

EFFECT OF PINE POLLEN ON SETTLING BEHAVIOR AND OVIPOSITION OF  
*FRANKLINIELLA OCCIDENTALIS* AND *FRANKLINIELLA FUSCA*  
(THYSANOPTERA: THIRIPIDAE) ON TOMATO AND PEANUT

by

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(Under the direction of David G. Riley)

ABSTRACT

The availability of pollen has been found to positively influence the host selection and fecundity of thrips species (Thysanoptera: Thripidae). Studies suggest that pine pollen which is deposited on plants each spring could increase the settling and reproduction potential of thrips on host plants of *Tomato Spotted Wilt Virus* potentially increasing its role as a vector of this virus. Effect of pine pollen (*Pinus elliottii* Engelm.) on the settling behavior and oviposition of *Frankliniella occidentalis* (western flower thrips) and *Frankliniella fusca* (tobacco thrips) was studied. Thrips settling behavior was increased by seven-fold with *F. occidentalis* and one-fold with *F. fusca* with the addition of pine pollen over both tomato and peanut. Oviposition was increased by three-fold with *F. occidentalis* and one-fold with *F. fusca* with pollen.

**INDEX WORDS:** Pine pollen, *Pinus elliottii*, settling behavior, oviposition,

*Lycopersicon esculentum*, *Arachis hypogaea*, Thrips, Thripidae,

*F. occidentalis*, *F. fusca*, tomato leaves, peanut leaves

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## **DEDICATION**

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## CHAPTER 1

### INTRODUCTION

Thrips are important crop pests on many agricultural and horticultural crops that cause damage by feeding on plant tissue (Kirk 1984). Of the 5500 species of thrips described only a few are serious crop pests (Ullman *et al.* 1998). At least ten species of thrips act as vectors of virus (Ullman *et al.* 1998) and of these, *Frankliniella occidentalis* (Pergande) (western flower thrips) and *Frankliniella fusca* (Hinds) (tobacco thrips) are important in the transmission of *Tomato Spotted Wilt Virus* (TSWV), family *Bunyaviridae* of genus *Tospovirus* in Georgia (Riley & Pappu 2000, 2004). These two thrips species are economically important vectors of virus infesting many vegetable, ornamental and fiber crops in tropical, sub-tropical, and temperate regions of the world (Culbreath *et al.* 2003).

Thrips are minute, slender insects generally ranging from 1-2 mm in length (Palmer *et al.* 1989). Thrips reproduce by parthenogenesis or by oviparity within a few weeks resulting in many generations per year. Thrips, being polyphagous minute creatures (Kirk 1997), have the ability to occur in large populations on a broad host range under varied climatic conditions (Terry 1997). The ability to generate large population levels makes thrips direct crop pests and also potentially serious vectors of virus (Ullman *et al.* 2002). Direct damage by thrips is caused by feeding on plant sap in the parenchyma with a consequent reduction of photosynthetic capacity (Royer *et al.* 1986, Hunter & Ullman 1989, Rosenheim *et al.* 1990). Indirect damage can occur by transmission of plant virus such as the tomato spotted wilt virus (Broadbent *et al.* 1987, Vierbergen 1990, German *et al.* 1992).

When TSWV was initially detected in 1920, the virus was detected in several plant families, especially in Solanaceae and Leguminaceae (Brunt *et al.* 1996). Recently, more than 600 plant species, belonging to 70 families have been reported to be susceptible to TSWV and 100 species are recorded within Solanaceae and Compositae (Peters & Goldbach 1995). Other important plant families where TSWV is found includes: Amaranthaceae, Apocynaceae, Amaryllidaceae, Asteraceae, Brassicaceae, Bromeliaceae, Chenopodiaceae, Caryophyllaceae, Cucurbitaceae, Convolvulaceae, Caryophyllaceae, Iridaceae, Malvaceae, and Papaveraceae (Brunt *et al.* 1996). TSWV causes a serious disease of tomato, *Lycopersicon esculentum* Mill (Peters & Goldbach 1995), tobacco (McPherson *et al.* 1999) and peanut (Chamberlin *et al.* 1992). TSWV is economically important in the southeastern United States in peanut (*Arachis hypogea* L.), pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.) and tobacco (*Nicotiana tobaccum* L.) causing millions of dollars in losses annually (Groves *et al.* 2001).

TSWV is endemic in Georgia where it affects major crops like tomato, peanut and peppers causing losses of approximately \$100 million per year (Bertrand 1997). In Georgia alone in 2000, TSWV reduced tomato yields resulting in an estimated loss of \$8.8 million (Riley & Pappu 2000). TSWV is primarily transmitted by western flower thrips, *Frankliniella occidentalis* (Pergande), and tobacco thrips, *Frankliniella fusca* (Hinds) in the tomato crop (Salguero Navas *et al.* 1991, Riley & Pappu 2000, 2004). Among the factors that contribute to the pest status of both these thrips species are high population growth (Watts 1934, Trichilo & Leigh 1988) and broad range of host plants (Sakimura 1932, Yudin *et al.* 1986, EPPO 1988). The reproduction of the two thrips species, tobacco thrips and western flower thrips are strongly affected by host plant quality (Chaisuekul 2004, Chaisuekul & Riley 2005). The results of a recent study

conducted at Tifton, GA to evaluate tomato and chickweed foliage for thrips reproduction indicated that host plant significantly affected the net reproduction of *F. fusca*. *F. occidentalis* reproduced poorly both on chick weed and tomato with only leaves as a food source while *F. fusca* reproduced significantly better on chick weed than on tomato (Chaisuekul 2004).

Variability in reproduction of *F. occidentalis* can be caused by many biotic and abiotic factors. The reproduction parameters of *F. occidentalis* can be affected by host plants and plant parts (Wijkamp *et al.* 1995) especially pollen (Gerrin *et al.* 1999, Hulshof *et al.* 2003), and temperature (Gaum *et al.* 1994). Host plants can potentially impact thrips oviposition based on host plant studies over a range of temperatures (Chaisuekul and Riley 2005). Temperature apparently affected reproduction parameters differently between different populations of *F. occidentalis* (Guam *et al.* 1994, Van Rijn *et al.* 1995) on the same plant species. Pollen is the natural food source for many flower thrips (Kirk 1984). The extent to which thrips are able to profit from the addition of pollen to the diet depends on the thrips species ability to feed on pollen and pollen characteristics like nutritional value, size, adhesiveness and the presence of feeding stimulants (Murai & Ishii 1982, Kirk 1985, Teulon & Penman 1991). The availability of pollen has been found to positively influence the growth, developmental time and fecundity of *F. occidentalis* (Trichilo & Leigh 1988, de Jager & Butot 1993, Van Rijn & Sabelis 1990).

In Georgia, pollen is highly seasonal, a major event occurring in spring that can be heavy enough to be nuisance to the public. Daily pollen counts collected from various plant sources for the past four years indicated that peak pollen counts occur during the spring season (Figure 2.3). Data recorded during 1999-2003, including peaks from previous years in the 1990's indicated very high pollen counts in April, mainly from trees such as pine (*Pinus spp*) (Figure 2.3).

Based on the data from Hulshof *et al.* (2003), that showed that pollen deposition on leaves can increase thrips reproduction, it is likely that peak pollination events such as flowering or deposition of large amounts of ambient pollen are affecting thrips population dynamics (Figure 2.3). Coincidentally, peak ambient pollen in Georgia (Figure 2.3) occurs just prior to peak thrips reproduction in tomato (Riley & Pappu 2000, 2004). Based on this observation, it is likely that ambient pollen is affecting thrips population dynamics on host crops in the spring.

### **Purpose of the study**

Previous studies indicated that pine pollen dusted onto leaves could increase the reproductive potential of *F. occidentalis* (western flower thrips) developing on the foliage of cucumber plants (Hulshof *et al.* 2003). It is therefore likely that pine pollen can affect the overall population dynamics of the vector population. The effect of pollen on feeding (Kirk 1997) as well as oviposition and reproduction (Loomans & Murai 1997) are well documented. The positive effect of pollen on thrips species other than *F. occidentalis*, *Thrips obscuratus* (Teulon & Penman 1991) and *F. bispinosa* (Tsai *et al.* 1996) has also been demonstrated. However, the effect of pine pollen on settling behavior and oviposition clearly needs to be investigated further. My study investigated possible connections between ambient pollen and thrips reproduction. Specifically two behavioral patterns, settling behavior and oviposition are studied using a leaf bioassay on intact tomato and peanut plants either treated (dusted) or untreated with pine pollen (*Pinus elliottii* Engelm.) I evaluated the effects of pine pollen on two thrips species *Frankliniella occidentalis* (western flower thrips) and *Frankliniella fusca* (tobacco thrips). The experiments were conducted at the Coastal Plain Experiment Station Tifton, GA under laboratory conditions.

To better understand these two behavioral patterns the following two main objectives were developed:

1. To test the effect of pine pollen on two species of thrips, and quantify the settling behavior on intact tomato and peanut leaves either treated (dusted) or untreated with pine pollen.
2. To test the effect of pine pollen on two species of thrips, and quantify the oviposition rate on intact tomato and peanut leaves either treated (dusted) or untreated with pine pollen.

### **Hypothesis and Experiments**

First, pine pollen when dusted on intact tomato and peanut leaves may increase the settling behavior of thrips and, consequently affecting thrips ability to vector *Tospovirus* since longer time on a host can increase percentage of transmission (Wijkamp *et al.* 1995). The simplest hypothesis to test here is whether or not pine pollen affects thrips settling behavior at all.

H-1<sub>0</sub> : Pine pollen does not increase thrips settling on intact tomato and peanut leaves.

H-1<sub>A</sub> : Pine pollen increases the thrips settling on intact tomato and peanut leaves.

For the whole plant versus pollen leaf experiment, the whole plant age was 4-5 weeks old. Thrips were exposed on 4 randomly selected intact tomato and peanut leaves in a bioassay designed in January 2003, to expose thrips on an equal portion of each treatment while keeping the treatment plants intact. Two treatment methods, choice (dusted with pine pollen) and no choice (no pine pollen) were used in this experiment conducted in 2005. The treated leaves were placed opposite to the plain leaves so that thrips could choose where to settle. Sixteen thrips were placed in the bioassay arena, 4 per leaflet to quantify the settling behavior and, the position of thrips was recorded for every 15 minutes for 2 hours 30 minutes.

Secondly, more oviposition is expected when thrips are allowed to settle, feed and oviposit for one week with the intact tomato and peanut leaves when treated (dusted) or untreated with pine pollen. The simplest hypothesis to test is whether or not pine pollen increases the thrips oviposition.

H-2<sub>o</sub> : Pine pollen does not increase the thrips oviposition on intact tomato and peanut leaves.

H-2<sub>A</sub> : Pine pollen will increase thrips oviposition on intact tomato and peanut leaves.

Thrips are allowed to feed for one week and oviposit in the same experiment, and then the thrips and leaves are removed. To evaluate the thrips oviposition, leaf samples are stained with lacto phenol-acid fuschin based on Parrella & Robb (1982) and Nuessly *et al.* (1995) to aid in identifying ovipositional scars and eggs under stereo microscopic inspection.

Also, a preliminary experiment was conducted to evaluate the effect of pine pollen on the percent acquisition of TSWV by *Frankliniella occidentalis* and *Frankliniella fusca* on tomato and peanut plants. TSWV can be acquired only by immature thrips (Ullman *et al.* 1992) and wingless immatures normally feed on the plants where their eggs were oviposited (Terry 1997). Thus, the proportion of TSWV vectors and consequently the level of epidemics are to some extent determined by the settling and ovipositional behavior of female thrips on TSWV host plants. The hypothesis tested under this experiment is whether or not pine pollen increases the percent acquisition of the TSWV in the thrips species.

H-3<sub>o</sub> : Pine pollen does not increase thrips percent acquisition of TSWV in tomato and peanut plants.

H-3<sub>A</sub> : Pine pollen will increase thrips percent acquisition of TSWV in tomato and peanut plants.

Thrips were released on virus infected plants dusted with pine pollen and allowed to feed for 2-3 weeks. New immature thrips and adults were collected off of the plants and tested the individual adults and immature thrips for TSWV using ELISA for thrips.

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## CHAPTER 2

### REVIEW OF LITERATURE

#### **Pollen vs. Plant Food Sources for Thrips**

Plant pollen is a major source of food for many herbivorous arthropods that use pollen to promote their survival and reproduction (Van Rijn *et al.* 2002). Some insect groups that benefit from pollen include chrysomelid and curculionid beetles (Jayanth *et al.* 1993, Jones *et al.* 1993), lycaenid and *Heliconus* butterflies (Gilbert 1972, Wagner & del Rio 1997), and many thrips species (Kirk 1997). The unique, piercing-sucking asymmetrical mouth parts that thrips have enable them to feed on a wide range of foods other than plant leaf tissue such as pollen grains or mite eggs (Kirk 1995).

The old idea that thrips feed by rasping, has been changed to mainly piercing and sucking behavior (Kindt 2004). Feeding in thrips is well designed for foods like leaf and petal cells, pollen grains, active stages of mites and small insects that can be viewed as liquid food enclosed by a thin cell wall. The wide variety of foods consumed by particular species of thrips can be determined in large part by host selection behavior, or where it lives. In case of leaf feeding thrips, feeding can be mainly on leaf cells while other thrips like flower thrips have easy access to foods like pollen, young buds and petals (Kirk 1995). However it has been reported that thrips feed on pollen, nectar and exposed liquids (Grinfeld 1959, Downey 1965, Heming 1978, and Chisholm 1983). For many flower thrips, pollen is the major food source (Kirk 1984). Kirk (1984) listed 26 species of thrips that fed on pollen grains, nectar or exposed liquids. The extent to which thrips are able to profit from the addition of pollen will depend on the biological and

ecological attributes of thrips species that allow them access to pollen and pollen characteristics like nutritional value, size, adhesiveness and presence of feeding stimulant (Murai & Ishii 1982, Kirk 1985, Teulon & Penman 1991). There are numerous claims that thrips feed on pollen, but relatively few studies are supported by direct observation (Kirk 1997).

Grinfeld (1959) detailed pollen-feeding in eight species of thrips. Thrips usually took 2-10 seconds and sometimes 15-20 seconds to feed on each grain, and can feed up to 20 or 30 grains in one session depending on the size of grain, thrips species and temperature (Grinfeld 1959). Kirk (1987) studied three different families of thrips which were fed on twelve different sources of pollen. All fed readily on pollen and it was determined that thrips can feed on as many as 106 grains (Kirk 1987). The pollen feeding thrips included *Thrips fuscipennis*, *T. major*, *T. imaginis*, *T. tabaci*, *Kakothrips pisivorous* and *Ceratothrips ericae* (Thripidae), *Aeolothrips intermidius* (Aelothripidae) and *Haplothrips leucanthemi* (Phaleothripidae). Mean feeding times of thrips on pollen ranged from 3-120 seconds varying with thrips species, instar, grain volume and temperature (Grinfeld 1959, Kirk 1984, 1987, Annadurai & Morrison 1987).

Pollen grains vary greatly in size between plant species and variation in feeding of thrips on pollen can be large, by a factor of 800 (Kirk 1984). Thus, not all plant pollens have equal value for thrips feeding. Studies indicate that adhesive or very sticky pollen grains reduce or prevent thrips feeding (Putman 1965, Kirk 1984, 1985). Even though there is a correlation between the body size and host flower size, there is no evidence that oligophagous flower thrips have adapted in body size to the pollen grain size on which they feed (Kirk 1991) and no feeding difficulties were observed when different species of pollen were tested (Hulshof *et al.* 2003).

## **Pollen - Nutrition and Effect on Insect Life Histories**

A pollen requirement in the diet is seen in a wide range of insects. The effect of pollen on insect population size may be through 1) increased survival, development and oviposition and 2) decreased rate of immigration out of crop (Van Rijn *et al.* 1990). Pollen is rich in protein (Todd & Bretherick 1942) and rearing experiments in thrips indicate that pollen can be necessary for adult fertility (Bournier *et al.* 1979), egg production (Andrewartha 1935, Murai & Ishii 1982) and larval growth (Murai & Ishii 1982). The main accepted criteria in assessing the nutritional value of food are growth, development, longevity, fecundity and population density (Dethier 1954). Plant tissues are just nutritionally adequate because insects have more protein nitrogen than the plants on which they feed (McNeil & Southwood 1978). Sugars and amino acids, which are the primary metabolites of proteins and nitrogen, stimulate thrips feeding when the host plants have a significantly high level (Ananthakrishnan 1984). Thus, thrips actively seek protein in diet.

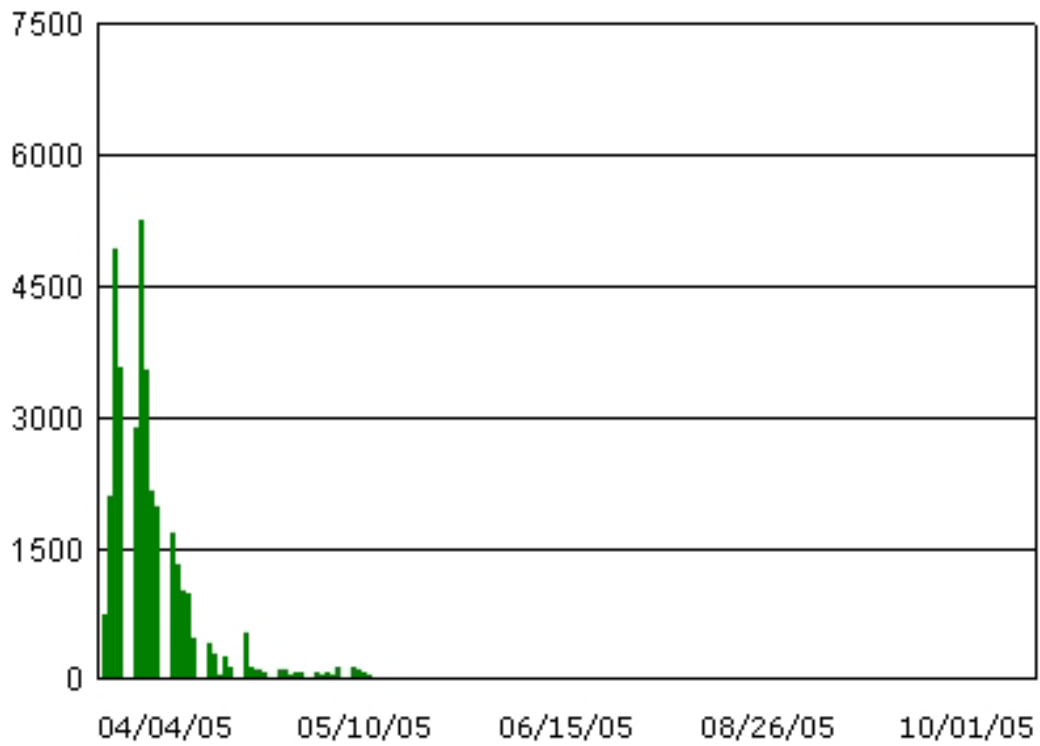
Plants species are heterogenous entities influenced by changes in time, space, growth conditions and climate. Significant differences in terms of nutrient concentration can be present in a plant leaf, the habitat forming the typically smallest resource unit for studies on herbivores. Fresh young leaves when pruned have been shown to be more acceptable for thrips because of high amino nitrogen content, suggesting a correlation between leaf amino nitrogen and thrips feeding (Ananthakrishnan 1993). Increase in nitrogen concentration improves nutrient assimilation and insect growth efficiency, inturn enhancing the rates of insect survival, growth and reproduction (Strong *et al.* 1984). Concentration of nitrogen in pollen is comparatively high (Slansky & Scriber 1985) and preference for this tissue is cited as one way to obtain a high protein diet (Kirk 1997).

