compared to 533.16 ± 52.88 in conventional production ($F=1.66$, $df =1, 52$, $p<0.2021$). However, the differences were not found to be significant. There was also no significant difference in vector species numbers between organic and conventional production ($F = 0.058$, $df = 1, 52$, $p<0.8112$) or with any other thrips species.

For all locations, vector species collected in individual flower blooms did positively correlate to what was collected on aerial sticky traps ($p<0.0001$).

**Individual weed and flower crop samples.** Over the course of this study, 1,170 total thrips were collected and identified to species on weed and crop flowers across all locations. There was a significant regional effect for vector species distribution ($F = 14.746$, $df = 33, 936$, $p<0.0001$), but no significant region effect existed for other species. The predominant species captured in both locations was *F. tritici*. *F. tritici* species comprised of 72.3% (n = 495) of the total population in the Piedmont region and 59% (n = 286) in the Coastal Plain region. Approximately 19% (n = 128) of the adult thrips captured were capable of transmitting both TSWV and INSV in the Piedmont region compared to 32.8% (n = 159) in the Coastal Plain region. Adult *F. fusca* was the predominate vector species in both regions,

comprising 95.3% (n = 122) of the total vectors followed by *F. occidentalis*, which comprised of 4.7% (n = 6) in the Piedmont and 98.1% (n= 156) and 1.9% (n = 3) in the Coastal Plain, respectively. The remaining 10% (n = 68) in the Piedmont and 8% (n = 40) in the Coastal Plain region comprised of *T. tabaci*, *L. cerealium*, *N. variabilis* and various species belonging to the Phlaeothripidae family.

Although the mean number of vectors found on floral crops was not significant between crop species, all but two floral crops contained vector species at any time during this study. Weeds supported significantly more vector species than flower crops ($F = 10.89$, $df = 1, 383$, $p<0.0011$) (Fig. 4). *Triodanis perfoliata* L., a weed found only during the early growing season, supported the most mean number of vector species (2.22 ± 1.01) followed by *Taraxacum officinale* (1.6 ± 0.33), *Oenothera laciniata* Hill (0.85 ± 0.38) and *Trifolium repens* L. (0.6 ± 0.18) (Figs. 5 & 6). Flower crops supporting the most mean vector species were *Centaurea cyanus* L. (2.2 ± 1.15), *Chrysanthemum spp.*(1.0 ± 0.58), *Antirrhinum majus* L. (1.0 ± 1.0), *Matricaria recutita* L. (1.0 ± 0.58), *Lilium - Asiatic hybrid* (1.0 ± 1.0), and *Achillea millefolium* L. (1.0 ± 0.6) (Fig. 7).

## Discussion

The results from this study show that there are regional differences in the abundance of different thrips species in North Carolina cut flower production. Because field cut flowers have only emerged in the last fifteen years as a commodity in North Carolina, there were few flower farms located in the western portion of the state and thus, this region was not included in the study. The prominent species captured in both the Piedmont and Costal Plain regions was the non-vector species *F. tritici.* Although the number of reported vectors of TSWV and

INSV caught on traps in both regions was relatively low compared to *F. tritici* catches, the Coastal Plain trap catches yielded 60% more vector species than the Piedmont region. Of those two vector species, *F. fusca* was predominate. These results are similar to previous studies by Groves *et al.* (2003), who reported that *F. fusca* appears to be the primary TSWV vector in the Piedmont and Coastal Plain North Carolina regions. Eckle *et al*. (1996) also reported that few *F. occidentalis* were found in the Coastal Plain region. Other than *T. tabaci*, the remaining thrips species compositions were relatively similar for both locations. In our study, *T. tabaci* were more predominant in the Coastal Plain and were captured later in the growing season, which is different from previous studies. Eckle *et.al*. (1996) found higher numbers of *T. tabaci* earlier in the growing season in the Piedmont region, and did not find many *T. tabaci* in the Coastal Plain. The differences in *T. tabaci* density and distribution, particularly later in the season, may be explained by thrips feeding preferences for cut flowers or because this species migrated to cut flowers from recently harvested crops in other cropping systems.

Although there were more thrips found in organic production systems compared to conventional production, there was no significant difference in vector species distribution between the two production systems. Salguero-Navas *et al.* (1991) found *F. occidentalis*, *F. fusca*, and *F. tritici* in tomato flowers but rarely on other plant parts. They suggested that insecticides may have reduced thrips populations in other plant structures. Also, Chau *et al.,* 2005, found that thrips disperse as flowers begin to open and that floral structures protect thrips from insecticides. Thus, conventional farms using insecticides may reduce dispersing thrips numbers on trap catches, but *F. occidentalis*, *F. fusca*, and *F. tritici* species are

attracted to floral parts which may explain the similarity of species composition between these two production systems.

Observed thrips population densities were similar to other North Carolina studies. Eckle *et al.* (1996), documented peak densities of *F. fusca* and *F. occidentalis* occurring from mid-May to early June in tobacco, tomato, and pepper crops. Groves *et al*. (2001) found that *F. fusca* and *F. occidentalis* began to disperse from weeds containing TSWV innoculum in early April, with peak flights occurring in mid to late May. In our study, the estimated peak flight occurred between early to mid June (Fig. 2). However, unseasonably cool temperatures at the beginning of this study may have resulted in a later dispersal flight. In fact, growers reported a two-week delay in their initial floral crop harvests while this study was taking place. Thus, it can be concluded that thrips species diversity is consistent among most field crops across the central and eastern portions of North Carolina.

A positive correlation was found between the number of thrips caught on sticky traps and the number collected using the individual flower sampling methods in field cut flower production. These findings are consistent with Cloyd & Sadof (2003), who found a significant correlation between thrips abundance on sticky cards with number of thrips in flowers in cut carnation greenhouse production. Unlike other field crops such as peanut and tobacco that are planted in the early spring and harvested in the summer, field cut flowers can be grown in the field for eight months of the year. For example, dutch iris (*Iris hollandica*) bulbs may be planted in the fall and flowers are harvested in the early spring. After harvest of the irises, later blooming crops such as sunflowers (*Helianthus annus* L.) or zinnia (*Zinnia elegans* L.) may be planted in the space once occupied by the irises. Also, a farmer may choose to maximize cultivar availability by staggering planting dates. In cut flower

production, tospovirus management may be complicated and longer in duration than in other cropping systems due to the continual availability of young, susceptible crops in the field as thrips may disperse to new plants shortly after harvest. In New York, Shelton & North (1986) found that thrips moved onto cabbage when nearby grain crops senesced or forage crops were cut. Utilizing sticky traps in field production may provide a useful tool when scouting for tospovirus vector prevalence in order to develop control strategies that limit tospovirus infection because there is a positive correlation to thrips found on cards compared to thrips in flower blooms. Growers may use sticky traps to monitor for seasonal peak flights and may incorporate strategies to reduce thrips numbers during those times. In other field crops, studies have suggested that pest management strategies can be employed during peak dispersal flights of vectors in order to reduce the risk of tospovirus infection in highly susceptible young plants (Salguero-Navas *et al.*, 1991).

There have been numerous studies involving host plant selection by flower thrips, particularly with *F. occidentalis.* For example, Chaisuekul & Riley (2005) found that *F. occidentalis* and *F. fusca* had different ovipositional preferences when given a choice between tomato and chickweed. Also, Allen & Matteoni (1991) observed plant feeding preferences with *F. occidentalis* in common greenhouse crops. Indirectly, these crops may influence species distribution because of thrips species feeding and ovipositional preferences for different crops. Yudin *et al.* (1988) found that all hosts of *F. occidentalis* contained twice as many thrips when flowers were present than those plants of the same age with the flowers removed. They also found that the least preferred pre-flowering host (*Verbesina encelioides* (Cav.) Benth. & Hook. f. ex Gray) supported 60 times more thrips when flowering. In similar studies involving *F. occidentalis*, similar results were found in chrysanthemum (de

Jager *et al*., 1993) and between flowering and non-flowering weed species (Bautista & Mau, 1994).

UV reflectance also plays a role in thrips host selection. Matteson & Terry (1992) found two peaks in photo reception occurring at 365 nm in the UV region and 540 nm in the green-yellow region with *F. occidentalis*. They also suggested that thrips first use this peak sensitivity to yellow-green in long-range orientation to plants and then use contrasts within plants to find flowers. They also suggested that thrips are able to locate blue spectral hues when both UV and yellow-green receptors are initiated. However, some thrips species show no preference for color such as *L. cerealium* (Lewis, 1997).

The results of our study also indicate that there are significant differences in vector species populations (*F. fusca* and *F. occidentalis*) seasonally and between weed and flower crop host plants. There was no difference between numbers of vector species found between the Piedmont and Coastal Plain regions. These finding are consistent with previous North Carolina studies in other cropping systems where no differences in vector numbers existed regionally (Groves *et al.*, 2001, Eckle *et al*., 1996). This shows that thrips species distribution in cut flower production is similar to other cropping systems.

Vector species overwinter on numerous weeds (Toapanta *et al.,*1996) and thrips from these hosts may disperse to crop plants as they become available (Groves et al. 2001). Groves *et al.* (2002) suggested that innoculum is able to remain in the field after the initial vector dispersal to crop plants via summer weed hosts. In order for a weed to serve as a bridge host between seasons of winter weeds, these plants must be susceptible to TSWV and/or INSV and able to support thrips reproduction (Groves *et al.,* 2001). We hypothesize that plants possessing continual blooms throughout the summer months may be more

attractive to thrips and thus, may contribute as another factor in identifying potential summer bridge innoculum sources. We chose to sample only the plant blooms because previous studies have shown that thrips are more likely to inhabit flowers than other plant parts (Cho *et al.*, 2000, Hansen, 2000, Salguero-Navas *et al.,* 1991). de Jager *et al*. (1993) showed that pollen production per flower head differs significantly among chrysanthemum cultivars and that cultivars with higher pollen production do not always support higher number of *F. occidentalis*. Gerin *et al*. (1999) found that flowers were a restrictive factor in *F. occidentalis* population growth and that flowers could serve as a mating/meeting site (Rosenheim *et al*., 1990). Thus, we also hypothesize that thrips densities may be attributed to floral preferences unique to specific plant species.

