RELATIONSHIP OF FLOWER THRIPS TO HARDLOCK OF COTTON

By

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To those who establish the foundation for future advances.

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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Hardlock is a limiting factor for cotton yields in Florida, and appears as a failure of the fiber to expand outward following boll opening. Annual losses are typically 30 to 70% of the crop. Prior research has identified *Fusarium verticillioides* as a causal agent. It is believed to infect the flowers on the day of bloom, and sustain itself within the developing boll. It was hypothesized flower thrips may increase the chance of infection by carrying the spores into flowers or allowing better access by the pathogen due to their feeding damage.

To determine the extent to which thrips in the field transport *F. verticillioides*, thrips were captured and placed on media to isolate *Fusarium*. Approximately 10% of cotton flowers contained thrips that were carrying *Fusarium*. *Frankliniella tritici* was the most commonly found thrips species, representing more than 99% of individuals. Similar results were noted in samples from Louisiana, Alabama, and South Carolina. It was also determined that the average relative humidity from 1900 on the day prior to 1000 on the day of sampling was negatively associated with the number of thrips in flowers at 1000.

Field trials were performed to test the ability of insecticide and fungicide applications to reduce thrips numbers and hardlock. Insecticides reduced thrips number and hardlock severity, and sometimes improved yields. The number of thrips in a plot was positively associated with

hardlock severity. Greenhouse experiments were performed to test the ability of *Fusarium* inoculation, thrips, or thrips exposed to *Fusarium* to cause hardlock. Thrips exposed to *Fusarium* resulted in the most severe symptoms. The effect of weather on hardlock was also explored. The average temperature between 0000 and 0600 on the day of bloom was negatively associated with hardlock severity in the resulting bolls. These findings demonstrate hardlock is associated with flower thrips and can be reduced by managing thrips numbers. Hardlock incidence is also influenced by temperature, and this information may allow control measures to be used when they would be most effective.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Cotton

Cotton, *Gossypium hirsutum* L., is an important crop in the southeastern US. Diseases and insects affecting its production are well documented, and it has been a topic of research since the 1800s. In 2003 cotton was produced on 38,000 hectares in Florida. The harvest of 117,000 bales each weighing 218 kg. yielded a market value of $33.49 million. Since 1995, yields have usually ranged from 450-670 kg/ha. These lower yields have been attributed to hardlock, a situation in which cotton fiber fails to fluff out of the boll after opening. From the early 1980s through the early 1990s, Florida yields were close to 785 kg/ha, and peaked in 1984 at 1105 kg/ha (Anonymous, 2005). Yields in Florida tend to be lower than other areas of the Southeast, resulting in smaller profit margins, and the potential for greater gains from new research.

Lower yields have occurred despite the release of improved varieties. Many of these varieties have been genetically modified to produce the *Bacillus thuringiensis* (Bt) endotoxin and to be resistant to glyphosate (Round Up Ready or RR). The most commonly used Bt toxin in cotton is the Cry1Ac protein, and it is highly effective against lepidopterans. Bt toxin expression is influenced slightly by parent variety background, but is sufficient for field conditions (Adamczyk and Sumerford, 2001). In addition to reducing insect damage to cotton, it has also allowed a substantial reduction in the number of insecticide applications during the season (Cattaneo, 2006). Resistance to glyphosate permits the herbicide to be used as a broad-spectrum herbicide while cotton seedlings are in the field. This is advantageous, since it allows control of broad-leaf weeds which would otherwise be difficult and require several other materials in a conventional control program. Absorption of the compound by humans is low, it is not metabolized, and does not accumulate in tissues. It is non-carcinogenic and has no impact on

fertility or reproductive parameters. Breakdown of the compound in the environment is also fairly rapid, with a half-life of 14 days under typical conditions (Accinelli, 2004).

The type of cotton produced in Florida and the southeastern US is known as "upland" cotton (*G. hirsutum*). This is in contrast to the long-staple Pima and Egyptian cottons (*G. barbadense*) that are sometimes produced in the southwestern US. Prior to 1920, Sea Island cotton (*G. barbadense*) was produced on the Florida peninsula and coastal islands of Georgia and the Carolinas. It was limited to these locations due to the long growing-season required for its production, and is still produced in the Caribbean. The eventual arrival of the boll weevil made its production unprofitable, and the industry collapsed. However, Sea Island production in the US preceded that of upland cotton, and its high profitability formed the basis for expansion of upland cotton into the interior of the southeast (May and Lege, 1999).

The profitability of cotton farming varies substantially from year to year. In 2003, the estimated return after all expenses was $236.62 per hectare, in the Southeast. From 1997 to 2004, only 3 of 8 years showed a profit, with total losses exceeding total profits. Production expenses, yields and prices received vary annually. In 2003, total per hectare operating costs were $793.14 and total allocated costs were $449.36. A lint yield of 932 kg/ha at $1.41/kg and a seed yield of 1507 kg/ha. at $0.09/kg yielded the $236.62/ha quoted above. The estimated farm size for the estimates listed above was 535 acres. Florida yields are typically lower than the rest of the cotton belt, and reducing the previous quoted yield to the actual planted yield for 2003 of 597 lbs/ac. results in a loss of approximately $123/ha (National Cotton Council of America, 2005, 2006).

Cotton is typically planted between April 20 and June 1. On average, emergence occurs at 7 days after planting (DAP), first square at 39 DAP, and first bloom at 62 DAP. Development is determined in large part by growing degree days, so warmer weather leads to faster maturation.

The normal developmental sequence of cotton bolls has been thoroughly documented. Cotton flowers require approximately 35 days to develop from carpels to anthesis, the stage of opening. The square appears 10-14 days into the process, and pollen mother cells are undergoing meiosis by days 13 and 14. Floral nectaries begin developing around day 10. Ovule number, which determines the number of locules per boll, is thought to be influenced by environmental factors, although varieties also differ (Gipson, 1982). On the day of bloom, indicated by a white flower, pollen germinates on the stigma and the pollen tube begins growing toward the ovary. This growth requires 12 to 30 hours to reach the ovary and accomplish fertilization.

By the second day of bloom, the flower has changed from white to a red-purple color. The flower usually detaches from the plant on the 3rd or 4th day after bloom. The zygote begins cell division 4 to 5 days after bloom (Pollock and Jensen, 1964). The initiation of flowering is primarily determined by the accumulated growing degree-days since planting. This is expressed by plants in warmer conditions initiating the first bloom on a lower branch (Roussopoulos et al, 1998). Some cotton varieties are photoperiod-sensitive, but they are not grown commercially in the US. Water stress prior to flowering was observed to increase the subsequent rate of flowering (Guinn, 1979). High night temperatures (25°C) have been reported to delay the onset of flowering (Mauney, 1966). Epidermal cells on the surface of the developing embryo undergo a period of extreme elongation for approximately 20 days. These elongated cells attain a length of 25 to 35 mm (Quisenberry and Kohel, 1975). They later enter a phase of secondary cell wall

thickening. At maturity the cells dry, leaving a hollow tube of cellulose which is utilized as fiber. The time required for this process is strongly influenced by temperature (Gipson and Joham, 1968). When about 60% of bolls have opened, a chemical defoliant is applied to the plants to speed development of unopened bolls and prepare the crop for harvest. Plants are defoliated to reduce contaminants in the harvested fiber, and this is performed 10 to 14 days before harvest. Approximately 155 days are required from planting till harvest in Florida (Wright and Brecke, 2002).

Cotton flowers provide many food resources for insects. Pollen provides a source of protein for egg development, resulting in increased fecundity (Trichilo and Leigh, 1988). Nectar is also an important resource for attracting pollinators and predatory insects to cotton plants (Wackers and Bonifay, 2004). In cotton, new flowers are rapidly colonized by thrips. Their role in cotton production and biology has not been investigated.

Flower thrips

Flower thrips, mainly *Frankliniella tritici* (eastern flower thrips), but also *F. occidentalis* (western flower thrips), and *F. bispinosa* (Florida flower thrips) rapidly colonize cotton flowers immediately after opening. More thrips accumulate as the day progresses, by the end of which flowers may contain 10-40 thrips. This occurs in many production areas, although the specific species involved may vary.

Thrips are a frequent problem on cotton seedlings in the US, resulting in distortion of expanding leaves, and small discolored spots. The most common species are *Frankliniella occidentalis* (western flower thrips), *Frankliniella fusca* (tobacco thrips) and *Thrips tabaci* (onion thrips). Thrips control is usually achieved using a granular insecticide at planting. Foliar insecticide applications are used if more than 2 to 3 thrips are present per plant and damage is observed (Sprenkel, 2005). Applications of jasmonic acid to cotton seedlings can reduce thrips

feeding by 80%, although leaf area is also reduced by 28% (Omer et al., 2001). However, this is not used as a management technique. Thrips feeding is generally not a problem when the plants are more mature. However, severe damage was reported in Turkey (Atakan and Ozgur, 2001a) by *Frankliniella intonsa* on mature cotton plants. Although more than 350 thrips were observed per flower, pollination was not adversely affected. Feeding by thrips larvae resulted in boll shedding, although ovipositioning by females in flower parts had a larger impact.

Several studies have examined the tendency of flower thrips to disperse in search of food resources. In British Columbia, *F. occidentalis* dispersal was shown to occur when windspeed was less than 15 km/h, although dispersal was most likely to occur in the absence of wind (Pearsall, 2002). In Turkey, *F. intonsa* was shown to follow a similar pattern. Red flowers were found to contain their highest numbers of thrips at 05:30, after which their numbers fell steadily until 11:30. This dispersal coincides with the opening of new white flowers, which were shown to accumulate between 20 and 200 individuals. The authors also suggested red flowers could serve as a refuge from insecticide applications (Atakan and Ozgur, 2001b).

Several flower-inhabiting thrips species are present in north Florida. In an 18-month study of 37 wild plant species, 78% of thrips were adults, and 87% of adults were from the genus *Frankliniella*. The most common species were *F. tritici*, *F. bispinosa*, *F. occidentalis* and *F. fusca*. The relative contributions of each species fluctuated substantially during the study. Cotton flowers were not examined, but during its bloom period of June, July and August, *F. tritici* and *F. bispinosa* were the most common species on wild hosts (Chellemi et al., 1994). The primary influence on *Frankliniella* populations appeared to be the availability of suitable flowers. *F. occidentalis* populations increased more rapidly than other species, probably due to its wider plant host range. It is non-native, and was not reported in the area until 1981 (Beshear,

1983). This is prior to the period of high yields (and presumably low hardlock) from the early 1980s to 1990s, so its arrival was probably not involved in the increased hardlock experienced since that time.

Thrips numbers are affected by predatory insects and parasites. *Orius insidiosus*, the minute pirate bug, is a predator of flower thrips that can consume 12.5 thrips per day (Tommasini and Nicoli, 1993). If a sufficient number of *Orius* are present in an area, localized extinction of flower thrips may occur. *Orius* will remain in the area, feeding on extrafloral nectaries or pollen and preventing the thrips population from rebounding. This diet is sufficient to allow development and oviposition by *Orius*. Small hair tufts called domatia are present on the underside of leaves of some plants, and are associated with larger numbers of predatory insects. However, cotton does not produce these structures. A parasitic nematode *Thripinema fuscum* was shown to infect *F. fusca* in north Florida. Among *F. fusca* found on peanut, as many as 51 and 67% of females on certain sampling dates were found to be infected, resulting in sterility. *T. fuscum* infection of *F. tritici* and *F. occidentalis* was far less common, occurring in only 2% of individuals (Funderburk et al., 2002; Stavisky et al., 2001; Ramachandran et al., 2001).

Species and sex differences have been observed in thrips behavior. On greenhouse pepper plants, movement of *F. occidentalis* was limited, while *F. tritici* and *F. bispinosa* were found to disperse relatively rapidly. Males of all three species were shown to be more mobile among plants (Ramachandran et al., 2001). Females of *F. occidentalis* have been shown to spend more time feeding and produce more feeding-associated scars on petunias than do males (van de Wetering et al., 1998). These differences in behavior resulted in *F. tritici* and *F. bispinosa* being less susceptible to predation than *F. occidentalis* (Ramachandran et al., 2001.

Thrips species also differ in their response to insecticides. *F. occidentalis* populations have been shown to increase following applications of acephate and esfenvalerate, while spinosad causes a reduction. However, acephate and esfenvalerate were highly toxic to *F. tritici* and *F. bispinosa*, while spinosad was less effective (Reitz et al., 2003). Other studies have shown spinosad to be equally effective against all three species (Eger et al., 1998). The effectiveness of insecticides on thrips numbers can be complicated by its effect on predators. Several insecticides have been evaluated for both lethal and sub-lethal effects on *Orius* which could affect its reproduction. Spinosad was found to have no effect, lethal or sub-lethal, on *Orius* populations (Studebaker and Kring, 2000). Cyhalothrin resulted in high mortality, but no sub-lethal effect was observed in survivors (Studebaker and Kring, 2000). Acephate was not tested, but is a broad-spectrum organophosphate which affects a larger number of arthropods.

The development rate of thrips is dependant on temperature (Lewis, 1973). It can best be modeled by the use of growing degree days (GDD), which is also used for predicting plant maturity (Toapanta, et al., 1996). This method was later confirmed and refined by Toapanta et al. (2001), demonstrating it is essential to use temperature measurements from within the plant canopy (where developing thrips are located) rather than above the plants. The life history of flower thrips differs slightly by species. Development time of *F. tritici* is approximately one day shorter than that of *F. occidentalis* (approximately 8.5 vs. 9.5 days). Oviposition rates and the number of offspring produced during the female lifetime are not statistically different. However, *F. tritici* does produce a larger portion of offspring early in its adult life. Despite these advantages, *F. tritici* does not show a greater rate of population increase in the field (Reitz et al., 2001).