Aromatic amino acids (AAA) could be the key compounds in determining the nutritional value of pollen for thrips. This is because molting takes place 4 times during the insect development process where the AAA's could be critical to the sclerotization of cuticles (Bernays & Woodhead 1984, Mollema & Cole 1996). The AAA's, phenyl alanine and tyrosine are critical insect nutrients and play key roles in various physiological processes of insects (Kirk 1997). In an early study conducted on pollen feeding in thrips by Grinfeld (1959), detailed observations on factors affecting thrips feeding were not recorded. Instead various reasons like pollen size, spines and other structures on the outer wall that inhibit feeding are given as possible sources of variation in the observed pollen-feeding. Results of more detailed observations of pollen – feeding in thrips by Kirk (1984) suggest that many factors could affect feeding in flower dwelling Thysanoptera.

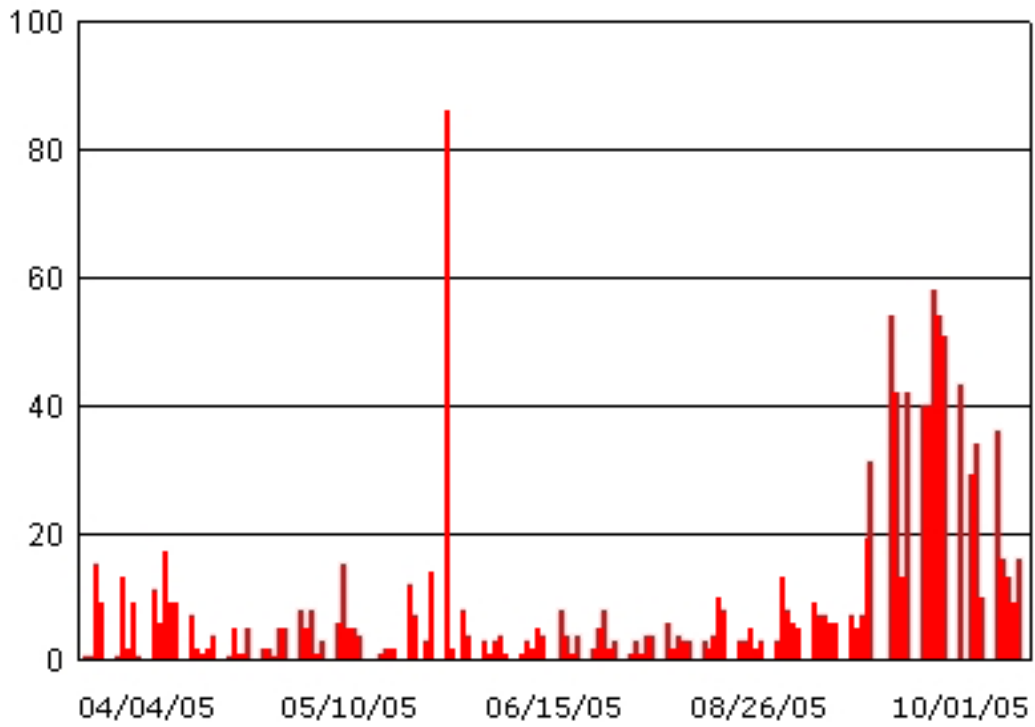
Several studies were conducted on thrips with different pollens and pollen added as supplemental diet in relation to feeding (Kirk 1984), pollination (Velayudhan & Annadurai 1986), fecundity (Annaduari & Morrison 1987), prey predator relationship (VanRijin & Sabelis 1990), fecundity and larval growth (de Jager *et al.* 1993), and reproductive capacity (Murai 2001). All indicated a significant positive effect on thrips population with pollen. In addition a recent study indicated that pine pollen could increase the reproductive potential of *F. occidentalis* developing on the foliage of cucumber (Hulshof *et al.* 2003). Thus, pollen only needs to be deposited on a leaf surface to have a major effect on thrips population. Also, Hulshof *et al.* (2003) suggested that pine pollen might be one of the best pollens for increasing *F. occidentalis* reproduction.

Pollen dispersed by wind from trees, weeds and grass is very common with significantly higher pollen counts during spring (NAB-National Allergy Bureau, Atlanta, GA). In Georgia,

pollen deposition is most common in the spring from trees (Figure 2.1) and to a lesser extent from weeds which tends to be more prevalent in the fall (Figure: 2.2). The annual total peak pollen counts are typically recorded during April-May (Figure 2.3).

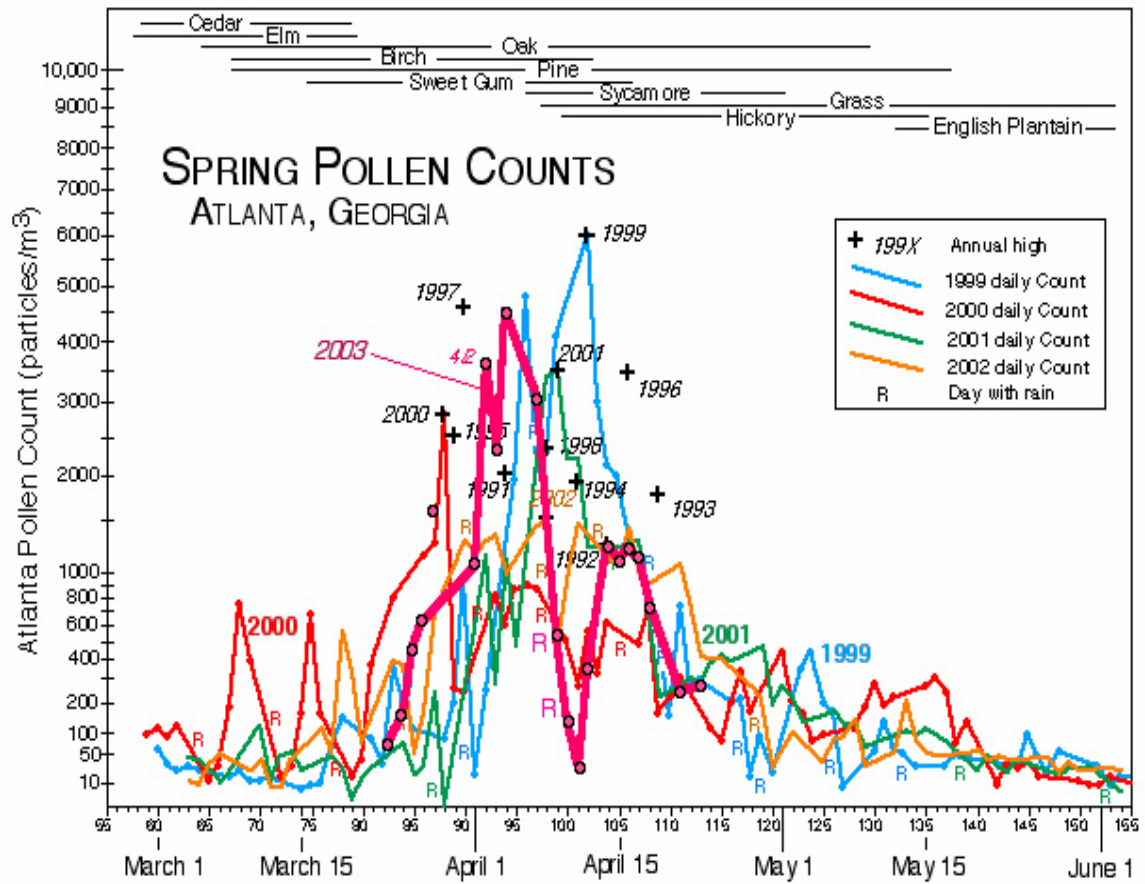


**Figure: 2.1** Total Tree Counts - Total pollen counts per cubic meter of air.  
Source: Anonymous 2005.



**Figure: 2.2** Total Weed Counts –Total weed pollen counts per cubic meter of air.

Source: Anonymous 2005.



**Figure 2.3** Spring Pollen Counts, Atlanta, GA.

Source: <http://www.gly.uga.edu/railsback/Atlpollen1.html> (Railsback 2003).

Slash pine (*Pinus elliottii* Engelm.) is one of the hard yellow pines which are indigenous to the southeastern United States. Slash begins shedding pollen around February (Dorman & Barber 1956) causing pollen deposition on a regional scale as it is highly airborne. Slash pine pollen shed is then followed by longleaf, *Pinus palustris*, loblolly, *Pinus taeda*, pond, *Pinus serotina*, and finally short leaf pine, *Pinus echinata* (Dorman & Barber 1956). Ambient pine pollen deposition in Georgia is high during spring (Figure 2.3) which occurs just prior to the peak reproduction time of thrips in tomato (Riley and Pappu, 2004). Based on this, and the fact that pine pollen increases reproduction in thrips (Hulshof *et al.* 2003) there is a likelihood that pollen is affecting thrips vector population dynamics on a regional basis. Various analyses of pine pollens showed high percentage of proteins and fats in the pollen grain Table 2.1 lists the percentiles of various components of pine pollens as reported by Todd and Brethrick (1942).

**Table: 2.1.** Percentile composition of various components of pine pollens.

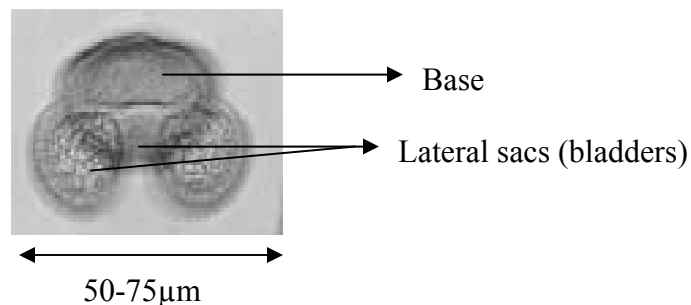
Protein	12.6-16.6
Fats	10-14.6
Sucrose	6-12
Starch	7.0
Ash	2.8-5.5
Reducing sugars	0-1.61

Source: Todd and Brethrick (1942).

*Pinus* pollen is bivesiculate pollen grain structure with a basal body of two sacs that are laterally placed with a maximum dimension of 50-75µm (Figure 2.4). Pine is monoecious and wind pollinated and is abundantly available in nature in the spring of each year. A study

conducted in Finland by Hulshof *et al.* (2003) reported that when *F. occidentalis* is caged on cucumber leaves and fed with seven different pollen food types, the intrinsic growth rate increased from 0.163 on just cucumber leaf to 0.240 with cucumber leaves dusted with pine pollen. The seven food sources used in this study included six different pollens *Betula pubescens* (birch), *Typha latifolia* (cattail), *Corylus avellana* (hazel) *Pinus sylvestris* (Scotch pine), hand and bee collected *Epilobium angustifolium* (fire-weed). The pollen species differing in the nutritional contents, with pine having more nutritional value which apparently favored thrips with a positive effect on reproduction. Pine pollen as an additional diet component for thrips had a positive effect on various life history components including oviposition.

Slash pine pollen grain



**Figure 2.4** Light micrograph of *Pinus elliotii* G. Engelmann var. *elliotti*

[http://pollen.usda.gov/Light\\_Micrographs/Pinaceae/Pinaceae.html](http://pollen.usda.gov/Light_Micrographs/Pinaceae/Pinaceae.html).

### **Pollen and Settling behavior**

Environmental versatility in insects is an essential biological characteristic to be reproductively successful. In this process insects develop specific structural, physiological and behavioral characteristics enabling them to behave and adopt diverse food habits at different times under varying environments (Ananthakrishnan 1984). Settling in insects is basically one

phase of the host finding process where they can optimize their feeding to maximize further development. Settling behavior is exhibited by many immature and adult insects and is mostly site specific (Madsen *et al.* 1962, Fennah 1963, Horton 1990, Berg *et al.* 1991, Lynch & Simmons 1993, McAuslane *et al.* 1993, Stamp & Bowers 1993). Intraspecific competition for feeding sites influences the insects to select and settle on specific plant parts (Chongratanameteekul *et al.* 1991). Limited immobility in the nymphs, particularly in the Order: Homoptera, where the late instar larvae have restricted ability to move to the new feeding sites increases the importance of initial settling behavior. As a result, adults through oviposition and the 1<sup>st</sup> instar nymphs initially select optimal feeding sites to continue the further developmental stages. Specific cues used by insects to assess specific settling sites are largely unknown (Luft & Paine 1997). Some potential cues like leaf roughness (Berg *et al.* 1991), gravity (Coombe 1982) and light (Hargreaves 1915) have been previously identified as important for site selection and settling in immature or adult stages of other insect species.

Settling behavior and probing was studied in relation to the effect of plant age, leaf age and imidachlopid on *Frankliniella occidentalis* and *Frankliniella fusca* (Joost 2003). This study reported that settling behavior was different for each species of the thrips. Both the thrips species preferred 3-4 week old plants to settle, but *F. fusca* settled more on 4 week old plants while no preference was shown by *F. occidentalis*. However, no information is available in the literature on the settling behavior of thrips when pollen is given as choice diet. The effect of pine pollen on the settling behavior of *F. occidentalis* and *F. fusca* has not been documented. The effect of pine pollen on the settling behavior is reported in the following chapter of this thesis.

## Pollen and Oviposition

Oviposition and reproductive success in thrips is influenced by nature of habitat and nutritional quality (Rivnay 1935, Lewis 1973, Ananthakrishnan 1984). Duration of development, size and fecundity of adults is determined partly by the nutritive value of food eaten by larvae (Lewis 1973). Fecundity in general ranges from 30-60 eggs per female thrips depending on available nutrition. Factors like temperature and humidity also influence the egg laying (Sakimura 1937, Watts 1936). The amount of protein in the diet of an insect is known to significantly affect the egg production (Englemann 1984).

Pollen is high in protein content (Mound *et. al.* 1980) and important for the egg production in several insect species (Gilbert 1972, Pesho & van Houten 1982) including thrips. Pollen, when supplied as a diet to thrips, exhibited highest and sustained levels of egg production compared to other diets (Teulon & Penman 1991, Tsai *et al.* 1996). Presence of pollen contributed as a significant factor in continuing the high levels of oviposition with *T. imaginis* (Andrewartha 1935), *Frankliniella intonsa* (Murai & Ishii 1982), *Frankliniella fuscipennis* (Kirk 1985), and *Frankliniella occidentalis* (Trichilo & Leigh 1988, Hulshof *et al.* 2003) when compared with diets like floral tissues, fruit and sugar solutions.

Pollen and its key role in the production of eggs was first reported by Andrewartha (1935) with *Antirrhinum* snapdragon pollen (stamen and anther) on two thrips species *Thrips imagines* and *Halothrips victoriensis*. A large influence in the average number of eggs produced from 19.8 to 209 was recorded with the addition of pollen, probably due to the protein content in the pollen (Andrewartha 1935). Studies on effect of pollen, in specific on oviposition rate were, reported on one generalist and two specialist flower thrips with three different pollens of *Vicia*, *Brassica* and *Calluna* (Kirk 1985). *Thrips fuscipennis*, a generalist, breeds in a wide range of flowers and the

specialist thrips *Kakothrips pisivorus* feeds in flowers of *Vicia*, *Pisum* and *Lathyrus* while *Ceratothrips ericae* feeds in flowers of *Calluna* and *Erica*. *T. fuscipennis* laid similar number of eggs when fed each of the pollens, but *K. pisivorus* laid significantly more eggs with its host *Vicia* and *C. ericae* laid more eggs with its host *Calluna*. Oviposition was significantly greater with the addition of pollen as a diet than with the addition of other floral parts. However the oviposition rate in this study was claimed not to be comparable between treatments as the species used for the tests were not at the same feeding and reproductive stage (Kirk 1985). Studies were conducted on the effect of pollen on various life history components in various thrips species. Pollen when added to the diet of different species of thrips in various experiments, influenced oviposition and other life history characteristics (Table 2.2).

Oviposition rate is highly dependent on the amount of pollen fed by the thrips and hence differences in the feeding rates could affect the oviposition rate (Kirk 1984). Studying the response of the thrips when a high quality food like pollen is available is important to understanding thrips population dynamics and for developing successful control strategies. The effect of pine pollen on the oviposition behavior of two thrips *F. occidentalis* and *F. fusca* on intact tomato and peanut leaves was investigated and the results are reported in the following chapter.

**Table: 2.2** References on the influence of different pollens on the life history characteristics of various thrips species.

