

PINE POLLEN EFFECTS ON *FRANKLINIELLA OCCIDENTALIS* AND *FRANKLINIELLA FUSCA* (THYSANOPTERA: THIRIPIDAE) REPRODUCTION

by

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(Under the Direction of David G. Riley)

ABSTRACT

The effects of pine pollen supplementation on reproduction in *Frankliniella occidentalis* (western flower thrips) and *F. fusca* (tobacco thrips) were investigated. For the first objective, pollen and thrips counts were taken in Tift Co., Georgia, USA, during years 2005, 2006, 2007, and 2008. Overall years combined showed significant positive correlation between pollen counts and thrips counts after a two week delay, or roughly one thrips generation time. For the second objective, bioassay methods analyzed *F. occidentalis* and *F. fusca* reproduction with and without pine pollen supplementation. A cage bioassay on tobacco yielded significantly higher mean offspring production per leaf on the pollen treatment. For the third objective, a micro caged onion bioassay was used for life table analysis of tobacco thrips. *Frankliniella fusca* mean net reproduction was significantly higher on onion with pollen supplementation. Results of all three objectives suggest that pine pollen stimulates *F. occidentalis* and *F. fusca* reproduction.

INDEX WORDS: *Frankliniella occidentalis*, *Frankliniella fusca*, reproduction, pollen

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CHAPTER 1

INTRODUCTION

Since the late twentieth century, thrips have become significant pests worldwide. Abetted by the growing global import-export exchange of food and ornamental products, through which thrips infiltrate new habitats, and by their increasing resistance to pesticides (Brødsgaard 1989), these thysanopteran insects have spread to span all continents in the temperate, sub-tropical and tropical regions (Lewis 1973, Ananthkrishnan 1984a, Ananthkrishnan 1984b). Thrips are difficult to control not only due to pesticide resistance, but because of a generally large host plant range, and an ability to rapidly increase population size in optimal conditions (Robb and Parrella 1989, Lewis 1997, Jan et al. 2003). These characteristics only exacerbate the amount of mechanical and pathological damage thrips cause plants.

In vegetable, fruit, fiber or floricultural crops, thrips can damage buds, leaves, flowers and fruit. Thrips attacking lateral buds causes stunted growth and distortion (Young et al. 1972, van Schoonhoven 1974, van Schoonhoven and Pena 1976). Leaves will turn silver or yellow-brown after thrips feeding damage (Lewis 1973), or if damage is severe enough, leaves will desiccate and shrivel (Esau 1961). Without ever opening, thrips-attacked flower buds can desiccate and shrivel, as can flower stigmas (Andrewartha and Kilpatrick 1951). Often, flowers will streak, silver, or distort, causing unmarketable aesthetic damage and economic loss in the floricultural industry (Robb and Parrella 1989, Oetting et al. 1993). In fruits and vegetables, thrips feeding or oviposition activity will leave silver or brown blemishes, pale halos, or deformity (Yokoyama 1977, Guerra-Sobrevilla 1989, Childers 1997). The degree of mechanical injury in crops, however, is often not as severe

as that sustained from thrips-vectored pathogens. Thrips-vectored crop pathogens are difficult to control because of thrips resistance to insecticide, or inaccessibility to spray (due to thrips small size and tendency to seek out plant crevices), and the relatively small or fleeting population needed to effectively inoculate a crop (Ullman et al. 1997). There are a few fungal and bacterial pathogens spread by thrips, but it is mainly thrips feeding injury that makes plants susceptible to invasion by these pathogens (Lewis 1973, Ananthkrishnan 1980, Bournier 1983). Likewise, there are a number of viral vectors that spread from pollen grains into open wounds in plants created by thrips feeding, like *Pelargonium flower break virus* (PFBV) (Krczal et al. 1995), or *Prunus necrotic ringspot virus* (PNRSV) (Childers 1997). However, the most economically important are the persistent *Tospovirus* pathogens that thrips vector to plant hosts.

The *tomato spotted wilt virus* (TSWV), a tospovirus, is responsible for particularly wide-ranging and costly economic damage in crops. TSWV can infect up to 900 plant species in 82 families worldwide (Prins and Goldbach 1998), including tomato, potato, tobacco, peanut, pepper, lettuce, papaya and ornamentals chrysanthemum, begonia, ageratum and impatiens (German et al. 1992). This virus causes necrosis, ring patterns, chlorosis, bronzing, speckling, silvering, stunting, wilting and lesions in plant tissues (German et al. 1992, Ullman et al. 1997, Mullis and Nischwitz 2005). The economic damage of TSWV in the world was estimated to be \$1 billion a decade ago (Prins and Goldbach 1998). It can cause up to \$8.8 million in losses in Georgia tomatoes alone in a single year (Riley and Pappu 2000). Although at least 13 species of thrips can transmit TSWV, the western flower thrips [*Frankliniella occidentalis* (Pergande)] is considered the most important vector worldwide, and in Georgia, the tobacco thrips [*Frankliniella fusca* (Hind)] is also a primary vector, especially in tobacco and peanut (Salguero

Navas et al. 1991, Riley and Pappu 2000, Riley and Pappu 2004, Campbell et al. 2005, Sherwood 2005).

In Georgia and other parts of the world, most TSWV damage occurs when young crops are infested with thrips vectors early in the growth season (Moriones et al. 1998, Chaisuekul 2003). Tobacco thrips and western flower thrips population densities build around this period of vulnerability, eventually peaking in mid-May in Georgia (Riley and Pappu 2000, Riley and Pappu 2004). During the critical period of high thrips population growth from the end of April to mid-May, it is important to understand what drives thrips population fluctuations in order that such increases may be preempted, or at least predicted.

One such event whose timing correlates with the spring increase in thrips population densities is the annual tree pollen deposition in the Southeast. The peak of the pine pollen event precedes the mid-May peak in population densities of tobacco and western flower thrips (Riley et al. 2007) by approximately one thrips generation time (2-3 weeks). O'Shaughnessy (1988) found pollen concentrations in northern Georgia start to increase in February, reach a sizeable peak in April, and then rapidly decrease again. *Pinus* pollen represents a large fraction of the total pollen at its peak in April (O'Shaughnessy 1988). Pollen is a nutritious food source and could conceivably be consumed by tobacco and western flower thrips after settling upon crops where thrips occur. As will be discussed in this research project, pollen has been shown to increase the reproductive rates of some thrips species after supplementation to a diet. Could the sudden plentiful availability of *Pinus* pollen to tobacco and western flower thrips help induce the population boom they exhibit in early spring by affecting their reproductive rates?

This study had three goals aiming to elaborate on this question. The first was to collect and analyze seasonal ambient pollen levels and thrips population dynamics. Next was to conduct

bioassays assessing pollen's effects on western flower and tobacco thrips reproduction, and lastly, to devise an experimental method to conduct a life table analysis of pine pollen effects on western flower and tobacco thrips. The hypothesis was that pine pollen supplementation to a plant leaf tissue diet would increase reproductive rates of tobacco and western flower thrips.

CHAPTER 2

LITERATURE REVIEW

Thrips Nutritional Requirements

To understand how pollen could potentially effect thrips reproduction, it is helpful to first examine thrips feeding and oviposition habits, and how they are affected by their nutritional requirements and the quality of nutrients in various food sources. Tobacco and western flower thrips are polyphagous. Their diets have differences, however; while tobacco thrips feed mainly on leaf tissue of crops (Newsom et al. 1953, Salguero Navas et al. 1991, Chamberlin et al. 1992, Eckle et al. 1996, Toapanta et al. 1996), grasses (Newsom et al. 1953, Toapanta et al. 1996), and broadleaf weeds (Stewart et al. 1989, Cho et al. 1995, Groves et al. 2002), western flower thrips are found feeding and/or ovipositing on leaves, flowers, and fruiting structures, and are considered mainly anthophilous (Terry 1997). Both species employ the same feeding mechanism, however. All thrips pierce a cell with their intermaxillary stylets and draw the liquid cytoplasm up through a feeding channel (Lewis 1989, Kirk 1997). To oviposit, all thrips in the suborder Terebrantia, including western flower and tobacco thrips, use a serrated ovipositor to place an egg within fruit, flower or leaf tissue (Lewis 1973, Brødsgaard 1989).

In order to find hosts upon which to feed or oviposit, western flower and tobacco thrips adults can catch air currents with fringed wings and drift great distances (Lewis 1989, Ananthakrishnan 1993, Lewis 1997). To identify the suitability of a host for feeding or oviposition, receptors on thrips mouthparts and antennae allow them to detect chemical, tactile and mechanical cues on plants (Lewis 1997). A variety of plant characteristics can have an effect on thrips host selection. These can include secondary compounds, plant architecture, or primary

metabolites. Secondary compounds may include defensive chemicals, like alkaloids or tannins (Schoonhoven et al. 1998), or sugars, which may stimulate feeding in western flower thrips (Morse 1995). Plant architecture might allow thrips to hide from predators (Brodbeck et al. 2002), or to avoid difficult substrates to navigate, like leaves with glandular trichomes (Kumar et al. 1995). Primary metabolites are chemicals, such as different forms of nitrogen, which are required for an organism to develop and reproduce.

Nitrogen (N) is considered an important requirement in the diets of all animals, including thrips. In general, animals are more than 50% protein (Hafez and Dyer 1969, DeFoliart 1975), or about 7-14% N by weight (Mattson 1980). Additionally, non-protein N, like the essential amino acids (which an animal or insect cannot synthesize), must be garnered from food sources. Insects and animals use N to construct genetic codes, cell structures and conduct metabolic processes (Mattson 1980). Nitrogen is especially important in thrips during a period of rapid growth or egg maturation (Terry 1997). Insects and other animals must find food sources to supply N for these functions, and replace the trace amounts that are excreted in wastes (Hale et al. 1978).

Plants are mainly comprised of carbohydrates (Mattson 1980). They will typically have 0.03-7.0% N by dry weight, depending on tissue type, seasonality or species (Mattson 1980). Plants' N levels are highest in storage tissues and in new tissues during growth seasons, when plants are rapidly increasing the number of cells in tissues or organs (Mattson 1980, Bernays and Chapman 1994). Leaf buds tend to be popular reproductive hosts for thrips, possibly due to higher N levels in the young, quickly-growing tissue (Kirk 1995). In general, however, seeds (inaccessible to thrips due to their protective shells) and pollen (accessible to thrips) have higher N levels while fleshy fruits and leaves have lower levels (Virtanen and Kari 1955, Kirk 1995). Because insects have higher protein and non-protein N levels, or a lower C:N ratio, than the bulk

of plant tissues (McNeill and Southwood 1978), many food sources may have less N than an insect needs for survival or reproduction.

To deal with a N-poor food supply, some insects can alter plant chemistry or evolve specialized alimentary canals or symbiotic relationships to supplement N stores (Mattson 1980). For others that can't, including western flower and tobacco thrips, behavioral alterations include increasing consumption rates, feeding/digesting/developing over longer periods of time, occasional cannibalization or predation, or switching among plants and plant tissues (Mattson 1980). Mound (1995) describes the opportunistic ability of thrips to utilize many different hosts for survival until a more nutritious host is available. With optimal nutrition, reproductive rates will skyrocket and population densities will boom. The lack of reproduction on unsuitable host plants may be due to inadequate nutrients for larval development, or for egg formation, as these are very nutritionally demanding. Thrips produce a great number of eggs, and each egg is large in proportion to thrips body mass (Lewis 1997). Furthermore, insects often prefer to lay eggs on the most nutritious hosts possible for their offspring (Thompson 1988). Alternate hosts lacking in nutrition may be used to supply water, sugar and other resources for basic survival, but with higher C:N ratios, thrips will not reproduce on them and development will slow (Janzen 1973, McNeill and Southwood 1978, Ananthakrishnan et al. 1982).

There are many examples of thrips using soluble plant N levels in host selection choice. For example, in a no-choice test, leaf N level correlated positively with tobacco thrips host choice (McNeill and Southwood 1978). Also, western flower thrips population densities were shown to seasonally correlate with soluble N levels in tomato flower tissue and total number of flowers (Brodbeck 2001). Studies by Brown (2002) showed that in leaf discs from various plants, western flower thrips consistently choose hosts with more soluble protein on which to feed and

reproduce. On poor host plants with high C:N ratios, the primarily phytophagous western flower thrips can even be induced to prey on mite eggs, a good source of soluble N (Faraji et al. 2002a, b, Janssen et al. 2002).

Similar to N, it is regarded that there is a significant correlation between thrips damage or egg production rates and leaf amino acid levels (Fennah 1963). The ten essential amino acids in insect diets are: arginine, histidine, isoleucine, leucine, lysine-HCL, methionine, phenylalanine, threonine, tryptophan, and valine (Rock and King 1966, Rock and King 1967). As with total N or soluble protein levels, amino acids tend to be higher in younger, growing plant tissues (Mattson 1980). Specific amino acid demands vary among species, and change throughout insect development (Rock and King 1966, Rock and King 1967). For example, phenylalanine and tyrosine, the aromatic amino acids (AAAs), have a central role in the tanning and hardening of cuticle proteins (Richards 1951, Bernays and Woodhead 1984, Mollema and Cole 1996). It has been found that AAA levels in lettuce, tomato, pepper and cucumber leaves, and in tomato flowers, correlated positively with host selection and larval damage by western flower thrips (Kirk 1997, Brodbeck 2001). In tobacco thrips, however, there is evidence that AAAs are not important in host selection, and that secondary compounds or plant architecture may be more important (Brodbeck et al. 2002). Thus, there may be some differences in nutritional requirements of western flower and tobacco thrips.