Thrips possess only one mandible which is used for cutting into plant tissue, and a stylet through which food is drawn. This results in open wounds to the plant, which could allow easier penetration of the tissue by pathogens. Females of *F. occidentalis* have been shown to feed more frequently and intensely than males, resulting in more tissue damage (van de Wetering et al., 1999). Pickett et al. (1988) reported 68% of adult *F. occidentalis* found on cotton plants occurred on fruiting structures, with most of these occurring in the flower itself. This is consistent with the concept that cotton pollen may be preferable to leaves as a food source for flower thrips (Agrawal et al., 1999). In tomato, amino acid analysis shows phenylalanine content to be associated with thrips numbers (Brodbeck et al, 2001).

Farrar and Davis (1991) investigated the relationship between *F. occidentalis* and fusarium ear rot of corn. Fusarium ear rot of corn is caused by *Fusarium verticillioides*, and can result in large yield losses in some years in addition to contamination of the crop with mycotoxins. Disease incidence had been connected previously to husk tightness and insect damage. Insecticide applications reduced the numbers of *F. occidentalis* observed and the incidence of disease. They concluded that thrips may be acting as vectors of *F. verticillioides* or promoting infection as wounding agents by feeding on the plant tissue.

Several observations regarding hardlock suggest flower thrips might be involved. First, there has been an increase in hardlock in recent years. Although hardlock rates have not historically been measured, this view is held by some cotton observers and is confirmed by the relatively lower yields experienced from the late 1990s (National Cotton Council of America, 2005). This coincides with reduced spraying of insecticides during the flowering stage, due to the use of genetically modified varieties producing the *Bacillus thuringiensis* endotoxin. Large numbers of flower thrips often occur within the flowers. Relatively higher rates of hardlock are

observed along field margins, and thrips numbers sometimes follow this same pattern. However, hardlock was reported to have been one of the major factors taking cotton out of the Southeast along with boll weevils in the last half of the 20th century (Wright et al., 2004).

Hardlock

Hardlock is characterized as a failure of the cotton fiber to expand outward after boll opening. Instead, it remains in compressed locules, similar to an orange slice, which may have a grey or occasionally faint pink color. These compressed locules are frequently missed or knocked to the ground by mechanical harvesters. The resulting yield loss often ranges from 20 to 60%, depending on the year. Until this dissertation, hardlock had not been formally recognized as a disease, although it could be considered a subset of the boll rot complex. Hardlock is most severe along the gulf coast, possibly due to the region's high temperatures and humidity. It is also associated with rainfall, high nitrogen, plant size and density. Attempts have been made to avoid harvest problems by the use of ultra-narrow row plantings and harvesting using a stripper, but this has not proven feasible due to higher production costs and lower fiber quality (Wright et al., 2004).

Previous research has shown hardlock to be associated with *F. verticillioides* (Marois et al., 2002). Prior to Michailieds and Morgan (1998), *F. verticillioides* was referred to as *F. moniliforme*. *F. verticillioides* commonly occurs in cotton fields, and can be found growing saprophytically on crop residue. It has been isolated from both seeds and peduncles. It can also sometimes be found on mature cotton fiber in the field, yielding the pink coloration described previously. It is hypothesized that *F. verticillioides* infects via the flowers, and colonizes the developing boll. Inoculation of flowers with a spore suspension of *Fusarium* has been shown to result in more hardlock (Marois et al., 2005).

F. verticillioides is capable of surviving for extended periods of time on crop debris. Cotten and Munkvold (1998) soaked maize stalk pieces in spore suspensions *of F. moniliforme*, *F. proliferatum*, and *F. subglutinans*, and left pieces in an Iowa field at the surface and several depths in the soil profile under several crop rotations. The recovery rate for all three species was over 50% during the first 300 days, and was around 10 to 20% after 600 days. Rohrbach and Taniguchi (1984) observed the rate of infection by *F. moniliforme* on pineapple during the flowering stage was best predicted by the number of hours per week the temperature was between 21 and 27°C. They also noted a significant negative correlation at 27 to 32°C and 32 to 38°C. Infections were also associated with rainfall, although a clear correlation was not demonstrated. Subbarao and Michailides (1995) determined the optimal temperature for *F. moniliforme* infection on pollinator figs is 30°C. It also resulted in the shortest incubation and latent periods (approximately 15 and 40 hours, respectively). At 35°C, these were increased to 70 to 90 and 90 to 120 hours. These periods were also increased at temperatures below 30°C, but less drastically.

Palmateer et al. (2004) found *F. moniliforme* to be relatively uncommon among *Fusarium* species isolated from living cotton plant tissue. *F. proliferatum* was also fairly uncommon. In contrast, Baird and Carling (1998) found *Fusarium* species to be present on 22 to 38% of dead cotton roots. Although *F. verticillioides* and *F. proliferatum* were not listed in the 6 most frequently isolated *Fusarium* species, 5 other unlisted species were isolated from 1 to 6% of samples. Bolkan et al. (1979) demonstrated *F. moniliforme* conidia to be short-lived (6 to 13 weeks) in the soil in the absence of host tissues. However, incorporating stem and leaf tissue from pineapple into the soil increased survival to at least 12 months. It should be noted cotton stems, roots, and fiber from the previous year are commonly found in cotton fields.

Other studies have examined *F. moniliforme* spore release under field conditions. Sanders and Snow (1978) found the amount of airborne spores increased for several weeks after first bloom, possibly due to saprophytic growth on shed flowers and other tissue, before finally declining approximately 6 weeks later. That trend was not observed in this study, although taking more samples might have showed it to be the case. It was later confirmed (Snow and Sanders, 1979) that shed flowers, bolls and squares were a suitable substrate to produce *Fusarium* spores. This was observed on 46 to 96% of flowers and 54 to 88% of bolls and squares. Ooka and Kommedahl (1977) observed a similar situation with *F. moniliforme* spores in corn fields. Spore numbers were lowest while the plant was actively growing, and increased as it reached maturity. They also observed wind-blown soil containing *F. moniliforme* spores which was likely to have traveled 300-400 km. However, Fernando et al. (2000) found more airborne spores of *Fusarium graminearum* within 1.5 m of an infected wheat field than at 5 m, suggesting local production of inoculum may be most important. Sanders and Snow (1978) found the release of boll-rotting pathogen spores (including *Fusarium*) to be highest between 18:00 and 06:00. Fernando et al. (2000) found spore release of *F. moniliforme* to be highest in wheat fields between 16:00 and 08:00. The availability of moisture is important for growth. Torres et al. (2003) examined the role of water activity (relative humidity expressed as a decimal, abbreviated a_w) and temperature on germination of *F. verticillioides* on maize kernels. Of the two temperatures (20 and 30°C) and three levels of a_w (0.92, 0.95 and 0.98) evaluated, the combination of 30°C and 0.98 resulted in the most rapid hyphal growth. Growth was substantially slower at lower temperature and levels of a_w.

Fusarium is associated with boll rots in cotton, and the symptoms of hardlock are often lumped with boll rots. Arndt (1950) reported an especially severe year of boll rots in South

Carolina, and a symptom he described as the "tight-lock condition." The prevalence of "tight-lock" ranged from 1 to 90%, with areas closer to the coast having a higher incidence. Bagga (1968) reported *F. monliforme* as the second most isolated boll rot species, occurring on 9 to 13% of samples. Fungal pathogens, including *Fusarium*, can be found within cotton bolls by the first several weeks of development (Roncadori, 1969). When inoculated directly into the pericarp of a developing boll, *F. moniliforme* causes disease on both adjacent carpels as well as the locule (Sparnicht and Roncadori, 1972). The possibility of inoculating flowers with spores of a pathogen to cause boll rot has been demonstrated (Edgerton, 1912). The incidence of boll rot is higher with a closed canopy, due to increased humidity, although this is sometimes alleviated by a lack of rainfall. Reduced nitrogen fertilizer rates sometimes resulted in less boll rot, although the canopy characteristics were not affected (Roncadori et al., 1975).

Boll rot is also influenced by the quantity of airborne inoculum available. At two locations in Louisiana, spore numbers were found to peak approximately 50 days after first bloom, and the first infected bolls were observed at 60 days. The spore concentration was considerably less 10-100 m from the cotton, so it is likely spores were originating in the crop. Spore concentration also varied considerably during the day, with the highest levels between 1800 and 0600 hr. Among boll-rotting fungi, *Fusarium* spores were second only to *Diplodia gossypina* in numbers. No relationship was found linking temperature, humidity or rainfall to spore numbers. It was suggested the increasing levels of spores could have been generated by fungal growth on naturally shed flowers, bolls, squares, and leaves (Sanders and Snow, 1978).

In the case of boll rot caused by *Colletotrichum capsici*, damage to lint can vary from a "tight-locked" condition to severe degradation of fiber (Roberts and Snow, 1984). Susceptibility to boll rot is also influenced by gossypol (a polyphenol in the Malvaceae), production of which

can be increased by mechanical damage of the plant (Bell, 1967). Alternative leaf shape has also been explored as an option for reducing boll rot incidence. Okra-leaf varieties have deeply lobed leaves, similar to that of okra. This results in less boll rot, probably due to a less humid plant canopy. These varieties also produce more flowers during a season, in the range of 150 to 210 per meter of row compared to 100 to 140 in conventional types. A more open plant canopy also improves the efficiency with which pesticides can be applied to the plants. Okra-leaf varieties produce better yields under adverse conditions, but lower yields under optimal conditions compared to conventional varieties. They have not been commercially successful in the US, although they account for approximately 50% of the cotton acreage in Australia (Heitholt and Meredith, 1998).

Marois and Wright (2004) reported a climate model for predicting hardlock incidence. The mean temperature and humidity from 0700 to 1900 on the day of bloom were recorded during 2002, and correlated to hardlock incidence in subsequent bolls originating from the white flowers of that particular day. The model was based on eight separate dates, and yielded a R^2 value of 0.935 and $p < 0.005$.

Since *F. verticillioides* was shown to be associated with hardlock (Marois et al., 2002), fungicide applications have been evaluated as a control measure. Marois and Wright (2004) showed significant reductions in hardlock and increases in yield during the 2002 growing season with applications of thiophanate-methyl. Benomyl applications were made to cotton under three nitrogen fertilizer regimes, but only reduced hardlock at the highest nitrogen rate (201 kg/ha). Applications to blooms instead of bolls were shown to be most effective. During the 2004 season, fungicide applications did not significantly affect hardlock or yield (Marois et al., 2005). However, significant increases in leaf area index (LAI) and decreases in leaf disease were noted.

Although significant yield differences were not noted, a positive correlation with LAI and a negative correlation to leaf disease were demonstrated. Other studies have failed to show reductions in hardlock due to fungicide applications. Seebold et al. (2004) examined the use of fungicide applications in five states in the Southeast during the 2003 season. Fungicides did not reduce hardlock, although it improved seed cotton yield in Georgia. The number of applications was shown to be more important than the rate or timing of those applications. The regional project was expanded to ten states for the 2004 season (Seebold and Kemerait, 2005). Fungicide applications had little impact on hardlock, regardless of the number of applications. No impact on yield was noted, except in Virginia. Yields in Louisiana, Florida and Georgia may have been lower due to inclement weather, which resulted in a complete loss of the Alabama study. In Florida, Georgia and Tennessee, fungicides increased LAI, and in Florida and Georgia this was determined by the number of applications. Marois et al. (2006) did not find improvements to LAI, hardlock, or yield from fungicide applications during the 2005 season in Florida.

Hardlock is a major limiting factor for cotton yields in Florida, and control strategies are not available. It is hypothesized that flower thrips may be involved in the problem, and that reducing their numbers could reduce the severity of hardlock. Studies were performed from 2003 to 2005 to investigate this possibility. The first objective was to quantify the density and diversity of arthropod species in cotton flowers and the effects of insecticides on their populations. The second objective was to determine if there was a relationship between thrips species and hardlock. The third objective was to assess if thrips numbers could be managed to reduced hardlock and improve yield.

CHAPTER 2
THRIPS ON FLOWERS OF COTTON

Introduction

Thrips are small insects, approximately 1mm in length (family Thripidae). They range in color from light tan to dark brown, depending on the species. They possess primitive wings that allow limited flight. Small hairs (setae) occur on some areas of the body, and along with antennae are characteristics useful for identifying the species. Most thrips of concern in cotton production belong to the genus *Frankliniella* (subfamily Thripinae). *Frankliniella fusca* is a pest of cotton seedlings and peanuts. Other species such as *Frankliniella occidentalis*, *Frankliniella tritici*, and *Frankliniella bispinosa* occur more often on mature vegetation or flowers.

Thrips are known vectors of plant viruses. *F. occidentais* (Sakimura, 1962), *F. fusca* (Pappu et al., 1998) and *F. bispinosa* (Avila et al., 2006) are capable of transmitting the tospovirus tomato spotted wilt virus. *F. fusca* has also been shown to transmit *Pantoea ananatis*, the bacterium responsible for center rot of onion (Gitiatis et al., 2003). Thrips can increase the incidence of corn ear rot, caused by *Fusarium verticillioides* (Farrar and Davis, 1991). Thrips can vector the pathogen and, by feeding, establish infection sites. Thrips can overwinter in the soil as larvae. *F. occidentalis* has been shown to survive whole-body freezing at temperatures as low as -16ºC if gradually acclimated (McDonald et al., 1997). In milder climates, adults are active during the entire year, although the maturation rate declines (Toapanta, 2001).

Several flower-inhabiting thrips species are present in north Florida. In an 18-month study of 37 wild plant species, 78% of thrips found were adults, and 87% of these adults were from the genus *Frankliniella* (Chellemi et al., 1994). The most common species were *F. tritici*, *F. bispinosa*, *F. occidentalis* and *F. fusca*. The relative contributions of each species fluctuated substantially during the study. Cotton flowers were not examined, but during its bloom period of

June, July and August, *F. tritici* and *F. bispinosa* were the most common species on wild hosts. The primary influence on *Frankliniella* populations appeared to be the availability of suitable flowers. *F. occidentalis* populations increased more rapidly during the spring than other species, probably due to its wider plant host range. It is non-native, and was not reported in the area until 1981 (Beshear, 1983).