Thrips Species	Pollen species used	Pollen species Influenced	Characteristics increased	Reference
<i>Thrips imaginis</i> <i>Halothrips victoriensis</i>	<i>Antirrhinum</i>	<i>Antirrhinum</i>	Egg production Longevity	Andrewartha, 1935.
<i>Thrips fuscipennis</i> <i>Ceratothrips ericae</i> <i>Kakothrips pisivorous</i>	<i>Vicia faba</i> <i>Calluna vulgaris</i> <i>Brassica napus</i>	All three <i>Calluna vulgaris</i> <i>Brassica napus</i>	Oviposition rate Feeding	Kirk, 1985.
<i>Ceratothrips cameroni</i> <i>Frankliniella schultzei</i>	<i>Solanum melangena</i> <i>S. trilobatum</i> <i>S. xanthocarpum</i> <i>S. nigrum</i> <i>Capsicum frutescens</i> <i>Physalis sp</i>	<i>Solanum melangena</i>	Fecundity Feeding	Annadurai and Morison, 1987.
<i>Frankliniella occidentalis</i>	<i>Gossypium hirsutum</i>	<i>Gossypium hirsutum</i>	Fecundity Longevity	Trichilo and Leigh, 1988.
<i>Frankliniella occidentalis</i>	Sweet Pepper	Sweet Pepper	Oviposition Larval growth	Van Rijin and Sabelis, 1990.
<i>Thrips obscuratus</i>	<i>Papaver nudicaule</i>	<i>Papaver nudicaule</i>	Egg production Low larval mortality Fast development	Teulon and Penman, 1991.
<i>Frankliniella occidentalis</i>	Chrysanthemum	Chrysanthemum	Fecundity Larval growth	de Jager <i>et al.</i> 1993.
<i>Frankliniella bispinosa</i>	<i>Bidens pinosa</i> <i>Typha domingensis</i> <i>Phoenix robelenii</i> <i>Pinus elliotii</i>	<i>Typha domingensis</i>	Egg production Net reproduction	Tsai <i>et al.</i> 1996.
<i>Taeniothrips inconsequens</i>	<i>Acersaccharum</i>	<i>Acer saccharum</i>	Adult longevity Total oviposition Oviposition rate	Leskey <i>et al.</i> 1997.
<i>Thrips hawaiiensis</i>	Camelia sinensis	Camelia sinensis	Fecundity	Muari, 2001.
<i>Frankliniella occidentalis</i>	<i>Betula pubescens</i> <i>Typha latifolia</i> <i>Coryllus avellana</i> <i>Pinus sylvestris</i> <i>Epilobium angutifolium</i>	<i>Pinus sylvestris</i>	Fecundity	Hulshof <i>et al.</i> 2003.
<i>Frankliniella occidentalis</i>	<i>Prunus brutia</i>	<i>Pinus brutia</i>	Oviposition	Whittaker and Kirk, 2004

## Thrips - Virus –Pollen Interactions

Thrips are the only known vectors of virus of *Tospovirus* (Ullman *et al.* 1992). One *tospovirus*, Tomato Spotted Wilt Virus (TSWV) infects as many as 1100 plant species causing plant damage in many agricultural horticultural crops and many weed species (Chatzivassiliou *et al.* 2001, Peters 2004). TSWV, belonging to the genus *Tospovirus*, family *Bunyaviridae*, is the only genus in this family of viruses infecting plants (Milne & Francki 1984, Francki *et al.* 1991). *Tospovirus* transmitting thrips species are polyphagous, piercing –sucking insects that feed on a variety of cells and different parts of the plant including leaf tissue and pollen (Mound 1996). At least seven thrips species are confirmed to be the vectors of virus by the 1990's (Todd *et al.* 1990) of which western flower thrips, *Frankliniella occidentalis* (Pergande) and tobacco thrips *Frankliniella fusca* (Hinds) are important in Georgia infesting tomato and peanut (Chamberlin *et al.* 1992). Currently there are 11 known vector species (Mortiz 2005).

The close link between thrips development and *Tospovirus* epidemiology is basic to understanding the *Tospovirus* infection cycle. Adult thrips are winged and disperse readily while the larvae are wingless. The female adults have to select and oviposit on host plants on which larvae can develop and acquire *Tospovirus* (Ullman *et al.* 1992). If the immigrating female is viruliferous, she can also infect the plant that she chooses for oviposition. Host suitability on which thrips feed significantly influences the virus acquisition and transmission (German *et al.* 1992). When a susceptible plant is infected, TSWV will spread systemically through the plant (Best 1968). The infection cycle for the thrips is initiated only when female adult thrips lays eggs on the virus infected host plants that favor egg and larval development. Acquisition of TSWV is dependent on the oviposition of the female thrips, since only early instars can acquire the virus and larvae develop on the host on which they are hatched (Maris *et al.* 2004). TSWV acquisition

occurs primarily when 1<sup>st</sup> instar larvae feed on virus infected plants (Van de Wetering *et al.* 1996). Virus acquisition by feeding larvae can occur in about 30 minutes (Razvyazkina 1953) but tends to increase with a longer access period (Wijkamp & Peters 1993). Tospovirus, once acquired, can be retained in thrips through molting, pupation, and until emergence to adult stage (Sakimura 1962), and this is often referred to as a persistent virus.

It could be direct if pollen grains become infected with *Tospovirus*. Thrips ingest the pollen and suck the liquids from the grains (Lewis 1973), but thrips transmitting a pollen-borne virus, such as occurs with Ilavirus (Mink 1992) while feeding on TSWV infected pollen or TSWV infected plant foliage has not been shown. In fact, TSWV has not been detected in plant pollen to date (S. Mullis personal communication). However, it has been claimed that thrips carry pollen and incidentally aids entry of virus into the plant when they feed (Gerber *et al.* 1991, Mink 1992, Hardy & Teakle 1992, Klose *et al.* 1996) for other virus systems. A more likely interaction is that pollen affects reproduction (Hulshof *et al.* 2003) which in turn affects acquisition of virus by immature thrips or increases settling of adults on plant tissue which in turn increases transmission of the virus to plants.

A study was initiated during summer 2005 in the Experiment station, to evaluate the effect of pine pollen on the percent acquisition of TSWV. This was to test for indirect effects, i. e., pollen increasing settling on infected plant tissue which in turn increases virus acquisition. The percent acquisition of the TSWV was tested with the two thrips species on virus infected tomato and peanut plants with choice (dusted pine pollen) and no choice tests (no pollen). The results of the test were reported in the following chapter.

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## CHAPTER 3

### EFFECT OF PINE POLLEN ON SETTLING BEHAVIOUR OF *FRANKLINIELLA OCCIDENTALIS* AND *FRANKLINIELLA FUSCA* (THYSANOPTERA: THIRIPIDAE) ON TOMATO AND PEANUT LEAVES

#### **Introduction**

Thrips cause direct damage by feeding on plants (Morgan *et al.* 1970) and indirectly by transmitting Tospovirus (German *et al.* 1992, Wijkamp *et al.* 1995, Ullman *et al.* 1992). Of the various thrips species described as competent vectors of *Tomato Spotted Wilt Virus*, family Bunyaviridae, genus Tospovirus, the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) (Pergande) (Sakimura, 1962) and the tobacco thrips *Frankliniella fusca* (Hinds) (Sakimura, 1963) are important vectors in the southeastern United States (Riley & Pappu 2000, 2004). *Tomato spotted wilt virus* is endemic in Georgia affecting peanut (Chamberlin *et al.* 1992), tomato (Greenough *et al.* 1990) and other solanaceous crops. TSWV incidence has increased in recent years with 80% disease incidence in individual fields (McPherson *et al.* 1999) with an estimated economic loss of \$5.8 million in peanut in 2001 (Brown & Todd 2002) and \$3 million in tomatoes in 2002 (Sparks & Riley 2002). High population growth of thrips (Watts 1934, Trichilo & Leigh 1988), broad host range (Yudin *et al.* 1986, EPPO 1988), and ability to transmit virus (Sakimura 1963, German *et al.* 1992) are among the factors contributing to the high pest status of thrips.

Thrips (Thysanoptera: Thripidae) are generally phytophagous but can take advantage of diverse food sources (Kirk 1997). Pollen can enhance the reproduction of thrips (Gerrin *et al.*

1999, Hulshof *et al.* 2003). Ambient pollen in Georgia peaks during the spring (Figure 3.1) which occurs prior to peak thrips numbers in the spring tomato crop (Riley & Pappu 2000, 2004). Thus, population peaks of thrips and peak counts of pollen recorded from the past years appear to coincide, suggesting a possible relationship between ambient pollen and thrips population dynamics. Based on this, and observations on pollen's positive effect on thrips reproduction (Hulshof *et al.* 2003) it has been suggested that pollen is affecting thrips vector population dynamics on a regional basis (Riley 2005). The proportion of TSWV vectors and the level of epidemics are to some extent determined by the settling behavior and oviposition of female thrips on virus infected host plants (Chaisukel & Riley 2005) which in turn might be affected by pollen deposition on the plant.

Settling in insects is one phase of host finding behavior where the females determine host suitability for optimal feeding and reproduction. Joost & Riley (2005) reported on the settling behavior of *Frankliniella occidentalis* and *Frankliniella fusca* as affected by imidacloprid insecticide treatment and demonstrated species specific responses. However no comparable information is available on the settling behavior of thrips using pollen as a treatment on the surface of host plants. In the current study we defined the term "settling behavior" as the amount of time thrips spent on the leaf tissue in a host choice study. The assumption was that thrips settle to feed or oviposit and thus these data would have important biological significance. Our study investigated the effect of pine pollen (*Pinus elliottii* Engelm.) on the settling behavior of two thrips species, the western flower thrips, *Frankliniella occidentalis* and the tobacco thrips, *Frankliniella fusca* on intact tomato and peanut leaves on potted plants. Settling behavior was analyzed by individual thrips species and crop. The null hypothesis was that pine pollen, when dusted on the leaf tissue does not increase thrips settling behavior on tomato and peanut leaves.

## Materials and Methods

Two experiments were conducted in the spring 2005 at the Coastal Plain Experiment Station, Tifton GA under laboratory conditions. Choice tests were conducted first with *F. occidentalis* on intact tomato and peanut leaves to test whether pine pollen dusted onto leaves would exert any influence on thrips settling. The second set of experiments tested *F. fusca* with the same choice treatment. Tomato plants, cv. ‘Sunny Hybrid’ (Asgrow Seed Co., Kalamazoo, MI), and peanut plants cv. ‘Sunoleic 97 R’ were reared in a green house using standard cultural practices. Seeds were allowed to germinate in transplant trays until the second true leaf stage, and then the plants were transplanted into 4-inch diameter pots using ‘Metro Mix 300’ soil media (Sun Gro Horticulture Dist. Inc, Bellevue, WA). Plants were visually checked to maintain them as insect free as possible and watered daily. Fully expanded terminal leaflets still attached to 4-5 week old plants were used for all the experiments.

Thrips settling behavior was evaluated by using a bioassay similar to that described by Joost & Riley (2005) but modified to a larger arena (Figure 3.2). In the bioassay thrips were exposed on an equal leaf portion of each treatment while keeping the treatments plants intact. An arena composed of 100×15 mm size petri dishes (Fisher brand ® Pittsburgh, PA) was used with a wooden square frame and 4 toggle bolts at the corners for tightening the arena onto the leaf. In the bioassay, thrips were exposed on an equal leaf portion of each treatment while keeping the treatment plants intact. A 3-mm thick, one sided adhesive foam (Nu-Calgon Wholesaler Inc. St. Louis, MO) was stuck on the sides of two 15× 14 cm squares of 1 cm thick poplar (*Populus*) wood plates. A 9-cm diameter circle is drilled through the center of one square and attached foam. This square is considered as the “top square”. The “bottom square” was left whole, covered with a solid sheet of foam. The 9-cm hole of the bioassay arena had the bottom rim of

petri dish glued in and was covered by the top dish. Four 7-mm diameter holes were drilled at the corners of each wooden square. Four 3-cm tall screws were inserted into the bottom square so that the foam side faced upwards. The top square was threaded through the screws with the foam side down. Wing nuts were used as a locking device. A thin layer (~2-mm) of Bacto agar (DIFCO Laboratories, Sparks, MD) was poured completely over the top of the bottom square and the bottom of the top cut out square and allowed to cool. After the leaves were sandwiched between the top and bottom plates of the arena, additional semi-liquid Bacto agar was used to seal the leaves and arenas together. The lid of the 100×15 mm tissue culture dish was sealed with yellow clay (Handi-Tak ® Super Glue Corp., Rancho Cucamonga, CA) and observations of thrips were made through the lid of the bioassay arena.

The plain leaf versus the pollen- treated leaf choice experiment were setup as follows: A terminal leaflet of four randomly selected 4-5 week old tomato or peanut plants were placed between the two wooden -foam squares, so that ~8 cm<sup>2</sup> triangle in the arena was laying on the agar. Crops were tested separately. The wing nuts were tightened, securing the leaf between the foam pads. The leaf's abaxial side was glued to the agar by adding agar droplets underneath the leaf. Ten mg of slash pine pollen, (*Pinus elliottii* Engelm.) collected from Tifton, Georgia in February 2005 was spread by dusting evenly on the leaf surface of the pollen-treated leaves in the arena. The untreated leaves were covered to prevent dusting during this process. The treated (dusted) leaves are placed opposite to the plain leaves so that thrips could equally choose where to settle and oviposit. Liquid agar was applied around the edges of the arena to seal the foam pads together and prevent escape by thrips.

Four individual females of *F. occidentalis* were placed into the bioassay arena, one in the middle of each leaflet using a zero number paint brush, and the lid was placed on the top of the

assay. To quantify oviposition behavior, thrips were allowed to oviposit for 8 days. The experiment was conducted in the same way on tomato and peanut using just female *F. fusca*. Two randomized complete block experiments with four replicates each were conducted for the pollen treatments for each combination of thrips species and crop.

The *F. occidentalis* colony was collected from the Plant Pathology Lab, University of Georgia, Athens, GA, USA and maintained on green beans (*Phaseolus vulgaris*) in plastic cups (Loomans & Muari 1997). The *F. fusca* colony was collected from peanut, *Arachis hypogaea* (L.), fields in Tifton, GA during the spring 2003 and maintained on whole peanut plants and green bean plants for use in these experiments. Before introduction into the arenas *F. occidentalis* and *F. fusca* were reared on green beans in 473 ml plastic deli cups. To have a synchronizing age for all the thrips in the experiments, first instar larvae of *F. occidentalis* were separated with a paint brush and transferred into separate plastic cups. Larvae were checked every day for development of wings pads, and 1-3 days old adults with wings were used for all the experiments. Since tobacco thrips were more difficult to synchronize, *F. fusca* adults of mixed ages were randomly picked from the colony and used for all the *F. fusca* experiments.

Data on the settling behavior was analyzed over days and over observation time using PROC GLM (SAS 1990). If interaction was significant over days and time repeated measures analysis of variance was performed. Fisher's least significant difference method was used for comparing the treatment means over days and time PROC GLM with  $\alpha = 0.05$ . Since identical experiments provided similar results, the analysis of variance was conducted over both experiments using all eight replicates.

## Results and Discussion

Combined results over both crops indicate that both thrips species, *F. occidentalis* and *F. fusca* preferred to settle more on pollen treated leaflets, but the effect was stronger for *F. occidentalis* (Figure 3.3). The settling behavior with the addition of pine pollen was increased by seven- fold with *F. occidentalis* and one- fold with *F. fusca*. The null hypothesis that pine pollen does not increase in the settling behavior of thrips was disproved with the data collected in the choice test (Figure 3.3).

***Frankliniella occidentalis*:** Settling behavior of *F. occidentalis* was significantly higher on pine pollen treated leaves than untreated leaves over both tomato and peanut ( $F= 425$ ,  $df= 1, 3$   $P= 0.0002$ ). A seven fold increase in the settling behavior was observed with pollen treated leaves (Figure 3.4) than leaves with no pollen. No significant crop- pollen interaction was observed ( $F= 1.46$ ,  $df= 1, 3$   $P= 0.31$ ). The direction and magnitude of the effect was the same for both the crops (Figure 3.5). Overall settling behavior was based on averages over all observation times over all days.

Data on the settling behavior was also analyzed for pollen treatment effects by days and time. Pollen –day treatment interaction in tomato was significant ( $F= 15.98$ ,  $df= 7, 194$   $P= <.0001$ ) that not only settling behavior significantly increased by the pollen treatment, but this effect changed over the course of eight days of observations. In tomato the mean number of thrips settling on pollen treated leaves exhibited a specific pattern. First there was an increase in the settling on pollen leaves from day 1 to day 3 and then slow decrease from day 4 to day 8 (Figure: 3.6). The gradual increase followed by gradual decrease in the mean settling was probably due to an initial host location effect followed by a decreasing quality of the pine pollen. As the days continued, even though thrips showed preference for settling on the pollen, it was

comparatively less over time due to the decreasing quality of pollen i.e., the effect of feeding on a limited supply of pollen. In the no pollen treatment, thrips settling behavior varied little and remained low all through the observation period. A similar pattern was noticed with settling behavior of thrips on peanut with pollen- treated leaves peanut ( $F= 10.24$ ,  $df= 7$ ,  $232$   $P= < .0001$ ) (Figure 3.7). A larger, more sustained increase in the settling behavior of thrips was noticed with the pollen treatment on peanut than on tomato. In the no pollen treatment, settling behavior was uniformly low.

The settling behavior over the 2.5 hour daily observation period revealed significant effects that were small but consistent with the overall effects on settling. Only days with significant, short term time observation effects were graphed (Figure: 3.8). Pollen-time interactions for *F. occidentalis* were observed only on peanut on the 4<sup>th</sup> day ( $F= 2.76$ ,  $df=9$ ,  $270$ ,  $P= 0.05$ ) and on the 6<sup>th</sup> day ( $F= 3.48$ ,  $df= 9$ ,  $261$ ,  $P=0.01$ ) of observation (Figure 3.8). The mean settling behavior of *F. occidentalis* on 4<sup>th</sup> day on peanut trended to increase on pollen while on day 6 there was movement towards non-pollen leaves early in the observation period. *F. occidentalis* showed significant pollen-time interaction on peanut. Overall results on settling behavior for *F. occidentalis* show strong increase in settling behavior on pollen treated leaves with the effect being slightly stronger in peanut.