Two of the four weeds, *T. officinale* and *O. laciniata*, hypothesized in Groves' 2002 study serving as both an innoculum source and supporting vector reproduction, possess blooms throughout the summer months. Based on these findings, we predicted that cut flower cultivars might also serve as summer bridge plant hosts of TSWV or INSV innoculum. Few studies have documented differences in cultivar bloom preference, but in Greece, Chatzivassiliou *et al.* (2000) found that 38.2% of field field-grown *Zinnia elegans* tested positive for TSWV, possessed both adult and immature stages of *F. occidentalis*, and that 12.9% of thrips collected from infected *Z. elegans* were able to transmit the virus. In Chatzivassiliou's study, TSWV infection of some field grown cultivars reached 100%. Although we found no significant differences in thrips preference and floral crop plants, mere presence may present an opportunity for innoculm to be maintained. In our study, floral crop plants with the highest mean number of vector species were *Centaurea cyanus*, *Chysanthemum spp., Antirrhinum majus, Matricaria recutita, Lilium - Asiatic hybrid,* and

*Achillea millefolium.* All of these aforementioned species are capable of TSWV and/or INSV infection, but it is unknown if these species are able to support vector populations. Future studies involving vector floral preferences, vector development, and TSWV/INSV infection prevelence between common cut flower cultivars in North Carolina may be beneficial in tospovirus management.

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# APPENDICES

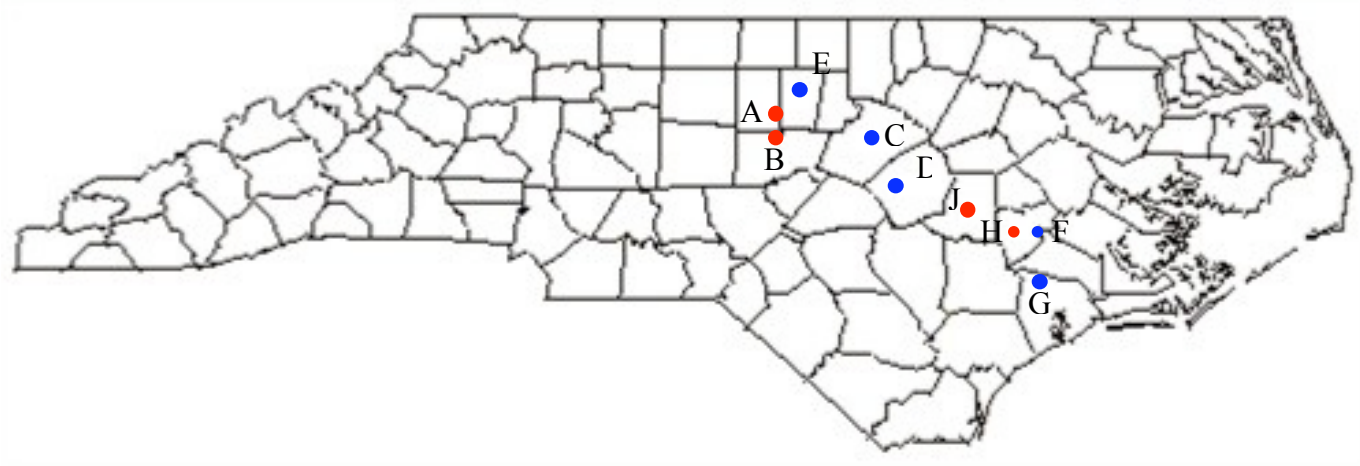


Figure 1: Map of North Carolina with farm locations. Red dots show organic farms and blue dots show conventional farms.

Farm A (Graham, Alamance Co.), farm B (Chapel Hill, Chatham Co.), farm C (Raleigh, Wake Co.), farm D (Clayton, Johnston Co.), farm E (Efland, Orange Co.), farm F (Kinston, Lenoir Co.), farm G (Jacksonville, Onslow Co.), farm H (LaGrange, Lenoir Co.), farm J (Goldsboro, Wayne Co.)

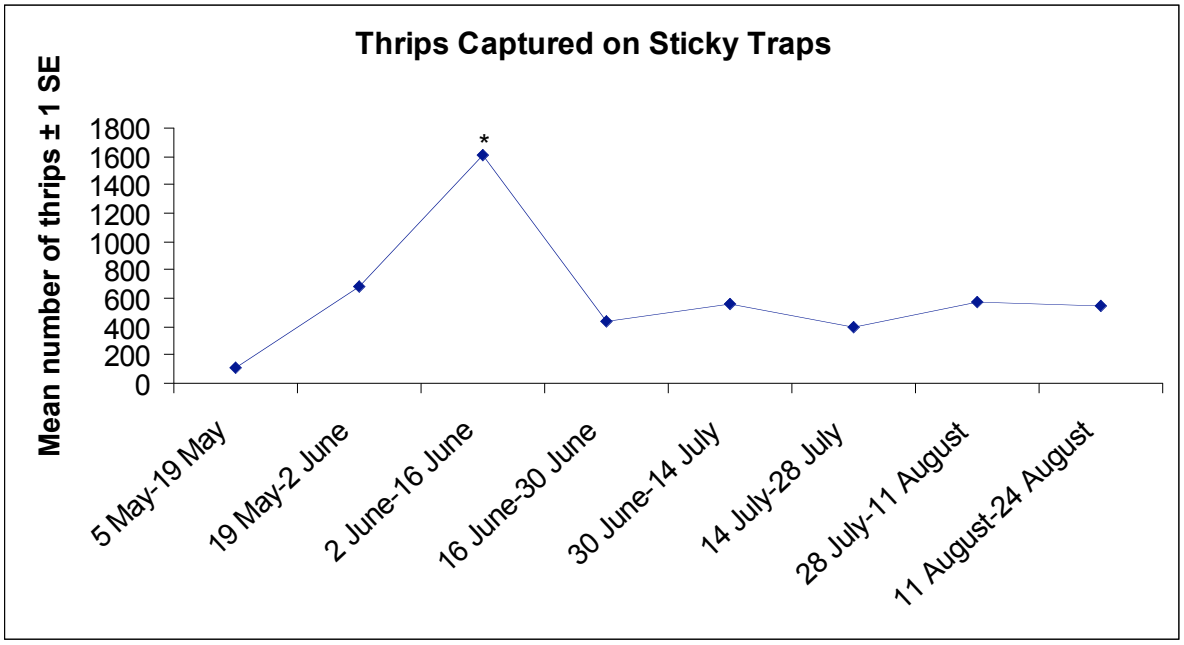


Figure 2: Thrips densities sampled at all locations

* = significantly different
($F = 5.3$, $df = 7, 46$, $p<0.0002$)

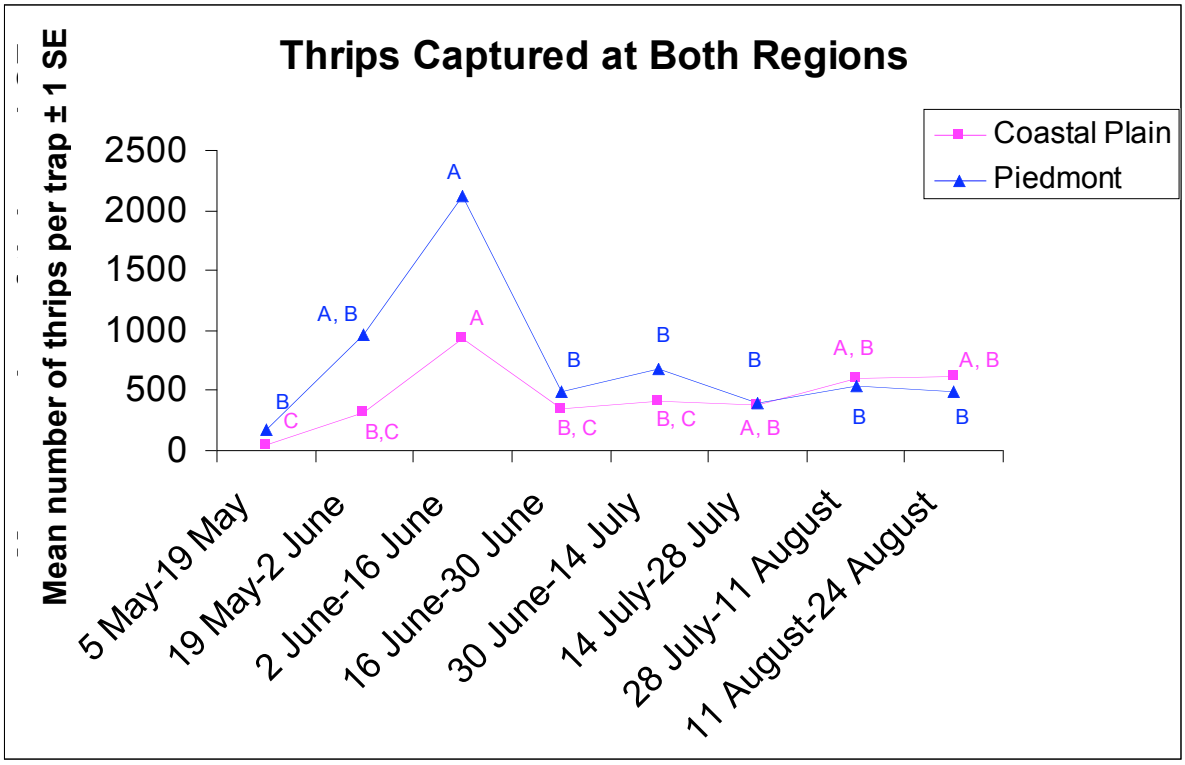


Figure 3: Thrips densities sampled at Piedmont and Coastal Plain locations

Piedmont region: ($F = 4.31$, $df = 7, 23$, $p<0.0035$), Coastal Plain region ($F = 7.87$, $df = 7, 15$, $p<0.0004$). Levels not connected by the same letter are significantly different.