Thrips numbers are affected by predatory insects and parasites. *Orius insidiosus* (minute pirate bug) is a common predator that can consume 12.5 thrips per day (Tommasini and Nicoli, 1993). If a sufficient number of *Orius* are present in an area, localized extinction of flower thrips may occur. *Orius* will remain in the area, feeding on extrafloral nectaries or pollen and preventing the thrips population from rebounding. This diet is sufficient to allow development and oviposition by *Orius*.

A parasitic nematode *Thripinema fuscum* was shown to infect *F. fusca* in north Florida. Among *F. fusca* found on peanut, as many as 51 and 67% of females on certain sampling dates were found to be infected, resulting in sterility. *T. fuscum* infection of *F. tritici* and *F. occidentalis* was far less common, occurring in only 2% of individuals (Funderburk et al., 2002; Stavisky et al., 2001; Ramachandran et al., 2001).

Species and sex differences have been observed in thrips behavior. On greenhouse pepper plants, movement of *F. occidentalis* was limited, while *F. tritici* and *F. bispinosa* were found to disperse relatively rapidly. Males of all three species were also shown to be more mobile among plants (Ramachandran et al., 2001). Females of *F. occidentalis* have been shown to spend more time feeding and produce more feeding-associated scars on petunias than do males (van de Wetering et al., 1998). These differences in behavior resulted in *F. tritici* and *F. bispinosa* being less susceptible to predation by *Orius* than is *F. occidentalis* (Ramachandran et al., 2001.

Thrips species also differ in their response to insecticides. *F. occidentalis* populations have been shown to increase following applications of acephate and esfenvalerate, while spinosad causes a reduction. However, acephate and esfenvalerate are highly toxic to *F. tritici* and *F. bispinosa*, while spinosad is less effective (Reitz et al., 2003). Other studies have shown spinosad to be equally effective against all three species (Eger et al., 1998). Spinosad is a macrolide that contains two active ingredients, spinosyn A and spinosyn D. They are derived from *Saccharopolyspora spinosa*, and result in hyperexcitation of neurons in the central nervous system resulting in eventual paralysis (Salgado, 1998). The effectiveness of insecticides on thrips numbers can be complicated by its effect on predators, which probably explains increases in *F. occidentalis*. Studebaker and Kring (2000) evaluated several insecticides for both lethal and sub-lethal effects on *Orius* which could affect its reproduction. Spinosad was found to have no effect, lethal or sub-lethal, on *Orius* populations. Cyhalothrin resulted in high mortality, but no sub-lethal effect was observed in survivors. Acephate was not tested, but is a broad-spectrum organophosphate which affects a large number of arthropods.

Thrips possess only one mandible which is used for cutting into plant tissue, and a stylet through which food is drawn. This results in open wounds to the plant, which could allow easier penetration of the tissue by pathogens. Females of *F. occidentalis* have been shown to feed more frequently and intensely than males, resulting in more tissue damage (van de Wetering et al., 1999). Pickett et al. (1988) reported 68% of adult *F. occidentalis* found on cotton plants occurred on fruiting structures, with most of these occurring in the flower itself. This is consistent with the concept that cotton pollen may be preferable to leaves as a food source for some thrips species (Agrawal et al., 1999). Pollen provides a source of protein for egg development, resulting in increased fecundity (Trichilo and Leigh, 1988). In tomato, amino acid

analysis showed phenylalanine content to be associated with thrips numbers (Brodbeck et al, 2001). Nectar is also an important resource for attracting pollinators and predatory insects to cotton plants (Wackers and Bonifay, 2004).

Thrips are a frequent problem on cotton seedlings in the US, resulting in distortion of expanding leaves, and small discolored spots. The most common species are *Frankliniella occidentalis* (western flower thrips), *Frankliniella fusca* (tobacco thrips) and *Thrips tabaci* (onion thrips). Thrips control is usually achieved using a granular insecticide at planting. Foliar insecticide applications are used if more than 2 to 3 thrips are present per plant and damage is observed (Sprenkel, 2005). Applications of jasmonic acid to cotton seedlings can reduce thrips feeding by 80%, although leaf area is also reduced by 28% (Omer et al., 2001). However, this is not used as a management technique. Thrips feeding is generally not a problem when the plants are more mature. However, severe damage was reported in Turkey (Atakan and Ozgur, 2001a) by *Frankliniella intonsa* on mature cotton plants. Although more than 350 thrips were observed per flower, pollination was not adversely affected. Feeding by thrips larvae resulted in boll shedding, although ovipositioning by females in flower parts had a larger impact.

In recent years there has been increasing interest in hardlock of cotton. Hardlock is a failure of the cotton fiber to expand outward from the boll after opening, and it instead remains in compact wedges. Affected locules will remain on the plant or be knocked to the ground during harvest. Fiber quality is not usually affected, but yields can be reduced considerably. Hardlock is associated with the fungus *Fusarium verticillioides*, and is believed to infect through the flower on the day of bloom (Marois et al., 2002). Most control strategies have focused on the application of fungicides to flowers and maturing bolls (Marois and Wright, 2004). It has also

been suggested flower thrips could be involved in hardlock. If that is the case, reducing their numbers may limit the severity of hardlock.

Previous studies have examined the thrips species associated with cotton plants. However these have been conducted outside the southeastern US, in drier climates, and the thrips species found differ from those in this area. *Frankliniella intonsa* has been reported as a pest of cotton in Greece (Deligeorgidis et al., 2002), Turkey (Atakan and Ozgur, 2001a). *Frankliniella schultzei* and *F. occidentalis* have been reported as pests of cotton flowers and leaves in Brazil (Monteiro, 2001). Other studies within the Southeast have focused on damage caused to cotton seedlings by thrips feeding. The objectives of this study are to describe the insect species found in cotton flowers and determine how they are affected by insecticide applications.

Materials and Methods

Field Plots

Two field studies were performed, approximately 40 miles apart, at branches of the North Florida Research and Education Center in Quincy and Marianna, Florida. Cultivar DPL 555 Bt/RR was used, and plots were maintained according to the recommendations of the University of Florida extension service unless otherwise noted. Acephate (Orthene) and lambda cyhalothrin (Karate) were used when needed to control *Nezara viridula* (southern green stink bug) and *Euschistus servus* (brown stink bug).

In Quincy, a large fungicide-insecticide study was utilized to evaluate thrips, hardlock, and yield for 2 years. It was a randomized complete block design, with 4 blocks. There were 28 treatments in 2004 and 10 treatments in 2005. Plots were 4 rows (0.9 m between rows) by 9 m long. Control and insecticide-treated plots (with or without fungicide, depending on the year) were sampled for thrips. Other treatments were varied rates and timings of fungicide applications, and were not sampled. In 2004, the insecticide treatment consisted of weekly

applications of 0.10 kg a.i. (active ingredient)/ha of spinosad (Tracer) on Mondays and 0.56kg/ha acephate + 0.04 kg a.i./ha lambda cyhalothrin (Warrior) on Thursdays. In 2005, 0.02 kg a.i./ha Karate (lambda cyhalothrin) was substituted for Warrior, and 0.9 kg/ha of thiophanate-methyl (Topsin M) was applied every 2 weeks.

The Marianna study examined the effects of insecticides and fungicides on thrips, hardlock and yield for 3 years. The site was part of a *Paspalum notatum* (bahiagrass) rotation, and the cotton was planted after peanuts each year. The plots were eight rows in width, with 0.9 m between rows, and 18 m in length. Rows were oriented north to south, and at each end a 6 m wide section of peanuts was planted. Peanuts support large numbers of *F. fusca*, and are often planted in proximity to cotton. It was suspected they could influence the species ratio found in cotton flowers. A randomized complete block design was used with 4 blocks and 4 treatments. The experiment included unsprayed control plots and three other treatments which were applied during the bloom period. The insecticide treatment consisted of spinosad at 0.07 kg a.i./ha alternated weekly with acephate at 0.9 kg a.i./ha. The fungicide treatment consisted of thiophanate-methyl at 1.1 kg a.i./ha applied weekly. A fourth treatment included a weekly application of both the insecticide and fungicide sprays listed above.

Sampling of Thrips

Cotton flowers are white on the first day they open, but by evening the fringes of the petals are often pink. On the second day, the petals have changed to a solid dark pink or red color. In this study, white, first day, flowers were collected and placed into individual 60 ml vials containing 70% ethanol, 30% deionized water. The flower was placed into the vial with the peduncle located at the opening. This allowed the flower contents to fall into the bottom of the vial. Flowers were sampled on a weekly basis from the two outer rows of each plot, between 11:00 and 13:00. In the Quincy study, 12 flowers were sampled from each of 4 control and 4

insecticide plots. During 2004, the interval between insecticide applications and sampling varied. In 2005, insecticide-only plots were not available, so an insecticide+fungicide treatment was substituted. The interval between treatment sprayings and sampling time was held constant at 2 days. In the Marianna study, 16 flowers were sampled from each of the 16 plots. The interval between insecticide applications and sampling varied between 2 and 6 days. In both locations, treatments were applied on the first week of bloom, and sampling began later that week. Sampling was discontinued in late August, since blooms after that time would not result in harvestable bolls. In 2004, a hurricane prevented sampling on the 5th week, and by the following week the plants had stopped flowering.

Identification of Thrips

Vials containing thrips were kept at room temperature until the sample was evaluated. During 2003 and 2004, the liquid from the sample was poured into a Petri dish and the flower was then immersed in the dish to dislodge any thrips present. The sample was then examined, and the insects recorded. In 2005, the flower was removed and insects were counted while still in the vial. Flowers were periodically dissected to ensure thrips were not remaining within the flowers. Thrips were recorded to species based on overall color, and antennae pigmentation and ornamentation, while sex was determined by presence or absence of the ovipositor, and abdomen width and curvature. Other commonly found or easily identified insects were classified to order or genus. Insects that were rarely observed were not recorded. The data were analyzed using the SAS GLM procedure, and means were separated using Tukey's Studentized Range Test.

Multi-State Species Survey

To determine if the species found in Quincy and Marianna were typical of the Southeast in general, or specific to north Florida, samples were taken in other states. Collaborators collected 50-80 flowers from Louisiana, Alabama and South Carolina and sent them to Quincy, FL for

identification. The Louisiana samples were collected at the Macon Ridge Research Station in Winnsboro, LA. The Alabama samples were collected at the Auburn University Gulf Coast Research Station located in Fairhope, AL. The South Carolina samples were collected at the Edisto Research and Education Center in Blackville, SC. Flowers were sampled for 3 years at each location.

Thrips Accumulation in Flowers

In 2004, white flowers were sampled at 2-hour intervals from 10:00 to 16:00 or 10:00 to 18:00 in Quincy, Florida. In 2005, this was repeated at two other sites within approximately 300 meters of the 2004 site. At each time interval, 15 flowers were collected from each site. Samples were processed as described previously. Sampling was discontinued on several days due to rainwater from thunderstorms remaining in flowers.

Results

Prevalent Species

Flower thrips species identified from Quincy and Marianna were consistent at both sites and across all years of the study (Tables 2.1 and 2.2). *F. tritici* (eastern flower thrips) was the most common species, and flowers contained an average of 1.4 to 4.2 individuals. Females outnumbered males, typically by a 2:1 to 5:1 ratio. *F. occidentalis* (western flower thrips) was rarely found in 2003 and 2004 (2 to 3 thrips per 1000 cotton flowers), and was not present in 2005. Although slightly more common (3 to 10 thrips per 1000 cotton flowers), the same pattern of diminishing prevalence was observed in *F. bispinosa* (Florida flower thrips). These three species are all flower feeders, and likely competitors for resources. *F. fusca* was observed at low levels (3 to 16 per 1000 flowers) in all years. It is a foliar-feeding species, and could have been found in the flowers as a result of dispersion in search of suitable host plants. Immature thrips were also present, and their numbers ranged from 0.05 to 0.10 per flower.

Orius species are predators of thrips, but can subsist on nectar when prey is unavailable. Observed ratios of *Orius* to thrips ranged from 1:35 to 1:220 depending on location and year (Tables 2.1 and 2.2). Previous research in field pepper (Funderburk et al. 2000) has shown thrips suppression to occur at 1:200, and several days after reaching 1:40 localized near-extinction occurred. Although low ratios were often observed, in cotton they were not sufficient to cause further reductions in thrips numbers.

Aphis sp. (aphids) were observed at levels of 1.2 to 5.3 per flower. They are known for secreting sticky honeydew, and if bolls are open this can result in "sticky cotton". This problem was not observed in the plots, and is distinct from hardlock. Members of the order Formicidae (ants) were also observed, with as many as 2.9 per 10 flowers. They were highly aggregated, and flowers containing ants typically contained 5 or more. Although they were not classified further, at least two species appeared to be present. In 2005, members of order Forficulidae (earwigs) were first recorded. They had been observed but not recorded previously due to their extremely low numbers. Beetles from order Staphilinidae were also first observed in high numbers (4 to 56 per hundred flowers) in 2005.

Similarities Across the Southeast

Samples from Louisiana, Alabama, and South Carolina were also examined to see if they were similar to those in Florida (Tables 2.3, 2.4, and 2.5). In all locations, *F. tritici* was most common. Their numbers ranged from 0.42 to 14.8 per flower. Louisiana in 2003 contained the highest number of *F. occidentalis* recorded (0.31 per flower), but they still constituted a minority of flower thrips present. One individual each of both *F. bispinosa* and *T. palmi* were found. The presence of *F. bispinosa* was unexpected since they are most common to peninsular Florida. The sex ratios varied considerably, with the highest being 1 male per 48 females. In a third of the location/years, males were more common than females. The large range of thrips numbers and

sex ratios observed may have been influenced by crop management practices. The number of immature thrips also varied considerably, from 0.01 to 0.96 per flower. *Orius* was generally uncommon, except in two instances where 0.15 and 0.21 per flower were found.