***Frankliniella fusca***: Settling behavior of *F. fusca* was significantly affected by pine pollen added on the surface of intact tomato and peanut ( $F= 1.32$ ,  $df= 1$ ,  $7$   $P= 0.28$ ) leaves but a lesser extent than the effect observed in *F. occidentalis* (Figure: 3.4). In crop-pollen interaction ( $F= 13.34$ ,  $df= 1$ ,  $7$   $P= .008$ ) the mean settling behavior was greater on peanut treated with pollen than with tomato treated with pollen (Figure: 3.9). The crop-pollen interaction with *F. fusca* showed the same trend for tomato and peanut, that is higher counts on pollen treated

leaves. Results suggested that this settling behavior effect, although significant was not as strong as the effect seen for *F. occidentalis*

Settling behavior for *F. fusca* analyzed over days indicated a marginally significant day-pollen treatment interaction. The mean number of thrips settling on pollen treated peanut over the eight days had a similar pattern to that observed for *F. occidentalis*, that is gradual increase in settling on pollen treated leaves from day 1 to day 4 and then a rapid decrease after day 5 (Figure: 3.10). The pollen-day interaction in peanut was significant ( $F= 5.13$ ,  $df= 7$ ,  $226$   $P= <.0001$ ). Again the gradual decrease in the mean settling behavior on pollen treated leaves was likely due to the decreasing quality of the pine pollen by 5<sup>th</sup> day. In the no pollen treatment, thrips settling behavior was not significantly affected and above the pollen treated level was low all through the observation days.

A similar pattern was noticed with settling behavior of thrips on peanut with pollen treated leaves (Figure 3.11), but a significant 2-fold increase in the settling on pollen treated peanut ( $F= 5.13$ ,  $df= 7$ ,  $226$   $P= <.0001$ ). Mean number of thrips on the pollen treatment gradually increased from day 1 to day 3 and then slowly decreased from day 4 to day 8 (Figure 3.11). There was a significant pollen-time interaction in tomato only on the 8<sup>th</sup> day observation ( $F= 3.35$ ,  $df=9$ ,  $270$ ,  $P=0.009$ ) (Figure: 3.12). This was characterized by a slight decrease in thrips on plain leaves over the observation interval. In peanut, the pollen-time treatment interaction was significant on day 1 ( $F= 3.21$ ,  $df= 9$ ,  $270$ ,  $P= 0.007$ ) and day 6 ( $F= 3.65$ ,  $df= 9$ ,  $270$ ,  $P= 0.03$ ) with some movement toward pollen on day 1 and then on day 6 (Figure: 3.13). Overall results on settling behavior for *F. fusca* indicated increased settling behavior on pollen treated leaves with a stronger effect in tomato than peanut.

Results of these studies with *F. occidentalis* and *F. fusca* indicate that pine pollen increased the settling behavior of both the thrips species on tomato and peanut leaves but the magnitude of the effect varied depending on the thrips species and host crop. A greater increase in settling of *F. occidentalis* was observed with the pollen treatment than for *F. fusca*. Both species increase settling on pollen treated peanut more so than on tomato. Even though there are slight preferences for settling behavior of the thrips based on the crop choice, results indicated that pine pollen significantly increased the settling behavior of both the thrips species on tomato and peanut leaves. Based on these results it is likely that other behaviors associated with settling behavior such as feeding and oviposition might also be affected by pollen deposition on host crop leaf surfaces. It appears that ambient pollen population dynamics is associated with thrips population dynamics on a regional scale.

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## Figure Captions

Figure 3.1 Eight-Year pollen counts for Savannah, GA (WOTC 2005, reproduced with permission from SAA).

Figure 3.2 Arrangement of intact leaves in a bioassay arena and pollen treatment in a choice test.

Figure 3.3 Settling and feeding of *F. occidentalis* adult on pine pollen in the bioassay arena.

Figure 3.4 Effect of pine pollen on settling behavior of *F. occidentalis* and *F. fusca*.

(a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

Figure 3.5 Effect of pine pollen on mean settling behavior of *F. occidentalis* on tomato and peanut (a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

Figure 3.6 Pine pollen on settling behavior of *F. occidentalis* on tomato over days (\* indicates significant treatment effect for that day).

Figure 3.7 Effect of pine pollen on settling behavior of *F. occidentalis* on peanut over days.

(\* indicates significant treatment effect for that day).

Figure 3.8 Effect of pine pollen on settling behavior of *F. occidentalis* on peanut over time.

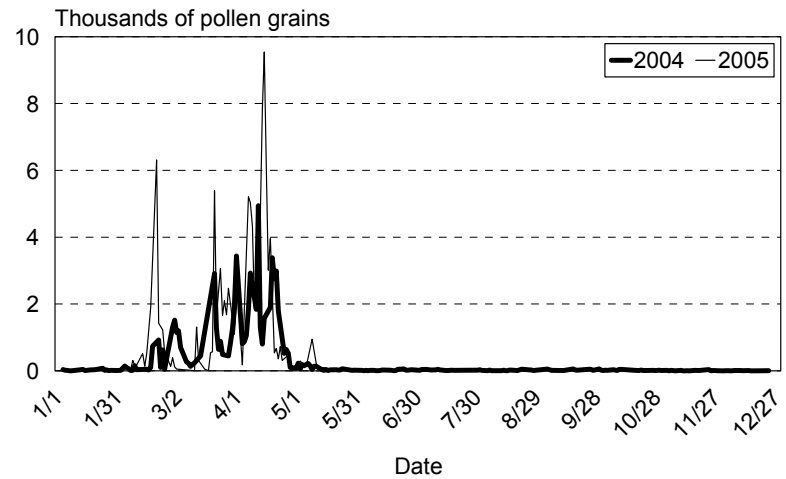
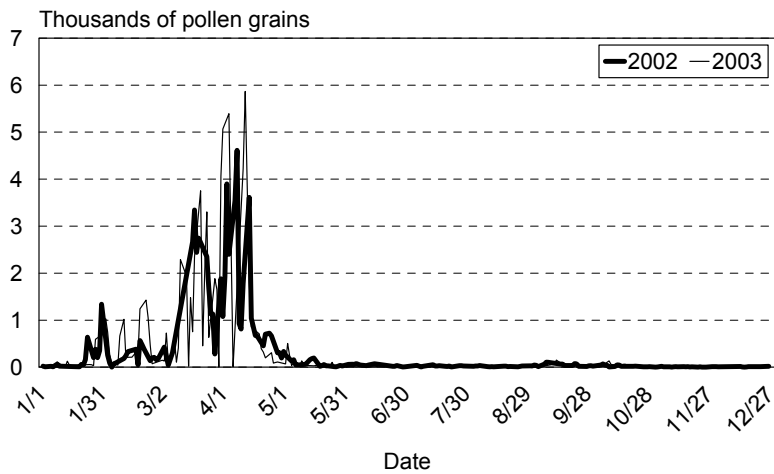
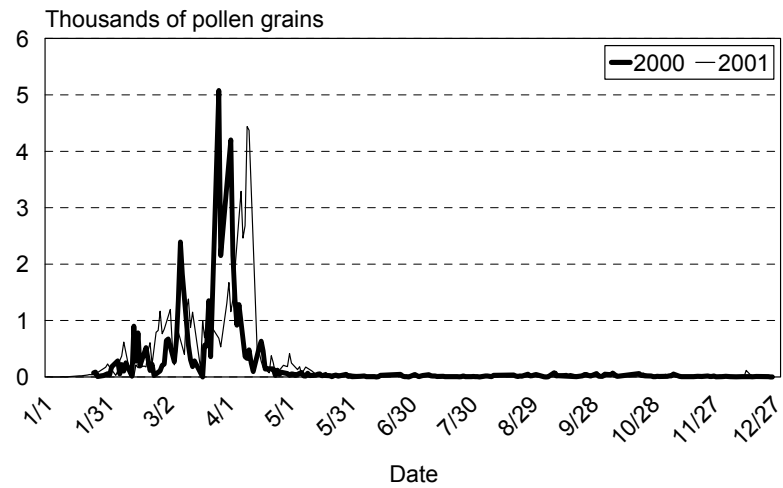
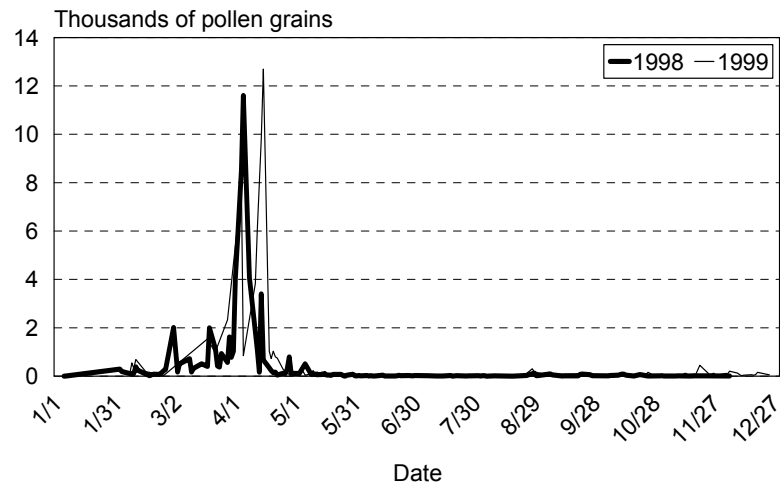
Figure 3.9 Effect of pine pollen on mean settling behavior of *F. fusca* on tomato and peanut.  
(a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

Figure 3.10 Effect of pine pollen on settling behavior of *F. fusca* on tomato over days.  
(\* indicates significant treatment effect for that day).

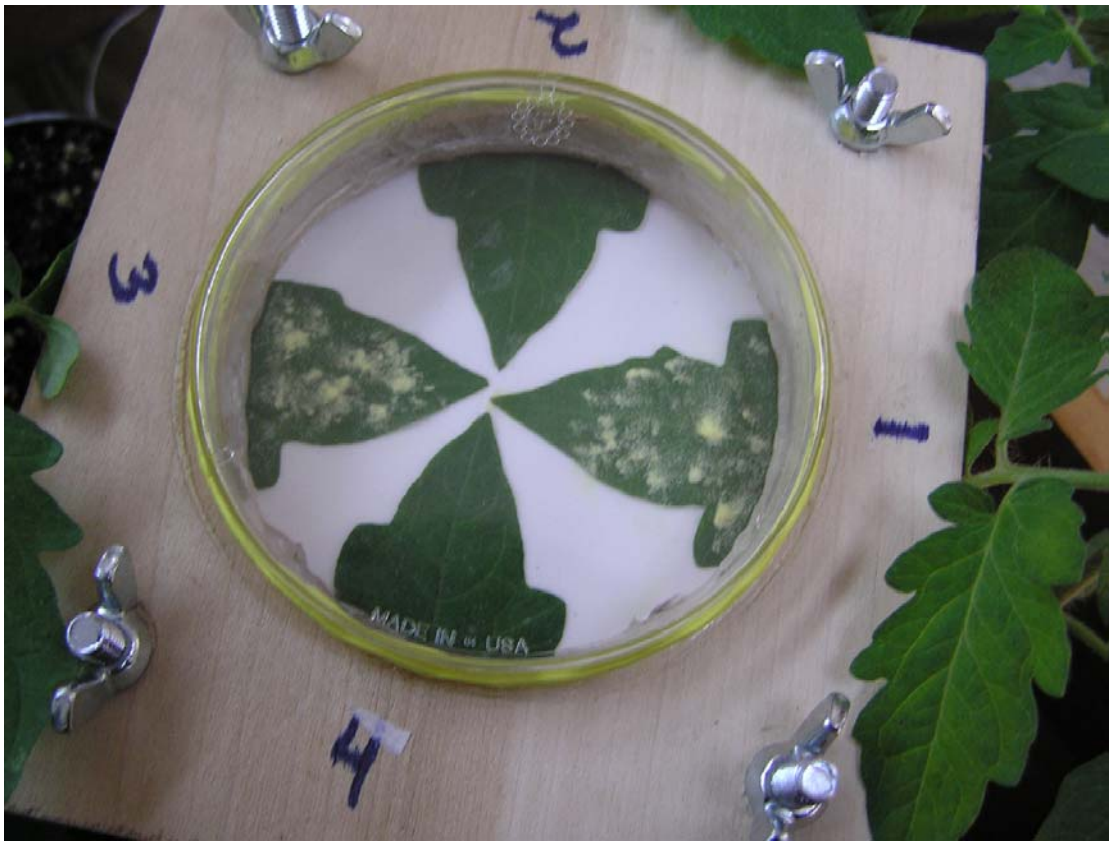
Figure 3.11 Effect of pine pollen on settling behavior of *F. fusca* on peanut over days.  
(\* indicates significant treatment effect for that day).

Figure 3.12 Effect of pine pollen on settling behavior of *F. fusca* on tomato over time.

Figure 3.13 Effect of pine pollen on settling behavior of *F. fusca* on peanut over time.



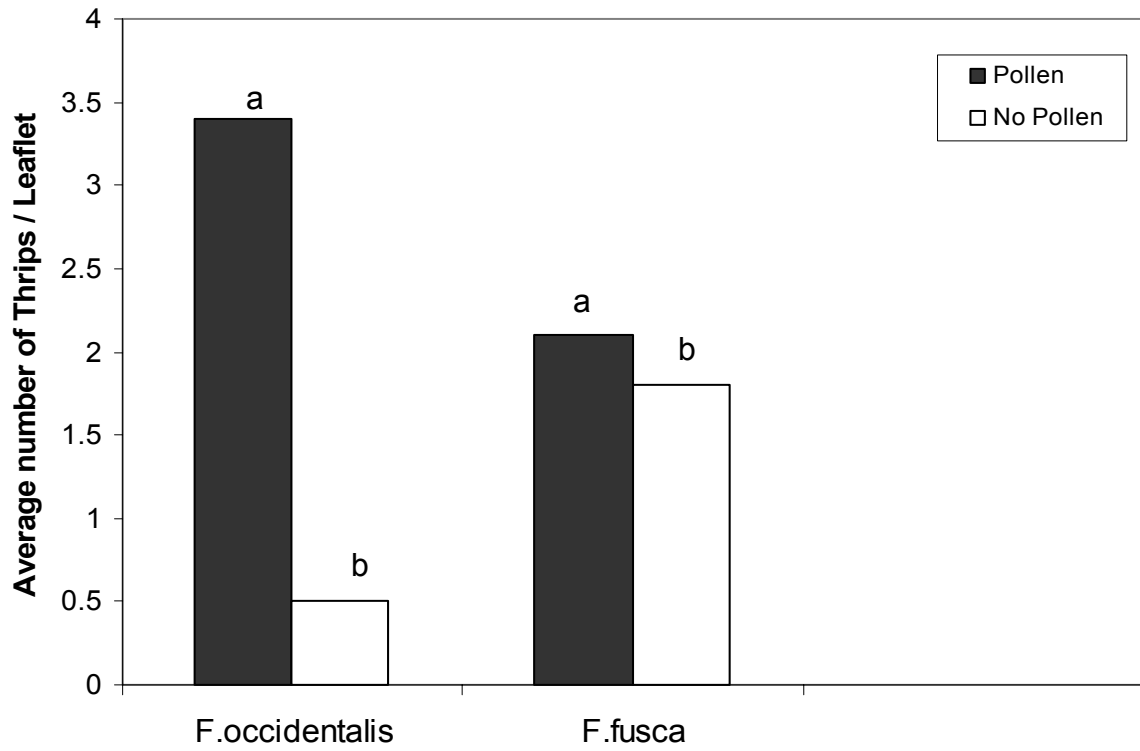
**Figure 3.1** Eight-Year Pollen Counts for Savannah, GA. (WOTC, 2005), reproduced with permission from SAA.



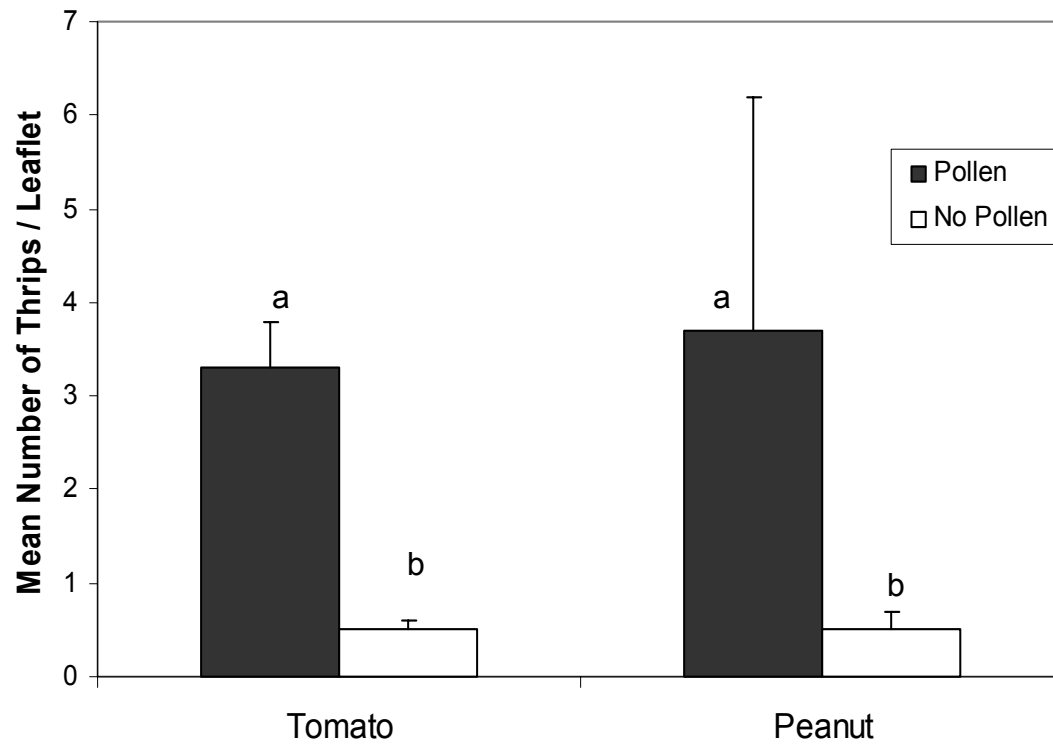
**Figure: 3.2** Arrangement of intact leaves in a bioassay arena and pollen treatment in a choice test.