Pollen Composition

Pollen is a plant tissue which tends to be high in soluble protein and amino acids. Total N levels in pollen vary among plant species, climate, and with the nutrients available to a plant (Stanley and Linkskins 1974). Gymnosperm pollen differs from angiosperm pollen as well. For example, gymnosperm pollen lacks a pollenkitt (Hesse 1984, Zavada 1984), where scented

compounds are generally found (Stanley and Linkskins 1974). It also tends to have lower levels of carbohydrates (Nielsen et al. 1955), and *Pinus* pollen may be lower in total N (Hulshof and Vänninen 2002). Pennsylvanian *Pinus resinosa* and North Carolinian *P. strobus* had N levels of 19.7 and 20.1 mg per gram of pollen, respectively (Doskey and Ugoagwu 1989, Doskey and Ugoagwu 1992), Scandinavian *P. strobus* and *P. montana* had 22 mg/g (Nielsen et al. 1955), and *Pinus sylvestris* pollen had 16.0 mg/g (Knight et al. 1972). In comparison, creeping willow, *Salix repens* L., had 44.2 mg/g, and rapeseed, *Brassica napus* L., had 36.4 mg/g (Knight et al. 1972). However, many of those comparative analyses examined total and not unbound N, which may not be reliable as unbound is the only N thrips can utilize. Amino acid levels in pollen will also vary among plant species, climate and with the nutrients available to plants (Stanley and Linkskins 1974). *Pinus* pollen has relatively higher levels of glutamic acid (an amino acid insects can convert into many others (Andersen et al. 1992) and lysine (Brodbeck 2001), while proline levels are generally high in all pollen (Virtanen and Kari 1955). However, all essential amino acids can be found in pollen at various levels (Stanley and Linkskins 1974).

Many insects consume pollen. Known predatory insects that consume pollen include heteropteran bugs (Alomar and Wiedemann 1996), ladybird beetles (Triltsch 1997, Cottrell and Yeargan 1998), hoverflies (Haslett 1989, Wratten et al. 1995), and green lacewings (Sheldon 1971), as well as predatory mites (van Rijn and Tanigoshi 1999). Some herbivorous insects that consume pollen include chrysomelid and curculionid beetles (Jayanth et al. 1993, Jones et al. 1993), mosquitoes (Hopla 1964-1965), collembolans (Scott 1963), and lycaenid and *Heliconius* butterflies (Gilbert 1972, Wagner and del Rio 1997).

Many thrips species consume pollen (Kirk 1997), though at rates depending somewhat on grain structure. To feed upon pollen, a thrips must stab a grain with the intermaxillary stylets and

ingest the cytoplasm through a feeding tube. Thrips' stylets are 1-2 μm in diameter (Grimaldi and Engel 2005), and the average pollen grain is 25-40 μm (Grimaldi and Engel 2005). A study found that thrips are able to feed on larger pollen grains, or a conglomerate of several grains, in less time (Kirk 1987), possibly because they are easier to probe or handle. Adhesive pollen grains will also affect thrips feeding rates by sticking to their body, inducing thrips to stop and groom (Kirk 1985b). The exine spines on pollen grains were not found to slow thrips feeding rates (Kirk 1985b). Thrips are also able to discern pollen grains from different plant species. Specialist thrips, in particular, are able to identify pollen from favored host plants, rarely consuming others (Kirk 1985a, Annadurai and Morrison 1987).

Pollen as a lone food source can result in high thrips mortality, as it cannot supply the water and carbohydrates needed. Pollen + water diets result in high mortality (Murai and Ishii 1982), while pollen + sucrose solution diets can have positive affects on thrips life history characteristics (Teulon and Penman 1991). Nevertheless, pollen can supply the protein and non-protein N necessary for normal thrips development and reproduction.

Pine Pollen Dehiscence

Pine pollen is ubiquitous in the South during the spring, covering virtually all exposed surfaces. The southern coniferous forest region is mainly comprised of slash (*Pinus elliottii* Engelm.), loblolly (*Pinus taeda* L.), longleaf (*Pinus palustris* Mill.) and shortleaf pine (*Pinus echinata* Mill.) (Harper and Dickenson 2004). Georgia has the largest percentage forest cover in the South at 67%, and of that forested area, 45% is loblolly-shortleaf and longleaf-slash pine forest (Harper and Dickenson 2004). The southern pines always dehisce in this order: slash pine first, then longleaf, loblolly, and shortleaf (Dorman and Barber 1956). Depending on latitude, elevation, or temperature (Dorman and Barber 1956, Stanley and Linkskins 1974), pines

generally begin dehiscence in the Gulf states in late January/early February and continue through April into May, and are fairly consistent (Wakeley 1954). A 2005 analysis of pollen counts in Georgia (Figure 1) shows ambient pollen counts follow this trend, with peaks occurring between April and May (Riley et al. 2007).

Pines can release great quantities of pollen which deposits over large areas. According to Snyder and Clausen (1973), 100 male *Pinus* spp. strobili contain around 150 cc of pollen. The number of male strobili in a hectare can number in the thousands; for example, one study found that depending on the diameter at breast height, *Pinus sylvestris* averaged from around 65,000 to 150,000 male strobili per hectare (Bilir et al. 2006). Climate and tree nutrition and light availability can also affect the quantity of pollen produced by a tree (Stanley and Linkskins 1974).

The distance a pollen grain can cover after dehiscence is affected by its size and shape. Pine pollen is relatively large; for example, slash pine has an average total grain diameter of 78-96 μm (Moss 1965). In spite of a larger size, its shape is conducive to wind dispersion. A pine pollen grain has three spheres: a solid central sphere with two air bladders attached (Stanley and Linkskins 1974). The air bladders increase surface area for wind currents or air resistance to act upon, thereby facilitating movement or decreasing the rate of settlement out of the air, respectively, and they add buoyancy to allow the pollen grains to float in water (Stanley and Linkskins 1974). In Sweden, Scots pine (*Pinus sylvestris* L.) was found to deposit most pollen within 700 m (Persson 1955), though some may drift farther after rising thousands of feet in the atmosphere (Stanley and Linkskins 1974). The grain shape allows pine pollen to be dispersed many meters from a pine stand.

Much of the ambient spring pollen in Georgia may originate from *Pinus* trees. O'Shaughnessy (1988) monitored ambient pollen levels in downtown Atlanta for a year, and found concentrations started to increase in February, reached a sizeable peak of about 350 grains/m³ in April, and then rapidly decrease again. *Pinus* pollen concentration peaked in April at 45 grains/m³ (O'Shaughnessy 1988), a relatively substantial fraction of the total pollen grains, which will eventually settle out onto surfaces such as crop fields.

Pollen's Effects on Thrips Life History Characteristics

The consumption of pollen has been shown to affect the behavior, population dynamics, development time and reproduction of various thrips species. Noticeable behavioral affects may include a tendency for thrips to settle on or remain in pollen-rich areas, or an increase in damage to pollen-rich crops due to higher feeding rates. Female western flower thrips are considerably more apt to occupy flowers with pollen (Ugine et al. 2006), and cotton pollen supplementation significantly decreased migration rates of western flower thrips larvae (Trichilo and Leigh 1988). In a study by Chitturi *et al.* (2006), the addition of slash pine pollen to peanut (*Arachis hypogaea* L.) and tomato (*Solanum lycopersicon* L.) leaves increased settling behavior of western flower thrips 7-fold, but had no significant effect on tobacco thrips. With the presence of flowers, western flower thrips populations will exponentially increase (Kirk 1997, Gerin et al. 1999), as they have also shown to do after the application of castor oil pollen to chrysanthemum (Skirvin et al. 2006), and birch and sweet pepper pollen to cucumber leaves (van Rijn and Sabelis 1993, van Rijn et al. 2002), respectively. These population growths may be largely attributable to the effects of pollen on thrips life history characteristics.

Development time in the larval stage has shown to be shortened by pollen supplementation in a number of species. Three species of predatory thrips from the tubuliferan

suborder are included: *Haplothrips victoriensis* Bagnall (Bailey and Caon 1986), *Haplothrips faurei* Hood (Putman 1965), and *Lepothrips mali* (Fitch) (Parrella et al. 1982). All three species, which require a high-N diet supplied by feeding on small arthropods or mite eggs, could not complete development on a diet of leaves alone, but were able to develop after leaves were dusted with pollen or anthers containing pollen were presented. In absence of prey, these predators can use pollen as an alternate food source to supply needed protein or non-protein N. A terebrantian predator, *Aeolothrips intermedius* Bagnall, must consume pollen as it cannot sexually mature without it in its diet (Bournier et al. 1979). There are many thrips from the subfamily Thripinae whose developmental rate increases with pollen supplementation: *Thrips obscuratus* (Crawford) (Teulon and Penman 1991), *T. imaginis* (Andrewartha and Kilpatrick 1951), *T. hawaiiensis* (Morgan) (Murai and Ishii 1982), *Frankliniella intonsa* (Trybom) (Murai and Ishii 1982), and western flower thrips (Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003). *T. imaginis* thrips must have pollen in their diets, or the females cannot produce eggs and the larvae will not develop. In the other thrips species mentioned, pollen supplementation markedly increased larval development rates. In fact, in one study by Hulshof et al. (Hulshof et al. 2003), western flower thrips development rate was affected by all five pollen types supplied: birch (*Betula pubescens* Ehrh.), hazel (*Corylus avellana* L.), fireweed (*Epilobium angustifolium* L.), common cattail (*Typha latifolia* L.) and Scots pine pollen (*Pinus sylvestris* L.). The birch and hazel pollens even significantly lengthened adult western flower thrips survival time (see Table 2.2). The addition of cotton pollen to cotton leaves significantly decreased western flower thrips development time from egg to adult (Trichilo and Leigh 1988), as well as apple pollen on green bean pods (Zhi et al. 2005), and van Rijn and Sabelis (1993) estimated that sweet pepper pollen supplementation on cucumber increased larval growth rate about 50%.

Similarly, most of the thrips species documented to achieve greater reproduction rates with pollen supplementation are in the sub-family Thripinae, with an exception (see Table 2.1). They include: *Ceratothripoides cameroni* Bagnall (Annadurai and Morrison 1987), *Ceratothrips ericae* (Haliday) (Kirk 1985a), *Kakothrips pisivorus* (Westw.) (Kirk 1985a), *Thrips obscuratus* (Teulon and Penman 1991), *T. imaginis* (Andrewartha 1951), *T. fuscipennis* Haliday (Kirk 1985a), and the flower thrips *Frankliniella schultzei* Trybom (Annadurai and Morrison 1987), *F. intonsa* (Trybom) (Murai and Ishii 1982), and *F. occidentalis* (Murai and Ishii 1982, Trichilo and Leigh 1988, van Rijn and Sabelis 1993, Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003, Zhi et al. 2005, Riley et al. 2007). The tubuliferan exception is *Taeniothrips inconsequens* (Uzel), which exhibits increased oviposition rates after sugar maple leaves are supplemented with pollen (Leskey et al. 1997).

As with developmental rates, generalist thrips tend to respond to pollen from a wide variety of plants with increased oviposition rates, while specialists may react only to host pollen. Thrips that are not associated with flowers may not benefit from pollen supplementation. *Thrips calcaratus* showed significant decreases in longevity and no change in fecundity with supplementation of pollen to the host plant (Rieske and Raffa 1996). There are many species of thrips, however, that react positively to pollen supplementation. In a study by Kirk (1985a), three specialist thrips, *K. pisivorus*, *C. ericae* and *C. cameroni*, and a generalist thrips *T. fuscipennis* were given a wide variety of pollens with a sucrose solution, after which feeding times and oviposition rates were noted. The generalist *T. fuscipennis* fed on all pollen grains. The different pollen species conferred similar and significantly higher oviposition rates than a diet of sucrose alone. The specialist thrips rarely fed upon non-host pollens offered, though the host pollen consumed significantly increased oviposition rates. Another generalist, *T. obscuratus*, was given

a variety of pollen species in a study by Teulon and Penman (1991). All four pollen species offered induced significantly higher oviposition rates. The generalist flower thrips *F. intonsa*, *F. schultzei* and western flower thrips follow a similar trend. All pollen species available to these thrips were consumed and boosted oviposition rates.

There is a particularly large body of evidence supporting that western flower thrips exhibits increased reproduction rates after diets are supplemented with many different types of pollen (see Table 2.1). Van Rijn and Sabelis (1993) added sweet pepper pollen to cucumber leaves for western flower thrips consumption. They found western flower thrips oviposition rate increased from an average 2.7 to 4.7 eggs per day. Cotton pollen added to leaves of a susceptible variety of cotton increased mean progeny produced per western flower thrips female from 4.4 on untreated leaves to 194.7 (Trichilo and Leigh 1988). This, combined with decreased larval development time and a significant increase in adult longevity induced a growth in the intrinsic rate of increase (r_m) by 40% (Trichilo and Leigh 1988). Additionally, green beans supplemented with apple pollen increased total eggs laid per female from about 80 to 100 (Zhi et al. 2005). In this case, factoring in the decreased larval development time, intrinsic rate of increase grew 12% (Zhi et al. 2005).

There is one particular study by Hulshof et al. (2003) that illustrates the potentially large affects of pine pollen on western flower thrips reproduction. In this analysis, pollen from Scots pine, birch, hazel, common cattail and fireweed was dusted on cucumber leaves and fed to newly-emerged adult females, after which fecundity (number of larvae per female) was recorded. While all pollen significantly increased fecundity rates, pine pollen affected by far the greatest increase. In turn, the overall intrinsic growth rate of thrips fed pine pollen had the greatest increase of all pollens over plain cucumber leaves, at 47%. (see Table 2.2)

If western flower thrips reproduction is greatly increased by the inclusion of pine pollen in spring diets as Hulshof et al. (2003) have shown, the annual pine pollen deposition in the Southeast may help induce western flower thrips annual large population increase shortly after pollen deposition. The effect of pollen on tobacco thrips reproduction has not been as extensively investigated. One investigation by Riley et al. (2007) found that while slash pine pollen dusted on tomato and peanut leaves significantly increased western flower thrips oviposition rates by about 200%, those of tobacco thrips were only increased significantly on peanut by about 100%, and not increased on tomato. This was expected, as western flower thrips are generally associated with flowers in the wild, while tobacco thrips are primarily foliage feeders. It is projected that on a diet of tobacco and onion foliage with pine pollen supplementation, both tobacco and western flower thrips will show an increase in reproductive rates over that on untreated foliage, with the latter species exhibiting the larger effect.