Impact of Insecticide Treatments on Flower-Inhabiting Insects

The influence of insecticide treatments on flower-inhabiting insects was examined (Tables 2.6 and 2.7). Insecticide applications, whether alone or in combination with fungicide, reduced thrips numbers. The reduction in thrips numbers varied depending on location and year. Thrips were reduced by 84 and 92% in Quincy, and 32, 20 and 36% in Marianna. Both males and females were affected, but males experienced larger percentage declines. Fungicide applications did not influence thrips numbers. Immature thrips were reduced by insecticides by 94 and 100% in Quincy. The number of immature thrips was not significantly reduced in Marianna. The number of male thrips per female was usually between 0.1 and 0.7 (Table 2.8). In Quincy the proportion of males was reduced in both years, by about 50%. In Marianna, insecticide applications significantly reduced the proportion of males in 2003, but not in 2004 or 2005. However, combining the data from all three years showed an overall significant reduction from 0.5 per female in the control to 0.4 in the insecticide-treated plots. The proportion of males varied considerably during the season (Fig. 2.1). *Orius* was adversely affected by insecticides in Quincy (Table 2.6). Adults were reduced by 83% during one year, and immatures by 80 and 100% in both years. The number of *Orius* observed was very low in all treatments, and considering the sample size, this result should be viewed with caution. Marianna, insecticides were less harmful to *Orius* (Table 2.7). Adults were not affected, while the number of immatures in the insecticide plot was reduced by 66% compared to the control during one of the three years. *Aphis sp*. were not consistently affected by the treatments. There was also little to

no impact on representatives from the order Formicidae (ants). The insecticide treatments appeared to reduce thrips numbers while having a small impact on other flower inhabitants.

Changes in Thrips Numbers During the Growing Season

The number of thrips observed varied considerably during the growing season (Fig. 2.3). At later sampling dates in Marianna in 2003 and 2005, thrips numbers were approximately 55 and 170% higher than earlier in the seasons and were associated with a declining number of white flowers available in the field. This may have caused crowding in the remaining flowers. The overall seasonal trend was similar in both of these years, although 2005 had fewer thrips. In 2004, flowering stopped unusually early, preventing continued sampling. This was preceded by a drastic decline in thrips numbers, from approximately 4.5 to 0.1 per flower. In Quincy in 2004, the season began with an unusually rapid increase in thrips numbers, followed by an abrupt decline. The control and insecticide plots declined by 70 and 80% from their mid-season peaks. In 2005, treatment differences were apparent. On the first sampling date, the control plots contained 13 thrips per flower, but declined to 4 by the next sampling date and declined for the remainder of the season. The insecticide-treated plots showed less variation between days, but thrips numbers were generally lower during the second half of the season.

Association of Thrips and *Orius*

Orius populations also fluctuated during the seasons (Fig. 2.2). In Marianna, control and insecticide plots generally showed similar fluctuations during the growing season for each year. In 2005, there was a rapid increase in the number of *Orius* present during the last two sampling dates. On the last sampling date of that year, *Orius* numbers were approximately 400% higher than before their increase (0.40 vs. 0.05 per flower). When the fluctuations in thrips thrips numbers (Fig. 2.3) are compared to *Orius* (Fig. 2.2), it is difficult to discern a clear relationship.

To determine if thrips and *Orius* were associated, the number of flowers containing either thrips or *Orius*, both species, or neither was calculated. The predicted distribution was determined (Table 2.9), and the significance of their association was calculated using a Chi-square test (Table 2.10). The number of flowers containing both thrips and *Orius* was higher than predicted, and this was significant ($p<0.05$) in 3 of 5 location-years. Three indexes of species association were used: Ochiai, Dice, and Jaccard. Values can range from 0 (not associated) to 1 (always associated), and those computed for this study were between 0.06 and 0.37. The covariation of thrips and *Orius* was evaluated by calculating their correlation on a per flower and a per plot*day basis for each location year (Table 2.11). On a per flower basis, the correlations were fairly low (≤ 0.20). By comparing the mean number of thrips and *Orius* for each plot and day, correlations as high as 0.50 were obtained. It appears that thrips and *Orius* are associated, but not strongly in cotton flowers. In a predatory relationship, a positive association suggests *Orius* fluctuates in response to thrips numbers. In contrast, a negative association would result if *Orius* resulted in a localized depression in thrips numbers. If both these scenarios occur, then the observed relationship would appear weak. There were several instances that may show the *Orius* population being influenced by thrips numbers. In the 2004 Marianna control plots (section A of Fig. 2.2 and 2.3), the decline in *Orius* numbers was less drastic than in thrips at the end of the season, suggesting they were depressing thrips numbers. In the 2003 Marianna insecticide plots (section B of Fig. 2.2 and 2.3) thrips reached their lowest numbers on the second week, then rebounded for the remainder of the season. This same pattern occurred in the *Orius* population, except it was delayed by one week. In the 2004 Quincy insecticide plots, a seasonal peak in thrips numbers occurred one week before the seasonal peak for *Orius*. It appears each species is capable of influencing the numbers of the other at certain times.

Thrips Accumulation in Flowers

Cotton flower buds are very tightly closed, and occasional dissections did not reveal any thrips within them prior to flower opening. The flower opens at approximately 09:00, and thrips begin arriving almost immediately (Fig. 2.4). On most days in 2005, there were 2 or fewer thrips found in flowers at the first sampling time of 10:00. At site 2 (2006), flowers at the 10:00 sampling time typically had 4 thrips per flower. Site 3 (2006) had slightly more, with 2 to 8 per flower. Their numbers usually increased until 14:00. At 14:00, flowers typically contained 4 to 14 (Site 1, 2005), 10 to 15 (Site 2, 2006), and 14 to 25 (Site 3, 2006) thrips. After 14:00, thrips numbers were generally as likely to decline as to increase further, but tended to remain in a similar range to the numbers at 14:00. When sampling days are compared to each other, there are obvious differences in the initial number of thrips, rate of increase, and time of maximum numbers. At sites 2 and 3 on 7/28/2006, thrips numbers at 10:00 were far higher than on any other day sampled that year. This was associated with lower humidity than on other days. There is considerable variation in thrips numbers between days, and it was hypothesized this was due to weather conditions. Temperature, relative humidity, solar radiation, wind speed and rainfall were compared to the number of thrips present. Correlations were performed between thrips numbers at 10:00 and temperature and relative humidity for each hour from 16:00 of the day prior to the time of sampling. Means were computed for temperature and relative humidity for the most highly correlated time periods. These means were then correlated to thrips numbers (Table 12.2). At sites 2 and 3, relative humidity from 19:00 on the day before sampling to 10:00 of the next day was strongly correlated to the number of thrips present in flowers at 10:00 (-0.87 and -0.85, respectively, $p<0.08$). Combining data from sites 2 and 3 resulted in a slightly lower, but highly significant correlation (-0.81, $p=0.0046$). Temperature from 8:00 to 10:00 was also correlated to thrips numbers in sites 2 and 3 (0.54 and 0.69, respectively, $p<0.35$). Combining

both days improved the relationship (0.60, p=0.07). Relative humidity and temperature were not correlated to thrips numbers at site 1. Sampling on those days was distributed over a 2-week period, while sites 2 and 3 were sampled on consecutive days. It is possible variations in the total thrips population during the course of sampling at site 1 obscured the role of weather. The sampling dates also showed narrower ranges of temperature (27.6º to 29.4ºC) and relative humidity (76.0 to 83.3%) in 2005 than in 2006 (26.6º to 30.5ºC and 66.3 to 84.7%). The narrower weather variable ranges in 2005 may have been insufficient to influence thrips numbers.

Regressions were performed comparing the weather variables described previously to thrips numbers (Table 2.13). Adjusted R^2 values were high for relative humidity and low for temperature. Using both variables to predict thrips numbers was less useful than relative humidity alone. Relative humidity appears to be the best predictor of thrips numbers at 10:00.

Solar radiation was not predictive of thrips numbers, possibly due to its limited ability to penetrate the plant canopy in early morning. Wind speeds were relatively low (0 to 11 km/h) on most days, and did not predict thrips numbers. Thrips numbers after 10:00 were not influenced by the weather variables examined. After 10:00, the best predictor of thrips numbers was the number observed at the previous sampling time. Rainfall also occurred during the dates sampled (Table 2.14). On one occasion (7/29/2006) rainfall slightly reduced thrips numbers, although this was only temporary. It is likely some thrips left the flowers, while others were sheltered between overlapping cotton petals. Overlapping cotton petals are also a favored position during normal weather conditions, and may harbor 40 to 80% of the thrips present in a flower.

Discussion

Cotton flowers contain valuable resources for insects, and regardless of what control measures are attempted, some organism is likely to utilize them. Flower inhabitants interact

through competition and predation, and their populations can fluctuate for unknown reasons. Several observations from these studies warrant further comment.

Based on its prevalence on vegetable crops and wild hosts prior to 2003, *F. occidentalis* was predicted to be a common species in cotton flowers. Instead, it was rare in 2003 and not found in 2005. This decline in *F. occidentalis* numbers was also observed on other crops. It may be that the ratio of flower thrips species found in cotton flowers is closer to an approximation of the species found in the surrounding area rather than being a unique ecological niche. If that is the case, fluctuations may occur in the ratio of thrips species. This could influence predators which feed on thrips. Some studies have suggested *Orius* feeds preferentially on *F. bispinosa* and *F. occidentalis* relative to *F. tritici.* This is probably due to the increased activity levels noted in *F. tritici*, which requires increased effort for predators (Reitz et al., 2001). Under certain conditions, predation may influence the species ratio. As time progresses, the most common species may face greater pressures as predator and parasite populations adjust themselves to better exploit it. It is possible this may prevent any one species from comprising a majority indefinitely. Having mostly one thrips species in a field does simplify management. Reitz et al. (2003) determined effects of insecticides varied according to species in pepper production. They found spinosad was effective against *F. occidentalis*, but not *F. tritici*. In contrast, esfenvalerate and acephate reduced populations of *F. tritici* and *F. bispinosa*, but resulted in higher populations of *F. occidentalis*. This suggests the spinosad component of the insecticide applications in this study may not have been as important. However, the high proportion of *F. tritici* in thrips populations occurred in multiple locations in the Southeast, and was present in plots not sprayed with spinosad. It is unlikely the use of spinosad biased the species ratio in this study.

Insecticide applications generally resulted in fewer males compared to females in the plots, and this could be relevant from a management perspective. Thrips are haplodiploid, with males being haploid and females being diploid. Sexual reproduction between male and female thrips results in exclusively female offspring, since sex is determined by ploidy level. Females also reproduce parthenogenically, which results in exclusively male offspring. Decreasing the number of males available for breeding might increase the likelihood of parthenogenic reproduction, resulting in fewer female offspring being produced. The reason for a reduction in males in the insecticide plots is unclear. Males tend to disperse more readily than females, suggesting they would re-colonize the insecticide plots faster than females. This is the opposite of what was observed. Research on tomato has shown males are more likely to occur in the upper portion of the plant canopy (Reitz et al., 2002). This increases their exposure to insecticide applications compared to females, which are more evenly distributed throughout the canopy. Greater exposure to spraying probably explains the lower proportion of males in insecticide-treated plots.

Alternating applications of spinosad and acephate in Marianna did not appear to harm *Orius* populations. However, *Orius* is highly mobile and may have re-colonized plots after treatment. It should be noted that spinosad is among the least harmful insecticides to *Orius* (Ramachandran, 2001; Reitz et al., 2001). Insecticide applications did reduce *Orius* numbers in Quincy, and this may be a result of using lambda cyhalothrin instead of acephate. In pepper production, *Orius* has been demonstrated to cause localized near-extinctions of thrips (Reitz et al., 2003). That did not occur in this study, possibly due to either different thrips species or host plant characteristics. *F. tritici* is more mobile, and more likely to evade *Orius* than is *F. occidentalis* (Reitz et al., 2001). Also, the near-extinctions observed in pepper do not occur in

tomato production and it is believed characteristics of the plant itself are involved. However, a positive association between thrips and *Orius* was observed.

In this study, the mean number of thrips declined each year in all treatments. In 2004, this was primarily due to a sharp decline in thrips numbers in the fourth week of bloom. The reason for that decline is unclear, but it preceded the unusually early end of flowering. Nitrogen concentration in plant tissue influences both thrips feeding and flowering. It is possible declining nitrogen availability within the plant led to reduced phenylalanine content, causing thrips to emigrate in search of better hosts (Brodbeck et al., 2001). Low nitrogen availability might also have caused premature termination of flowering. This seems unlikely considering the abruptness with which this sequence occurred. Another possibility is planting date. Due to weather and technical problems, planting dates were slightly later each year, with the onset of flowering ranging from June 20 in 2003 to about July 5 in 2005. Thrips populations are at their peak in the spring, but decline rapidly in May (Chellemi et. al, 1994). It is possible delayed planting of cotton resulted in more of a gap in food availability between the wild spring-hosts and the cotton flowers, reducing thrips numbers. It is also possible that various parasites and predators more suited to *F. tritici* have increased since the time of its presumed replacement of *F. occidentalis* in early 2003. Parasitic nematodes from the genus *Thipinema* are common worldwide. *T. fuscum* infects 40-80% of *F. fusca* females in north Florida, but fewer than 2% of *F. occidentalis* and *F. tritici* (Funderburk et al., 2002; Stavisky et al., 2001; Ramachandran et al., 2001). It likely there are other such parasites which more commonly affect *F. tritici*.