**Figure 3.3 Settling and feeding of *F. occidentalis* adult on pine pollen in the bioassay arena**



**Figure: 3.4** Effect of pine pollen on settling behavior of *F. occidentalis* and *F. fusca*.



**Figure 3.5** Effect of pine pollen on mean settling behavior of *F. occidentalis* and *F. fusca* on tomato and peanut.

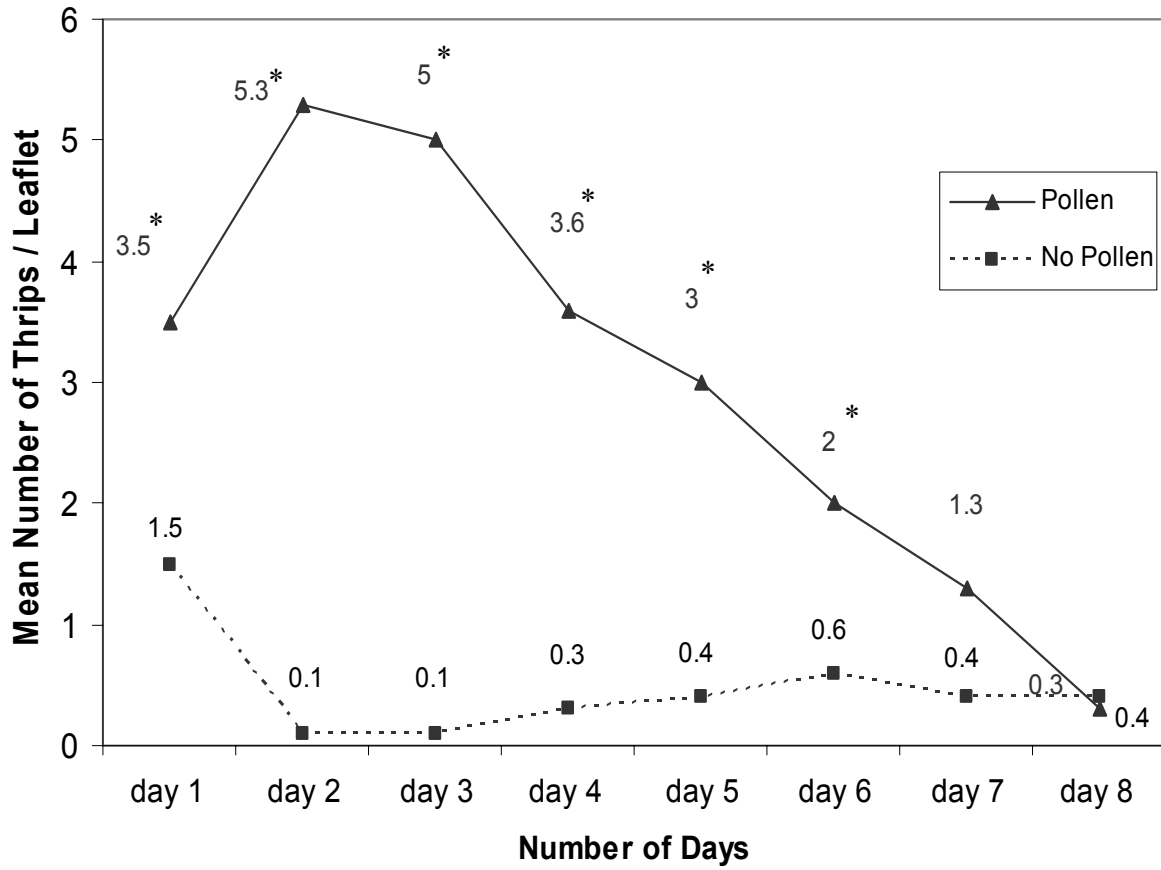
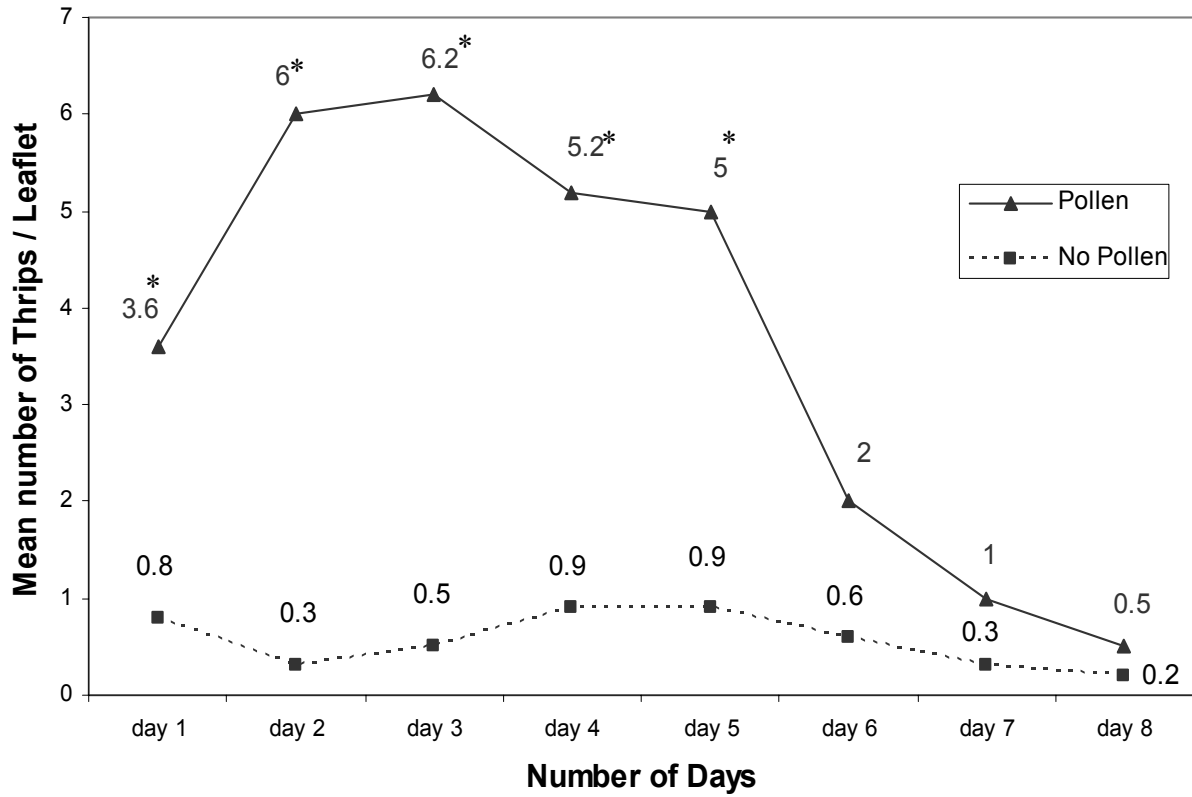
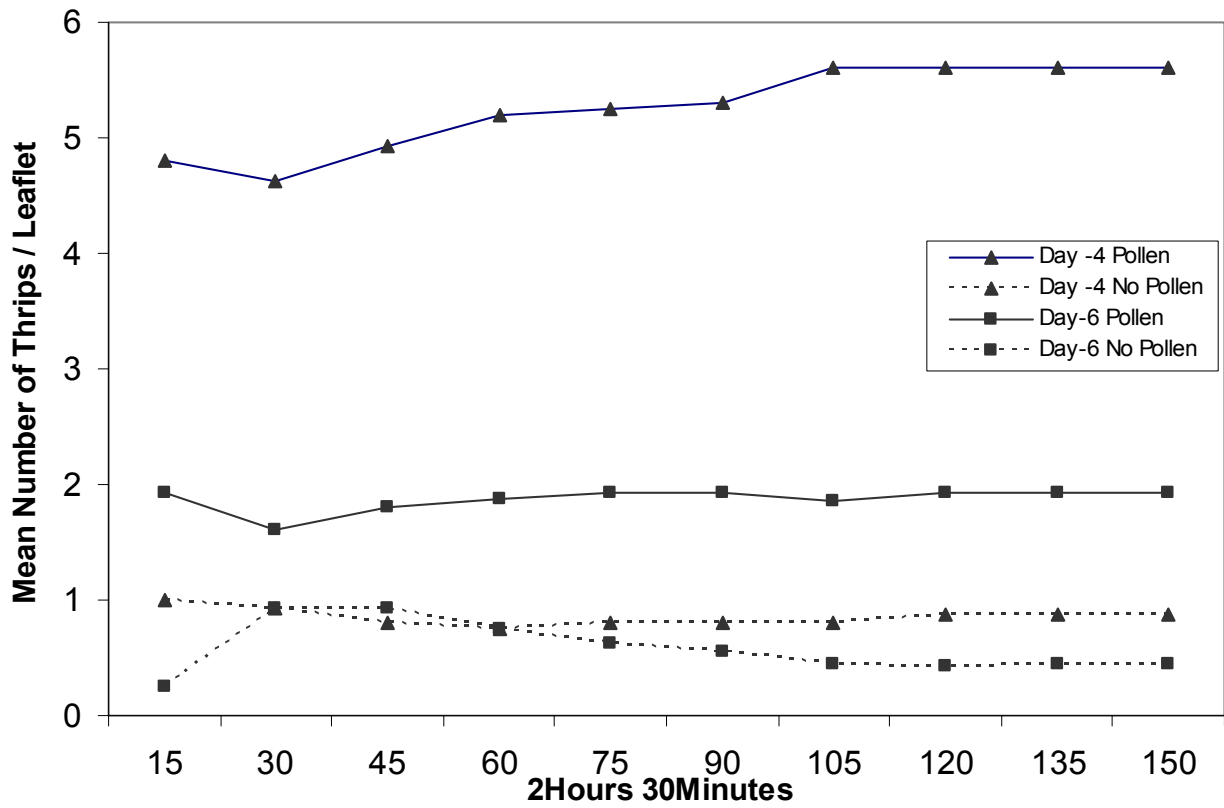


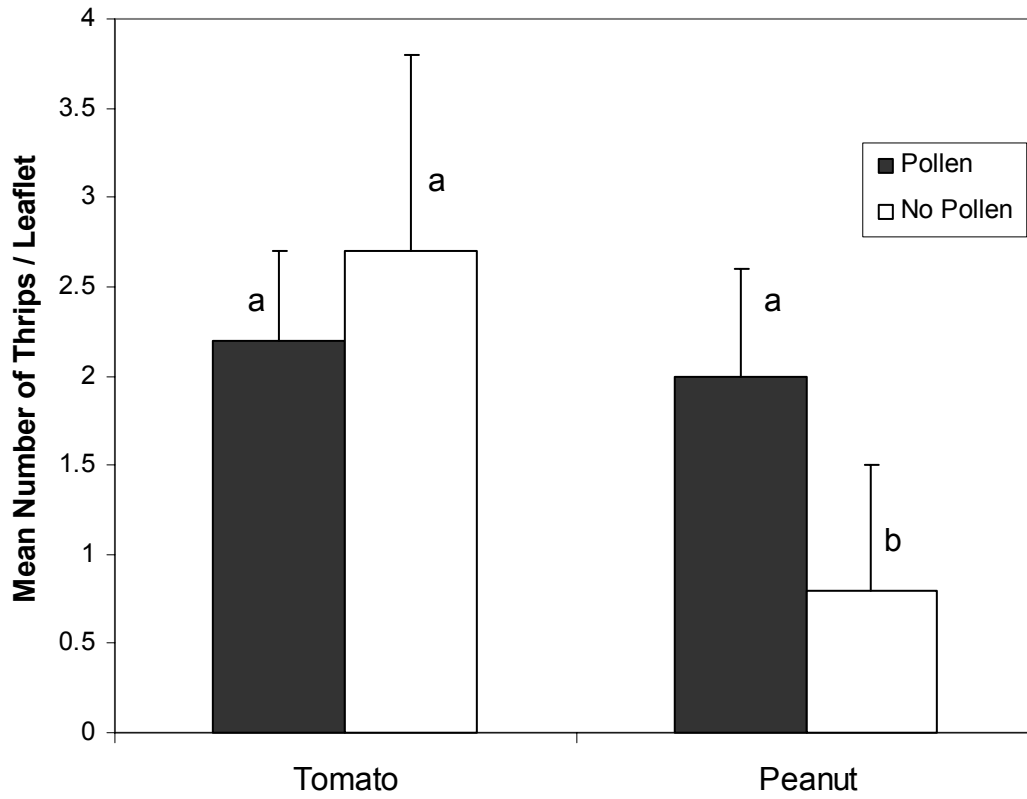
Figure: 3.6 Effect of pine pollen on settling behavior of *F. occidentalis* on tomato over days.



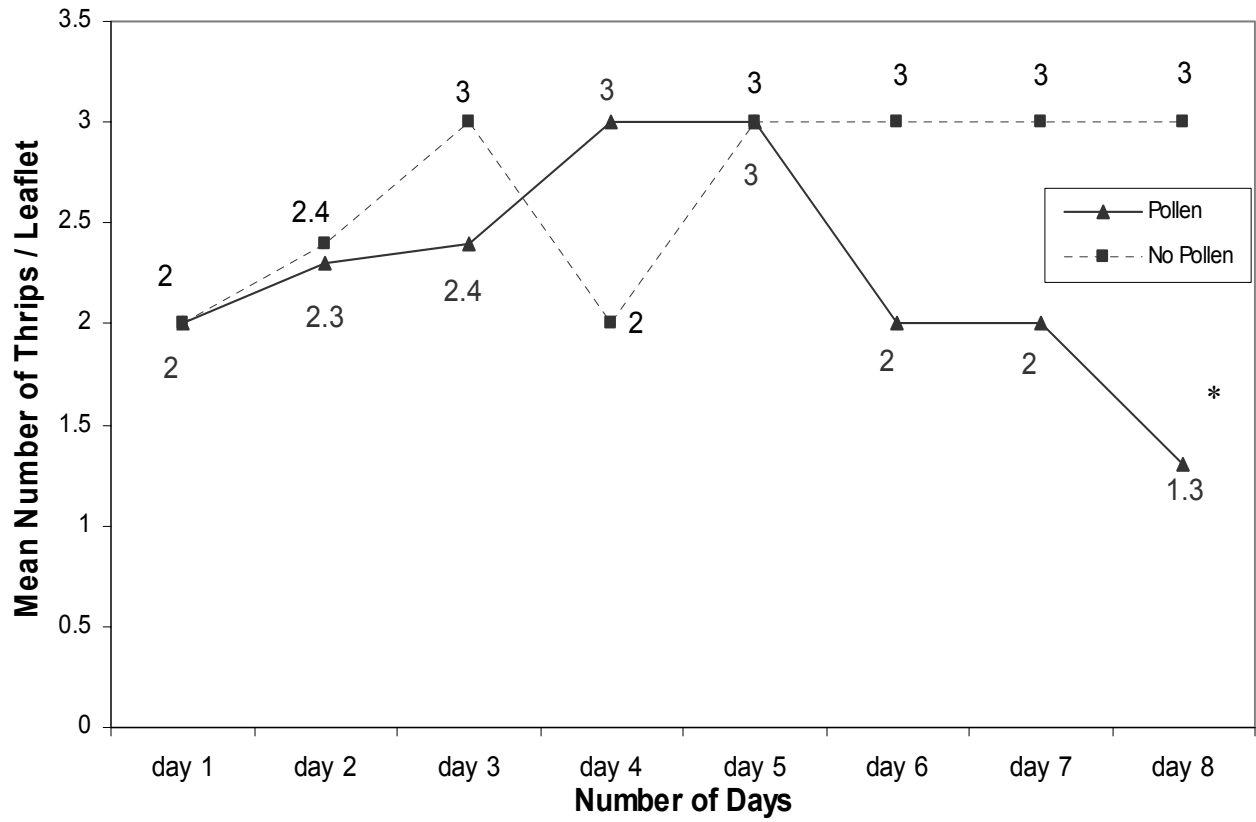
**Figure: 3.7** Effect of pine pollen on settling behavior of *F. occidentalis* on peanut over days.



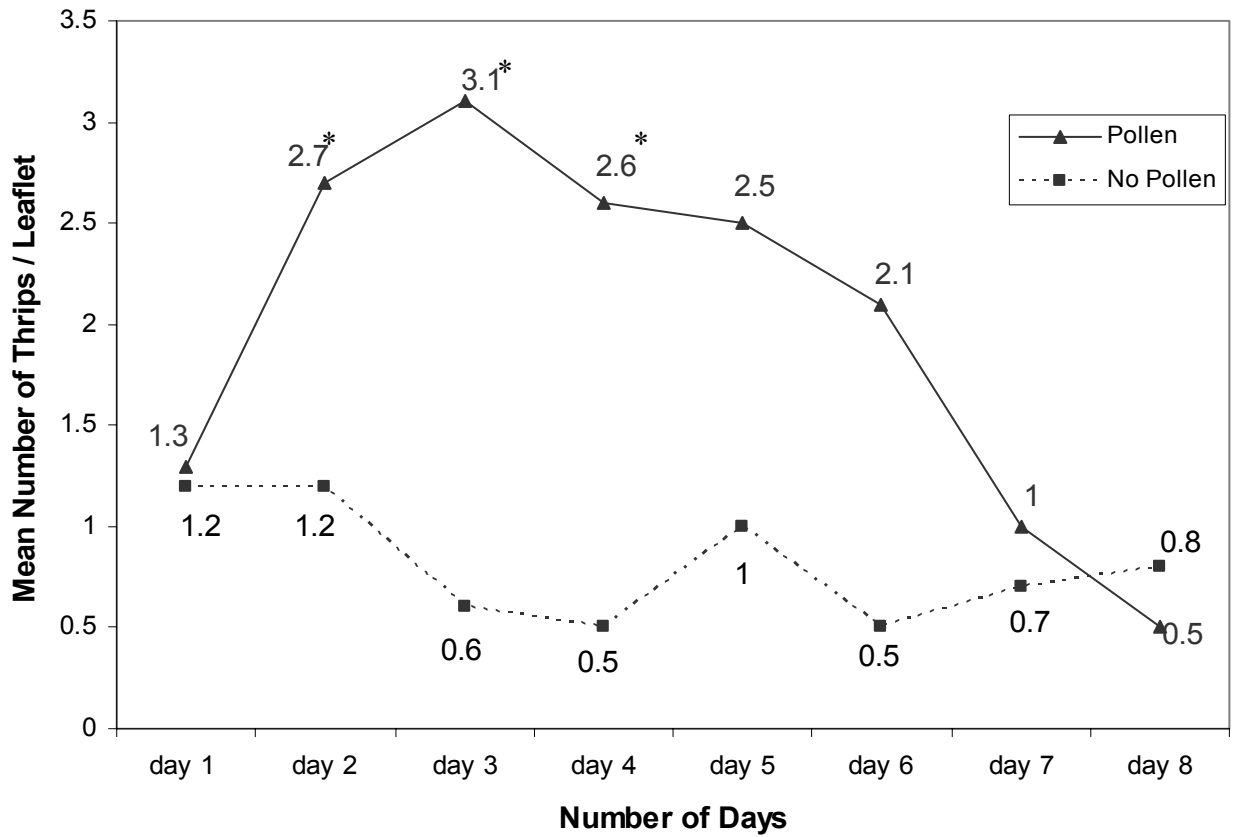
**Figure: 3.8** Effect of pine pollen on settling behavior of *F. occidentalis* on peanut over time.