Table 2.1 Documentation of thrips species in which reproduction is affected by pollen supplementation.

Thrips species	Reference(s)
<i>Ceratothripoides cameroni</i> Bagnall	(Kirk 1985a)
<i>Ceratothrips ericae</i> (Haliday)	(Kirk 1985a)
<i>Frankliniella intonsa</i> (Trybom)	(Murai and Ishii 1982)
<i>Frankliniella occidentalis</i> (Pergande)	(Murai and Ishii 1982, Trichilo and Leigh 1988, van Rijn and Sabelis 1993, Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003, Zhi et al. 2005, Riley et al. 2007)
<i>Frankliniella schultzei</i> (Trybom)	(Annadurai and Morrison 1987)
<i>Kakothrips pisivorous</i> (Westw.)	(Kirk 1985a)
<i>Taeniothrips inconsequens</i> (Uzel)	(Leskey et al. 1997)
<i>Thrips fuscipennis</i> Haliday	(Kirk 1985a)
<i>Thrips imaginis</i> Bagnall	(Andrewartha and Kilpatrick 1951)
<i>Thrips obscuratus</i> (Crawford)	(Teulon and Penman 1991)

Table 2.2 Life history characteristics of *F. occidentalis* on various diets (Hulshof et al. 2003).

Diet	Developmental time (days) larva to adult	Adult survival (days)	R_0 , Net reproduction per female	r_m , Intrinsic growth rate/day
Cucumber leaf	8.9b	21b	32	0.163
Scots pine pollen + cucumber leaf	8.4a	24b	141	0.240
Birch pollen + cucumber leaf	8.4a	34a	91	0.206
Hazel pollen + cucumber leaf	8.4a	37a	98	0.211
Fireweed pollen + cucumber leaf	8.5a	24b	77	0.204
Fireweed bee- collected pollen + cucumber leaf	8.6a	24b	73	0.207
Cattail pollen + cucumber leaf	8.4a	23b	66	0.201
Milk powder + yeast + cucumber leaf	9.0b	14c	21	0.157

Means within columns with the same letter are not statistically significant ($P < 0.05$)

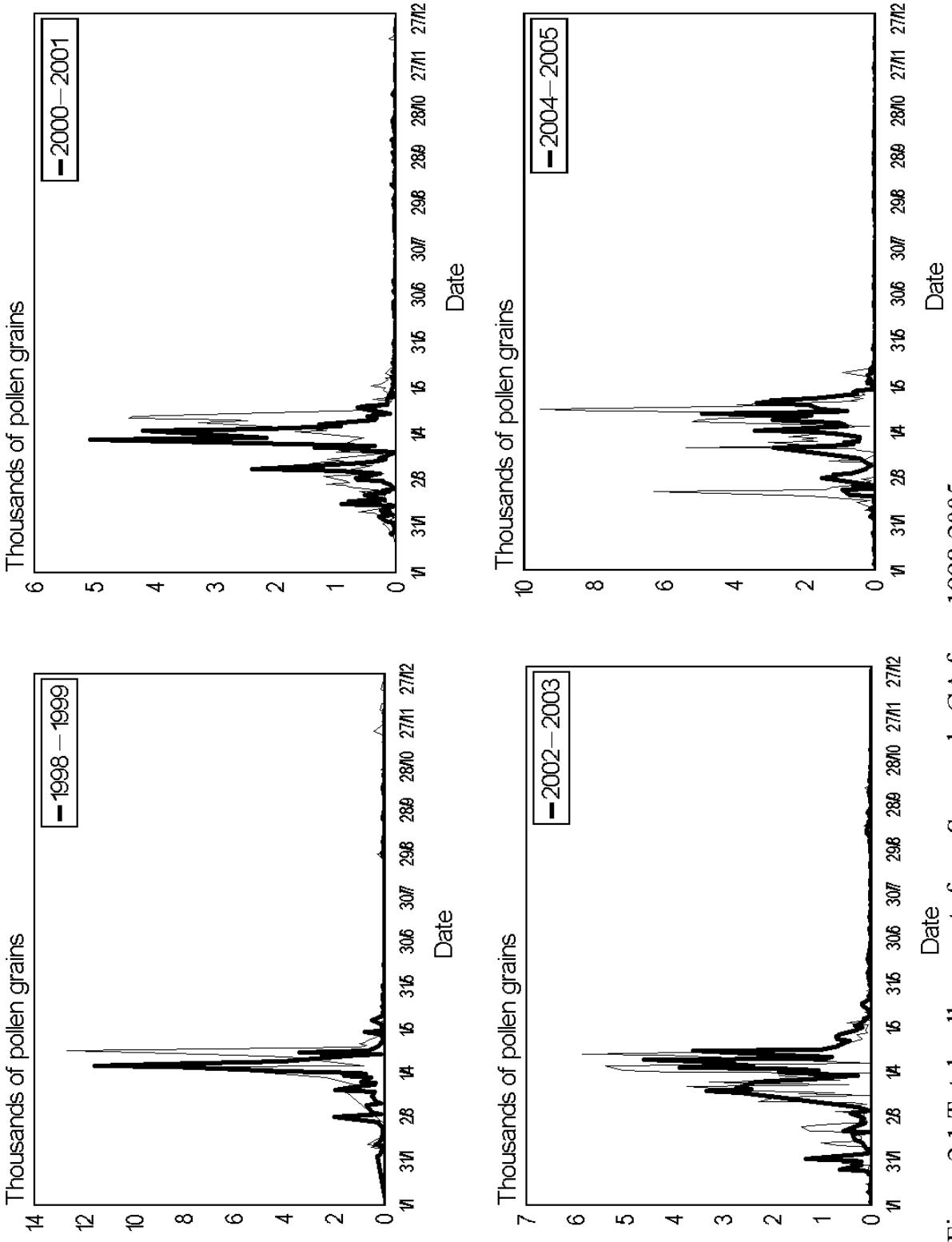


Figure 2.1 Total pollen counts from Savannah, GA from 1998-2005

CHAPTER 3

AMBIENT POLLEN AND THRIPS POPULATION DYNAMICS¹

¹ Angelella, G.M. and D.G. Riley. 2008. To be submitted to *Ecological Applications*.

Abstract

A study was conducted to correlate tree pollen dehiscence and thrips population dynamics in the field. Ambient pollen and thrips trap count data were collected in Tift Co., Georgia, USA, from early spring to late summer/early fall in 2005, 2006, 2007 and 2008. Correlation analyses were conducted by year, as well as for overall years combined using weekly means of pollen and thrips from the same week, or one-, two-, three-, four- and five-weeks offset with the pollen week advancing while the thrips week remained stationary. With the exception of 2006, analyses suggested a significant positive correlation between pollen and thrips counts. Correlations varied in strength depending on the weekly data alignment and year. Significant, positive correlations occurred after a two-week time lag in 2005, a one-to-two-week offset in 2007, and a two-to-four-week offset in 2008. Results overall showed a positive correlation between pollen and thrips counts after a two week offset, i.e. thrips populations increased two weeks following an increase in pollen dehiscence.

Introduction

Thrips populations are known to fluctuate seasonally, presumably in reaction to changing host availability, temperatures and other factors (Lewis 1973). Two important crop pests in the southeastern USA that exhibit seasonal population fluctuations are the tobacco thrips (*Frankliniella fusca* (Hinds)) and western flower thrips (*Frankliniella occidentalis* (Pergande)). Tobacco thrips populations increase significantly in the spring, peaking sometime in April to June (Riley and Pappu 2000, Groves et al. 2003, Riley and Pappu 2004). Some seasonal factors referenced as inducing changes in thrips populations include temperature (Lowry et al. 1992), photoperiod (Whittaker and Kirk 2004, Chaisuekul and Riley 2005), humidity (Kirk 1997), and availability of nutritious hosts for food and oviposition (Lewis 1973, Mound and Teulon 1995).

Springtime pine pollen deposition may also be responsible in part for stimulating a significant increase in population growth in tobacco thrips and western flower thrips (Riley et al. 2007).

In the Southeastern U.S., large amounts of pine tree pollen are deposited every year (Dorman and Barber 1956). The peak of this event in Raleigh, North Carolina is around April 1st-15th (Dorman and Barber 1956). It precedes the seasonal tobacco thrips population peak in North Carolina as measured by Groves et al. (2003) by two-to-five weeks. In Georgia, tree pollen concentrations start increasing in February, build to a peak in April, and thereafter decline (O'Shaughnessy 1988). A sizeable portion of the tree pollen during peak concentrations represents the pine genus, *Pinus* (O'Shaughnessy 1988). In the Gulf States, pine species generally begin dehiscence in late January/early February and continue through May (Wakeley 1954), with peak tree pollen concentrations occurring between April and May (Riley et al. 2007). The southern coniferous forest region is mainly comprised of slash (*Pinus elliottii*), loblolly (*Pinus taeda*), longleaf (*Pinus palustris*) and shortleaf pine (*Pinus echinata*) (Harper and Dickenson 2004). Thus, these species provide the bulk of ambient pine pollen in Georgia.

Pollen is a nutritious food source, benefiting many thrips species. Especially among flower thrips from the genera *Thrips* and *Frankliniella*, pollen supplementation in a diet can induce an increase in both developmental and reproduction rates (Andrewartha and Kilpatrick 1951, Murai and Ishii 1982, Annadurai and Morrison 1987, Trichilo and Leigh 1988, Teulon and Penman 1991, van Rijn and Sabelis 1993, Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003, Zhi et al. 2005, Riley et al. 2007). These changes can in turn generate rapid population growth. In western flower thrips (*Frankliniella occidentalis*), intrinsic rate of population increase grew by 47% after Scots pine supplementation to cucumber leaves (Hulshof et al. 2003). The effect of pollen on tobacco thrips reproduction has not been as extensively studied. Western

flower thrips may be affected more greatly because they feed primarily on flower tissues, while tobacco thrips are associated primarily with foliage. Riley et al. (2007) found that slash pine pollen dusted on tomato and peanut leaves increased western flower thrips oviposition rates more than that of tobacco thrips, but did record an increase in tobacco thrips oviposition on peanut by 100% with the addition of pollen.

In this study, correlations between population fluctuations of total thrips, tobacco thrips and other thrips species and total tree pollen dehiscence levels in the spring was investigated. Correlations with pine pollen alone were also conducted. To do so, field surveys monitored tobacco thrips and other thrips species counts in Tift Co., Georgia, where ambient pine and other tree pollen counts were simultaneously collected. The hypothesis was that after a thrips generation time lag (approximately two weeks), thrips population dynamics would positively correlate with tree pollen shed dynamics.

Materials and Methods

Thrips population density field sampling. Four sticky traps (3 x 5 inch yellow, Olson Products, Medina, OH) were placed 1-2 m around a pollen collector located in Tift Co., Georgia, and were replaced on a weekly basis from January to June of 2006 and 2007, and from January to September in 2008. The location was an open field at the Coastal Plains Experiment Station in Tift Co. Pollen counts were retrieved from the same location. Tobacco thrips and total number of thrips were counted on traps (both sides of each trap) and compared to Burkard pollen count data at the Tift Co. location. Tobacco thrips were tallied separately beginning in 2006, because they are easily identifiable on sticky cards, while other specimens were identifiable as thrips but not down to the species level.

Burkard sampler. A Burkard air sampler (Burkard Manufacturing Co., Limited, Rickmansworth, Hertfordshire, England) collected daily recordings of ambient pollen. Method of collection was similar to that described first by Hirst (1952), and later by Lacey & West (2006). In a Burkard air sampler, a lightly greased plastic tape is slowly rotated over the course of a week. Air is drawn in, with suspended particles depositing on the tape. Calberla's fuchsin dye solution (Fisher Scientific, Pittsburg, PA, USA) was added to stain pollen grains, which were identified under a microscope (Anonymous 2008). The amount of pollen deposited was then analyzed in hourly sections. Total number of pollen grains and pine pollen grains collected per hour were summed daily for population dynamics and then weekly for correlation with weekly thrips totals.

Statistical analysis. Total thrips numbers were correlated with the total pollen counts from the Burkard air sampler by year and over four years (SAS Institute 1990). Cumulative pollen counts were adjusted to reflect the same week, a difference of the pollen week + 1, pollen week + 2, pollen week + 3, pollen + 4, and pollen week + 5 weeks and then correlated again to weekly thrips counts on traps.

Results and Discussion

The total pollen counts and thrips counts for years 2005, 2006, 2007, and 2008 were graphed over time. Although total thrips count data in 2005 is incomplete, having carried through only the months of April and May, it displays one peak in mid-May with a small peak in late April (see Figure 3.1b). Pollen counts in 2005 exhibited one early major peak in mid-February (see Figure 3.1a). In 2006, a small pollen count peak occurred in early February, and the larger peak in mid-March (see Figure 3.2a). Thrips counts had trimodal peaks, in early April, early May and mid-May (see Figure 3.2b). A smaller peak in pollen counts occurred in later January of

2007 with a larger peak pollen count occurring again in mid-March (see Figure 3.3a). Thrips numbers followed to peak in beginning April and mid-May (see Figure 3.3b). In 2008, a pollen peak occurred in mid-February, followed by a second peak in early March, and a large peak in later March (see Figure 3.4a). Thrips counts peaked first in early April and again in mid-May (see Figure 3.4b). The seasonal distributions of the pollen and thrips counts suggested correlation, in that peaks in thrips numbers appeared to follow peaks in pollen counts during all four years.