The observed increase in thrips numbers per flower during the day is consistant with previous studies. In Turkey, *F. intonsa* was shown to follow a similar pattern. Red flowers (day after bloom) were found to contain their highest numbers of thrips at 05:30, after which their

numbers fell steadily until 11:30. This dispersal coincides with the opening of new white flowers, which were shown to accumulate between 20 and 200 individuals. The authors also suggested red flowers could serve as a refuge from insecticide applications (Atakan and Ozgur, 2001b). In British Columbia, it was determined *F. occidentalis* dispersal could occur if windspeed was less than 15 km/h, although it was most common in the absence of wind (Pearsall, 2002). Wind suppressing thrips movement was not observed in this study, probably because wind speed in this study did not exceed 16 km/h, and was typically below 8 km/h. The association of lower temperature and relative humidity prior to 10:00 with higher thrips numbers was unexpected. A prior study (Toapanta, 2001) has shown thrips generation lengths to be determined by growing degree days, with warmer temperatures resulting in faster maturity. Higher morning temperatures in this study decreased thrips movement, perhaps because the temperatures encountered were already above the optimum. The lower humidity might result in less dew on the plants, allowing thrips to move more easily within the plant canopies.

Thrips control in cotton flowers had not been attempted previously, and its potential effectiveness was unknown. Insecticide applications reduced total thrips numbers by approximately 20 to 90% depending on the year and location. This study demonstrates insecticide applications are an effective strategy for reducing thrips numbers in cotton flowers. This reduction in thrips numbers was associated with reduction in hardlock severity, and is described in Ch.3.

Table 2.1. Mean number of inhabitants per flower across all treatments in Quincy, FL.

Thrips			2004		2005	
	Juvenile		0.10 ± 0.03		0.06 ± 0.02	
	Adult		Male	Female	Male	Female
		Frankliniella tritici	1.10 ± 0.12	2.41 ± 0.18	0.39 ± 0.06	1.88 ± 0.17
		F. bispinosa	<0.01 ± 0.00	<0.01 ± 0.00	0	0
		F. fusca	0	<0.01 ± 0.00	0	0.01 ± 0.00
		F. occidentalis	<0.01 ± 0.00	0	0	0
		Thrips palmi	0	0	0	0
Other insects observed						
	Juvenile	*Orius sp.*	0.03 ± 0.01		0.01 ± 0.01	
	Adult	*Orius sp.*	0.05 ± 0.01		0.03 ± 0.01	
		Aphis sp.	3.84 ± 0.36		5.32 ± 0.32	
		Formicidae	0.25 ± 0.06		0.08 ± 0.03	
		Forficulidae	---		0	
		Staphilinidae	---		0.04 ± 0.01	

Table 2.2. Mean number of inhabitants per flower across all treatments in Marianna, FL.

Thrips			2003		2004		2005	
	Juvenile		0.13 ± 0.02		0.05 ± 0.01		0.02 ± 0.00	
	Adult		Male	Female	Male	Female	Male	Female
		Frankliniella tritici	1.16 ± 0.08	3.21 ± 0.08	0.68 ± 0.04	2.10 ± 0.08	0.40 ± 0.03	1.04 ± 0.04
		F. bispinosa	0.01 ± 0.00	0.07 ± 0.00	<0.01 ± 0.00	0.01 ± 0.00	0	0
		F. fusca	0	0.02 ± 0.00	0	0.01 ± 0.00	0	0.01 ± 0.00
		F. occidentalis	<0.01 ± 0.00	<0.01 ± 0.00	<0.01 ± 0.00	0	0	0
		Thrips palmi	<0.01 ± 0.00	<0.01 ± 0.00	0	0	0	0
Other insects observed								
	Juvenile	*Orius sp.*	0.06 ± 0.00		0.04 ± 0.00		0.04 ± 0.00	
	Adult	*Orius sp.*	0.10 ± 0.00		0.05 ± 0.00		0.15 ± 0.00	
		Aphis sp.	1.20 ± 0.09		1.83 ± 0.16		5.48 ± 0.37	
		Formicidae	0.10 ± 0.02		0.29 ± 0.03		0.25 ± 0.03	
		Forficulidae	---		---		<0.01 ± 0.00	
		Staphilinidae	---		---		0.56 ± 0.03	

Table 2.3. Mean number of inhabitants per flower in Winnsboro, LA.

Thrips			2003		2004		2005	
	Juvenile		0.08 ± 0.03		0.13 ± 0.07		0.01 ± 0.00	
	Adult		Male	Female	Male	Female	Male	Female
		Frankliniella tritici	1.30 ± 0.27	1.51 ± 0.24	0.83 ± 0.15	4.30 ± 0.53	0.01 ± 0.01	0.41 ± 0.15
		F. bispinosa	0	0	0	0	0	0
		F. fusca	0	0	0	0	0	0
		F. occidentalis	0.12 ± 0.06	0.19 ± 0.05	0	0	0	0
		Thrips palmi	0	0.01 ± 0.01	0	0	0	0
Other insects observed								
	Juvenile	*Orius sp.*	0		0.02 ± 0.02		0	
	Adult	*Orius sp.*	0.01 ± 0.01		0		0	
		Aphis sp.	3.02 ± 0.65		18.06 ± 2.61		0.04 ± 0.02	
		Formicidae	0		0		2.34 ± 0.45	
		Forficulidae	---		---		0.13 ± 0.05	
		Staphilinidae	---		---		0.51 ± 0.22	

Table 2.4. Mean number of inhabitants per flower in Fairhope, AL.

Thrips			2003		2004		2005	
	Juvenile		0.96 ± 0.26		0.29 ± 0.11		0.62 ± 0.12	
	Adult		Male	Female	Male	Female	Male	Female
		Frankliniella tritici	2.96 ± 0.36	1.52 ± 0.21	6.32 ± 0.78	8.50 ± 1.01	1.88 ± 0.25	1.31 ± 0.14
		F. bispinosa	0	0.02 ± 0.02	0	0	0	0
		F. fusca	0	0	0	0	0	0
		F. occidentalis	0.02 ± 0.02	0	0	0	0	0
		Thrips palmi	0	0	0	0	0	0
Other insects observed								
	Juvenile	*Orius sp.*	0.14 ± 0.05		0.07 ± 0.03		0.09 ± 0.03	
	Adult	*Orius sp.*	0		0.21 ± 0.07		0.03 ± 0.02	
		Aphis sp.	0.40 ± 0.23		0.13 ± 0.07		0.43 ± 0.12	
		Formicidae	0.20 ± 0.07		0.09 ± 0.05		0.01 ± 0.01	
		Forficulidae	---		---		0	
		Staphilinidae	---		---		0	

Table 2.5. Mean number of inhabitants per flower in Blackville, SC.

Thrips			2003		2004		2005	
	Juvenile		0.14 ± 0.06		0.02 ± 0.02		0.18 ± 0.06	
	Adult		Male	Female	Male	Female	Male	Female
		Frankliniella tritici	3.00 ± 0.45	1.35 ± 0.20	0.18 ± 0.06	0.98 ± 0.16	1.28 ± 0.23	2.73 ± 0.28
		F. bispinosa	0	0	0	0	0	0
		F. fusca	0	0.01 ± 0.01	0	0	0	0
		F. occidentalis	0	0	0	0	0	0
		Thrips palmi	0	0	0	0	0	0
Other insects observed								
	Juvenile	*Orius sp.*	0		0		0.07 ± 0.04	
	Adult	*Orius sp.*	0		0.07 ± 0.04		0.15 ± 0.04	
		Aphis sp.	0.83 ± 0.29		0.54 ± 0.16		14.66 ± 2.62	
		Formicidae	0.18 ± 0.01		0.20 ± 0.09		0.03 ± 0.02	
		Forficulidae	---		---		0	
		Staphilinidae	---		---		0.01 ± 0.01	

Table 2.6. Mean numbers of flower inhabitants by treatment in Quincy, FL.

			F. tritici			*Orius sp.*			
year	treatment	N	male	female	immature thrips	immature	adult	*Aphis sp.*	*Formicidae*
2004	C	188	2.05 a	4.03 a	0.19 a	0.05 a	0.06 a	3.43 a	0.51 a
2005	C	288	0.73 b	3.43 a	0.11 a	0.03 a	0.06 a	3.78 a	0.13 b
2004	C	188	2.05 a	4.03 a	0.19 a	0.05 a	0.06 a	3.43 a	0.51 a
2004	I	190	0.15 b	0.81 b	0.01 b	0.01 b	0.04 a	4.25 a	0.00 b
2005	C	288	0.73 a	3.43 a	0.11 a	0.03 a	0.06 a	3.78 b	0.13 a
2005	IF	280	0.04 b	0.28 b	0.00 b	0.00 b	0.01 b	6.91 a	0.03 a

Numbers within a column between horizontal lines followed by the same letter are not significantly different according to Tukey's Studentized Range Test Range Test ($p \leq 0.05$). Treatment "C" is a control, "I" is a weekly application of lambda cyhalothrin and spinosad insecticides (separate days), and "IF" is a combination of the fungicide and insecticide treatments.

Table 2.7. Mean number of inhabitants per flower by treatment in Marianna, FL.

			F. tritici			*Orius sp.*			
year	treatment	N	male	female	Immature thrips	immature	adult	*Aphis sp.*	*Formicidae*
2003	all	1052	1.16 a	2.72 a	0.13 a	0.04 a	0.11 b	1.41 b	0.09 b
2004	all	1023	0.68 b	2.10 b	0.05 b	0.04 a	0.05 c	1.83 b	0.29 a
2005	all	1190	0.40 c	1.04 c	0.02 b	0.04 a	0.15 a	5.48 a	0.25 a
all	C	838	0.92 a	2.21 a	0.06 a	0.05 a	0.10 a	2.73 a	0.25 a
all	F	803	0.86 a	2.05 a	0.09 a	0.04 a	0.13 a	3.21 a	0.25 a
all	IF	792	0.57 b	1.72 b	0.04 a	0.03 a	0.10 a	3.32 a	0.16 a
all	I	832	0.56 b	1.68 b	0.06 a	0.03 a	0.09 a	2.85 a	0.20 a
2003	C	278	1.54 a	3.06 a	0.10 a	0.04 a	0.10 a	0.85 c	0.15 a
	F	247	1.35 a	2.90 a b	0.17 a	0.04 a	0.15 a	1.57 a b	0.08 a
	IF	244	0.94 b	2.62 a b	0.08 a	0.03 a	0.09 a	2.03 a	0.05 a
	I	283	0.82 b	2.33 b	0.15 a	0.03 a	0.08 a	1.28 c b	0.10 a
2004	C	256	0.77 a	2.47 a	0.06 a b	0.03 a	0.04 a	1.68 a	0.34 a
	F	255	0.72 a	2.20 a b	0.09 a	0.03 a	0.08 a	1.59 a	0.20 a
	IF	256	0.56 a	1.81 b	0.02 b	0.04 a	0.04 a	1.79 a	0.28 a
	I	256	0.66 a	1.91 b	0.02 b	0.05 a	0.04 a	2.25 a	0.34 a
2005	C	304	0.48 a	1.20 a	0.03 a	0.06 a	0.14 a	5.34 a	0.25 a
	F	301	0.58 a	1.21 a	0.01 a	0.04 a b	0.15 a	5.93 a	0.44 a
	IF	292	0.28 b	0.88 b	0.02 a	0.02 b	0.15 a	5.74 a	0.15 a
	I	293	0.24 b	0.84 b	0.01 a	0.02 b	0.15 a	4.88 a	0.17 a

Numbers within a column between horizontal lines followed by the same letter are not significantly different according to Tukey's Studentized Range Test ($p \leq 0.05$). Treatment "C" is control, "F" is thiophanate-methyl fungicide, "I" is a weekly application of acephate or spinosad insecticides, and "IF" is a combination of the fungicide and insecticide treatments.

Table 2.8. Number of males per female by treatment for each year of sampling.

	Quincy, FL	
Treatment	2004	2005
C	0.52 a	0.21 a
IF		0.10 b
I	0.25 b	
P-value	0.0178	0.1574

	Marianna, FL			
Treatment	2003	2004	2005	all years
C	0.63 a	0.29 a	0.45 a b	0.48 a
F	0.57 ab	0.31 a	0.50 a	0.47 a
IF	0.40 b	0.35 a	0.33 a b	0.36 b
I	0.40 b	0.39 a	0.27 b	0.36 b
P-value	0.0295	0.4766	0.0402	0.0155

Numbers within a column between horizontal lines followed by the same letter are not significantly different according to Tukey's Studentized Range Test ($p \leq 0.05$). Treatment "C" is control, "F" is thiophanate-methyl fungicide, "I" is a weekly application of acephate or spinosad insecticides, and "IF" is a combination of the fungicide and insecticide treatments.