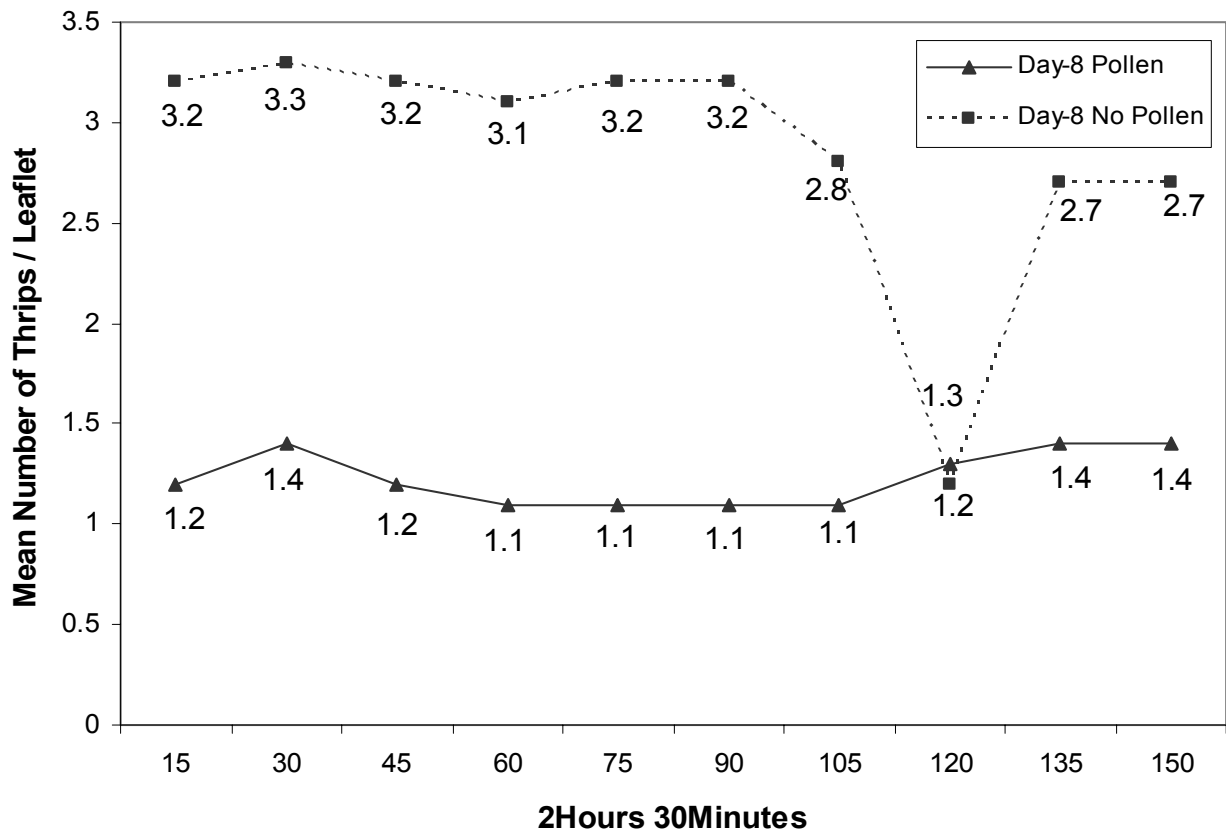
**Figure: 3.9** Effect of pine pollen on mean settling behavior of *F. fusca* on tomato and peanut.



**Figure: 3.10** Effect of pine pollen on settling behavior of *F. fusca* on tomato over days.



**Figure: 3.11** Effect of pine pollen on settling behavior of *F. fusca* on peanut over days.



**Figure: 3.12** Effect of pine pollen on settling behavior of *F. fusca* on tomato over time.

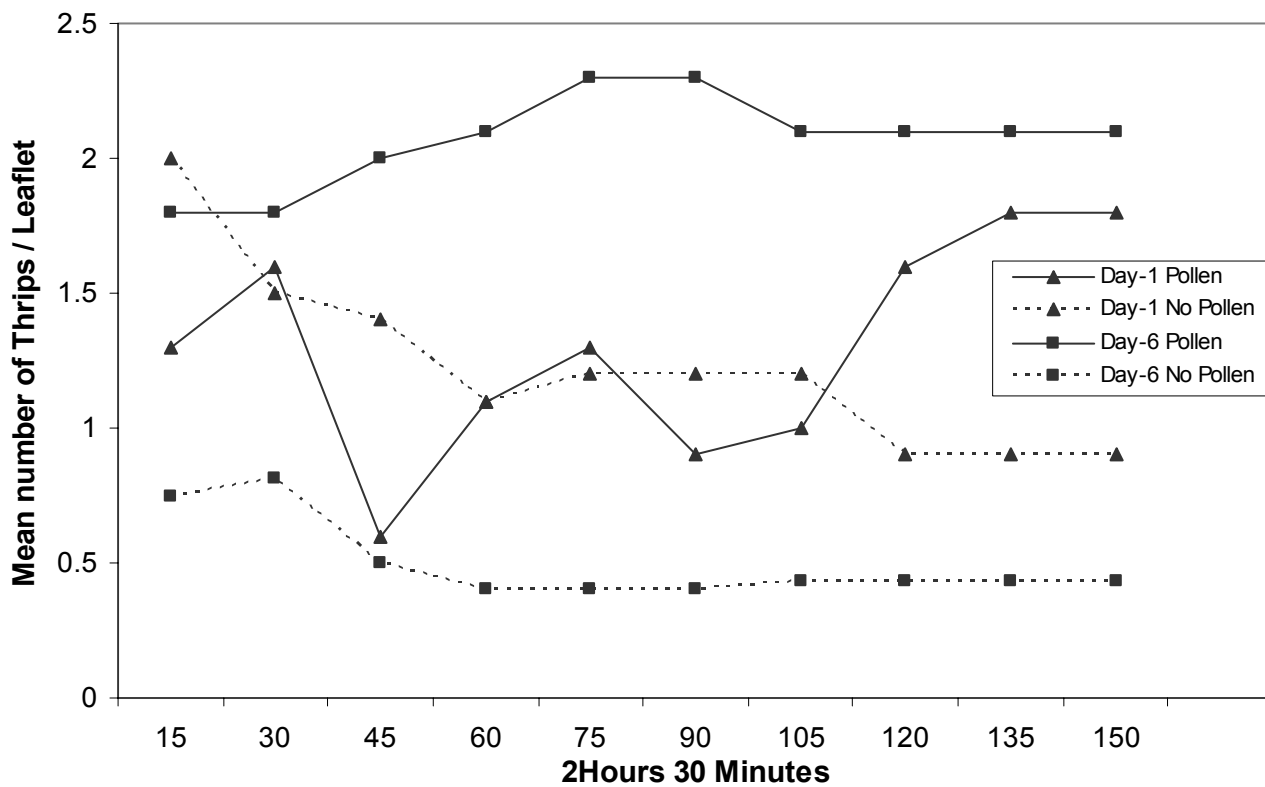


Figure: 3.13 Effect of pine pollen on settling behavior of *F. fusca* on peanut over time.

## CHAPTER 4

### EFFECT OF PINE POLLEN ON OVIPOSITION OF *FRANKLINIELLA OCCIDENTALIS* AND *FRANKLINIELLA FUSCA* (THYSANOPTERA: THIRIPIDAE) ON TOMATO AND PEANUT LEAVES

#### **Introduction**

Oviposition and reproductive success in thrips is influenced by nature of habitat and nutritional quality (Rivnay 1935, Lewis 1973, Ananthakrishnan 1984). The amount of protein in the diet of an insect significantly effects the egg production (Englemann 1984), and is important in several insect species (Gilbert 1972, Pesho & van Houten 1982) including thrips. Pollen is high in protein content (Mound *et al.* 1980) and when it is supplied as a diet to thrips, promotes high levels of egg production compared to other diets (Teulon & Penman 1991). Presence of pollen is a significant factor in reproduction in many species of thrips (Kirk 1984). Pollen as a diet for *Frankliniella* species of thrips has been shown to influence oviposition and other life history characteristics (Trichilo & Leigh 1988, Tsai *et al.* 1996).

A study conducted by Hulshof *et al.* (2003), showed that when *F. occidentalis* was engaged on cucumber leaves and then supplementaly fed with seven different pollen food types, the intrinsic growth rate increased from 0.163 on a plain leaf to 0.240 on leaves with pine pollen in a choice test. The pollen species were known to differ in the nutritional contents, specifically pine pollen was known to be a good source of protein (Todd 1942) high in nutritional value for thrips. Pine pollen as a supplemental diet to *F. occidentalis* had a positive effect on various life history components, in particular oviposition (Hulshof *et al.* 2003). Even so, this effect of pine pollen on

the reproductive potential of western flower thrips, *F. occidentalis* on foliage of cucumber was not verified for other species of *Frankliniella* on other host plants. Only a few studies have been conducted on the reproduction behavior of *F. fusca* (Lowry *et al.* 1992, Chaisuekul 2004) and none with pollen. In this study we investigated the effect of pine pollen on the oviposition of *Frankliniella occidentalis* and *Frankliniella fusca* in two crops, tomato and peanut. We hypothesized that the presence of pine pollen would increase the oviposition in *F. occidentalis* and *F. fusca* on intact tomato and peanut leaves.

### **Materials and Methods**

Two experiments were conducted in the spring 2005 at the Coastal Plain Experiment Station, Tifton GA under laboratory conditions. Choice and no choice tests were conducted first with *F. occidentalis* on intact tomato and peanut leaves to test whether pine pollen dusted onto leaves would exert any influence on thrips preference for oviposition. The second set of experiments tested *F. fusca* with the same choice and no choice treatments. Tomato plants, cv. ‘Sunny Hybrid’ (Asgrow Seed Co., Kalamazoo, MI), and peanut plants cv. ‘Sunoeleic 97 R’ were reared in a green house using standard cultural practices. Seeds were allowed to germinate and grow in transplant trays until the second true leaf stage, and then the plants were transplanted into 4-inch diameter pots using ‘Metro Mix 300’ soil media (Sun Gro Horticulture Dist. Inc, Bellevue, WA). Plants were visually checked to maintain them as insect free as possible and watered daily. Fully expanded terminal leaflets still attached to 4-5 week old plants were used for all the experiments.

Thrips oviposition was evaluated by using a bioassay similar to that described by Joost & Riley (2005) but modified to a larger arena (Figure 3.2). In the bioassay, thrips were exposed on an equal leaf portion of each treatment while keeping the treatments plants intact. An arena

composed of 100×15 mm size petri dishes (Fisher brand ® Pittsburgh, PA) was used with a wooden square frame and 4 toggle bolts at the corners for tightening the arena onto the leaf.

A 3- mm thick, one sided adhesive foam (Nu-Calgon Wholesaler Inc. St. Louis, MO) was stuck on the sides of two 15× 14 cm squares of 1 cm thick poplar (*Populus*) wood plates. A 9-cm diameter circle was drilled through the center of one square and attached foam. This square was considered as the “top square”. The “bottom square” was left whole, covered with a solid sheet of foam. The 9-cm hole of the bioassay arena had the bottom rim of petri dish glued in and was covered by the top dish. Four 7-mm diameter holes are drilled at the corners of each wooden square. Four 3-cm tall screws were inserted into the bottom square so that the foam side faced upwards. The top square was threaded through the screws with the foam side down. Wing nuts were used as a locking device. A thin layer (~2-mm) of Bacto agar (DIFCO Laboratories, Sparks, MD) is poured completely over the top of the bottom square and the bottom of the top cut out square and allowed to cool. After the leaves were sandwiched between the top and bottom plates of the arena, additional semi-liquid Bacto agar was used to seal the leaves and arenas together. The lid of the 100×15 mm tissue culture dish was sealed with yellow clay (Handi-Tak ® Super Glue Corp., Rancho Cucamonga, CA) and observations of thrips were made through the lid of the bioassay arena.

The plain leaf versus the pollen- treated leaf choice experiment were setup as follows: A terminal leaflet of four randomly selected 4-5 week old tomato or peanut plants were placed between the two wooden -foam squares, so that ~8 cm<sup>2</sup> triangle in the arena was laying on the agar. Crops were tested separately. The wing nuts were tightened, securing the leaf between the foam pads. The leaf's abaxial side was glued to the agar by adding agar droplets underneath the leaf. Ten mg of slash pine pollen, (*Pinus elliottii* Engelm.) collected from Tifton, Georgia in

February 2005 stored in a refrigerator, was spread by dusting evenly on the leaf surface of the pollen-treated leaves in the arena. The untreated leaves were covered to prevent dusting during this process. The treated (dusted) leaves are placed opposite to the plain leaves so that thrips could equally choose where to settle and oviposit. Liquid agar was applied around the edges of the arena to seal the foam pads together and prevent escape by thrips.

Four individual females of *F. occidentalis* were placed into the bioassay arena, one in the middle of each leaflet using a zero number paint brush, and the lid was placed on the top of the assay. To quantify oviposition behavior, thrips were allowed to oviposit for 8 days. The experiment was conducted in the same way on tomato and peanut using just female *F. fusca*. Two randomized complete block experiments with four replicates each were conducted for the pollen treatments for each combination of thrips species and crop.

The *F. occidentalis* colony was collected from the Plant Pathology Lab, University of Georgia, Athens, GA, USA and maintained on green beans (*Phaseolus vulgaris*) in plastic cups (Loomans & Murai 1997). The *F. fusca* colony was collected from peanut, *Arachis hypogaea* (L.), fields in Tifton, GA during the spring 2003 and maintained on whole peanut plants and green bean plants for use in these experiments. Before introduction into the arenas, *F. occidentalis* and *F. fusca* were reared on green beans in 473 ml plastic deli cups. To have a synchronizing age for all the thrips in the experiments, first instar larvae of *F. occidentalis* were separated with a paint brush and transferred into separate plastic cups. Larvae were checked every day for development of wings pads, and 1-3 days old adults with wings are used for all the experiments. Since tobacco thrips were more difficult to synchronize, *F. fusca* adult females of mixed ages were randomly picked from the colony and used for all the *F. fusca* experiments.

The oviposition sites were counted by following the lacto phenol -acid fuschin staining technique for both tomato and peanut leaves detailed by Nuessly *et al.* (1995) and Parella & Robb (1982). After the week of oviposition, thrips were removed and any immatures that had already emerged out from each leaflet were counted and collected in 70% ethyl alcohol. The intact leaflets were pinched off the plant and then they were decolorized by boiling for 3-5 minutes in the lacto phenol acid fuschin solution while working under a fume hood by using all standard safety precautions. Stained leaves were cooled for 3-5 hours, and excess stain was removed with warm water and examined under a stereo microscope for oviposition sites which were indicated by purple rings. Data on the oviposition behavior was analyzed using PROC GLM (SAS Institute 1990). Fisher's least significant difference method was used for determining treatment differences using PROC GLM with  $\alpha = 0.05$ . Since identical experiments provided similar results, the analysis of variance was conducted over both experiments using all eight replicates.

### **Results and Discussion**

Oviposition was evaluated by individual thrips species and by crop. However combined results over both crops indicate that both thrips species *F. occidentalis* (Figure 4.1) and *F. fusca* oviposited more on pollen- treated leaflets (Figure 4.2), but that the oviposition increase was stronger for *F. occidentalis*. A significant crop-pollen interaction over both thrips species indicated a strong effect of pollen on oviposition ( $F= 28.04$ ,  $df = 1, 7$ ,  $P= 0.0011$ ) in peanut than tomato (Figure 4.3). No choice test results on oviposition rate over both crops, showed increase in oviposition for *F. occidentalis* when all leaves were dusted with pollen (Table 4.1).The hypothesis that pine pollen increases the oviposition in both the thrips species on intact tomato and peanut leaves was supported by the data collected in these tests (Figure 4.2).

***Frankliniella occidentalis***: Oviposition rate in *F. occidentalis* was significantly higher on pollen- treated leaves over both crops ( $F= 25.22$ ,  $df = 1, 7$ ,  $P= 0.0015$ ). A three- fold increase in the oviposition rate was observed on pollen- treated leaves (Figure 4.2). A significant crop- pollen interaction was observed ( $F= 19.26$ ,  $df = 1, 11$ ,  $P= 0.0011$ ) indicating different effects of pine pollen on the oviposition rate across crops (Figure 4.4). Mean oviposition rate was higher on pollen- treated leaves in peanut than with tomato treated with pollen. A four- fold increase in mean oviposition rate was observed on pollen treated leaves to plain leaves ( $277 \pm 49$   $69 \pm 32$ ) in peanut while in tomato there was a two- fold increase in mean oviposition on pollen treated leaves to plain leaves ( $186 \pm 78$   $88 \pm 20$ ) (Figure 4.4). In no choice tests, oviposition rate was stronger for *F. occidentalis* when all the leaflets were dusted with pollen with a five-fold increase ( $260 \pm 48$   $49 \pm 25$ ) in the oviposition rate being observed in tomato (Table 4.1). We also observed high number of *F. occidentalis* nymphs emerging on pollen treated leaves at end of one week in both tomato ( $n= 450$ ) and peanut ( $n=430$ ). Over all results on oviposition rate in *F. occidentalis* revealed a strong increase in oviposition on pollen treated leaves with effect being stronger in peanut.

***Frankliniella fusca***: Oviposition rate in *F. fusca* was significantly affected by pine pollen, but to a lesser extent than the effect observed in *F. occidentalis*. There was a significant increase in oviposition on pollen- treated leaves by *F. fusca* ( $F= 25.22$ ,  $df = 1, 7$ ,  $P= 0.0015$ ) (Figure 4.2). A crop-pollen interaction was significant for *F. fusca* ( $F= 28.04$ ,  $df = 1, 7$ ,  $P= 0.0011$ ). The mean oviposition was greater in peanut than in tomato treated with pollen (Figure 4.5). A two- fold increase in mean oviposition rate was observed on pollen- treated leaves compared to plain leaves ( $124 \pm 58$   $66 \pm 22$ ) in peanut, and less than or one- fold increase on pollen treated to plain leaves ( $66 \pm 32$   $48 \pm 24$ ) was observed in tomato (Figure 4.5). No choice tests showed no

significant treatment effect when leaves were not dusted with pine pollen (Table 4.1). Overall results on oviposition rate in *F. fusca* indicated increased oviposition on pollen- treated leaves with a stronger effect on peanut than tomato.