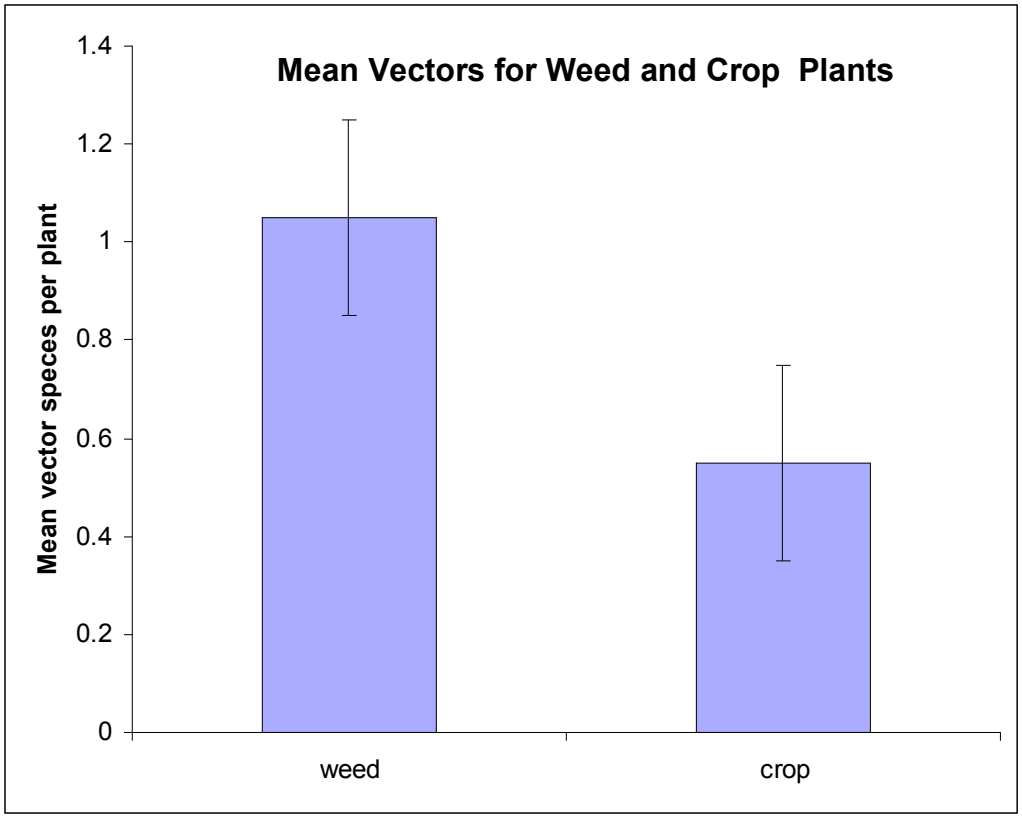


Figure 4: Mean vectors for weed and crop plants

Weeds harbored more vector species than crop plants ($F = 10.89$, $df = 1, 382$, $p = 0.0011$).

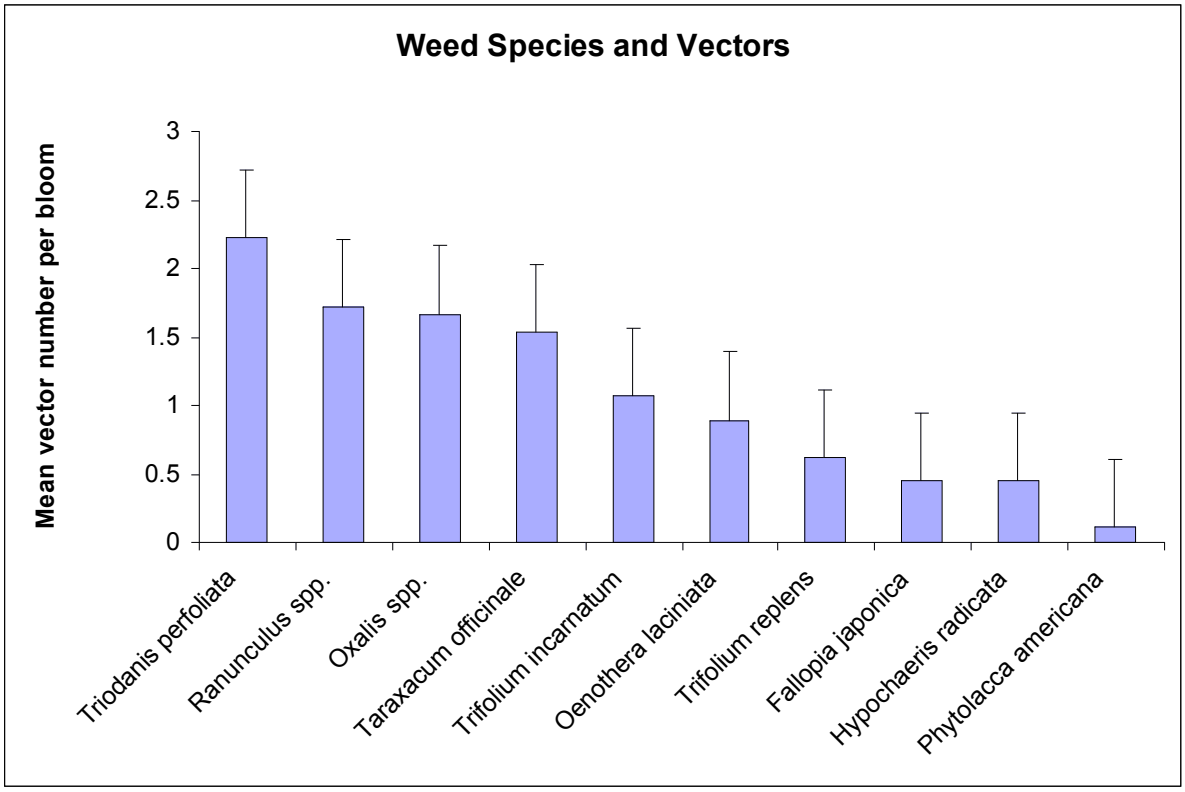


Figure 5: Mean number of vectors on weed species

*T. perfoliata* harbored significantly more vector species than *O. laciniata*, *T. replens , F. japonica, H. radicata,* and *P. americana* ($p<0.0477$)

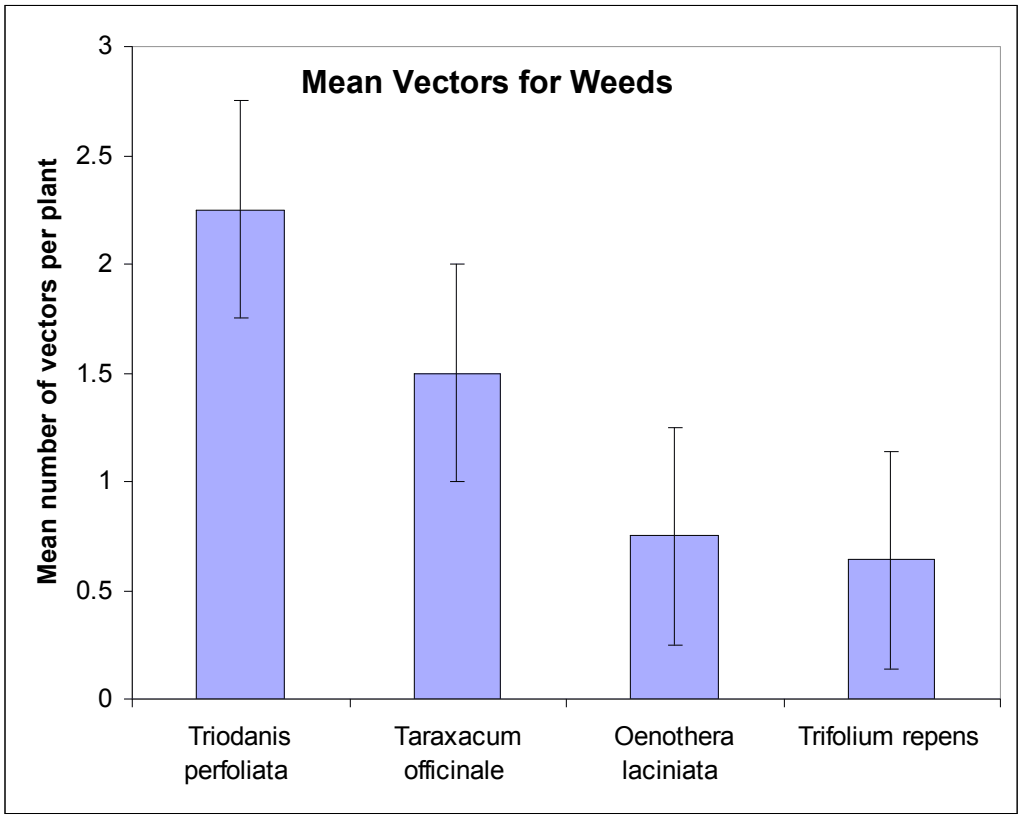


Figure 6: Susceptible weeds supporting vector reproduction

*T. repens* harbored significantly less vectors than other weed species ($p<0.0387$)