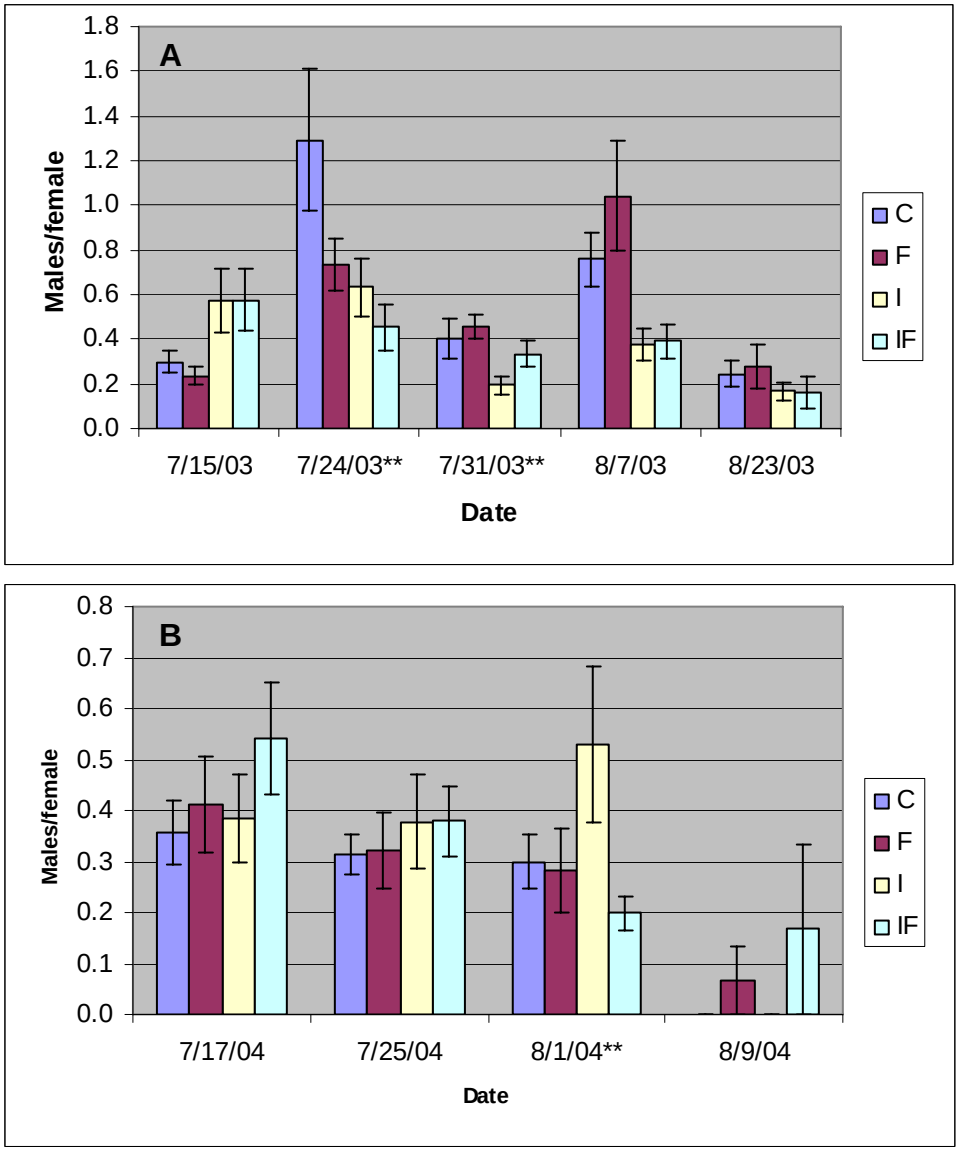
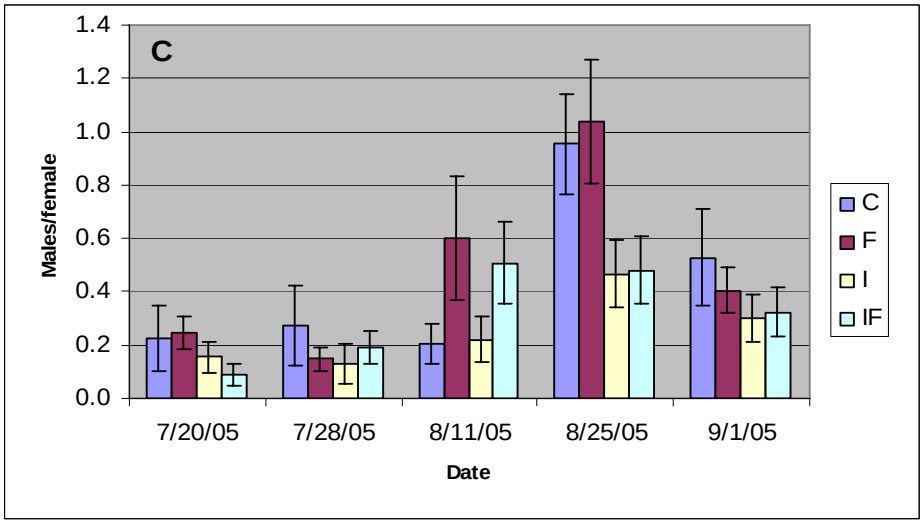
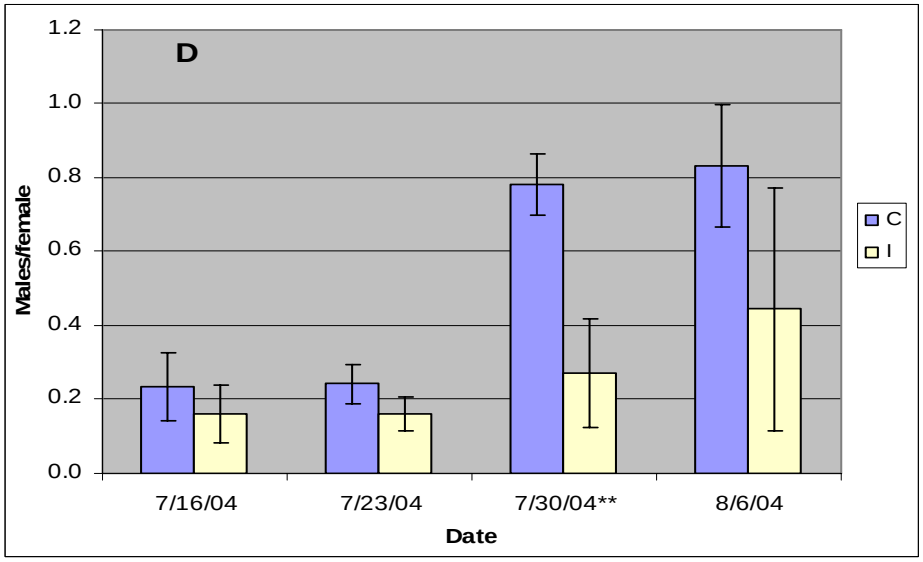


Figure 2.1. Number of males per female by treatment on each day of sampling. **A**, Marianna, 2003. **B**, Marianna, 2004. **C**, Marianna, 2005. **D**, Quincy, 2004. **E**, Quincy, 2005. Treatment "C" is control, "F" is thiophanate-methyl fungicide, "I" is a weekly application of acephate or spinosad insecticides, and "IF" is a combination of the fungicide and insecticide treatments. A double asterisk (**) indicates the control and insecticide treatments differed significantly ($p < 0.05$) according to Duncan's multiple range test for that specific date.





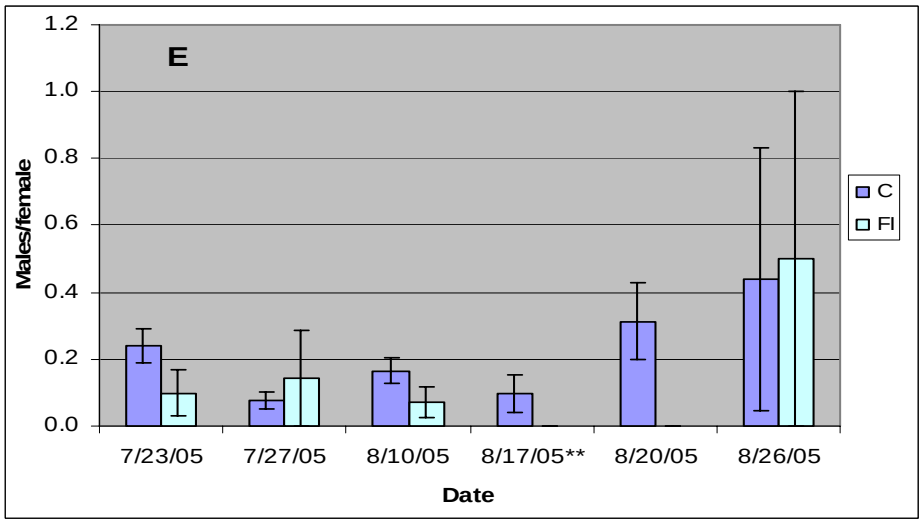


Figure 2.1. Continued

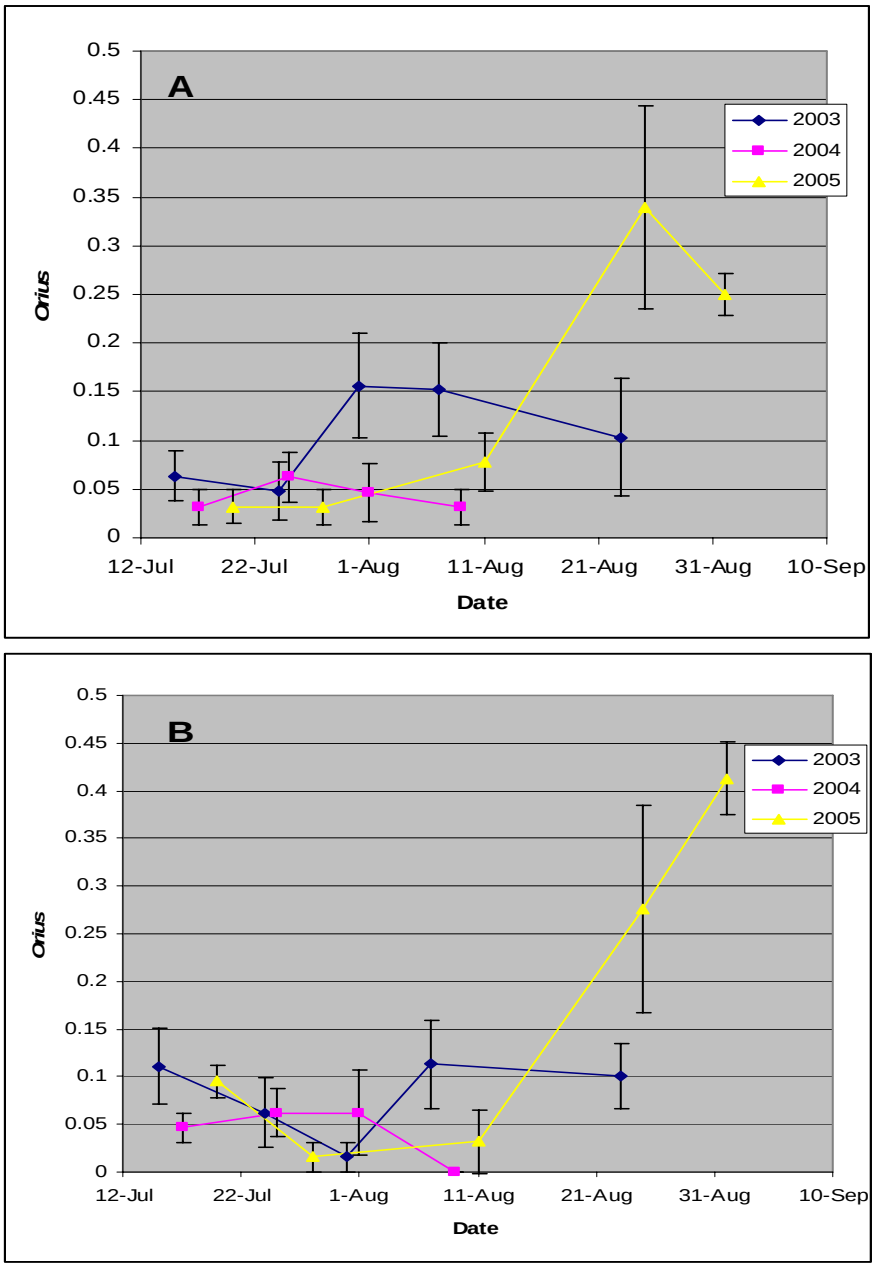
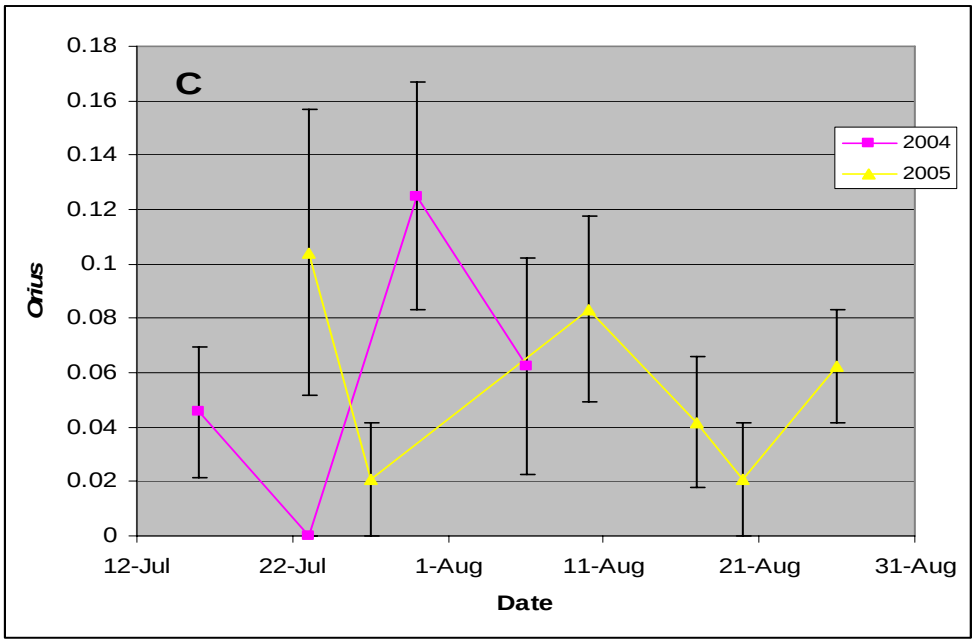


Figure 2.2. Variation in adult *Orius* (minute pirate bug) numbers over time. **A**, Marianna, FL unsprayed control. **B**, Marianna FL insecticide treatment. **C**, Quincy, FL unsprayed control. **D**, insecticide treatment.