In summary, results of oviposition studies with *F. occidentalis* and *F. fusca* indicate that pine pollen increased the oviposition rate of both thrips species on plant leaf tissue, but that the oviposition rate varied depending on the thrips species and host crop. A greater oviposition rate with pollen treatment was observed for *F. occidentalis* than for *F. fusca*. Both species of thrips showed a stronger increase in oviposition on pollen- treated peanut than tomato, showing a specific crop- pollen interaction relative to thrips choice for oviposition. Also, based on these results and the results on settling in chapter 3, oviposition behavior is positively correlated with settling behavior. These findings strongly support the hypothesis that settling and oviposition behavior patterns of thrips are directly affected by pollen deposition. We suspect that the ambient pollen reported by Railsback (2003) for Georgia is affecting thrips population dynamics in the same region with respect to settling and oviposition on pine pollen dusted leaf tissue.

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### Figure Captions

Figure 4.1 Stained leaf tissue showing eggs of *F. occidentalis* deposited on the tomato leaves at two magnifications.

Figure 4.2 Effect of pine pollen on oviposition of *F. occidentalis* and *F. fusca* (a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

Figure 4.3 Effect of pine pollen on mean oviposition of *F. occidentalis* and *F. fusca* on tomato and peanut (a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

Figure 4.4 Effect of pine pollen on mean oviposition of *F. occidentalis* on tomato and peanut. (a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

Figure 4.5 Effect of pine pollen on mean oviposition of *F. fusca* on tomato and peanut (a, b indicates a significant difference within the same species, LSD Test,  $P < 0.05$ ).

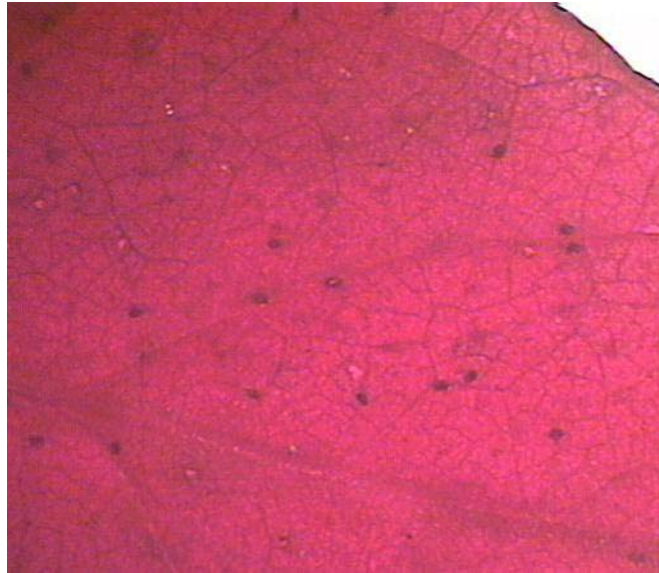
**Table 4.1 Effect of pine pollen on oviposition and percent scar fraction of *F. occidentalis* and *F. fusca* in tomato and peanut**

Species	Crop	Treatment	Eggs	% Scar fraction
<i>F. occidentalis</i>	Tomato	Pollen	260.1± 48.9 a	0.75 ± 0.36a
		No Pollen	49.2 ± 25.5 b	0.76 ± 0.35a
	Peanut	Pollen	151.6 ± 80.9 a	0.00 ± 0.00a
		No Pollen	0.00 ± 0.00 b	0.00± 0.00 a
<i>F. fusca</i>	Tomato	Pollen	47.3 ± 25.7a	0.06 ± 0.19a
		No Pollen	52.3 ± 23.5 a	0.48 ± 0.14b
	Peanut	Pollen	126.2 ± 47.1 <sup>2</sup>	0.02 ± 0.02 <sup>2</sup>

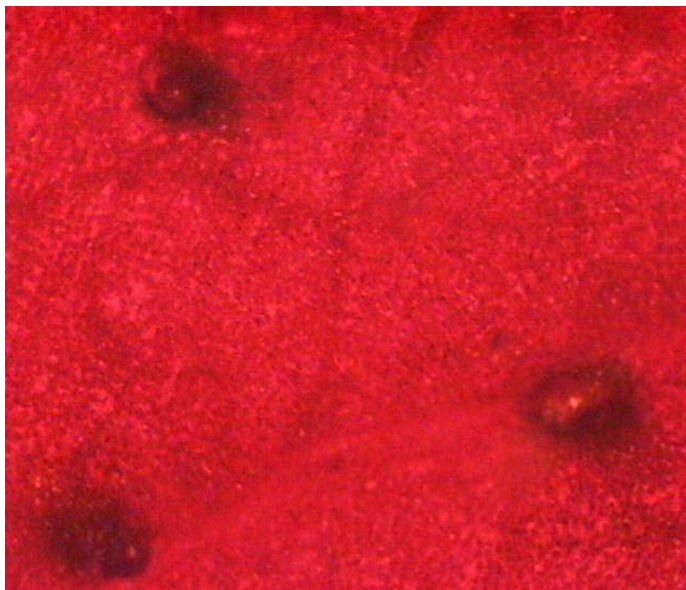
<sup>1</sup>Means within columns within a crop and thrips species are not significant if followed by the same letter, LSD Test,  $P < 0.05$ .

<sup>2</sup>*F. fusca* was not tested on peanut without pollen.

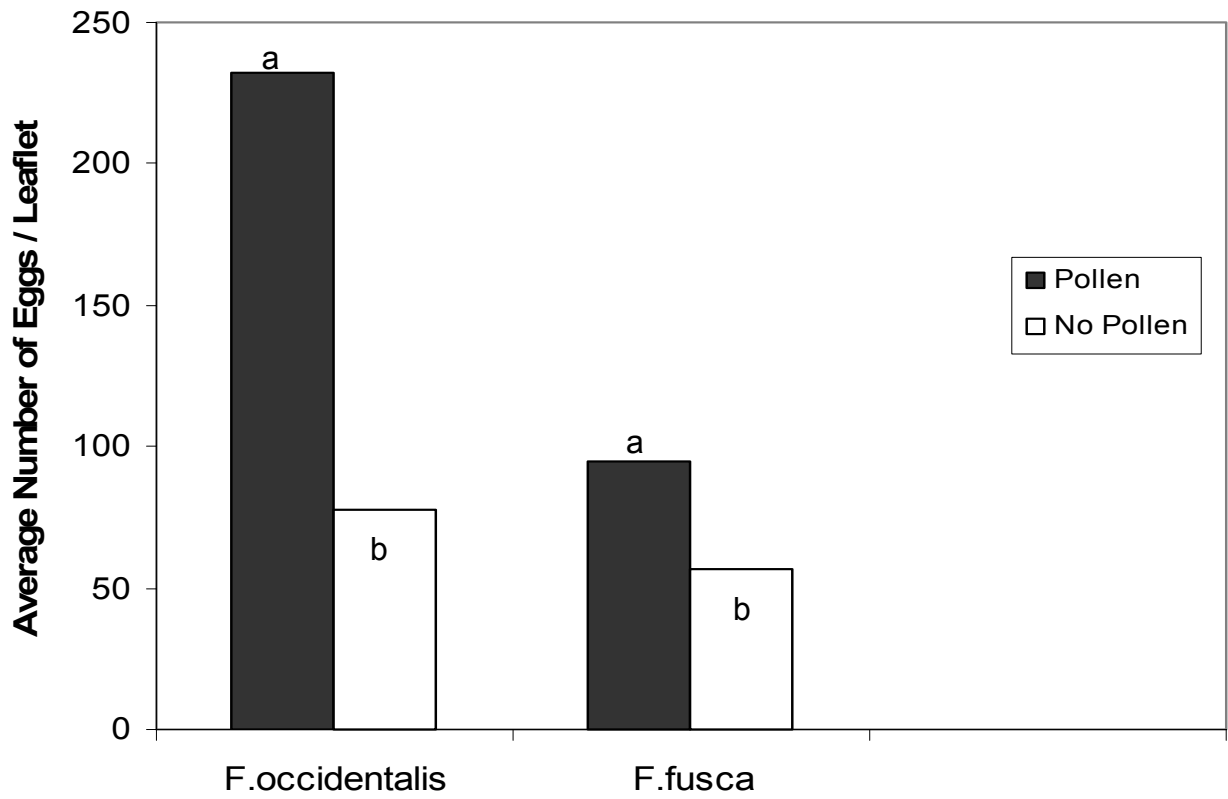
A. Low magnification



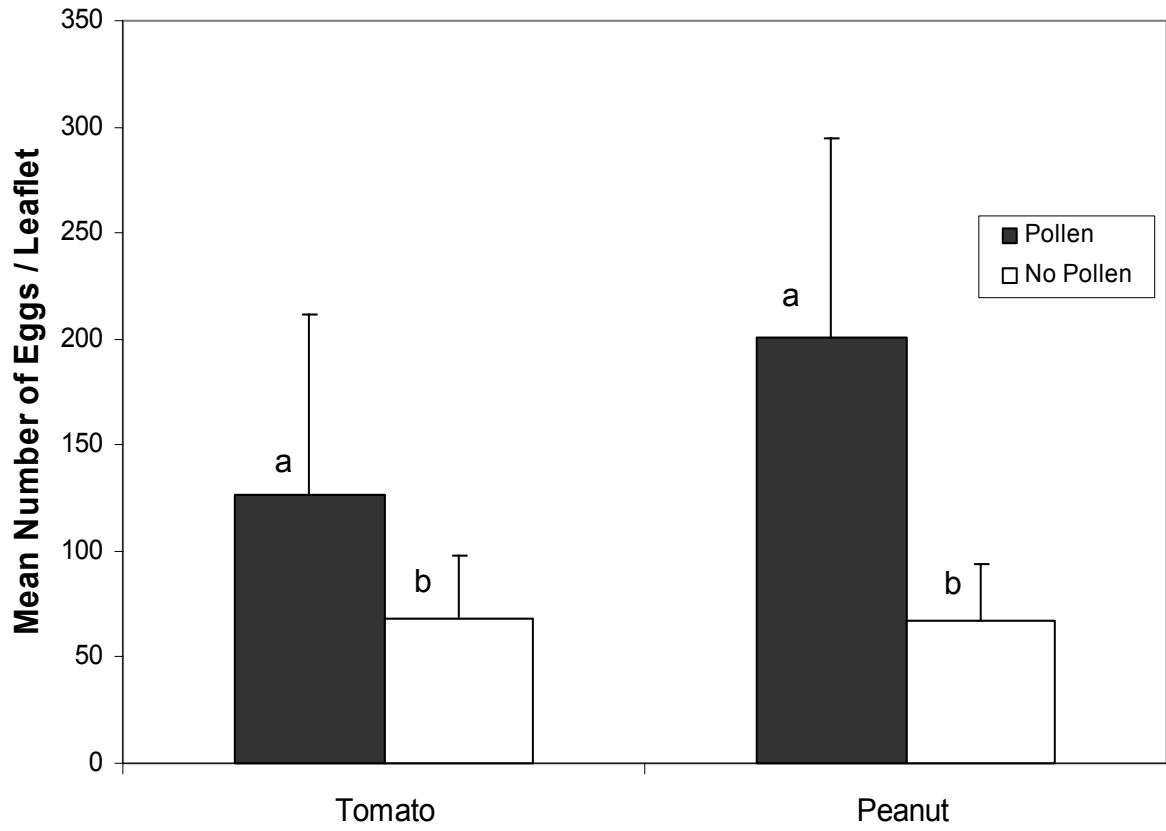
B. High Magnification



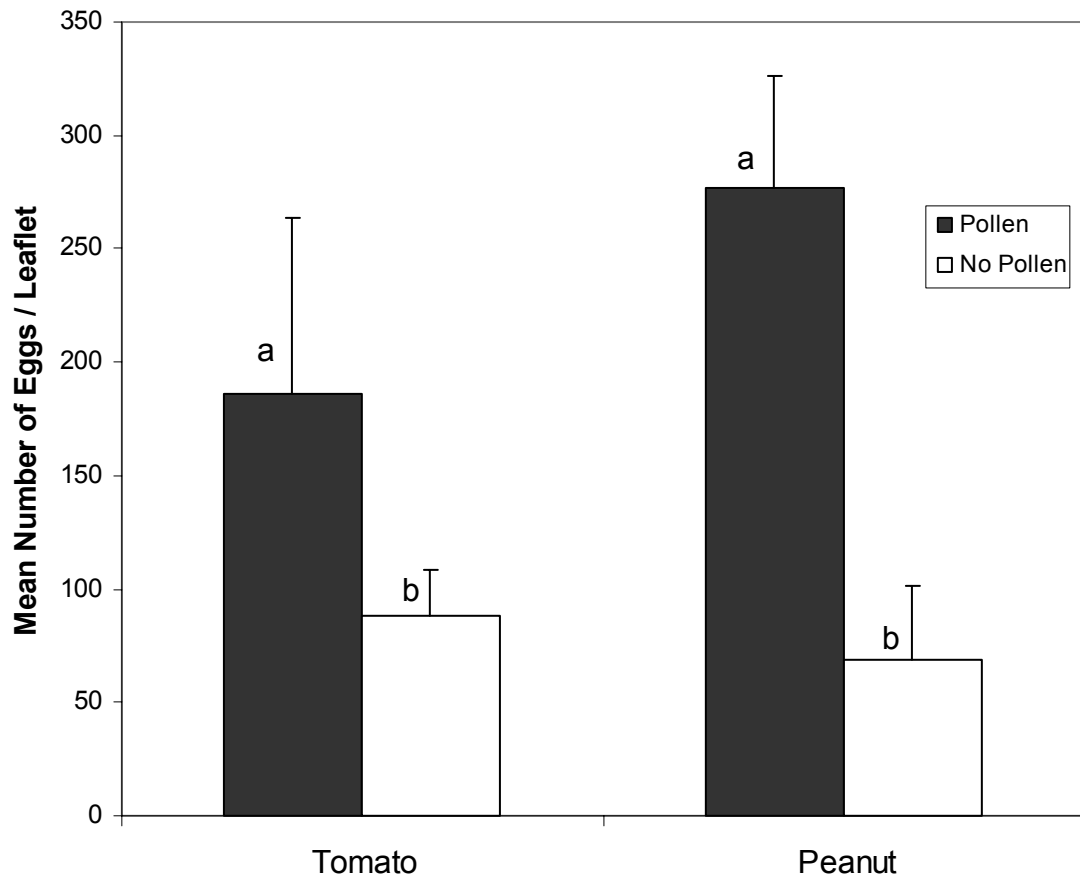
**Figure 4.1 Stained leaf tissue showing eggs of *F. occidentalis* deposited on the tomato leaves at two magnifications.**



**Figure 4.2** Effect of pine pollen on oviposition of *F. occidentalis* and *F. fusca*.



**Figure 4.3** Effect of pine pollen on mean oviposition of *F. occidentalis* and *F. fusca* on tomato and peanut.



**Figure 4.4** Effect of pine pollen on mean oviposition of *F. occidentalis* on tomato and peanut.

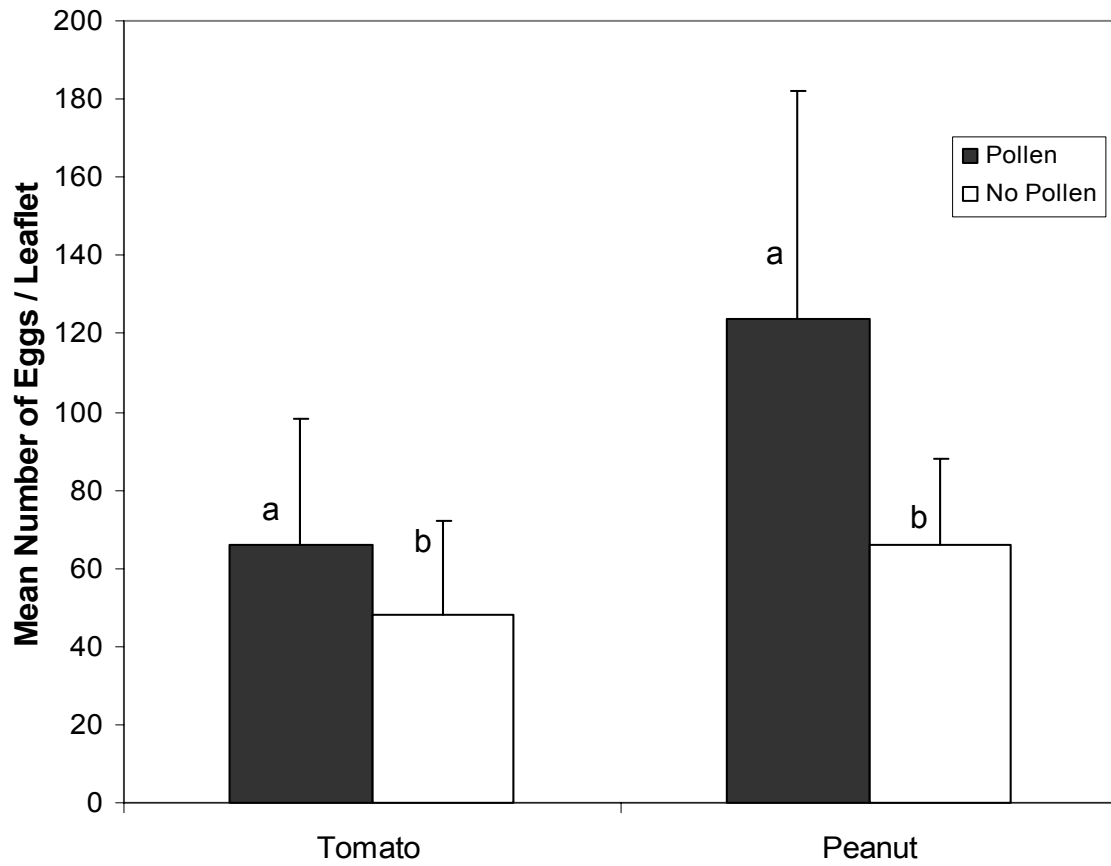


Figure 4.5 Effect of pine pollen on mean oviposition of *F. fusca* on tomato and peanut.