Overall, pine pollen counts were only marginally positively correlated with thrips counts in pollen week + 2 ($R=0.238$, $P=0.080$), and were negatively correlated with the same pollen week alignment ($R=-0.276$, $P=0.045$). Pine pollen counts significantly positively correlated with total pollen overall ($R=0.936$, $P<0.0001$), and represented 94% of overall mean total pollen. This reinforces how relatively large a proportion pine pollens represent in peak pollen counts.

Correlation values between pollen counts and thrips counts varied depending on the year and on the time delay of the pollen-thrips count week alignment in comparison (see Table 3.1). In 2005, correlation between pollen and thrips counts was not significant when the same pollen week, pollen week + 1, pollen week + 3, pollen week + 4, or pollen week + 5 alignments were analyzed (see Table 3.1). The pollen week + 2 alignment showed a significantly positive correlation in 2005 ($R=0.956$, $P=0.011$, $n=5$). None of the correlation values of 2006 pollen week alignments were significant (see Table 3.1). In 2007, pollen week + 1 ($R=0.609$, $P=0.001$, $n=26$) and pollen week+2 ($R=0.579$, $P=0.002$, $n=26$) both showed a significant positive correlation between pollen and thrips counts, with pollen week + 1 having a slightly stronger significant correlation. The remaining pollen week alignments of 2007 were not significant (see Table 3.1). In 2008, the same pollen week, pollen week + 1 and pollen week + 5 alignments were

not significant (see Table 3.1), while pollen week + 2, pollen week + 3 and pollen week + 4 alignments were significantly positively correlated ($R=0.446$, $P=0.012$, $n=31$; $R=0.416$, $P=0.022$, $n=30$; $R=0.531$, $P=0.003$, $n=29$, respectively). Pollen week + 2 had the second strongest levels of significance and positive correlation, while pollen week + 4 had the strongest significant positive correlation. Lastly, in the overall correlation between pollen and thrips counts combining all 5 years, only pollen week + 2 and pollen week + 4 were significantly and positively correlated (Table 3.2, $R=0.389$, $P=0.001$, $n=77$; $R=0.267$, $P=0.021$, $n=75$, respectively). Week+2 exhibited both the greatest significance and the strongest positive correlation. Same week, week+1, week+3 and week+5 correlation were not significant (see Table 3.1). Tobacco thrips counts on traps correlated significantly and positively with the overall total pollen week + 2 counts (Table 3.2, $R=0.278$, $P=0.014$). The mean tobacco thrips value represented 34% of mean total thrips counts. Other thrips species correlated well with overall pollen in pollen week + 2, pollen week + 3, and pollen week + 4 counts (Table 3.2, $R=0.365$, $P=0.002$; $R=0.252$, $P=0.033$; $R=0.322$, $P=0.007$, respectively).

Overall, the two week pollen delay (pollen week + 2) comparison drew the strongest support for a positive correlation between pollen and thrips counts. It was strongly and positively correlated in analyses for 3 out of 4 years, as well as in the cumulative analysis of all years combined for both total thrips and tobacco thrips counts. Other thrips species, which are likely predominantly flower thrips, may express increased reproduction in response to pollen deposition over a longer period of time, as the other thrips species trap counts significantly correlated positively with later week alignments of pollen counts as well. Fourteen days is about the length of time needed for a western flower thrips to hatch and mature to adulthood at 25°C, and slightly longer than the time needed for tobacco thrips (Lowry et al. 1992). As thrips

intercepted with sticky traps must be airborne, it follows that they likely have wings, and so have adult morphology. So, peak thrips trap counts likely represent peak numbers of winged adults. The correlation between pollen counts and thrips counts suggests a possible association between thrips reproduction and tree pollen deposition. As peak numbers of adult thrips induced peak thrips trap counts two weeks after peak pollen counts, peak reproduction may have occurred roughly one thrips generation time, or two weeks, earlier. This relationship would need to be vigorously tested in the field, however, to determine that it is an increase in reproduction triggered by pollen deposition, and not fluctuations in temperature, predation, or some other variable interacting with thrips population dynamics.

The correlation between ambient pollen levels and thrips population numbers supports laboratory studies demonstrating that pollen supplementation in thrips diets increased net reproduction, and thus population intrinsic rates of growth (Chapters 4 & 5). In the future, this correlation will need to be strengthened with field studies directly investigating pollen supplementation effects on thrips reproduction. Furthermore, the relationship between ambient pollen levels and the rate of pollen deposition onto substrates should be investigated, as well as the species of thrips affected by such depositions as dietary supplement. However, the positive correlation could imply that spring pollen depositions in the southeastern USA give field populations of thrips a reproductive boost, which manifests itself in larger thrips trap counts in the field.

If spring pollen deposition is responsible for increasing thrips populations, this may have implications for pest thrips management. The growth seasons of several economically important crops, such as tomato, tobacco and pepper, coincide with the timetable of increased pollen and thrips counts from February through May. Increased levels of pest thrips such as western flower

and tobacco thrips can transmit damaging Tospoviruses, and have caused up to \$1 billion in crop losses in the past (Prins and Goldbach 1998). Pollen has been shown to increase reproduction in western flower thrips (Murai and Ishii 1982, Trichilo and Leigh 1988, van Rijn and Sabelis 1993, Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003, Zhi et al. 2005, Riley et al. 2007) and oviposition rate in tobacco thrips (Riley et al. 2007). It has also been associated with an increase in the number of viruliferous tobacco thrips (Riley et al. 2007). If pest species of thrips such as tobacco and western flower thrips respond to pollen deposition with increased reproduction and population growth, monitoring of pollen levels may prove useful in augmenting predictive models for scheduling precise thrips control tactics to suppress damaging pest thrips population densities.

Table 3.1 Correlation of cumulative thrips (all species) on traps per week to cumulative total pollen collected per week on the same week and advancing pollen weeks (Tifton, GA).

Thrips Comparison to Pollen	Year 2005	Year 2006	Year 2007	Year 2008
Same Week	R=0.124, P=0.843, n=5	R=-0.464, P=0.081, n=15	R=0.061, P=0.767, n=26	R=-0.138, P=0.445, n=33
Week + 1	R=0.828, P=0.084, n=5	R=-0.418, P=0.121, n=15	R=0.609, P=0.001, n=26	R=0.111, P=0.545, n=32
Week + 2	R=0.956, P=0.011, n=5	R=-0.028, P=0.920, n=15	R=0.579, P=0.002, n=26	R=0.446, P=0.012, n=31
Week + 3	R=0.146, P=0.815, n=5	R=-0.270, P=0.330, n=15	R=0.163, P=0.417, n=27	R=0.416, P=0.022, n=30
Week + 4	R=-0.545, P=0.342, n=5	R=0.038, P=0.897, n=14	R=0.085, P=0.672, n=27	R=0.531, P=0.003, n=29
Week + 5	R=-0.766, P=0.076, n=6	R=0.132, P=0.668, n=13	R=0.135, P=0.502, n=27	R=0.284, P=0.143, n=28

Table 3.2 Correlation of cumulative total thrips, *Frankliniella fusca*, and thrips species other than *F. fusca* on traps per week to cumulative total pollen collected per week on the same week, and advancing pollen weeks over all years combined (Tifton, GA 2005-08).

Thrips Comparison to Pollen	All Thrips	<i>Frankliniella fusca</i>	Other Thrips Species
Same Week	R=-0.133, P=0.243, n=79	R=-0.163, P=0.267, n=48	R=-0.137, P=0.330, n=74
Week + 1	R=0.095, P=0.410, n=78	R=0.146, P=0.219, n=73	R=0.091, P=0.445, n=73
Week + 2	R=0.389, P=0.001, n=77	R=0.360, P=0.002, n=72	R=0.365, P=0.002, n=72
Week + 3	R=0.218, P=0.569, n=77	R=0.070, P=0.557, n=72	R=0.252, P=0.033, n=72
Week + 4	R=0.267, P=0.021, n=75	R=0.068, P=0.577, n=70	R=0.322, P=0.007, n=70
Week + 5	R=0.144, P=0.220, n=74	R=0.119, P=0.333, n=68	R=0.161, P=0.189, n=68

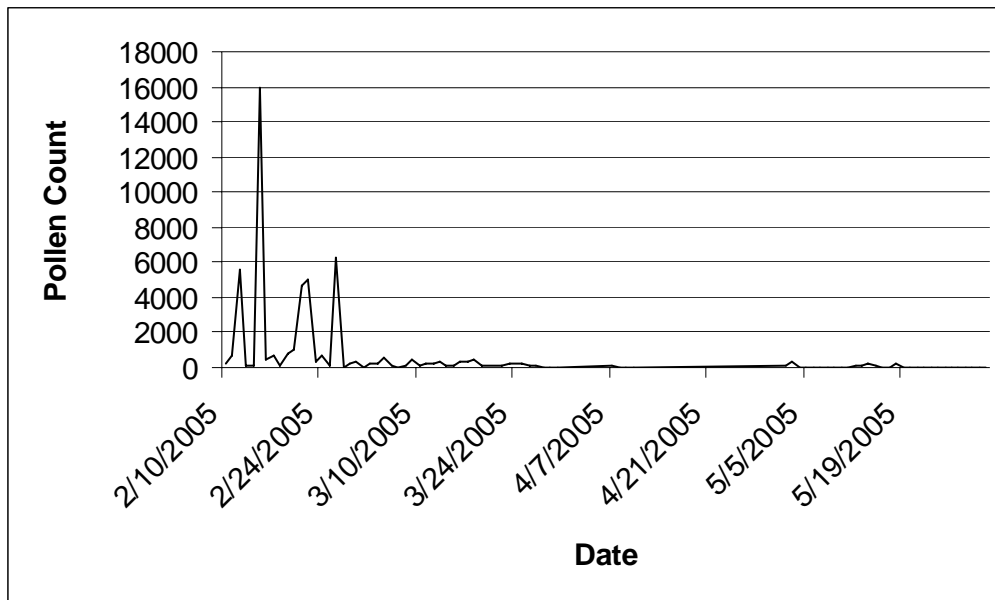


Figure 3.1a Pollen count dynamics in 2005 (Tifton, GA).

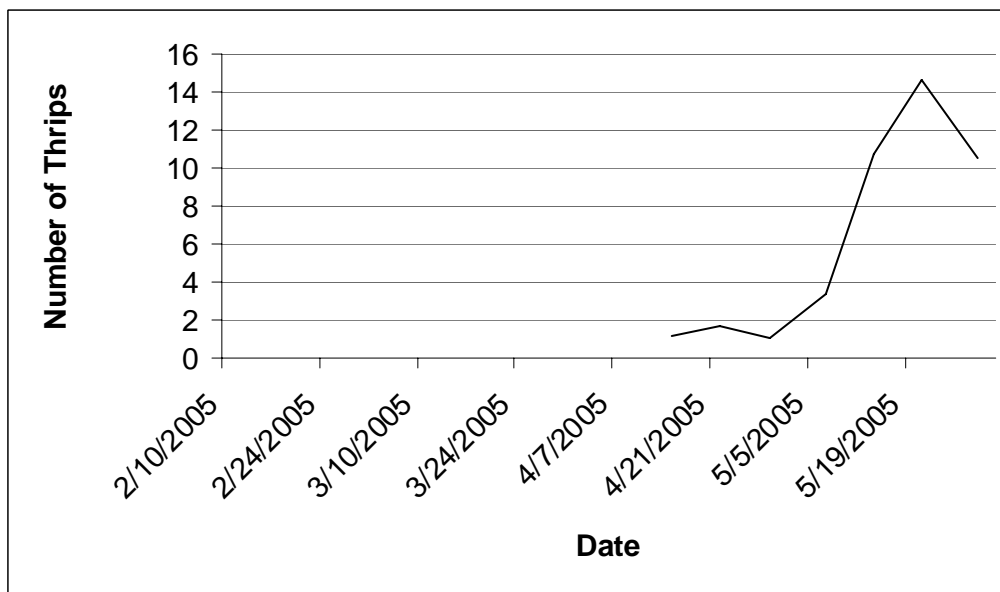


Figure 3.1b Thrips count dynamics in 2005 (Tifton, GA).

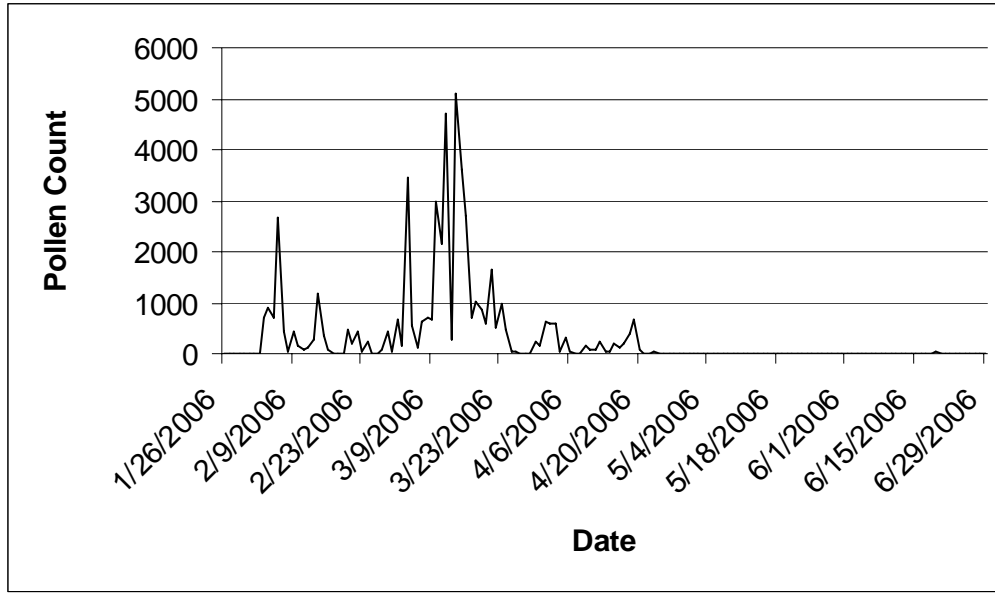


Figure 3.2a Pollen count dynamics in 2006 (Tifton, GA).