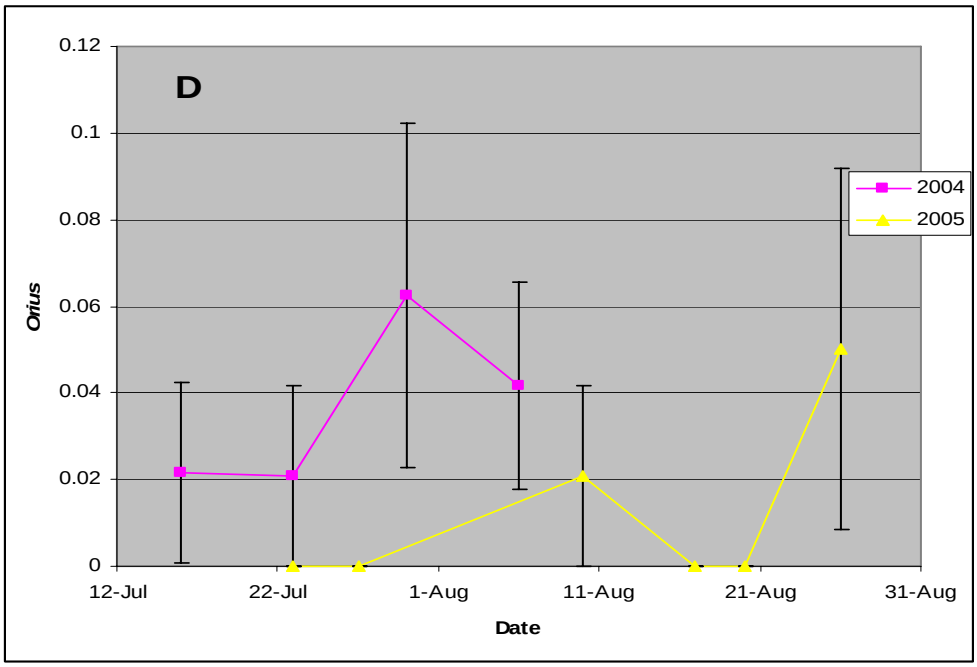


Figure 2.2. Continued

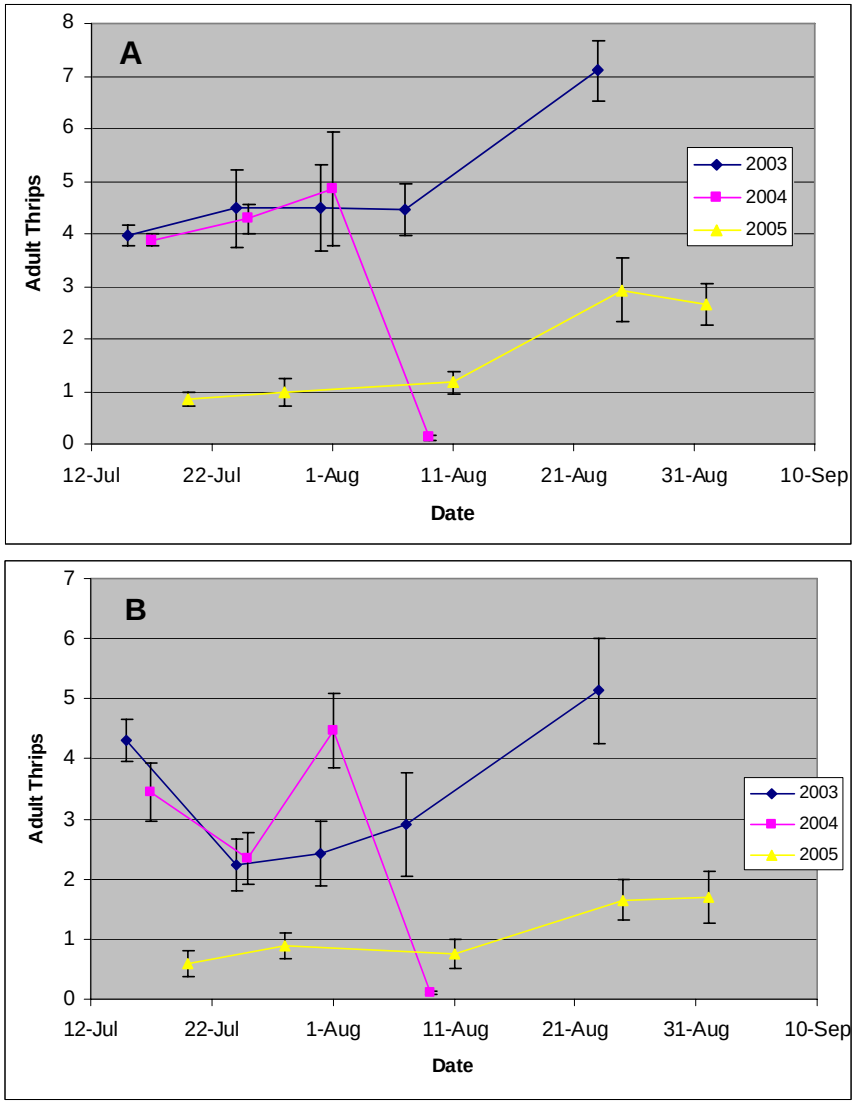


Figure 2.3. Variation in thrips numbers over time. **A**, Marianna, unsprayed control. **B**, Marianna, insecticide treatment. **C**, Quincy, unsprayed control. **D**, Quincy, insecticide treatment.

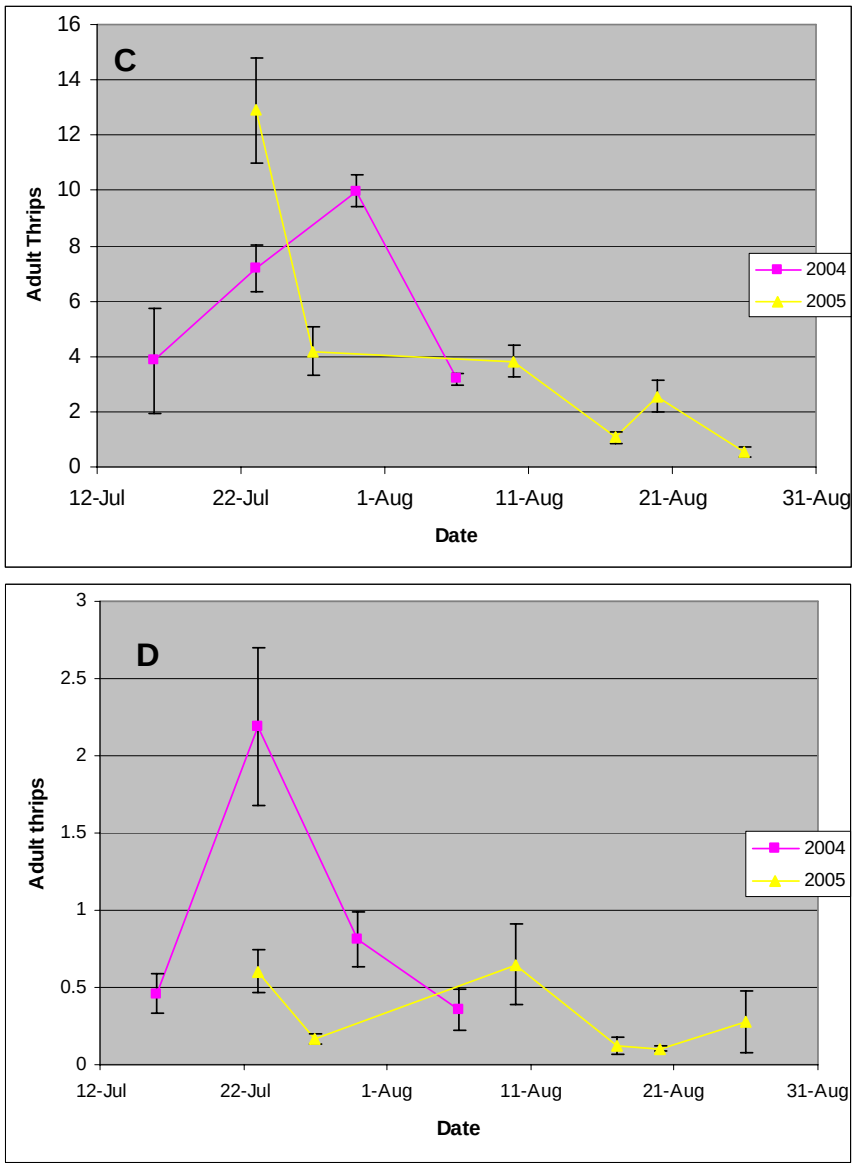


Figure 2.3. Continued

Table 2.9. Occurance of adult thrips and *Orius* in flowers sampled.

		thrips + *Orius*	thrips alone	*Orius* alone	neither present	N
Quincy 2004	actual	14	225	4	135	378
	predicted	11	228	7	132	378
Quincy 2005	actual	15	247	4	302	568
	predicted	9	253	10	296	568
Marianna 2003	actual	89	831	7	125	1052
	predicted	84	836	12	120	1052
Marianna 2004	actual	41	670	8	304	1023
	predicted	34	677	15	297	1023
Marianna 2005	actual	118	561	31	480	1190
	predicted	85	594	64	447	1190

Table 2.10. Interspecific association of thrips and *Orius*.

	association	p	Ochiai	Dice	Jaccard
Quincy 2004	positive	<0.25	0.21	0.11	0.06
Quincy 2005	positive	<0.005	0.21	0.11	0.06
Marianna 2003	positive	<0.25	0.30	0.18	0.10
Marianna 2004	positive	<0.05	0.22	0.11	0.06
Marianna 2005	positive	<0.005	0.37	0.29	0.17

Table 2.11. Interspecific covariance of thrips and *Orius*.

	correlation	p	N	grouping
Quincy 2004	0.0446	0.3876	378	
Quincy 2005	0.2011	<0.0001	568	per flower
Quincy	0.1341	<0.0001	946	
Marianna 2003	0.0651	0.0347	1052	
Marianna 2004	0.0932	0.0029	1023	
Marianna 2005	0.1855	<0.0001	1190	
Marianna	0.0754	<0.0001	3265	
Quincy 2004	0.1905	0.2965	32	
Quincy 2005	0.4568	0.0011	48	per day*plot
Quincy	0.3512	0.0014	80	
Marianna 2003	0.1785	0.0263	155	
Marianna 2004	0.1936	0.0285	128	
Marianna 2005	0.5067	<0.0001	160	
Marianna	0.1291	0.0065	443	

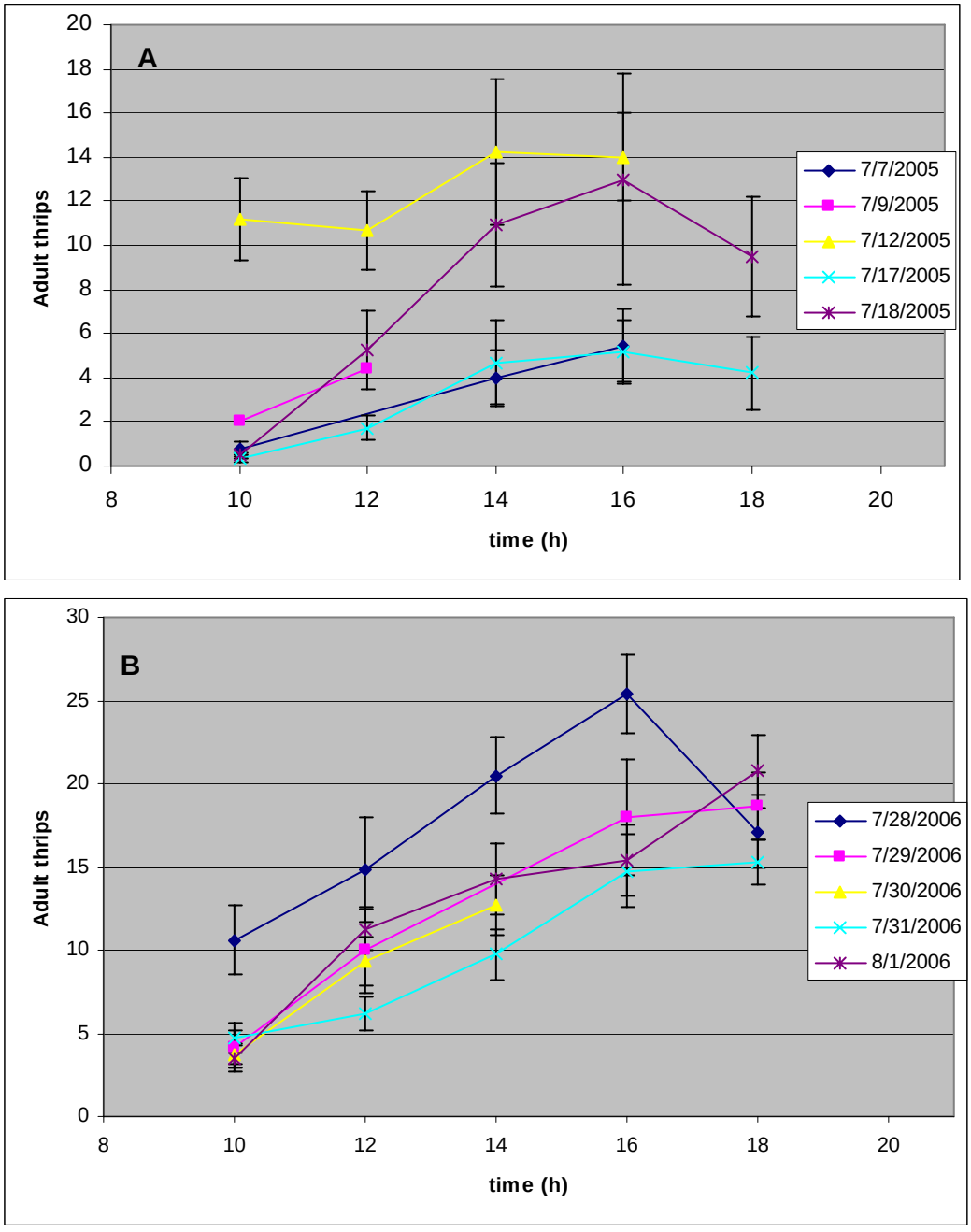


Figure 2.4. Increase in thrips numbers during first day of bloom in Quincy, FL. **A**, Site 1, 2005. **B**, Site 2, 2006. **C**, Site 3, 2006.