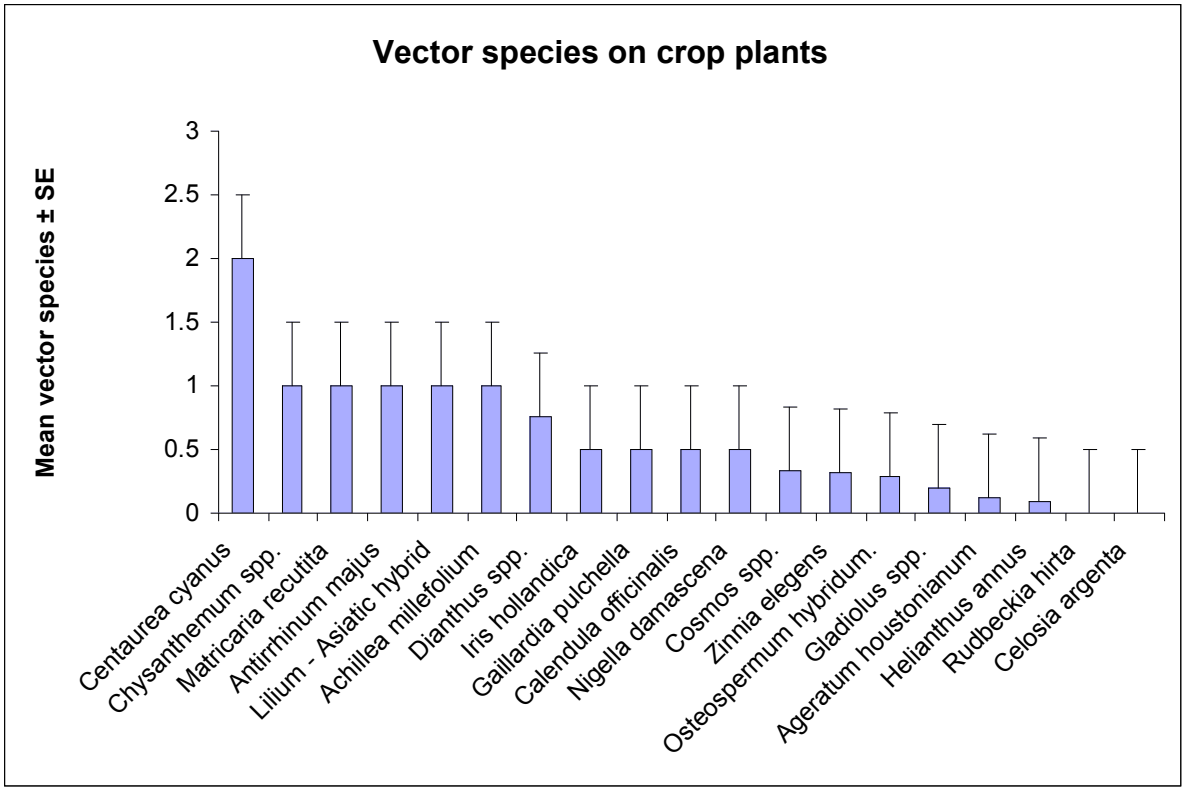


Figure 7: Mean number of vectors on crop plant species

There was no significant difference in vector species on crop plants ($p<0.1$)

Table 1: Weed species and TSWV/INSV infection

| **Plant family**<br>**Species** | **TSWV infection** | **INSV infection** | **Location** |
|---|---|---|---|
| Campanulaceae | | | |
| *Triodanis perfoliata* | Groves *et al.*, 2002 | | **A**, D, F, **H** |
| Compositae | | | |
| *Taraxacum officinale* | Stobbs *et al.* 1992 | | **A**, **B**,C, D, E, G, **J** |
| *Hypochaeris radicata* | | | D, F, G, **H**, **J** |
| Fabaceae | | | |
| *Trifolium incarnatum* | Edwardson & Christie, 1997 | | **A**, **B**, C, E |
| *Trifolium replens* | Stobbs *et al.* 1992 | | **A**, **B**, C, D, E, F, G |
| Lamiaceae | | | **B** |
| *Lamium purpureum* | Chatzivassiliou *et al.* 1998 | | **A**, **B** |
| Onagraceae | | | |
| *Oenothera laciniata* | Groves *et al.*, 2002 | | **A**, **B**, C, F, G, **H**, **J** |
| Oxalidaceae | | | |
| *Oxalis spp.* | | Hausbeck *et al.* 1992 | **A**, G, **J** |
| Phytolaccaceae | | | |
| *Phytolacca americana* | | | **B**, D, **H**, **J** |
| Polygonaceae | | | |
| *Fallopia japonica* | Kaminska & Korbin, 1994. | | **A**, **B**, C, F, G |
| Ranunculaceae | | | |
| *Ranunculus spp.* | | Hausbeck *et al.* 1992 | **A**, **B**, D |

Farm A (Graham, Alamance Co.), farm B (Chapel Hill, Chatham Co.), farm C (Raleigh, Wake Co.), farm D (Clayton, Johnston Co.), farm E (Efland, Orange Co.), farm F (Kinston, Lenoir Co.), farm G (Jacksonville, Onslow Co.), farm H (LaGrange, Lenoir Co.), farm J (Goldsboro, Wayne Co.). Letters in bold represent organic farms.

Table 2: Crop plant species and TSWV/INSV infection

| Plant family<br>Species | TSWV infection | INSV infection | Location |
|---|---|---|---|
| Amaranthaceae | | | |
| *Celosia argenta* | Adam & Kegler, 1994 | | **A**, D, **J** |
| Asteraceae | | | |
| *Achillea millefolium* | Gognalons *et al.*, 1996 | | **A**, F |
| *Ageratum houstonianum* | Ruter & Gitaitis 1993 | Roggero *et al*. 1999 | **A**, **B**, C, D, E |
| *Calendula officinalis* | Tehrani *et al*. 1990 | | **B** |
| *Centaurea cyanus* | Stobbs & Broadbent, 1992 | | **A** |
| *Chysanthemum spp.* | Calpas & Penner, 1993 | | F |
| *Cosmos spp.* | | | **B** |
| *Gaillardia pulchella* | | Marchoux *et al.* (unpublished) | G |
| *Helianthus annus* | Edwardson & Christie,1997 | | **A**, C, D, F, G, **H**, **J** |
| *Matricaria recutita* | Chatzivassiliou *et al.* 1998 | | **H** |
| *Osteospermum hybridum.* | Hausbeck *et al.* 1992 | | C |
| *Rudbeckia hirta* | Schuster & Halliwell 1994 | | A |
| *Zinnia elegens* | Tehrani *et al.* 1990 | de Avila *et al*., 1992 | **A**, **B**, C, D, E, G, **H**, **J** |
| Caryophyllaceae | | | |
| *Dianthus spp.* | Ghotbi *et al.*, 2005 | Ghotbi *et al.,* 2005 | **B**, C, E, F, G |
| Iridaceae | | | |
| *Iris hollandica* | Derks & Lemmers 1996) | Derks & Lemmers 1996) | **B** |
| *Gladiolus spp.* | Derks & Lemmers 1996) | Derks & Lemmers 1996) | G |
| Liliaceae | | | |
| *Lilium - Asiatic hybrid* | Hausbeck *et al.* 1992 | Hausbeck *et al*. 1992 | F, G, **H** |
| Ranunculaceae | | | |
| *Consolida ajactis* | | | D |
| *Nigella damascena* | | | D |
| Scrophulariaceae | | | |
| *Antirrhinum majus* | Tehrani *et al.* 1990 | Daughtrey, 1996 | C, D, G |

Farm A (Graham, Alamance Co.), farm B (Chapel Hill, Chatham Co.), farm C (Raleigh, Wake Co.), farm D (Clayton, Johnston Co.), farm E (Efland, Orange Co.), farm F (Kinston, Lenoir Co.), farm G (Jacksonville, Onslow Co.), farm H (LaGrange, Lenoir Co.), farm J (Goldsboro, Wayne Co.) Letters in bold represent organic farms.

Table 3: Thrips species identified in Coastal Plain region across all dates.

| | *F. Fusca* | *F. occidentalis* | *F. tritici* | *Thrips tabaci* | Phlaeothripidae | *L. cerealium* | *N. variabilis* |
|---|---|---|---|---|---|---|---|
| 5 May- 19 May | 9.13 ± 5.15 | 1.55 ± 0.72 | 24.25 ± 13.15 | 4.52± 3.39 | 0.53 ± 0.38 | 9.83 ± 8.85 | 6.33 ± 3.58 |
| 19 May- 2 June | 17.0 ± 5.42 | 1.53 ± 0.79 | 236.66 ± 22.21 | 6.24± 0.85 | 1.77 ± 1.17 | 10.68 ± 4.01 | 43.77 ± 1.27 |
| 2 June-16 June | 99.76 ± 29.64 | 0 | 649.49± 143.63 | 72.1± 32.55 | 19.63 ± 6.25 | 63.47 ± 32.96 | 29.2 ± 13.09 |
| 16 June-30 June | 12.64 ± 1.4 | 0.52 ± 0.52 | 264.73± 21.32 | 85.33± 42.81 | 2.67 ± 0.55 | 5.99 ± 3.31 | 6.7 ± 3.58 |
| 30 June- 14 July | 11.76 ± 4.98 | 0 | 176.96± 10.91 | 205.39± 95.17 | 5.9 ± 1.95 | 4.03 ± 2.47 | 6.61 ± 3.78 |
| 14 July-28 July | 3.91 ± 1.73 | 0.47 ± 0.47 | 128.89± 15.25 | 221.6± 163.4 | 6.41 ± 2.04 | 0 | 37.8 ± 0.7 |
| 28 July- 11 August | 4.42 ± 0.98 | 0.49 ± 0.49 | 245.4± 74.1 | 241.8± 107.33 | 12.22 ± 7.75 | 8.1 ± 4.63 | 101.2 ± 36.96 |
| 11 August- 24 August | 0.81 ± 0.81 | 1.13 ± 1.13 | 312.03± 3.62 | 195.67± 25.0 | 8.15 ± 2.87 | 4.23 ± 3.1 | 93.37 ± 32.51 |

Table 4: Thrips species identified in Piedmont region across all dates.

| | *F. Fusca* | *F. occidentalis* | *F. tritici* | *Thrips tabaci* | Phlaeothripidae | *L. cerealium* | *N. variabilis* |
|---|---|---|---|---|---|---|---|
| 5 May- 19 May | 14.21 ± 6.76 | 3.35 ± 1.81 | 115.5 ± 42.09 | 10.5 ± 5.8 | 1.76 ± 0.12 | 2.78 ± 1.53 | 17.62 ± 9.41 |
| 19 May- 2 June | 28.37 ± 8.15 | 6.84 ± 4.51 | 405.83 ± 75.52 | 2.48 ± 1.03 | 1.16 ± 1.16 | 0 | 68.38 ± 28.06 |
| 2 June-16 June | 66.74 ± 12.79 | 3.58 ± 3.58 | 1778.33 ± 570.26 | 40.89 ± 30.09 | 26.34 ± 11.24 | 28.76 ± 19.8 | 200.78 ± 67.92 |
| 16 June-30 June | 11.38 ± 3.78 | 0.42 ± 0.42 | 430.25 ± 69.93 | 3.9 ± 3.0 | 2.91 ± 1.6 | 2.37 ± 0.39 | 36.87 ± 14.09 |
| 30 June- 14 July | 16.39 ± 6.13 | 0 | 590.56 ± 81.63 | 18.75 ± 13.52 | 6.24 ± 3.14 | 12.61 ± 11.06 | 38.85 ± 22.49 |
| 14 July-28 July | 2.04 ± 0.99 | 0 | 366.67 ± 61.78 | 7.16 ± 4.78 | 3.47 ± 1.32 | 0.86 ± 0.86 | 17.96 ± 11.22 |
| 28 July- 11 August | 1.88 ± 1.08 | 0 | 466.36 ± 130.07 | 31.91 ± 13.99 | 6.65 ± 3.13 | 3.2 ± 2.26 | 32.85 ± 9.17 |
| 11 August- 24 August | 3.07 ± 2.48 | 0 | 356.92 ± 62.86 | 86.05 ± 48.08 | 2.74 ± 2.74 | 9.91 ± 9.39 | 26.06 ± 10.44 |