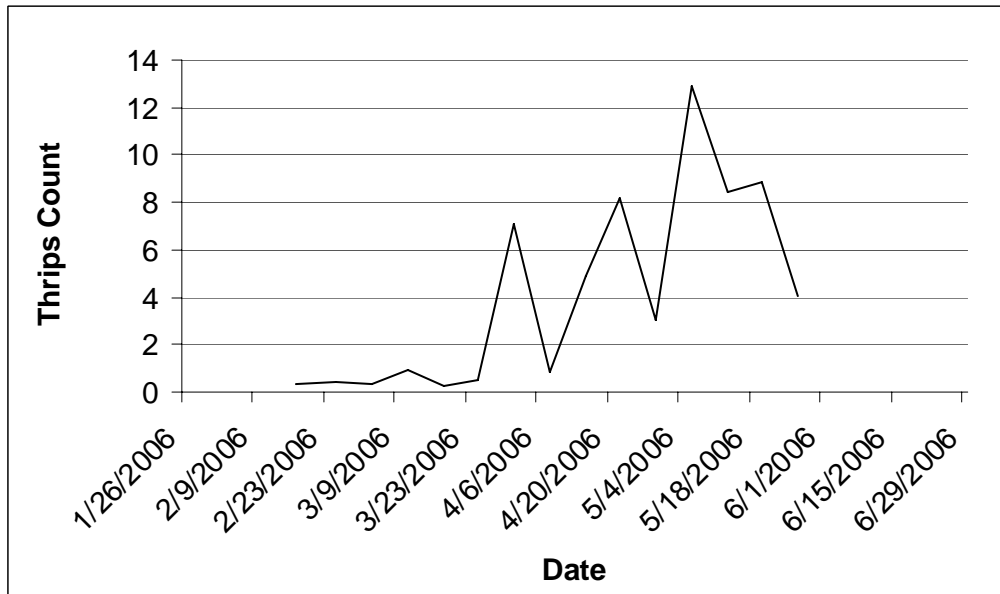


Figure 3.2b Thrips count dynamics in 2006 (Tifton, GA).

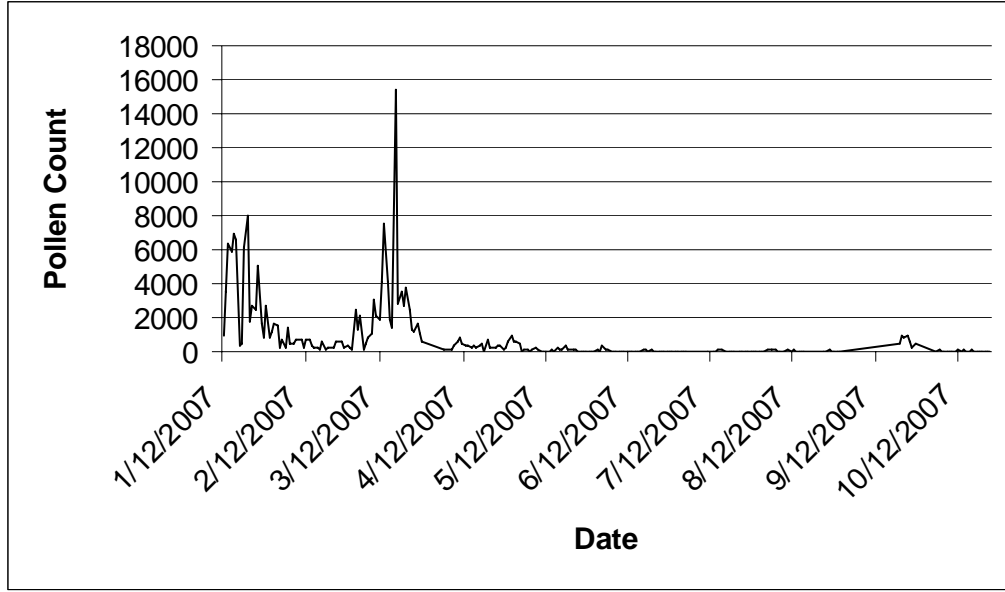


Figure 3.3a Pollen count dynamics in 2007 (Tifton, GA).

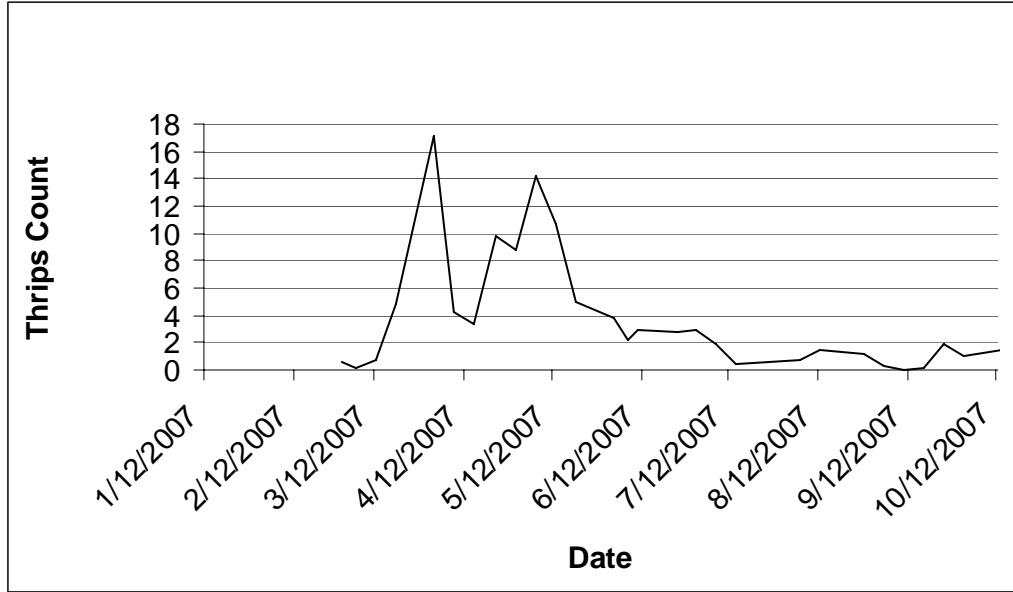


Figure 3.3b Thrips count dynamics in 2007 (Tifton, GA).

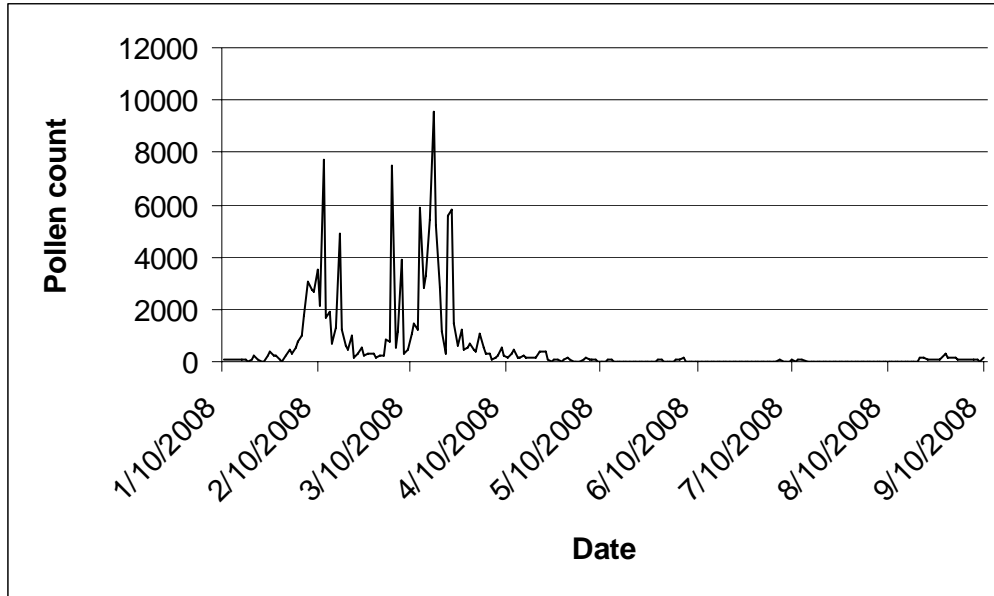


Figure 3.4a Pollen count dynamics in 2008 (Tifton, GA).

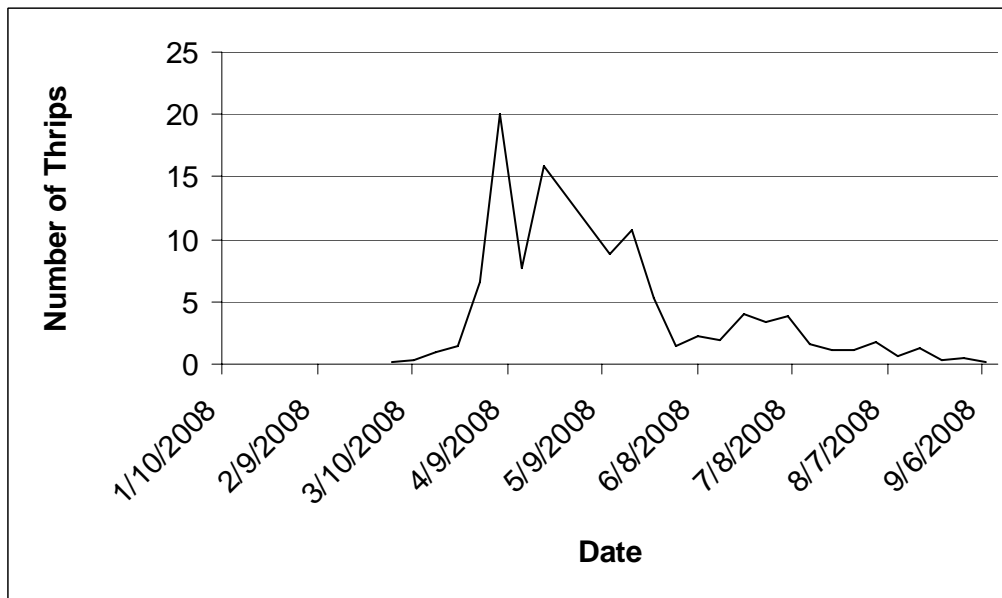


Figure 3.4b Thrips count dynamics in 2008 (Tifton, GA).

CHAPTER 4

LEAF CAGE BIOASSAY OF PINE POLLEN EFFECTS ON THRIPS REPRODUCTION¹

¹ Angelella, G.M. and D.G. Riley. 2008. To be submitted to *Journal of Entomological Science*.

Abstract

Pine trees (*Pinus* spp.) dehisce annually throughout spring in the Southeastern United States, depositing large quantities of pollen. Several methods were designed and tested to evaluate the effects of a diet supplementation of pine pollen, mimicking such pollen deposition on a plant leaf, on thrips reproduction. Two leaf sleeve cage methods were tested on tobacco (*Nicotiana tabacum* L.), one under field and one under laboratory conditions. Also, a gelatin capsule micro cage method was tested, cemented to peanut (*Arachis hypogaea* L.), tomato (*Solanum lycopersicum* L.), and cabbage (*Brassica oleracea* L.) leaves under laboratory conditions. The field sleeve cage and micro cage bioassay methods proved too problematic to be effective. However, the laboratory tobacco sleeve cage method showed slash pine (*P. elliottii* Engelm.) pollen supplementation to significantly increase the number of offspring produced per *Frankliniella occidentalis* or *F. fusca* female on a diet of tobacco leaves. Total offspring produced increased 400% in *F. fusca* and 2125% in *F. occidentalis* on the pollen treatment compared to no pollen supplementation.

Introduction

Pollen is consumed by many thrips species (Kirk 1997). Due to its richly nitrogenous protein and amino acid concentrations (Stanley and Linkskins 1974), pollen supplementation in thrips diets could provide essential nutrients otherwise lacking in a host plant foliage. Many thrips species have benefited from pollen supplementation under laboratory conditions. Among these, the flower thrips (of the *Thrips* or *Frankliniella* genera) have produced a large number of species exhibiting increase in reproduction after pollen supplementation: *F. intonsa* (Trybom) (Murai and Ishii 1982), *F. occidentalis* (Pergande) (Murai and Ishii 1982, Trichilo and Leigh 1988, van Rijn and Sabelis 1993, Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003, Zhi et

al. 2005, Riley et al. 2007), *F. schultzei* (Trybom)(Annadurai and Morrison 1987), *T. fuscipennis* Haliday (Kirk 1985a), *T. imaginis* Bagnall (Andrewartha and Kilpatrick 1951), and *T. obscuratus* (Crawford) (Teulon and Penman 1991). Thrips more closely associated with blooms may exhibit stronger effects than foliage-dwelling thrips. One study by Riley *et al.* (2007) found a significant increase in the western flower thrips (*F. occidentalis*) oviposition rates by nearly 200% with slash pine (*Pinus elliottii* Engelm.) supplementation on peanut (*Arachis hypogaea* L.) and tomato (*Solanum lycopersicum* L.). Those of the tobacco thrips [*F. fusca* (Hinds)], more commonly associated with foliage, increased only 100% on peanut, and not at all on tomato (Riley et al. 2007).

Relatedly, in the Southeastern United States, pine pollen is deposited in massive quantities every spring (Dorman and Barber 1956). A substantial fraction of the total tree pollen count originates from pines during the spring (O'Shaughnessy 1988). Georgia's land is 67% forested, and 45% of the forested area is loblolly-shortleaf and longleaf-slash pine forest (Harper and Dickenson 2004). In the Gulf States, pines begin dehiscence in late January/early February and proceed in this order: slash (*Pinus elliottii* Engelm.), then longleaf (*P. palustris* Mill.), loblolly (*P. taeda* L.), and lastly shortleaf (*P. echinata* Mill.) (Dorman and Barber 1956). The annual growth curve of tobacco thrips (*F. fusca*) and western flower thrips (*F. occidentalis*) begins around the end of March and peaks in mid-May (Riley and Pappu 2000, Groves et al. 2003, Riley and Pappu 2004). This build-up and decline seems to track ambient pine pollen concentrations in the spring (Chapter 3).