## CHAPTER 5

### EFFECT OF PINE POLLEN ON THE PERCENT ACQUISITION OF *TOMATO SPOTTED WILT VIRUS* IN *FRANKLINIELLA OCCIDENTALIS* AND *FRANKLINIELLA FUSCA* (THYSANOPTERA: THIRIPIDAE)

#### **Introduction**

Tomato Spotted Wilt Virus (TSWV) transmitted by thrips infects 1100 plant species causing damage in many agricultural and horticultural crops plus many weed species (Chatzivassiliou *et al.* 2001, Peters 2004). TSWV, belonging to the genus *Tospovirus*, family *Bunyaviridae* is the only genus in this family of virus infecting plants (Milne & Francki 1984, Francki *et al.* 1991). At least seven thrips species are confirmed to be the vectors of virus by the 1990's (Todd 1990) of which western flower thrips, *Frankliniella occidentalis* (Pergande) and tobacco thrips *Frankliniella fusca* (Hinds) are important in Georgia infesting tomato and peanut (Chamberlin 1992). Currently there are 11 known vector species (Mortiz 2005).

The close link between thrips development and *Tospovirus* epidemiology is necessary to understand the *Tospovirus* infection cycle. Adult thrips are winged and disperse readily while the larvae are wingless. The female adults have to select host plants on which larvae can develop and the developing adults finally disperse (Ullman *et al.* 1992). Host suitability on which thrips feed significantly influences the virus acquisition and transmission (German *et al.* 1992). When a susceptible plant is infected, TSWV will spread systemically through the plant (Best 1968). The infection cycle is initiated only when female adult thrips lays eggs on a virus infected host plant that also favors egg and larval development. Acquisition of TSWV is dependent on the

oviposition of the female thrips, since only early instars can acquire the virus and larvae develop on the host on which they are hatched (Maris 2004). TSWV acquisition can occur only when young larvae feed on virus infected plants or adults that arise from such immatures (Van de Wetering *et al.* 1996). TSWV is endemic in Georgia, the two common thrips, *Frankliniella occidentalis*, western flower thrips and *Frankliniella fusca*, tobacco thrips are the vectors of virus (Chamberlin 1992).

Thrips ingest the pollen and suck the liquids from the grains (Lewis 1973), but thrips transmitting a pollen- borne virus, such as occurs with Ilavirus (Mink 1992) while feeding on TSWV infected pollen or TSWV infected plant foliage has not been shown. In fact TSWV, has not been reported in plant pollen. However, it has been claimed that thrips carry pollen and incidentally aids entry of the virus into the plant when they feed (Gerber *et al.* 1991, Mink 1992, Hardy & Teakle 1992, Klose *et al.* 1996) for other virus systems. A study was initiated during the summer of 2005 at the Coastal Plain Experiment station in Tifton Georgia, to evaluate the effect of pine pollen on the percent acquisition of TSWV. In this test we investigated the percent acquisition of TSWV by *F. occidentalis* and *F. fusca* on virus infected tomato and peanut leaves with choice (dusted with pine pollen) and no choice (no pollen) feeding trials.

### **Materials and Methods**

Two tests were conducted in the summer of 2005 at the Coastal Plain Experiment Station, Tifton GA in growth chambers. Peanut plants cv. ‘Sunoelic 97 R’ were reared in a green house under standard cultural practices. Seeds were allowed to germinate in transplant trays until the second true leaf stage, and then the plants were transplanted into 4- inch diameter pots using ‘Metro Mix 300’ soil media (Sun Gro Horticulture Dist. Inc, Bellevue, WA). Plants were

visually checked to maintain them as insect free as possible and watered daily. Peanut plants were grown in the green house when the plants were one month old they were exposed to a population of viruliferous *F. fusca*. The individual test plants were tested with an enzyme-linked immunosorbent assay (ELISA) to determine if they were positive for TSWV infection. The colony of *F. occidentalis* was collected from the Plant Pathology Lab, University of Georgia, Athens, GA, USA and maintained on green beans (*Phaseolus vulgaris*) in plastic cups (Loomans & Murai 1997). The *F. fusca* colony was collected from peanut, *Arachis hypogaea* (L.), fields in Tifton, GA during the spring 2003 and maintained on whole plants for use in these experiments. The colonies of *F. occidentalis* and *F. fusca* were reared free of TSWV. Ten mg of ambient pine pollen, slash pine (*Pinus elliottii* Engelm.), collected from southern Georgia in February 2005 was spread by dusting evenly on the leaf surface of the pollen-treated leaves.

In the first test, twenty peanut plants that were confirmed positive for TSWV with ELISA were placed into 4-inch plastic pots. Choice test with ten peanut plants dusted with pine pollen and a no choice test of plain plants (no pine pollen) were taken. Five *F. occidentalis* and five *F. fusca* adults were placed on each of the peanut plants and a fine-screened, 8×8 cm vented, gallon zipper seal plastic storage bag (26.8 ×27.9 cm) (Mktd. Walmart Stores Inc. Bentonville, AR) was placed over the entire plant. The peanut plants were maintained for 2-3 weeks. Both the original number of thrips and the new immature thrips coming off of the plants were collected. The individual adults and the immature thrips were tested for TSWV using the ELISA.

In the second test, eight confirmed TSWV infected peanut plants were placed in 4-inch pots. A choice test, dusted with slash pine pollen on four peanut plants and no choice test, with no pollen on the remaining four peanut plants were taken. Five adults of *F. occidentalis* were placed on each of the peanut plants and a fine-screened vented plastic bag was placed over the

entire plant. Plants were maintained for 2-3 weeks and original number of thrips and the new immature thrips coming off of the plants were collected and tested for TSWV using the ELISA for thrips. Data on the percent acquisition of tomato spotted wilt virus was analyzed using PROC GLM (SAS Institute 1990).

### **Results and Discussion**

Percent acquisition of TSWV in both thrips species was evaluated with choice and no choice tests in peanut. Percent acquisition was calculated over adults and nymphs with *F. occidentalis* and *F. fusca*. Results indicate a significant percent acquisition of TSWV over both adults and nymphs when pollen was added on peanut leaves. The percent acquisition of virus was higher with pollen treated plants with nymphs and adults than with no pollen added (Figure 5.1). Results analyzed by growth stage indicated that the pollen- treatment effect was marginally significant with *F. fusca* adults ( $F= 2.45$ ,  $df= 1, 6$ ,  $P= 0.16$ ) and nymphs ( $F= 3.46$ ,  $df= 1, 6$ ,  $P= 0.11$ ). Sufficient data was not available for *F. occidentalis* to conduct an analysis of variance due to low reproduction of this species on peanut, but the percent acquisition of TSWV was reported for the few thrips collected. A square root transformation of the data was applied to the percent viruliferous thrips for *F. fusca* to normalize the data for analysis of variance, but means were reported in the original percentage. Percent viruliferous thrips were reported over both nymphs and adults for *F. fusca* (Figure 5.2) and *F. occidentalis* (Figure 5.3), but only *F. fusca* data indicated a significant increase in the percentage of virus acquisition with plants dusted with pollen ( $F= 6.14$ ,  $df= 1, 9$ ,  $P= 0.03$ ).

These data demonstrate that the presence of pollen significantly affected virus acquisition, at least with the thrips species that was able to successfully reproduce on the host plant, i.e., *F. fusca*. In addition, these data suggest that pollen dusted onto host plant leaves

could significantly increase the percent acquisition in the generation of thrips resulting from an ovipositional event. This observation could be very important in trying to understand the population dynamics of thrips vectors and the epidemiology of TSWV in the field. It is possible that pollination events, such as pine pollen shed that occurs systematically each year in the spring in the Southeastern USA (Dorman & Barber 1956) could increase the acquisition of TSWV by thrips vectors in the field.

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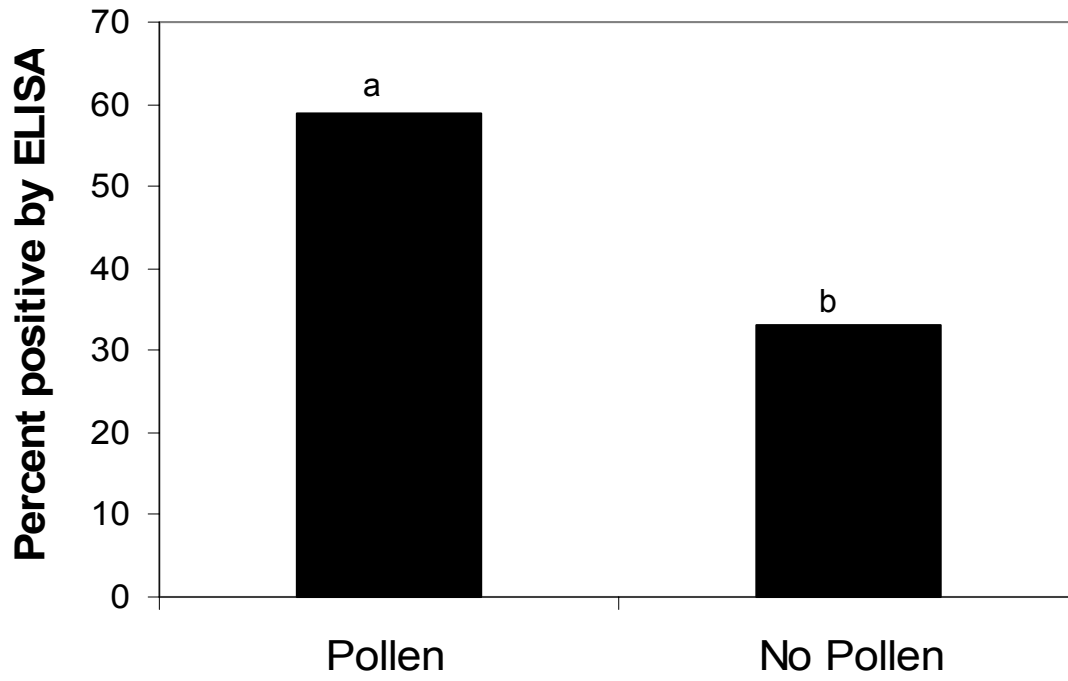
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## Figure Captions

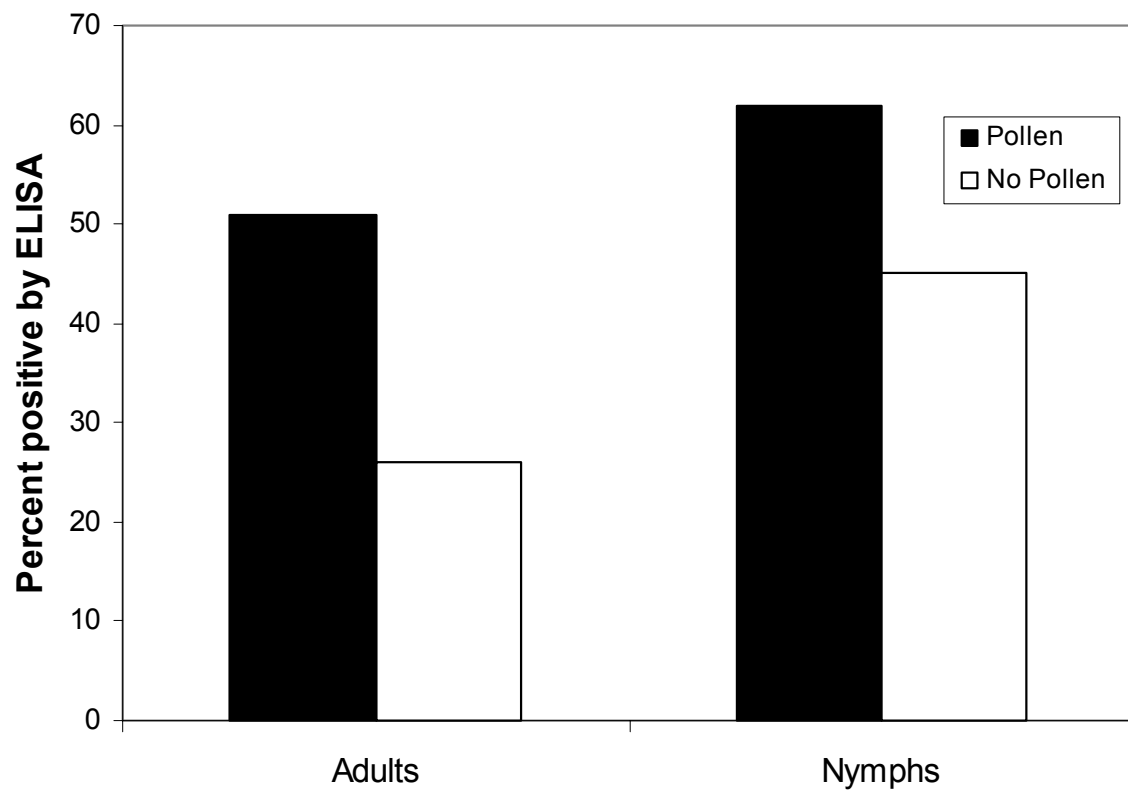
Figure 5.1 Effect of pine pollen on the percent viruliferous *F. fusca* adults and nymphs reared on peanut (a, b indicates significant difference in the treatment, LSD Test,  $P < 0.05$ , based on square root transformed percentages, reported in original percentages).

Figure 5.2 Effect of pine pollen on percent acquisition of TSWV in *F. fusca* by adults and nymphs reared on peanut (LSD Test,  $P < 0.05$ ).

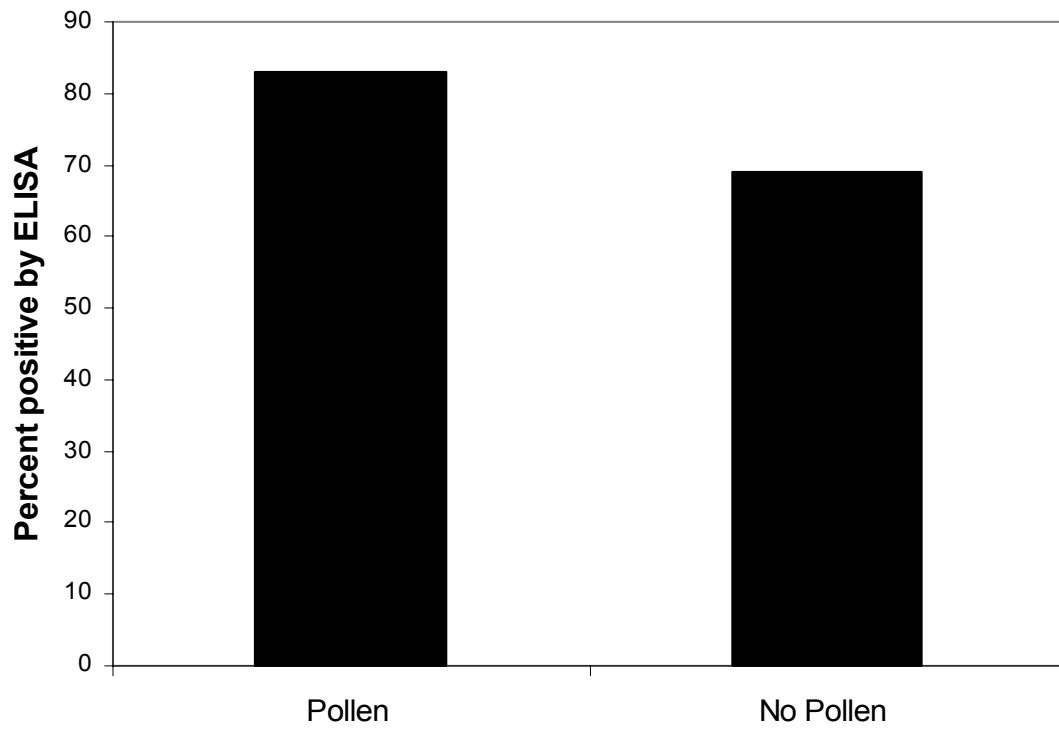
Figure 5.3 Effect of pine pollen on the percent viruliferous *F. occidentalis* by adults and nymphs reared on peanut (LSD Test,  $P < 0.05$ ).



**Figure 5.1** Effect of pine pollen on the percent viruliferous *F. fusca* by adults and nymphs reared on peanut.



**Figure 5.2** Effect of pine pollen on percent acquisition of TSWV in *F. fusca* by adults and nymphs reared on peanut.



**Figure 5.3** Effect of pine pollen on the percent viruliferous *F. occidentalis* by adults and nymphs reared on peanut.

## CHAPTER 6

### CONCLUSIONS

In the experiments on thrips settling behavior, results indicated that pine pollen increased the settling behavior of both the thrips species on tomato and peanut leaves but the magnitude of the effect varied depending on the thrips species and host crop. A greater increase in settling of *F. occidentalis* was observed with the pollen treatment than for *F. fusca*. Both species increase settling on pollen treated peanut more so than on tomato. Even though there are slight preferences for settling behavior of the thrips based on the crop choice, results indicated that pine pollen significantly increased the settling behavior of both the thrips species on tomato and peanut leaves. Based on these results it is likely that other behaviors associated with settling behavior such as feeding and oviposition might also be affected by pollen deposition on host crop leaf surfaces.

In the experiments on thrips oviposition results of these studies indicated that pine pollen increased the oviposition rate of both thrips species on plant leaf tissue, but that the oviposition rate varied depending on the thrips species and host crop. A greater oviposition rate with pollen treatment was observed for *F. occidentalis* than for *F. fusca*. Both species of thrips showed a stronger increase in oviposition on pollen- treated peanut than tomato, showing a specific crop-pollen interaction relative to thrips choice for oviposition. Also, based on these results and the results on settling in chapter 3, oviposition behavior is positively correlated with settling behavior. These findings strongly support the hypothesis that settling and oviposition behavior patterns of thrips are directly affected by pine pollen deposition.

In the experiment the effects of pollen on TSWV acquisition, the presence of pollen significantly affected virus acquisition, at least with the thrips species that was able successfully reproduce on the host plant, i.e., *F. fusca*. In addition, these data suggested that pollen dusted onto host plant leaves can significantly increase the percent acquisition in the generation of thrips resulting from an ovipositional event. This observation could be very important in trying to understand the population dynamics of thrips vectors and the epidemiology of TSWV in the field. I suspect that pollination events, such as pine pollen shed that occurs systematically each year in the spring in the Southeastern USA (Dorman & Barber 1956) could increase the acquisition of TSWV by thrips vectors in the field.

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