CHAPTER 3

# Evaluation of thrips Feeding Preferences of indicator plants and other crop plants as a Tospovirus Management Tool

THE TOSPOVIRUSES impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV) have caused significant economic loss in the greenhouse industry (Daughtrey *et al.,* 1997, Fiedorow, 1999). *Frankliniella fusca* (Hinds) and *Frankliniella occidentalis* (Pergande) have been determined to be the primary thrips vector species of both TSWV and INSV in North Carolina (Eckle *et al.*, 1996, Daughtrey *et al.*, 1997, Naidu *et al.*, 2001). *F. occidentalis*, western flower thrips (WFT), is considered to be the primary vector in greenhouse floriculture production (Daughtrey *et al.*, 1997) while *F. fusca* is the primary vector in field crop production (Eckle *et al.*, 1996). The symptoms of TSWV and INSV are variable and differ between time of year (field crops), age of plant, plant species, and virus strain (Jones & Baker, 1991). Typical symptoms of both viruses include necrotic spots, stunting, and/or wilting of the host plant, but virus detection can be difficult because tospovirus symptoms often mimic symptoms associated with fungal, bacterial, or nutritional disorders (Ullman *et al.,* 1998). Some infected plants are asymptomatic while in other plants, symptoms may take several weeks to develop (Allen & Matteoni, 1991). Since there is no cure for INSV or TSWV, infected plants must be discarded, so, early detection of virus may reduce economic losses. Enzyme-linked immunosorbent assay (ELISA) quick test kits for detection of INSV and TSWV in plants are commercially available, and are useful to confirm that a plant is infected rather than having another disease or nutritional disorder. However, it

would be more useful for growers to know that viruliferous thrips were present before disease symptoms are observed. The use of indicator plants may alert the grower to the presence of viruliferous thrips before plant infection so that immediate tospovirus management strategies can be implemented, reducing economic loss.

Inexpensive indicator plants can be used to alert growers to the presence of viruliferous thrips because these plants show symptoms within two to seven days, while crop plants may take two weeks or more to become symptomatic (Ullman *et al.*, 1998, Allen & Matteoni, 1991). Allen & Matteoni (1991) describe the ideal indicator plant to be highly attractive to the vector when in competition with other crops, express characteristic viral symptoms quickly, not become systemically infected, and to be widely available, easily propagated, and easy to maintain. In their study, they identified petunia (*Petunia* x *hybrida* 'Calypso') as a successful indicator compared to seven other potential indicator plants. Growers have been reluctant to use petunia indicator plants primarily because there is little evidence to support thrips feeding preference for petunia compared to other crop plants. Also, germination of petunia seed can take over a month and plants can be difficult to maintain. Fava bean (*Vicia faba* L.) has been suggested as a potential indicator for INSV-infected thrips because germination takes three to four days and necrotic lesions develop within three to four days when exposed to viruliferous thrips feeding (Casey, 2000). However, relative feeding preferences of WFT for petunia, fava bean, and key greenhouse flower crops are unknown.

The first objective of this study was to determine the relative feeding preference of western flower thrips for two indicator plant species, petunia and fava bean. The second

objective was to determine the relative feeding preference of western flower thrips between indicator plant feeding and other crop plant feeding. The third objective was to evaluate feeding preferences for different petunia varieties and the fourth objective was to evaluate viral expression among petunia varieties.

## Materials and Methods

**Plant and insect materials**. Test plants were either grown from seed or were asexually propagated. Seed from petunia varieties 'Purple Wave', 'White Swan', 'Red Halo', 'Dreams-Midnight Blue', and heirloom varieties 'Mix' and 'White Bedder'; fava bean 'Aqua Dulce'; and garden impatiens (*Impatiens walleriana* Hook, f.) 'Busy Lizzy' were planted separately into 6-inch (15.2 cm) plastic pots containing Sun Gro metro-mix 200 (Sun Gro Horticulture Canada CM Ltd.). Only one seed was planted in each pot. The pots were held under greenhouse conditions at 27 ± 1° C. Once germination occurred, a thrips exclusion cage was placed over each potted plant to prevent insect contamination and feeding. The cages were made from two-liter clear plastic bottles with the bottoms cut off. The large opening at the bottom of the bottles was buried about 2 cm into the soil. To allow for ventilation, a 6 $cm^2$ hole was cut into the side of the cage and thrips-proof screening (Bioquip, Rancho Dominguez, CA) was glued into place to cover the hole. Thrips proof screening was also attached to the tops of the thrips exclusion cages. Test plants not grown from seed were purchased from a local nursery several weeks prior to experimentation. These plants were placed in isolation in thrips-proof cages to insure that there was no prior or current insect feeding damage. When possible, cuttings of these plants were rooted in water

and placed in a thrips-proof cage to encourage new growth without possible insecticide contamination. Plants that could not be asexually propagated were cut back to encourage new growth so that new leaf material without possible insecticide contamination could be obtained.

Thrips for the experiments were provided from laboratory colonies reared on bean pods (*Phaseolus vulgaris* L.) held on a laboratory bench at a constant temperature of 24 ± 1 °C under fluorescent lighting 16:8 (L:D) h. The thrips culture was originally started with adults that were collected from an infestation on chrysanthemum found in a greenhouse on the North Carolina State University campus in 1998 and the colony was subsequently infused with thrips from flowers of *Capsicum annuum* L. found in Sampson Co., NC in 2002. Viruliferous thrips were infected by feeding first-instar larvae with TSWV infected *Emilia sonchifolia* (L.) DC. var. *javanica* (Burm. f.) Mattf.. The TSWV isolate RG2 was isolated from a field-infected tobacco plant (*Nicotiana tabacum* L.) in Carteret Co., NC in 1997 and has been maintained in *E. sonchifolia* via *F. fusca* transmission.

All choice tests were conducted in 12-quart clear arcrylic containers with lids (Rubbermaid Commercial Products Inc., Winchester, VA) (Fig. 1). To allow for ventilation, 10 x 7 cm holes were cut into each lid. Thrips-proof screening was glued into place to cover the hole. Thick foam weather stripping, 3/16-inch (0.48 cm) (M-D Building Products, Inc, Oklahoma City, OK) was attached along the perimeter lid lip to prevent thrips escape.

**Choice Test Experimental Design.** Single leaves of relatively equal size were cut from each plant species. The stem was then inserted through a parafilm (American National Can, Neenah, WI) cover into a 5-dram vial of distilled water to maintain plant turgidity. A 6-cm²

square was cut from blue plastic (polystyrene plates, Solo Cup Company, Highland Park, IL) in order to attract thrips (Ullman, *et al.*, 1998). A hole was punched at the center bottom of the square and the disk was attached to the vial using 6 mm blue pipe cleaners. The disks were oriented behind the leaf-cutting in the choice test arena. All experiments were conducted using a randomized block design with ten replicates. One plant of each of the species tested was placed in each replicate of the choice test arena. All experiments were performed on a laboratory bench under fluorescent lighting 16:8 (L:D) h at a constant temperature of 24 ± 1 °C. At the end of the experiment, a video computer image analysis system (CIAS) was used to measure the total leaf area (Sorenson *et al.*, 2000) and damage was assessed as scars per cm². Each scar was defined as a ~1 mm² area of silvery tissue (Agrawal *et al.*, 1999). The feeding scars per cm² data were square-root transformed to normalize distribution and analyzed via ANOVA. Means were separated using Tukey-Kramer HSD in all choice tests except for choice tests involving petunia varieties (SAS JMP 6, Cary, North Carolina, 2005).. Means presented in results and figures were not transformed.

The following choice tests were performed: choice test between two indicator plant species, choice test between two indicator plants and four crop species, choice test between two indicator plant species and six crop species, choice test between six petunia varieties with non-viruliferous thrips, and choice test between six petunia varieties with viruliferous thrips.

**Experiment 1: Thrips feeding preference between two indicator plant species.** The first choice test was performed to determine thrips feeding preferences between two accepted indicator plants, petunia 'Mix' and fava bean. This test was performed in order to

determine thrips feeding preferences when there was no competition with other crop plants. Detached leaves were obtained from different plants of the mother stock. The randomized block design consisted of two treatments were blocked by similar leaf size with ten replicates of each indicator plant species. Plants were randomly assigned locations within each study arena. Approximately 10 female non-viruliferous WFT were released in the center of the container and the number of feeding scars per leaf was recorded at 1d, 2d, 4d and 7d intervals.

**Experiment 2: Thrips feeding preference between two indicator plant species and four crop plant species.** The second choice test was performed to evaluate thrips feeding preferences between indicator plants (petunia and fava bean) and other crop plants: chrysanthemum (*Chrysanthemum morifolium* Ramat), impatiens (*Impatiens walleriana*), gloxinia (*Sinningia speciosa* cv.), and begonia (*Begonia rex* hybrids). Detached leaves were obtained from different plants of the mother stock. The six treatments were blocked by similar leaf size with ten replicates. Plants were randomly assigned locations within each study arena. Approximately 10 female non-viruliferous WFT were released in the center of the container and the number of feeding scars per leaf was recorded at 1d, 2d, 4d and 7d intervals.