Tobacco and western flower thrips are important economic pests, responsible for the bulk of *tomato spotted wilt virus* (TSWV) transmission in vegetable and peanut (Salguero Navas et al. 1991, Riley and Pappu 2000, Riley and Pappu 2004, Campbell et al. 2005, Sherwood 2005). As

such, it is important to understand what seasonal stimuli may contribute to population growth to greater allow for prediction of thrips dynamics in vulnerable crops.

Several methods were tested under laboratory or field conditions to investigate the effects of various crop plant leaf diets supplemented with pine pollen on the reproduction of both western flower and tobacco thrips. Attempted methods include, 1) a gelatin capsule micro cage technique in peanut (*Arachis hypogaea* L.), tomato (*Solanum lycopersicum* L.) and cabbage (*Brassica oleracea* L.) leaves, 2) a sleeve cage technique used on peanut (*Arachis hypogaea* L.) in the field, and 3) a sleeve cage technique used on tobacco (*Nicotiana tabacum* L.) under laboratory conditions. The hypothesis was that both western flower and tobacco thrips would respond to pollen supplementation with increased reproduction, as measured by the number of offspring in one generation.

Materials and Methods

Thrips colonies. The western flower thrips colony used for these experiments was obtained from field populations at Tifton, Tift Co., GA, USA and maintained on green beans in 473 ml plastic deli cups (Loomans and Murai 1997). The tobacco thrips colony was initiated from thrips collected from peanut fields in Tifton, Tift Co., GA during spring 2006 and summer 2008 and maintained on green beans in plastic deli cups for use in these experiments. To ensure synchronous age of thrips adults used in the experiment, pupal or prepupal thrips of each species were collected by a round size 3 craft brush (Robert Simmons, New York City, New York, USA) into separate containers, with several adult males added. Pupae were allowed 3 d to molt and one additional day to ensure opportunity for females to mate. Thus, 1-3 day-old adult females with wings were used as progenitors for all experimental trials. A sub-sample of these thrips were used to verify species identification and a sub-sample of thrips from the 2006 colonies used were

used as vouchers in the vegetable entomology research lab collection at the Coastal Plain Experiment Station in Tift Co., GA, USA.

Pollen. Two species of pollen were used: loblolly pine (*Pinus taeda* L.), and slash pine (*P. elliottii* Engelm.). Slash pine strobili were collected just prior to dehiscence on IV-II-2008, from live trees in Tift Co., GA, USA. Loblolly pine strobili were collected III-IX-2007 in Tift Co., GA, USA. The strobili were allowed to dry, crumbled by hand, and passed through a sieve of 150 μm openings (Fisher Scientific, Pittsburg, PA, USA) to isolate pollen. The pollen was stored in sealed glass vials at 3°C.

Gelatin capsule micro cage. Experiments were conducted in the summer of 2007 at the Coastal Plain Experiment Station, Tifton GA in the Vegetable Entomology Research Laboratory. The ends of gelatin capsules were snipped and an open capsule end cemented to a leaf with yellow clay (Handi-Tak[®], Super Glue Corp., Rancho Cucamonga, CA, USA) (Fig. 4.1). Leaves were dusted with roughly 3.4 mg pollen or left untreated. A single age-synchronized female was placed in a capsule on each plant: tobacco thrips on peanut, and western flower thrips on tomato, cabbage and tobacco in capsules. There were 8 reps of each plant-treatment type. Every 3 days over 16 days total the female progenitor thrips was moved into a new capsule on the same plant and number of thrips in all other capsules tallied.

Data on the thrips reproduction were analyzed using PROC GLM (SASInstitute 1990). Fisher's least significant difference method was used for determining treatment differences using PROC GLM with $\alpha = 0.05$.

Sleeve cage on field peanut. Experiments were conducted during summer, 2007, at the Coastal Plain Experiment Station Horticultural Fields in Tifton, GA. Eight plots of 20 ft with approximately 60 peanut plants per plot were equally divided into pollen-dusted and untreated

types. Before each trial, 10 terminal leaflets were collected from each plot and analyzed for total initial thrips population using PROC GLM with $\alpha = 0.05$. Leaves were misted with tap water before pollen applications to facilitate pollen adhesion. Several clusters of peanut leaves from a single plant per plot were placed inside sleeve cages. Sleeves were constructed from nylon stockings fitted over roughly 16x7 cm wire frames (Fig. 4.2). In the first trial, pollen was applied at a rate of 5 cc per 2 plots, and 6 age-synchronized female plus 3 male tobacco thrips were inserted into cages. The adults were collected after 7 days, and after 15 days the cages and leaves were collected and number of thrips present counted. In the second trial, pollen was applied at a rate of 10 cc per 2 plots, and 4 age-synchronized female western flower thrips were inserted into cages, and sleeves tied shut with plastic ribbon. After 3 days, western flower thrips adults were collected. After an additional 10 days cages and leaves were collected and number of thrips present tallied.

Data on the thrips reproduction were analyzed using PROC GLM. 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 replicates.

Sleeve cage on excised tobacco leaf. Experiments were conducted in spring 2008 through fall 2008 at the Coastal Plain Experiment Station, Tifton GA in the Vegetable Entomology Research Laboratory. No-choice tests were conducted first with tobacco thrips on intact tobacco leaves to test whether pine pollen dusted onto leaves would exert any influence on thrips preference for oviposition. Young 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 the soil was kept moist. Fully

expanded leaves of age equivalence (of equivalent distance relative to apical meristems) were used, at either 3rd, 4th, or 5th leaf position. Variations due to differently aged leaves were analyzed using PROC GLM with $\alpha = 0.05$.

The plant bioassay used thrips arenas consisting of plastic sleeves, fitted on one end with a fine copper mesh screen with 76 μm openings (TWP Inc., Berkeley, CA) (Fig. 4.3). Insects generally cannot pass through screens with holes smaller than their thorax diameter (Bethke and Paine 1991). Because female tobacco and western flower thrips thoracic diameters are about 145-150 μm (Johansen 2002, Robb et al. 2005) with males' thoraxes only slightly smaller, the mesh screen allowed air to circulate without allowing thrips to escape the cage. Leaf stems were placed in beakers with water. To prevent thrips from falling into the water and drowning, cotton was stuffed around the stems above the water line. Leaves under the pollen treatment were lightly dusted with approximately 0.02 mg pollen, and then plastic sleeves were slid over the leaves and taped to the beakers with duct tape. Five female thrips adults were introduced with a paintbrush directly onto each of the leaves in the cage via a slit cut in the plastic, then sealed with a glue gun. Adults were removed from the cage after 7 d, and subsequently 15 d later the total thrips on the leaves were counted, and their developmental stage and sex noted. Reproduction was measured as the total number of offspring produced in a given treatment per initial female progenitor.

Data on the thrips reproduction were analyzed using PROC GLM. 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 replicates. A crude estimation of mean net

reproduction was generated using PROC GLM, combining female pupa and adult offspring to generate mean offspring produced per female, by treatment and species.

Results and Discussion

Gelatin capsule micro cage. The capsule bioassays yielded high thrips mortality; although, data for all thrips revealed a significantly higher reproduction with pollen. The western flower thrips model was not significant, indicating excessive variability in the experiment. Even so, the treatment effect for reproduction was approximately 4x greater with pollen (Table 4.1, $F=5.56$, $df=1,31$, $P=0.023$). For tobacco thrips on peanuts, the mean number of nymphs on pollen treatment (1.63) was significantly greater than the untreated (0.406). The western flower thrips mean nymphs on the pollen treatment (0.213) was marginally significant ($F=3.78$, $df=1,79$, $P=0.054$) compared to the mean nymphs on untreated leaf (0.063). Thrips mortality was deemed excessive with this bioassay.

To investigate the cause of thrips mortality during the capsule bioassay, a simple study was conducted with a single adult western flower thrips placed inside either a gelatin capsule or centrifuge tube. A whole peanut leaf dusted with pollen was also inserted. The 4 treatment types were: entire capsules, halves of capsules sealed with plumbers' putty, and halves of capsules sealed with yellow clay. Gelatin capsule cages had significantly lower mean survival time than centrifuge tube cages ($F=277$, $df=5,23$, $P<0.0001$) (see Table 4.2).

An unknown property of the gelatin capsules induced a very high mortality rate in the thrips progenitors, resulting in limited reproduction throughout the experiment. Despite this, the mean offspring produced per female in the tobacco thrips was significantly greater on slash pine pollen-treated peanut than on untreated peanut. Although the mean offspring produced per female in the western flower thrips showed no significant treatment effect, mean offspring

produced per female was greater on all plant leaf cages under the pollen treatment than on untreated leaf tissue. Furthermore, western flower thrips have been documented as responding to pine pollen supplementation in leaf tissue diets with increased reproduction (Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003), and to slash pine pollen supplementation in peanut and tomato leaf diets with significantly increased oviposition rates (Riley et al. 2007). It is likely, therefore, that with proper cage design methods, both western flower thrips and tobacco thrips would express increased reproduction with slash pine pollen supplementation in a leaf tissue diet.

Sleeve cage on field peanut. In the sleeve cage data there was no significant effect of pollen treatment on thrips reproduction, although the trend was toward higher reproduction with pollen. In tobacco thrips, there was a mean of 19.25 offspring counted on pollen-treated peanut, and 10.24 offspring on untreated peanut ($F=1.93$, $df=1,3$, $P=0.259$, see Table 4.3). In western flower thrips, means were 10.5 offspring on pollen-treated peanut and 7.5 offspring on untreated peanut ($F=1.00$, $df=1,3$, $P=0.500$, see Table 4.3). Total initial thrips populations in terminal peanut leaflets was not significant ($P=0.608$).

A large proportion of the adults inserted into sleeve cages were not recovered. It is likely that the nylon material comprising the sleeve had holes large enough to allow thrips escape. Also, the sleeve tie may not have effectively sealed the cage off from thrips escape. It proved difficult to tie the cages tightly without damaging the leaf tissue. Although there were no significant treatment effects in either thrips species, both generated higher mean offspring counts per leaf on the pollen treatment. However, because of an ineffective cage design, the data indicating mean offspring produced per leaf with and without pine pollen supplementation on tobacco in western flower thrips and tobacco thrips reproduction were unreliable. To more accurately assess thrips reproduction in a sleeve cage, a cage must be designed with fine enough

openings to prevent thrips escape and cage contamination from surrounding thrips in the field. Also, a better method of sealing the cage is needed, which will prevent thrips escape while not harming the plant tissue or constricting its vascular flow.

Sleeve cage on excised tobacco leaf. Both tobacco and western flower thrips exhibited greater numbers of offspring on pollen-treated versus untreated tobacco. Larger mean male and female adult, pupal and larval offspring values under the pollen treatment suggested a trend toward higher reproduction under a pollen treatment, although not all were significant (see Tables 4.4, 4.5). Tobacco thrips mean female adult ($F=20.4$, $df=1,15$, $P=0.003$), male adult ($F=13.2$, $df=1,15$, $P=0.008$), and female pupal offspring produced per leaf ($F=16.1$, $df=1,15$, $P=0.005$) were significantly higher under the pollen-treatment of tobacco leaves. Western flower thrips female pupal offspring produced per leaf ($F=11.1$, $df=1,15$, $P=0.013$) as the only stage with significantly greater offspring produced under pollen-treatment of tobacco leaves. The age difference among leaves used was not significant ($F=2.05$, $df=2,31$, $P=0.153$).

Mean total offspring of both tobacco thrips ($F=15.6$, $df=1,7$, $P=0.006$) and western flower thrips ($F=8.17$, $df=1,7$, $P=0.024$) were significantly greater under the pollen treatment using a log+1 transformation (see Tables 4.6 and 4.7). Mean adult and pupal males produced per female after a log transformation was significantly higher in the pollen treatment ($F=45.3$, $df=1,7$, $P=0.0003$), although the effect on mean adult and pupal females produced per female was not significant after log transformation ($F=0.11$, $df=1,7$, $P=0.751$). Western flower thrips male adults and pupae produced per female ($F=5.98$, $df=1,7$, $P=0.044$) as well as female adults and pupae produced per female ($df=1,7$, $F=7.61$, $P=0.028$) were significantly greater in the pollen treatment after log transformation. There were no western flower thrips offspring on the untreated tobacco, suggesting that tobacco leaves are not a reproductive host for western flower

thrips. This analysis does not project an accurate picture of males and females produced per western flower thrips female, but does indicate a treatment effect.

Crude net reproduction analysis showed that both species exhibited significantly higher rates of female offspring production per female on the pollen treatment (see Tables 4.6, 4.7). Tobacco thrips produced 2.68 female offspring per female on pollen-treated tobacco, versus 0.125 female offspring per female on untreated tobacco ($F=17.7$, $df=1,15$, $P=0.004$). Western flower thrips produced 1.35 female offspring per female on pollen-treated tobacco, versus 0.10 female offspring per female on untreated tobacco ($F=8.24$, $df=1,15$, $P=0.024$).

The mean total offspring produced per tobacco leaf and crude net reproduction values strongly suggests an effect of pollen supplementation in a tobacco leaf diet on the reproduction of both tobacco and western flower thrips. Tobacco thrips produced a more than 400% increase in offspring per leaf, and western flower thrips produced a 2125% increase in offspring per leaf. This, along with the marked increase in female offspring produced per female on the pollen-treated tobacco, insinuates a trend toward population increase with a pollen-supplemented leaf diet. Should pine pollen be deposited at similar rates on tobacco field crops, it is likely that a field population of tobacco or western flower thrips would increase.

Table 4.1 Mean offspring per female on pollen-dusted and untreated leaves (*F. occidentalis* on tomato, tobacco and cabbage; *F. fusca* on peanut).