**Experiment 3: Thrips feeding preference between two indicator plant species and six crop plant species.** The third choice test performed expanded the number of crop plants to include New Guinea impatiens (*Impatiens hawkeri* Hook. f.), and kalanchoe (*Kalanchoe blossfeldiana* Poelln.) in addition to the plants in second choice test experiment. Detached leaves were obtained from different plants of the mother stock. The eight treatments were blocked by similar leaf size with ten replicates. Plants were randomly

assigned locations within each study arena. Approximately 10 female non-viruliferous WFT were released in the center of the container and the number of feeding scars per leaf was recorded at 1d, 2d, 4d and 7d intervals.

**Experiment 4: Thrips feeding preference between six petunia varieties.** The fourth choice test was performed to determine thrips feeding preferences among six petunia varieties: 'Purple Wave', 'White Swan', 'Red Halo', 'Dreams–Midnight Blue', and heirloom 'Mix' and 'White Bedder' petunia. Detached leaves were obtained from different plants of the mother stock. The six treatments were blocked by similar leaf size with ten replicates. Plants were randomly assigned locations within each study arena. Approximately 10 female non-viruliferous WFT were released in the center of the container and the number of feeding scars per leaf was recorded at 1d, 2d, 4d and 7d intervals.

**Experiment 5: Viruliferous thrips feeding preference between petunia varieties.** A fifth choice test experiment was performed using the same petunia varieties in the fourth choice test, but viruliferous thrips were used to determine if feeding preferences differed between viruliferous and non-viruliferous thrips. Viruliferous adult thrips were obtained by placing first instar larvae into a 100 X 15 mm Perti dish (Becton Dickinson Labware, Franklin Lakes, NJ) containing a TSWV-infected *Emilia sonchifolia* leaf and after a 48 h acquisition period were transferred to healthy green bean pods to complete development (Abamburu *et al.*, 2000).

## Results

**Experiment 1: Thrips feeding preference between two indicator plants, petunia and fava bean.** Petunia had significantly more feeding scars per $cm^2$ than fava bean ($F = 15.14$,

df = 1, 76, $p<0.0002$) across all times. When separated by time, the means between the two treatments were significant at four days ($F=10.52$, df = 1,18, $p<0.0045$) with a mean of 6.25 ± 1.86 feeding scars per cm² of leaf area for petunia and 1.28 ± 10.5 for fava bean and at seven days ($F = 10.74$, df = 1,17, $p<0.0044$) 10.84 ± 12.34 for petunia and 1.28 ± 10.5 for fava bean (Fig. 2).

**Experiment 2: Thrips feeding preference between two indicator plant species and four crop plant species.** In the second choice test comparing indicator plants (petunia and fava bean) and four crop plants, the indicator plant (petunia) and the crop plant (begonia) had significantly more feeding scars than all other plants tested ($F = 12.02$, df = 5, 234, $p<0.0001$) across all times. When separated by time, significant differences between the six treatments were observed at two days ($F = 4.52$, df = 5, 54, $p<0.0016$) with begonia having significantly more feeding scars than all other treatments except for petunia. Differences were more profound at seven days ($F = 5.81$, df = 5, 54, $p<0.0002$) with petunia measuring 15.2 ± 3.31 feeding scars per cm² of leaf area and begonia measuring 13.76 ± 3.14 compared to the next highest mean measurement of 5.56 ± 3.5 feeding scars per cm² of leaf area for chrysanthemum (Fig. 3).

**Experiment 3: Thrips feeding preference between two indicator plant species and six crop plant species.** In the third choice test experiment involving indicator plants and six crop plants, petunia had significantly more feeding scars per cm² than all other plants tested ($F = 31.24$, df = 7, 312, $p<0.0001$) across all times. When separated by time, significant differences between the eight treatments were observed at day one ($F = 7.58$, df = 7, 72, $p<0.0001$) with petunia having significantly more feeding scars per cm² of leaf area than all other treatments. However, differences were measurable at 2 days ($F = 6.65$, df = 7, 72,

$p<0.0001$) with petunia measuring 3.25 ± 1.2 feeding scars per cm² of leaf area and fava bean measuring 1.32 ± 0.92 compared to the next highest mean measurement of 0.33 ± 0.33 feeding scars per cm² of leaf area for New Guinea impatiens (Fig. 4).

**Experiment 4: Thrips feeding preference between six petunia varieties.** Feeding preferences among petunia varieties with non-viruliferous thrips indicated that 'Mix' (heirloom) and 'Red Halo' varieties had significantly more feeding scars than all other varieties ($F = 10.37$, $df = 5, 234$, $p<0.0001$) across all times. Although feeding preferences showed significance between varieties when separated by time (Fig. 5), differences were apparent at four days ($F = 3.85$, $df = 5, 54$, $p<0.0046$) with 'Red Halo' measuring a mean of 11.7 ± 2.64 scars per cm² per leaf area and 'Mix' with 9.24 ± 1.154 compared to the next highest mean measurement of 3.79 ± 0.91 for 'Purple Wave'

**Experiment 5: Viruliferous thrips feeding preference between petunia varieties.** Feeding preferences among the same petunia varieties with viruliferous thrips indicated that there were no significant differences in feeding scars per cm² of leaf area for all petunia varieties ($F = 2.73$, $df = 5, 234$, $p<0.05$) across all times or when separated by time. Although feeding scars per cm² of leaf area were not significant, there were noticeable trends in mean number of scares per cm² at seven days. For example, 8.44 ± 2.64 feeding scars per cm² of leaf area were measured for 'Purple Wave' which was almost twice the number of of feeding scars per cm² than 'Mix' (4.01 ± 1.15), 'White Swan' (3.65 ± 1.24), or 'Red Halo' (3.22 ± 0.03) (Fig. 6). Of the ten replicates, the following varieties showed necrotic lesions and tested positive for TSWV (Table 1): 'White Bedder', 2 lesions on one leaf and 1 wilted leaf testing positive; 'Mix' (Heirloom), 2 lesions on one leaf; 'Purple Wave', 1 lesion on one leaf, 1 wilted leaf that tested positive; 'Dreams - Midnight Blue', 1 wilted leaf testing

positive; ‘White Swan’, 2 wilted leaves testing positive; ‘Red Halo’, 2 wilted leaves testing positive.

## Discussion

No studies have evaluated thrips feeding preferences for petunia indicator plants relative to other crop plants. Pundt (1991) found the petunia ‘Calypso’ to be a successful indicator for viruliferous thrips in greenhouse bedding production in New York State. Ullman *et al.* (1998) found that petunia indicator plants placed on a blue background in conjunction with directional sticky traps were useful in the detection of viruliferous thrips in outdoor ranunculus production. In Allen and Matteoni’s (1991) study, potential indicator plants were ranked as to the extent of feeding damage; pepper ranked the highest followed by petunia, tomato, amaranth, gloxinia, *Nicotiana glutinosa*, *N. benthemiana*, and *N. tabacum*. Although pepper ranked higher than petunia in feeding damage, it was not considered to be an ideal indicator plant because viral symptoms were difficult to detect during the early stages of infection.

Although petunia and fava bean have been determined to be acceptable indicator plants, there have been no studies conducted to determine thrips relative feeding preference for the two species. Neither petunia nor fava bean exhibit all of the characteristics of an ideal indicator plant as suggested by Allen and Matteoni (1991). Both indicators are capable of expressing virus symptoms quickly and both are widely available, yet both plants can be difficult to maintain. While petunia is consistently preferred by thrips relative to other crops, shows characteristic lesions, and is not systemically infected, germination can take up to six weeks. In contrast, fava bean is attractive to WFT in the presence of other crop plants and is

easily propagated, but is susceptible to fungal growth, which may be confused with viral lesions. Also, fava bean does become systemically infected and must be discarded if found infected. Because thrips show a significant feeding preference for petunia over fava bean, we conclude that petunia may be a more useful indicator plant as a tospovirus management tool than fava bean. However, significant differences in feeding preferences in choice tests between both indicator plants and among crop plants were observed only after four days suggesting that from a practical standpoint (for a grower utilizing this system), indicator plants should be checked for viral symptoms twice weekly.

Since petunia showed significant feeding damage compared to other indicator and crop plants, further studies were conducted to determine if there were thrips feeding preferences between petunia cultivars. As public demand grows for new petunia varieties each year, several of the petunia varieties suggested for use as indicator plants in previous scientific journal articles are no longer available. Discontinued varieties include: 'Calypso', 'Super Blue Magic', and 'Super Magic Coral', three of eight varieties suggested by Robb *et al.* (1998) and Daughtrey *et al.* (1995) as excellent indicator plants for the detection of tospoviruses. Other varieties suggested by Robb *et al.* (1998) and Daughtrey *et al.* (1995) that are still available are 'Blue Carpet', "Cascade Blue', 'Summer Madness', 'Burgundy Madness' and 'Red Cloud'. Many of these varieties are not available in retail seed catalogs and can only be special ordered commercially. Studies to determine thrips feeding preferences between these varieties have not been performed. Petunia varieties chosen in our study were based on retail availability. We were particularly interested in the open-pollinated (heirloom) 'Mix' and 'White Bedder' varieties because their availability is not likely to change over time, they have a compact growth habit, and are able to reseed. We

also found that the 'Mix' variety germinated within a few weeks, compared to other varieties that took much longer (unpublished data), thus eliminating the negative attribute of petunia not being easy to propagate. Although the 'Mix' variety only showed significant thrips feeding preference relative to four other petunia varieties tested with non-viruliferous thrips, this variety did exhibit characteristic TSWV viral lesions. The varieties 'Purple Wave' and 'White Bedder' also showed characteristic viral lesions and preferential viruliferous thrips feeding compared to other varieties. Presence of characteristic viral lesions and thrips feeding preference suggest these varieties are suitable as tospovirus indicator plants. The 'Mix' variety was significantly more attractive to non-viruliferous thrips when compared to other petunia varieties, but was not significantly attractive to viruliferous thrips. Possible reasons for the insignificant results in choice tests using viruliferous thrips may be due to thrips fitness when infected with TSWV. For example, Belliure *et al.* (2005) found that virus-infected plants were of higher quality for a potential vector's offspring than non-infected plants and that plant pathogens such as TSWV may have evolved in order to overcome plant defenses against potential vectors. Also, Maris *et al.* (2004a) found that when WFT were released on thrips-resistant or thrips-susceptible pepper (*Capsicum annuum* L.) phenotypes, thrips dispersed at significantly higher rates from the thrips-resistant phenotype. They also found that there was a negative effect on ovipostion and larval survival with the thrips-resistant phenotype compared to the thrips-susceptible phenotype.

Whitfield *et al*. (2005) suggests that different TSWV isolates may differ in pathogenicity to thrips and may also cause nutritional changes in host plants, thus affecting thrips mortality. It has also been suggested that some viral isolates such as TSWV-BR01

have no effect on WFT mortality (Maris *et al.,* 2004b) yet, another TSWV isolate was found to decrease the life span of infected thrips (Whitfield *et al.,* 2005). Stumpf and Kennedy (2005) examined the effect of two TSWV isolates (CFL and RG2) on *F. fusca*. They found differences in thrips survival between the two TSWV isolates when reared on different infected host plants (*Datura stramonium* and *Emilia sonchifolia*). Thrips survival significantly increased with thrips infected with CFL reared on *E. sonchifolia* compared to those reared on *D. stramonium*. However, the percent survival of non-infected thrips and thrips infected with RG2 decreased on *E. sonchifolia* compared to those reared on *D. stramonium*. The decrease of survival with thrips infected with the RG2 was slightly less than with non-infected thrips. These differences in host plant material combined with the effects on thrips of different TSWV isolates may explain why the feeding preferences of viruliferous thrips were less significant. Perhaps the RG2 isolate used in the experiment combined with the host plant species may have had a detrimental effect on the thrips and thus, limited thrips movement. Future studies involving different isolates of TSWV or INSV may further clarify the interaction of petunia indicator plants and thrips feeding preferences. Also, other heirloom cultivars have not been tested in addition to other commercially available cultivars. Therefore it is possible that other heirloom and hybrid petunia varieties are more attractive to thrips feeding and exhibit viral symptoms more frequently than 'Mix', 'White Bedder' and 'Purple wave'.

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# APPENDICIES

Figure 1: Choice test arena.

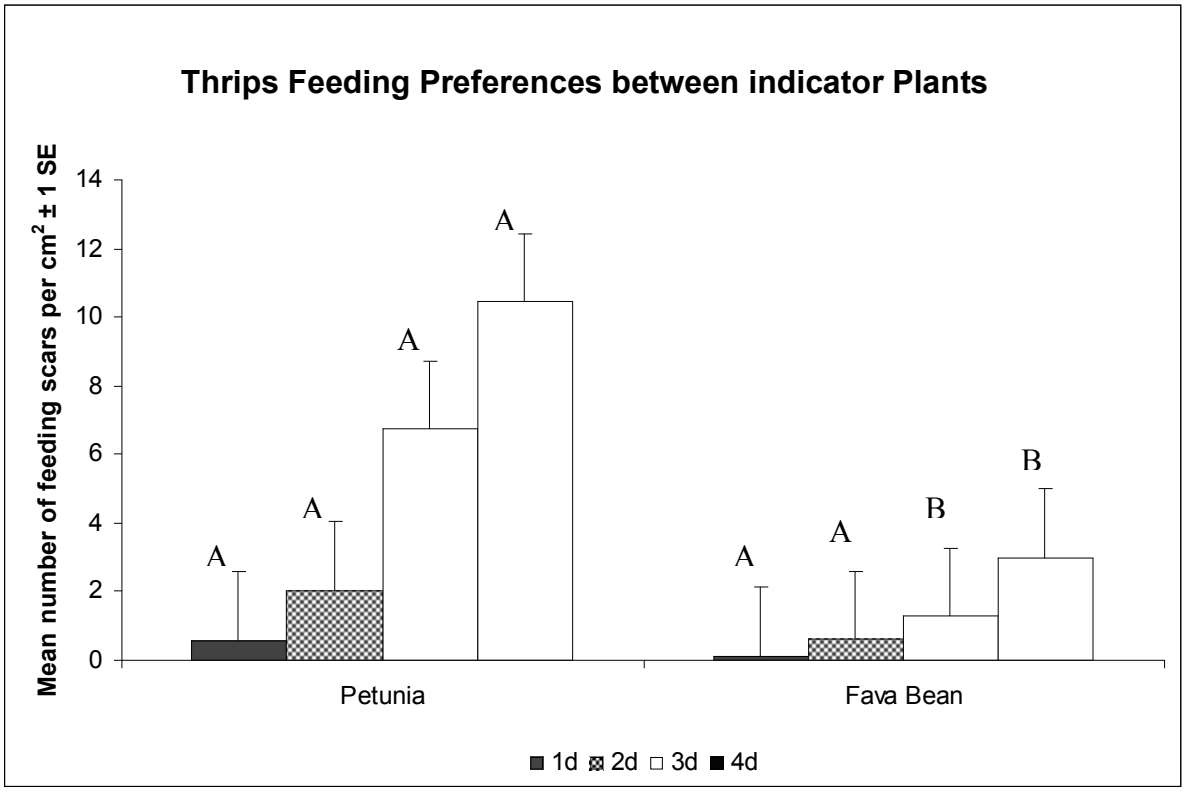


Figure 2: Western flower thrips feeding preferences between petunia and fava bean. Petunia exhibited significantly more feeding scars per cm² across all times than fava bean ($F = 15.14$, $df = 1$, $p<0.0002$).

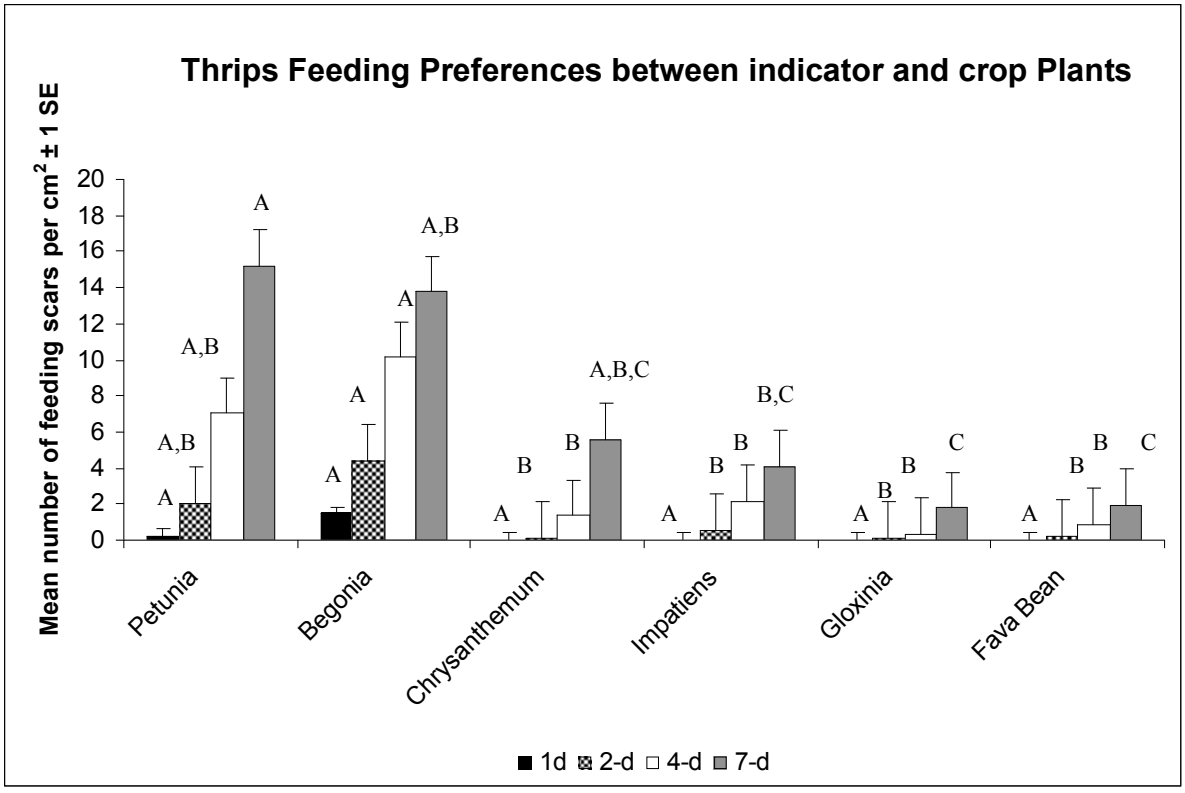


Figure 3: Western flower thrips feeding preferences between petunia, fava bean, begonia, chrysanthemum, garden impatiens, and gloxinia across all times. Petunia and begonia exhibited significantly more feeding scars than other crop plants ($F = 12.02$, $df = 5$, $p<0.0001$).