Treatment	<i>F. fusca</i> - peanut	<i>F. occidentalis</i> -overall	<i>F. occidentalis</i> -tomato	<i>F. occidentalis</i> -tobacco	<i>F. occidentalis</i> -cabbage
Pollen	1.63b	0.213a	0.250a	0.125a	0.250a
Untreated	0.406a	0.063a	0a	0.083a	0.094a

Means within columns with the same letter are not significantly different ($P < 0.05$)

Table 4.2 Mean thrips survival (days) on pollen-dusted peanut leaves under various cage construction treatments.

Treatment	Mean Thrips Survival (days)
Entire Gelatin Capsules	1.75 ± 0.50c
Gelatin Capsules with Plumbers' Putty	1.25 ± 0.50c
Gelatin Capsules with Yellow Clay	1.75 ± 0.50c
Entire Centrifuge Tubes	7.00 ± 0.00b
Centrifuge Tubes with Plumbers' Putty	16.75 ± 1.89a
Centrifuge Tubes with Yellow Clay	17 ± 2.00a

Means within columns with the same letter are not statistically significant ($P < 0.05$)

Table 4.3 Mean thrips counts on pollen-treated and untreated peanut.

Treatment	<i>F. fusca</i>	<i>F. occidentalis</i>
Pollen	19.3 ± 1.29a	10.5 ± 1.41a
Untreated	10.3 ± 3.50a	7.50 ± 3.54a

Means within columns with the same letter are not statistically significant ($P < 0.05$)

Table 4.4 Mean *F. fusca* offspring on tobacco leaf – counted day 15.

Treatment	♀ Adults	♂ Adults	♀ Pupae	♂ Pupae	Larvae
Pollen	3.38a	2.38a	10.0a	2.63a	16.1a
Untreated	0.250b	0.125b	0.375b	0.875a	5.13a

Means within columns with the same letter are not statistically significant ($P < 0.05$)

Table 4.5 Mean *F. occidentalis* offspring on tobacco leaf – counted day 15.

Treatment	♀ Adults	♂ Adults	♀ Pupae	♂ Pupae	Larvae
Pollen	3.25a	2.13a	3.50a	0.625a	1.63a
Untreated	0.500a	0a	0b	0a	0a

Means within columns with the same letter are not statistically significant ($P < 0.05$)

Table 4.6 *F. fusca* total offspring analysis and female offspring produced per female.

Treatment	Mean total offspring per leaf	Female offspring (pupae + adults) per female
Pollen	34.5 ± 3.65a	2.68 ± 1.85a
Untreated	6.75 ± 1.56b	0.125 ± 0.183b

Means within columns with the same letter are not significantly different, using log transformation ($P < 0.05$)

Table 4.7 *F. occidentalis* total offspring analysis and female offspring produced per female.

Treatment	Mean total offspring per leaf	Female offspring (pupae + adults) per female
Pollen	11.1 ± 3.64a	1.35 ± 1.30a
Untreated	0.500 ± 0.535b	0.100 ± 0.107b

Means within columns with the same letter are not significantly different, using log transformation ($P < 0.05$)



Figure 4.1 A centrifuge tube and gelatin capsule micro cages (left to right) cemented to leaf with yellow clay.



Figure 4.2 A sleeve cage on peanut.

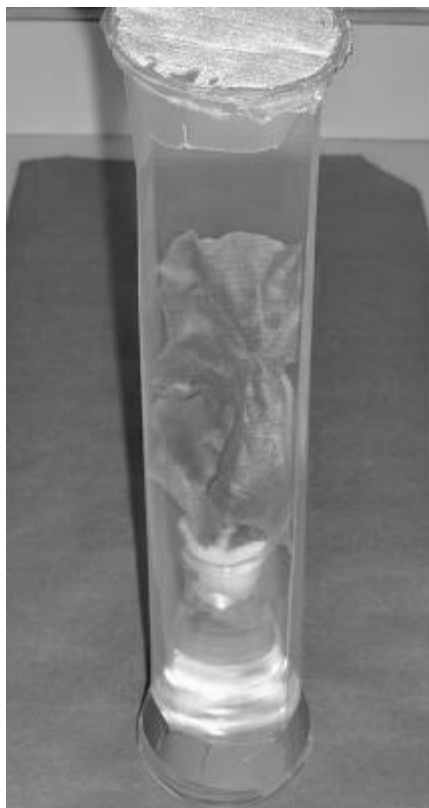


Figure 4.3 A sleeve cage on an excised tobacco leaf.

CHAPTER 5

LIFE TABLE ANALYSIS OF PINE POLLEN EFFECTS ON *FRANKLINIELLA FUSCA* REPRODUCTION¹

¹ Angelella, G.M. and D.G. Riley. 2008. To be submitted to *Environmental Entomology*.

Abstract

A micro cage method was developed to test the effect of pollen supplementation to an onion (*Allium cepa* L. var. *Pegasus*) diet on thrips (Thysanoptera: Thripidae) reproductive parameters. *Frankliniella fusca* (Hinds) females were placed on 3-leaf stage onion seedling under a treatment of either slash pine (*Pinus elliottii* Engelm.) pollen dusting (a pollen supplement) or no pollen treatment. Adult survival, net oviposition and offspring produced over a series of ten, 2-day intervals were recorded. From these values, l_x , $l_x m_x$ and R_0 values were constructed. A trimodal distribution of oviposition was observed with the pollen supplement. Increased oviposition rates led to higher female offspring production per female, and to a 340% increase in mean net reproduction.

Introduction

Thrips have been described as ‘opportunists’: able to survive on poor hosts until nutritionally superior plants are available, whereupon their copious reproduction leads to rapidly accelerating population growth (Mound and Teulon 1995). Pollen, due to its high amino acid and nitrogen content (Stanley and Linkskins 1974), could be a nutritious supplement to a thrips diet, and many species do in fact consume pollen (Kirk 1995). Pollen supplementation has increased developmental and reproductive (and sometimes survival) rates in many thrips species. For example, pollen’s effects on the life history characteristics of the western flower thrips [*Frankliniella occidentalis* (Pergande)] have been well documented (Murai and Ishii 1982, Trichilo and Leigh 1988, van Rijn and Sabelis 1993, Hulshof and Vänninen 1999, 2002, Hulshof et al. 2003, Zhi et al. 2005, Riley et al. 2007).

Western flower thrips, along with tobacco thrips [*Frankliniella fusca* (Hinds)], are responsible for the bulk of tomato spotted wilt virus transmission to crops (Salguero Navas et al.

1991, Riley and Pappu 2000, Riley and Pappu 2004, Campbell et al. 2005, Sherwood 2005), resulting in nearly \$1 billion in losses (Prins and Goldbach 1998). Understanding the stimuli that induce population growth in pest thrips such as western flower and tobacco thrips can result in better prediction of population dynamics. This may be especially pertinent to the southeastern United States, as there is an annual pollen deposition event in early spring, the peak of which precedes western flower and tobacco thrips population peaks by roughly one thrips generation time, or 2 weeks (Chapter 3)(Riley and Pappu 2000, Riley and Pappu 2004). This time of year coincides with the early stages of plant growth in several important crops such as tobacco (*Nicotianum tabacum* L.), tomato (*Solanum lycopersicum* L.) and peanuts (*Arachis hypogaea* L.) – at a time when plants are most vulnerable to TSWV damage (Moriones et al. 1998, Chaisuekul 2003).

Many previous studies involving observation of thrips life history characteristics have utilized excised leaves, or leaf discs (Murai and Ishii 1982, Hulshof et al. 2003) in order to facilitate the containment of thrips. The minuscule size and thigmotactic tendencies of thrips make them difficult to confine. This, along with the need to maintain thrips and host health over a length of time sufficient for the observation of life table parameters, led to many problems in constructing a life table bioassay using whole, live plants. This study attempted to initiate a new life table bioassay method using whole, live micro plants, in which the effects of pollen supplementation on thrips life history characteristics could be evaluated. A micro plant, such as a two-leaf onion seedling (*Allium cepa* L. var. *pegasus*), held inside a micro centrifuge tube to prevent the escape of thrips could provide live plant material long enough to measure thrips life table parameters. It was hypothesized that net reproduction of tobacco thrips would increase on the onion tissue diet with a supplement of slash pine pollen (*Pinus elliottii* Engelm.).

Materials and Methods

The tobacco thrips colony was initiated from thrips collected from peanut fields in Tifton, Tift Co., GA during spring 2006 and summer 2008 and maintained on green beans (*Phaseolus vulgaris* L.) in 473 ml plastic deli cups (Loomans and Murai 1997) for use in these experiments.

The pollen used was slash pine, *Pinus elliottii* Engelm. Slash pine strobili were collected just prior to dehiscence on IV-II-2008, from live trees in Tift Co., GA. The strobili were allowed to dry, crumbled by hand, and passed through a sieve with 150 μm openings (Fisher Scientific, Pittsburg, PA, USA) to isolate pollen from debris. The pollen was stored in sealed glass vials at 3°C.

Thrips were placed in an enclosed cage on a two-leaf onion (*Allium cepa* L. var. *pegasus*) seedling using a round size 3 craft brush (Robert Simmons, New York City, New York, USA), moved every two days for two weeks, and the number of eggs oviposited and pupal or adult offspring quantified. Centrifuge tubes (Central Research, Athens, GA) with a 0.5 cm diameter window covered with copper mesh screen (TWP Inc., Berkeley, CA) with 76 μm openings were used to contain live onions. Insects generally cannot pass through screens with holes smaller than their thorax diameter (Bethke and Paine 1991). Because female tobacco and western flower thrips thoracic diameters are about 145-150 μm (Johansen 2002, Robb et al. 2005) with males' thoraxes only slightly smaller, the mesh screen allowed air to circulate without allowing thrips to escape the cage. Damp cotton was placed under the trimmed onion roots in the tube bottom to keep the onions alive. Onions under the pollen treatment were lightly dusted at an approximate rate of 1mg for every 70 onions before placement into the tubes. To synchronize age, pupal and prepupal thrips of both sexes were separated by brush into separate plastic deli cups. The thrips remained in the cup for 4 d: three to allow time for adults to emerge and one additional to ensure

opportunity for females to mate. Two adult females were placed directly onto onion foliage with a paintbrush. Each tubular micro cage was stored in a growth chamber, under 25°C and 94% r.h. and 24 h daylight. Two days following thrips placement on an onion, they were transferred to a tube with a fresh onion under similar treatment. Every 2 days upon transference, progenitor mortality was noted.

In 40% of the treatment replicates (8 onions from each treatment and 2 d time interval), eggs in the onion tissue were stained using a lacto phenol-acid fuchsin staining technique similar to (Nuessly et al. 1995) and first described by (Simonet and Pienkowski 1977). Onion leaflets were separated from the bulb, and all onion tissue boiled for 3-4 minutes in a 1:2:1:1:1 solution of 10% lactic acid, 50% glycerin solution, distilled water, saturated phenol buffered at pH 4.3 and 1g/l of acid fuchsin high purity biological stain. Chemicals were obtained from Fisher Scientific, Pittsburg, PA, USA. Stained leaves were allowed to cool for 3-5 hours and excess stain rinsed off with warm tap water. Stained eggs were observed in onion tissue under a stereo microscope.

Due to the thrips' tendency to crawl into onion crevices, offspring were not easily observable to monitor development rates. In order to view all possible thrips on an onion, the leaves must be gently opened and separated, thus damaging or killing the plant. To avoid damaging the onion, a single observation of nymph stage thrips was made. Based on life table data compiled by Lowry et al. (1992) describing development time of *F. fusca* on peanut (*Arachis hypogaea* L.), a time period was estimated that would ensure that all larvae would have had ample time to hatch from eggs. We estimated that 8.1 (+/- 0.2) days after egg deposition would be required for 100% hatch at 25° C. So, nine days following the end of the 2 d ovipositional period, onions were taken apart and all larvae that had hatched were removed from the old onion and placed onto a fresh onion under similar treatment, to ensure the presence of

living tissue on which to feed. After 18 d, the duration of time needed for thrips to develop into pupae (Lowry et al. 1992), the remaining 60% of treatment replicate onions (12 onions from each treatment and 2 d time interval under) were removed and taken apart to count all male and female progeny.

Mean eggs and offspring per female. Mean eggs, larvae, and male plus female adults produced per female were calculated for each 2-day interval under both treatments. Analysis of variance for the effect of pollen treatment, (mainplot) and interval (subplot) on the eggs, larvae, and male and female adults produced per female were analyzed using PROC GLM (SASInstitute 1990) with a split plot design model. Fisher's least significant difference method was used for determining interval differences using PROC GLM with $\alpha = 0.05$.

R₀ calculations. R₀ values were calculated in one of two ways. In the first, one R₀ value was generated per treatment. In the second, four R₀ values were calculated, and then a mean R₀ was generated per treatment.

In order to generate one R₀ value for each treatment, l_x and m_x values first had to be calculated. The l_x values were calculated for each 2-day interval under each treatment. To determine l_x values, the percent surviving progenitors during a given time interval was multiplied by the percentage of thrips surviving from egg to adult emergence. The percent surviving progenitors were calculated for every 2-day interval. The percentage of thrips surviving from egg to adult for each two day interval was determined by dividing mean total offspring surviving through adult emergence per female by the mean eggs produced per female.

To determine $l_x m_x$ values, l_x was multiplied by m_x , which was the product of an average sex ratio and mean eggs per female for a 2-day interval. Sex ratio values were determined by dividing total adult female offspring by total adult offspring per time interval.

Lastly, the $l_x m_x$ values for each time interval in a treatment were added together. This yielded one R_0 value for each treatment.

The second way involved the calculation of four R_0 values per treatment, and the subsequent generation of a mean R_0 for each treatment. The l_x values were calculated for each time interval in a treatment, in the same way as described above. Next, however, four m_x values per time interval were generated from the product of egg count data and an average sex ratio. These values were multiplied by the l_x values of the corresponding time interval in a treatment. This produced four $l_x m_x$ values per time interval in a treatment, and subsequently, four R_0 values for each treatment. A mean R_0 value per treatment could then be generated.

The four m_x values were found by generating four separate average sex ratios and four individual mean eggs produced per female values for each time interval in a treatment. There were twelve replicates of onions from which the offspring sex ratios were calculated. Four average sex ratios were generated for each time interval by grouping the replicates the following way: the first group contained replicates 1-3, the second contained 4-6, the third contained 7-9 and the fourth contained 10-12. Additionally, the egg count data was collected from eight replicates of onions. Four mean eggs produced per female values were generated for each treatment by processing the egg count data in groups of four by replicates: the first group contained replicates 1-2, the second contained 3-4, the third contained 5-6 and the fourth contained 7-8. To generate the m_x values for each time interval per treatment, average sex ratios and mean eggs produced per female values generated from each corresponding replicate group were multiplied. For example, the average sex ratio value generated from the first replicate group was multiplied by the mean eggs produced per female generated from the first replicate group, and then the second replicate's average sex ratio was multiplied by the second replicate group's

mean eggs produced per female value, and so on. Each of the $l_x m_x$ values from one of the four replicate groups in each treatment's time interval were added together for an R_0 value.

The four R_0 values were analyzed using PROC ANOVA (SAS Institute, 1990). To determine treatment differences, Fisher's least significant difference method was implemented using PROC ANOVA with $\alpha = 0.05$. Since identical experiments provided similar results, the analysis of variance was conducted over both experiments using all replicates.

Results

Mean eggs and offspring per female. Under the pollen treatment, mean values of eggs ($F=17.5$, $df=1,159$, $P=0.004$), larvae ($F=23.8$, $df=1,217$, $P=0.0005$), and male adults produced per female ($F=24.4$, $df=1,215$, $P=0.0004$) were significantly greater under the pollen treatment (see Table 5.1). The eggs, larvae, adult females and adult males per female during each time interval were analyzed in bulk with both treatments combined (see Table 5.2). The distribution of mean eggs produced per female was trimodal throughout the 10 time intervals (Figs. 5.1 and 5.2). Eggs produced per female varied significantly with each 2-day time interval ($F=38.4$, $df=1,159$, $P<0.0001$), as did larvae produced per female ($F=18.1$, $df=1,217$, $P<0.0001$), female pupae and adults produced per female ($F=17.0$, $df=(1,215)$, $P<0.0001$), and male pupae and adults produced per female ($F=5.74$ $df=1,215$, $P=0.019$).

Comparing means by pollen treatment (see Figs. 5.1, 5.2), both data sets held roughly the same trimodal distributions. However, the egg production per female peaks under the pollen treatment were almost 3x higher than those on untreated onions.

R_0 calculations. There was a marked difference in the data between the two treatments (see Tables 5.3, 5.4). Under the pollen treatment the R_0 value was 4.68, nearly 3.4x larger than the untreated R_0 value of 1.39. Using the four subgroups per treatment, the mean R_0 value for the

pollen treatment was significant at 4.84 ($F=27.03$, $df=1,7$, $P<0.05$) (see Table 5.5). Mean R_0 value for the pollen treatment was 4.4x larger than that of untreated onion, which was 1.10 (see Table 5.5).

Discussion

The slash pine supplementation in an onion diet greatly increased reproduction in tobacco thrips. Although under both treatments oviposition rates of a female over the ten, 2-day time intervals fluctuated to form a trimodal distribution, the oviposition rate peaks were much larger in pollen-treated onions than in untreated onions. This increase led to greater numbers of female offspring produced, and thus, a significantly larger net reproduction value (R_0).

It is not known what the rate of pollen deposition is on field crops. If it is comparable to the light dusting applied to these onions, it could follow that tobacco thrips in the field would react with a similarly increased intrinsic growth rate. To validate the effect of slash pollen on tobacco thrips reproduction in the field, the rate of pollen deposition would need to be determined. Concurrent thrips population fluctuations would also need to be observed. Furthermore, the species composition of deposited pollen would be of interest, as would the investigation of different pine species pollen effects on tobacco thrips reproduction.

Table 5.1 Mean eggs, larvae, ♀ adults and ♂ adults per female, for each treatment (pollen + untreated).

Treatment	Mean eggs per female	Mean larvae per female	Mean ♀ adults per female	Mean ♂ Adults per female
Pollen	4.21 ± 4.22a	2.32 ± 2.82a	0.503 ± 1.06a	0.537 ± 1.11b
Untreated	1.33 ± 1.78b	0.876 ± 1.54b	0.199 ± 0.797a	0.079 ± 0.238a

Means within columns with the same letter are not statistically significant ($P < 0.05$)

Table 5.2 Mean eggs, larvae, ♀ adults and ♂ adults per female, for each interval (2 days) under both treatments combined (pollen + untreated).

Interval (2 day)	Mean eggs per female	Mean larvae per female	Mean ♀ adults per female	Mean ♂ adults per female
1	2.66abc	2.52a	0.521ab	0.396abc
2	4.25a	0.833b	0.146ab	0.146c
3	3.00ab	1.00ab	0.042b	0.104c
4	1.41bc	1.19ab	0.087ab	0.391abc
5	3.53a	2.03ab	0.354ab	0.367abc
6	2.44abc	1.98ab	0.565a	0.217bc
7	0.625c	2.14ab	0.432ab	0.364abc
8	2.94ab	1.62ab	0.477ab	0.857a
9	4.24a	0.833b	0.056b	0.111c
10	2.60abc	1.79ab	0.423ab	0.731ab

Means within columns with the same letter are not significantly different (LSD, $P < 0.05$)

Table 5.3 Mean eggs per female, sex ratio, l_x , $l_x m_x$ and R_0 for each time interval (2 days) on untreated onions.

Treatment	Time interval (2 days)	Mean eggs per female	Sex ratio	l_x	$l_x m_x$
Untreated	1	2.20	0.538	0.310	0.209
Untreated	2	3.27	0.500	0.302	0.291
Untreated	3	4.47	1.00	0.279	0.732
Untreated	4	2.67	0	0.248	0
Untreated	5	3.66	.	0.248	.
Untreated	6	1.85	0.333	0.233	0.097
Untreated	7	1.16	0.286	0.209	0.022
Untreated	8	0.756	0.25	0.202	0.189
Untreated	9	3.02	0	0.155	0
Untreated	10	2.19	0.167	0.140	0.020
					Sum= R_0 : 1.39

Table 5.4 Mean eggs per female, sex ratio, l_x , $l_x m_x$ and R_0 for each time interval (2 days) on pollen-treated onions.

Treatment	Time interval (2 days)	Mean eggs per female	Sex ratio	l_x	$l_x m_x$
Pollen	1	4.06	0.581	0.340	0.802
Pollen	2	6.56	0.375	0.315	0.774
Pollen	3	3.38	0	0.298	0
Pollen	4	1.50	0.333	0.281	0.140
Pollen	5	5.69	0.400	0.264	0.599
Pollen	6	3.63	0.826	0.255	0.764
Pollen	7	0.875	0.643	0.230	0.129
Pollen	8	5.50	0.300	0.213	0.351
Pollen	9	6.60	0.667	0.179	0.786
Pollen	10	4.33	0.455	0.170	0.335
					Sum= R_0 : 4.68

Table 5.5 Mean R_0 calculated for each treatment.

Treatment	N	Mean R_0
Pollen	4	$4.84 \pm 1.40a$
Untreated	4	$1.10 \pm 0.331b$

Means within columns with the same letter are not significant ($P < 0.05$)

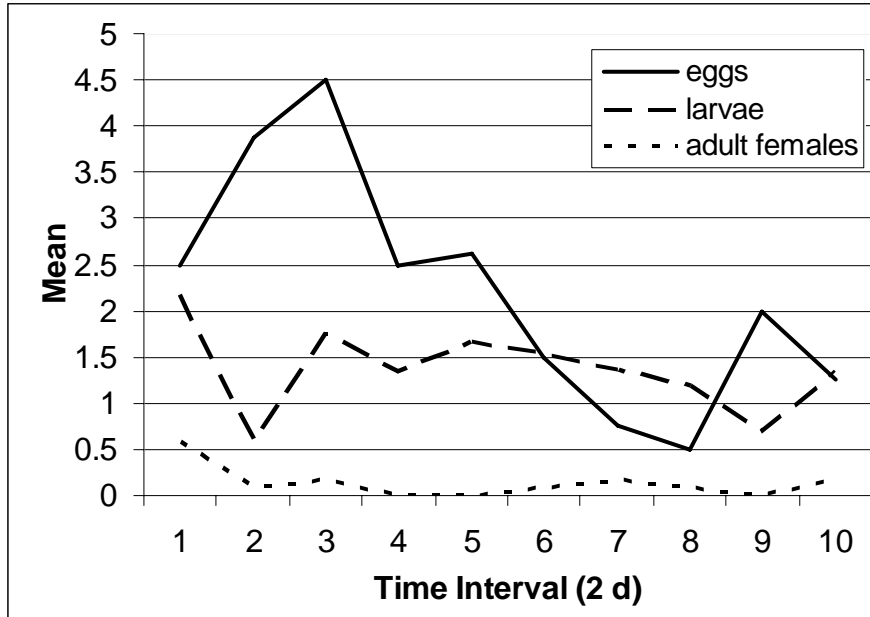


Figure 5.1 Mean eggs, larvae and adult females per time interval (2 d) on untreated onions.

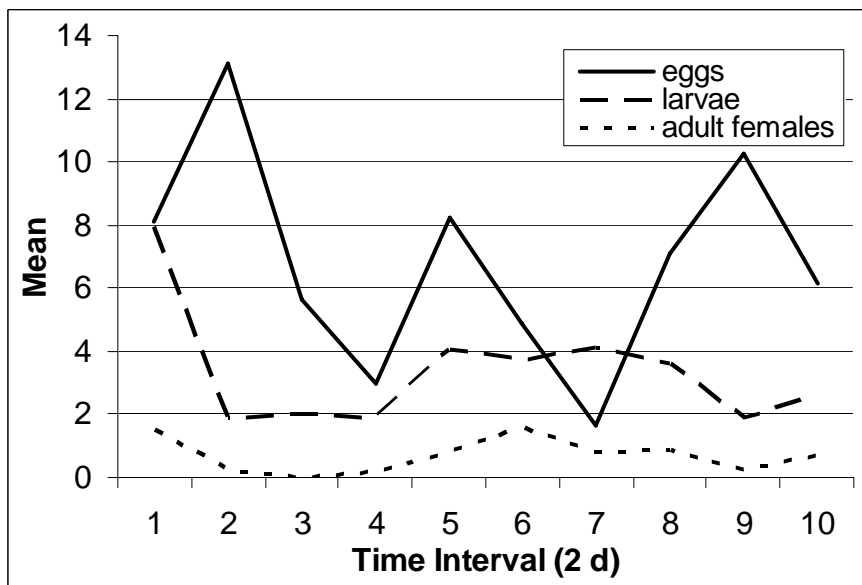


Figure 5.2 Mean eggs, larvae and adult females per time interval (2 d) on pollen-treated onions.

CHAPTER 6

SUMMARY

Three objectives were set up to investigate whether pine pollen supplementation in the spring could affect reproduction in western flower and/or tobacco thrips. The first was to collect and analyze seasonal ambient pollen levels and thrips population dynamics. The second was to conduct bioassays assessing pollen supplementation effects on western flower and tobacco thrips reproduction, and the last was to devise an experimental method to conduct a life table analysis of pine pollen supplementation effects on western flower and tobacco thrips. The hypothesis was that pine pollen supplementation in the leaf tissue diet of western flower and tobacco thrips would increase reproduction in both species.

For the first objective, pollen counts and thrips counts on sticky traps were taken in Tift Co., GA, USA during years 2005, 2006, 2007, and 2008. The data were graphed, and correlation analyses were run with the same pollen count week lined up with thrips count data, the pollen week + 1, pollen week + 2, pollen week + 3, pollen week + 4 and pollen week + 5. Though there was variation depending on the year and the pollen week alignment, when all years were combined, a significantly strong positive correlation was observed between pollen counts and thrips counts after a pollen week + 2 alignment. This is roughly the time required for one of a thrips generation.

For the second objective, three bioassay methods were designed to analyze western flower and tobacco thrips reproduction with pine pollen supplementation, including a gelatin capsule micro cage, a sleeve cage on peanut, and a sleeve cage on excised tobacco leaf. All had

two treatments: a light pollen dusting, or no pollen treatment. The first two methods had a number of problems with thrips containment and mortality rates, but suggested a trend toward greater thrips reproduction on pollen treatments. In both thrips species examined, the third method yielded significantly higher mean offspring production per leaf on the pollen treatment, and estimates suggested net reproduction was higher on the pollen treatment.

For the third objective, a micro cage on onion bioassay was designed to analyze tobacco thrips reproduction on an onion diet with or without a pine pollen treatment. The two treatments were: a light dusting of pine pollen and no pollen treatment. Under the pollen treatment, there were significantly greater mean numbers of eggs, larvae, and female adults produced per female. In the graphed distribution of eggs produced per female per 2 day time interval, the trimodal peaks were considerably higher under the pollen treatment. Net reproduction was higher under the pollen treatment, as well as mean net reproduction, which was significant.

The results of all three experiments lead to the conclusion that pine pollen deposition affects thrips reproduction. Both western flower and tobacco thrips showed an increase in reproduction with pine pollen supplementation. Additionally, there was a significant positive correlation between thrips numbers and pollen counts offset by 2 weeks in Georgia, which could suggest an increase in thrips reproduction caused by increased levels of pollen deposition. For further investigation into the effects of the spring pine pollen deposition event on thrips reproduction, future studies could examine pine pollen supplementation on various other common Georgian thrips species, or could improve the current bioassay methods. Studies examining pine pollen deposition rates, and the changes in thrips reproduction in the field could also be beneficial.

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