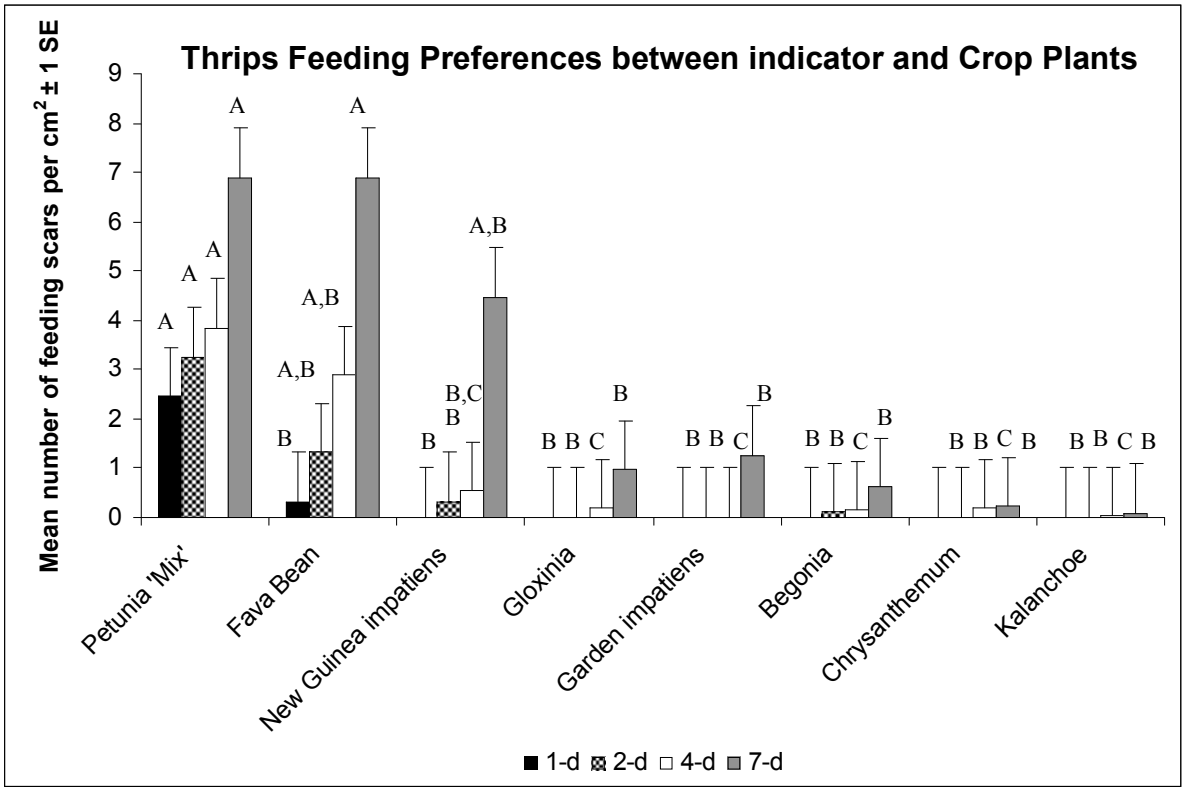


Figure 4: Western flower thrips feeding preferences between petunia, fava bean, begonia, chrysanthemum, garden impatiens, New Guinea Impatiens, Kalanchoe, and gloxinia across all times. Petunia exhibited significantly more feeding scars than other crop plants ($F = 31.24$, $df = 7$, $p<0.0001$).

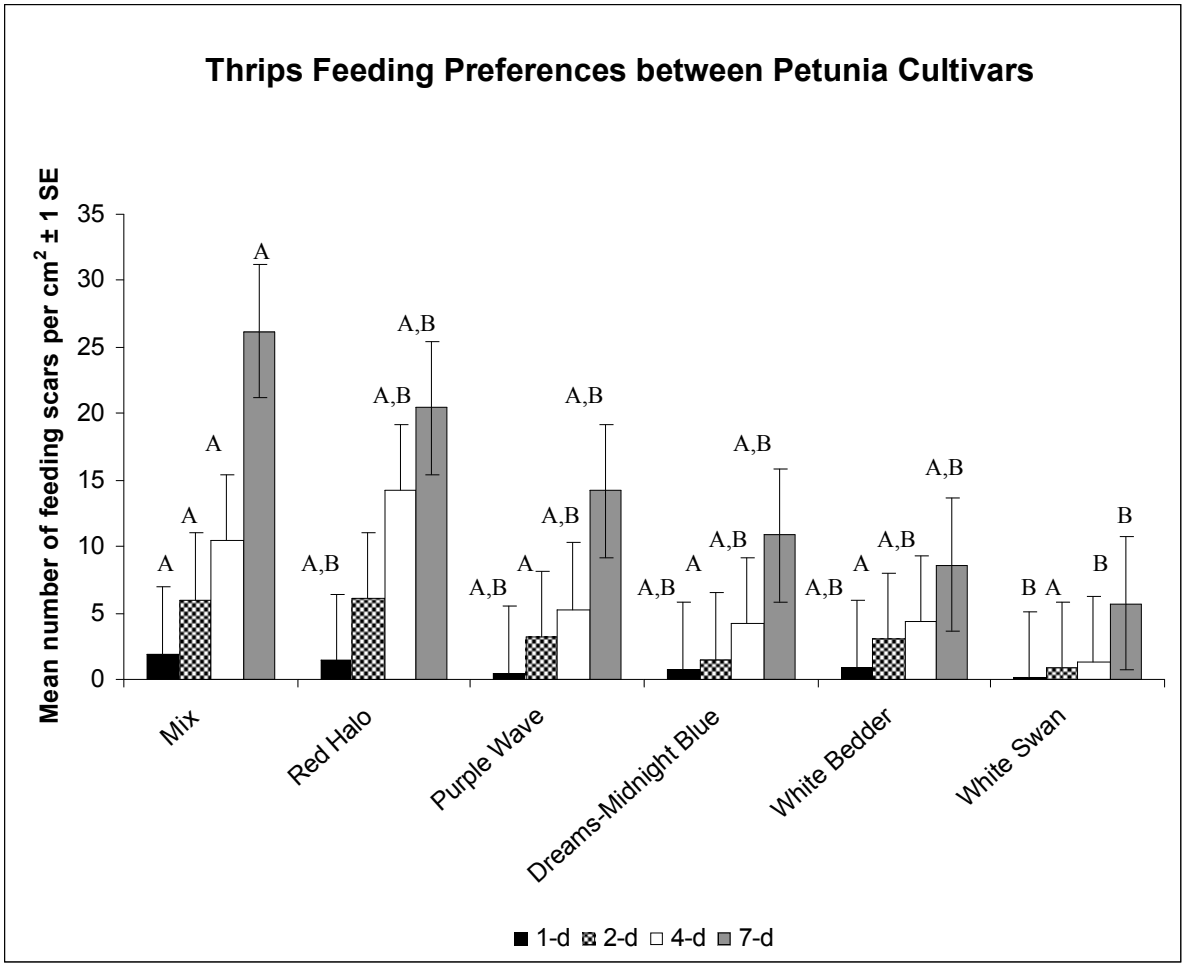


Figure 5: Western flower thrips feeding preferences for six petunia varieties with non-viruliferous thrips across all times. 'Mix' (heirloom) and 'Red Halo' varieties had significantly more feeding scars than all other varieties ($F = 10.37$, $df = 5$, $p<0.0001$).

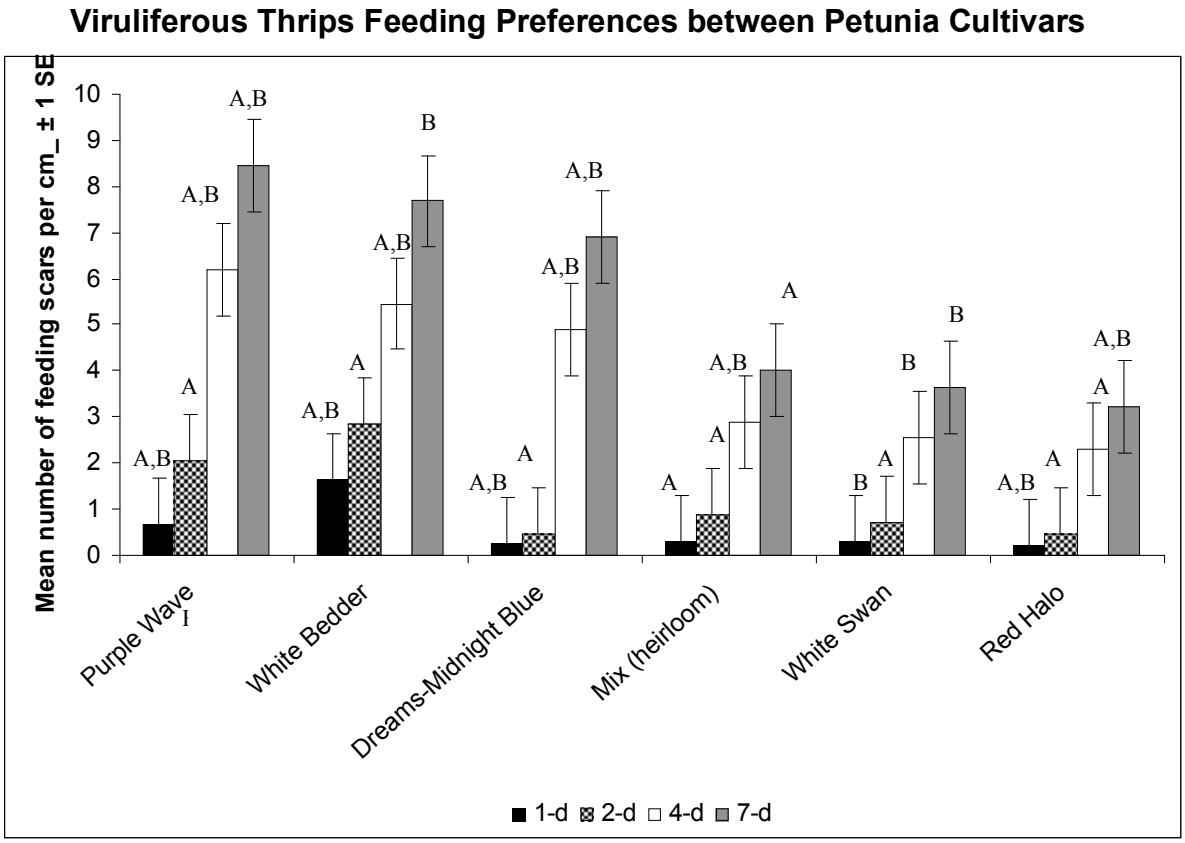


Figure 6: Western flower thrips feeding preferences for six petunia varieties with viruliferous thrips across all times. White Bedder' and 'Purple Wave' varieties had significantly more feeding scars than all 'Red Halo' or 'White Swan', but were not significantly different from 'Dreams-Midnight Blue' or 'Mix' ($F = 2.73$, $df = 5, 234$, $p<0.05$).

Table 1: TSWV occurrence and symptom description in viruliferous thrips in petunia choice test.

| Petunia Variety | Positive TSWV occurrence | Symptom description on individual leaf |
|---|---|---|
| 'Mix' | 1/10 | 2 large ringed lesions |
| 'Purple Wave' | 2/10 | wilted |
| | | 1 large necrotic lesion |
| 'Dreams-Midnight Blue' | | |
| 'White Swan' | 2/10 | wilted |
| 'White Bedder' | 2/10 | 2 small necrotic lesions |
| | | wilted |
| 'Red Halo' | 2/10 | wilted |