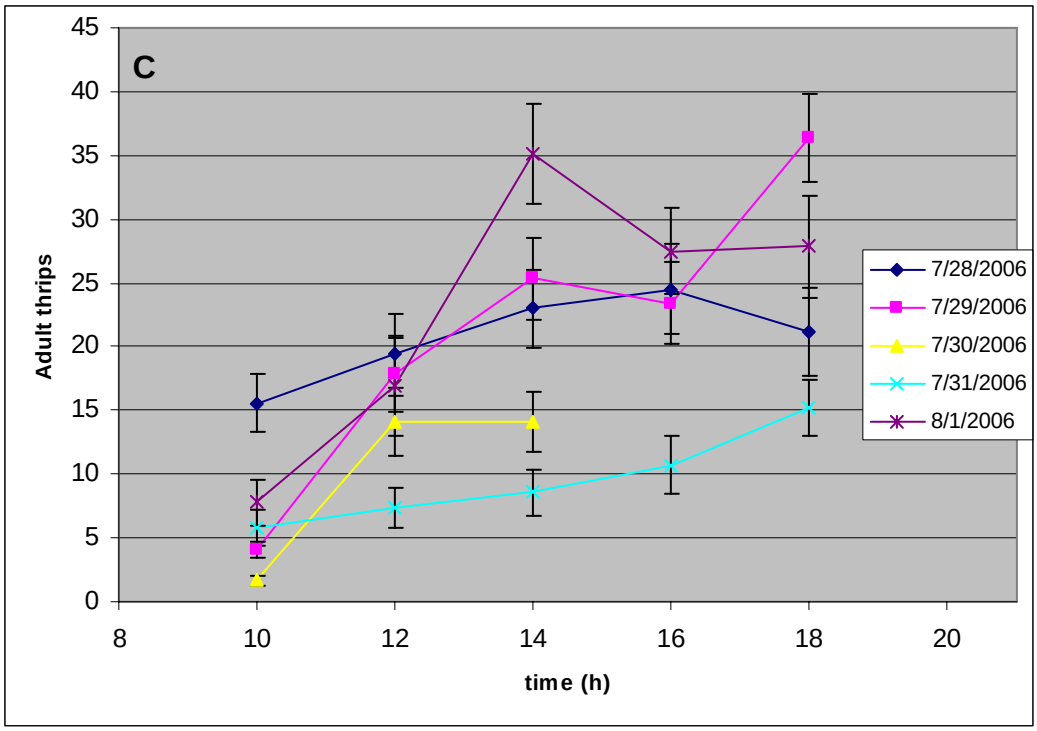


Figure 2.4. Continued

Table 2.12. Correlation of mean weather conditions to thrips numbers at 1000.

			Temperature 0800 to 1000		RH 1900 to 1000	
Site	Year	N	Corr	p	Corr	p
1	2005	5	-0.4146	0.4877	0.1221	0.8449
2	2006	5	0.5437	0.3436	-0.8727	0.0535
3	2006	5	0.6906	0.1967	-0.8523	0.0666
2+3		10	0.6001	0.0667	-0.8089	0.0046
1+2+3		15	0.4211	0.1180	-0.6049	0.0169

Table 2.13. Relationship of mean temperature from 0800 to 1000 and relative humidity from 1900 to 1000 to thrips numbers at 1000

Site	N	Equation	R^2	adj.-R^2	p
1	5	thrips = 82.93826 -T*2.88495	0.1719	-0.1041	0.4877
2	5	thrips = -26.52871 +T*1.12867	0.2956	0.0608	0.3436
3	5	thrips = -64.85198 +T*2.54632	0.4769	0.3026	0.1967
2+3	10	thrips = -45.69035 +T*1.83749	0.3601	0.2801	0.0667
1+2+3	15	thrips = -39.65781 +T*1.59549	0.1773	0.114	0.118
1	5	thrips = -8.83217 +RH*0.12930	0.0149	-0.3134	0.8449
2	5	thrips = 35.52886 -RH*0.34261	0.7616	0.6821	0.0535
3	5	thrips = 59.40033 -RH*0.59429	0.7264	0.6351	0.0666
2+3	10	thrips = 47.46459 -RH*0.46845	0.6543	0.6111	0.0046
1+2+3	15	thrips = 42.51782 -RH*0.41942	0.366	0.3172	0.0169
1	5	thrips = 74.90710 -T*3.71156 +RH*0.33889	0.2602	-0.4795	0.7398
2	5	thrips = 69.29711 -T*0.81540 -RH*0.46463	0.8192	0.6385	0.1808
3	5	thrips = 52.78204 +T*0.15981 -RH*0.57037	0.7271	0.4541	0.2729
2+3	10	thrips = 61.03958 -T*0.32780 -RH*0.51750	0.6586	0.5611	0.0232
1+2+3	15	thrips = 38.22897 +T*0.11002 -RH*0.40594	0.3664	0.2608	0.0647

Table 2.14. Rainfall during the sampling times shown in Figure 2.4.

date	hour	rain (cm)
7/9/2005	13	1.27
7/9/2005	18	0.71
7/29/2006	13	0.20
7/29/2006	18	1.40
7/30/2006	14	0.15
7/30/2006	15	0.74
7/30/2006	16	0.15

CHAPTER 3
RELATIONSHIP OF FLOWER THRIPS TO FUSARIUM HARDLOCK

Introduction

Hardlock is expressed as a failure of the locule of fiber to expand outward after boll opening. Instead, it remains in compressed locules, which may have a faint pink or orange color. These compressed locules are frequently missed or knocked to the ground by mechanical harvesters. The resulting yield loss often ranges from 20 to 60%, depending on the year. It has not been formally recognized as a disease, although it could be considered a subset of the boll rot complex. Hardlock is most severe along the gulf coast, possibly due to the region's high temperatures and humidity. It is also associated with rainfall, high nitrogen, plant size and density. Attempts have been made to avoid harvest problems by the use of ultra-narrow row plantings and harvesting using a stripper, but this has not proven feasible due to higher production costs and lower fiber quality (Wright et al., 2004).

Previous research has shown hardlock to be associated with *F. verticillioides* (Marois et al., 2002). Prior to Michailieds and Morgan (1998), *F. verticillioides* was referred to as *F. moniliforme*. *F. verticillioides* commonly occurs in cotton fields, and can be found growing saprophytically on crop residue. It has been isolated from both seeds and peduncles. It can also sometimes be found on mature cotton fiber in the field, yielding the coloration described previously. It is hypothesized that *F. verticillioides* infects via the flowers, and colonizes the developing boll. Inoculation of flowers with a spore suspension of *Fusarium* has been shown to result in more hardlock (Marois et al., 2005).

Fusarium is strongly associated with boll rots in cotton, and the symptoms of hardlock are often lumped with boll rots. Arndt (1950) reported an especially severe year of boll rots in South Carolina, and a symptom he described as the "tight-lock condition". The prevalence of

"tight-lock" ranged from 1 to 90%, with areas closer to the coast having a higher incidence. Bagga (1968) reported *F. monliforme* as the second most isolated boll rot species, occurring on 9 to 13% of samples. Fungal pathogens, including *Fusarium*, can be found within cotton bolls by the first several weeks of development (Roncadori, 1969). When inoculated directly into the pericarp of a developing boll, *F. moniliforme* causes disease on both adjacent carpels as well as the locule (Sparnicht and Roncadori, 1972). The possibility of inoculating flowers with spores of a pathogen to cause boll rot has been demonstrated (Edgerton, 1912). The incidence of boll rot is higher with a closed canopy, due to increased humidity, although this is sometimes alleviated by a lack of rainfall. Reduced nitrogen fertilizer rates sometimes resulted in less boll rot, although the canopy characteristics were not affected (Roncadori et al., 1975). Boll rot is also influenced by the quantity of airborne inoculum available. At two locations in Louisiana, spore numbers were found to peak approximately 50 days after first bloom, and the first infected bolls were observed at 60 days. The spore concentration was considerably less 10-100 m from the cotton, so it is likely spores were originating in the crop. Spore concentration also varied considerably during the day, with the highest levels between 1800 and 0600 hr. Among boll-rotting fungi, *Fusarium* spp. spores were second only to *Diplodia gossypina* in numbers. No relationship was found linking temperature, humidity or rainfall to spore numbers. It was suggested the increasing levels of spores could have been generated by fungal growth on naturally shed flowers, bolls, squares, and leaves (Sanders and Snow, 1978). In the case of boll rot caused by *Colletotrichum capsici*, damage to lint can vary from a "tight-locked" condition to severe degradation of fiber (Roberts and Snow, 1984). Susceptibility to boll rot is also influenced by gossypol (a polyphenol in the Malvaceae), production of which can be increased by mechanical damage of the plant (Bell, 1967).

Cotton flowers usually open between 09:00 and 09:30, and are rapidly colonized by flower thrips. In north Florida, *Frankliniella tritici* (eastern flower thrips) are most common, but *F. occidentalis* (western flower thrips) and *F. bispinosa* (Florida flower thrips) are also present (Mailhot, 2006). Their numbers increase as the day progresses, and flowers eventually contain 10 to 40 thrips. Predatory insects such as *Orius insidiosus* (minute pirate bug) can reduce thrips numbers (Ramachandran et al., 2001), but can sometimes be harmed by insecticide applications used to control thrips (Studebaker and Kring, 2000). Acephate is highly toxic to *F. tritici* (Reitz et al., 2003). Spinosad is less toxic to *F. tritici* but non-toxic to *O. insidiosus* (Studebaker and Kring, 2000).

Thrips possess only one mandible which is used for cutting into plant tissue, and a stylet through which food is drawn. This results in open wounds to the plant, which could allow easier penetration of the tissue by *Fusarium* spores. Females of *F. occidentalis* have been shown to feed more frequently and intensely than males, resulting in more tissue damage (van de Wetering et al., 1999). Farrar and Davis (1991) investigated the relationship between *F. occidentalis*, and Fusarium ear rot of corn. They concluded that thrips may be acting as vectors of *F. verticillioides* or as wounding agents by feeding on the plant tissue. Pickett et al. (1988) reported 68% of adult *F. occidentalis* found on cotton plants occurred on fruiting structures, with most of these occurring in the flower itself. This is consistent with the concept that cotton pollen may be preferable to leaves as a food source for thrips (Agrawal et al., 1999).

In order to demonstrate the association of thrips with Fusarium hardlock, a series of studies were performed. The objectives were to determine the prevalence of thrips exposed to *Fusarium* in the field, whether artificially-exposed thrips would cause hardlock in a controlled setting, and if field applications of insecticide would reduce hardlock.

Materials and Methods

Isolation of Fusarium from Thrips

White cotton flowers were collected from a crop rotation study in Quincy, FL. In 2004, cultivar DPL 458 BG/RR was used, producing the *Bacillus thuringiensis* endotoxin and providing resistance to glyphosate herbicide. In 2005, DPL 555 BG/RR was used, providing the same benefits. The plants were maintained according to recommendations of the University of Florida extension service, and treated with insecticides when necessary. Fungicides were not applied during the growing season. Flowers were collected from border rows of plots between 10:00 and 11:00. Each flower was placed into a separate plastic bag, and sealed shut to contain any thrips which were present. The sample bags were then refrigerated for approximately three hours to reduce thrips mobility. All thrips from the particular bag were then placed onto Petri dishes of one-quarter strength acidified potato dextrose agar. The dish was then covered and sealed with parafilm to prevent any thrips from escaping. The thrips were permitted to move around the dish, allowing any spores on them to be spread across the plate. In 2005 the procedure was modified. Thrips were collected from flowers in the field using an aspirator, placed directly onto APDA Petri dishes, and sealed with parafilm. The thrips survived for approximately three days, after which time fungal colonies were counted, and suspected *Fusarium* colonies were marked. *Cladosporium* and *Trichoderma* were also recorded due to their frequency and distinctive appearances. After seven to ten days, suspected *Fusarium* colonies were re-examined to verify the original count. Between 20 and 100 flowers were sampled on each date, depending on the number of thrips present and weather conditions. The data were analyzed using the SAS GLM procedure, and means were separated using Duncan's multiple range test.

Influence of Thrips and Fusarium on Hardlock Incidence in Greenhouse

Cotton was planted and maintained in a greenhouse at the North Florida Research and Education Center in Quincy, FL. The temperature varied between 25° and 45°C and the plants were watered as necessary. A potting mix including peat moss and composted bark was used. Each cotton seedling was treated with 0.17 ml of Admire (imidacloprid) to prevent insect infestations. Approximately 5 g of water-soluble 15-30-15 fertilizer was applied to each plant early in the experiment. Pix (mepiquat chloride) was applied at a rate of 0.01 ml/plant 1 or 2 times, as needed to reduce plant height. Excessive height and boll weight required some plants to be attached to bamboo stakes to remain upright. Shortly before flowering, plants were divided into four groups: a control, thrips-only, thrips exposed to *Fusarium*, and *Fusarium*-inoculated. The thrips-only treatment consisted of 10 thrips being placed into each open flower. *Fusarium verticillioides* cultures (2, 5, and 6) isolated from infected bolls were transferred to ¼ strength acidified potato dextrose agar. These isolates have been shown previously to result in hardlock (Marois and Wright, 2004) if inoculated into flowers. One transfer from each was used to produce a mixed quantity of inoculum to better approximate that found in the field. Later work analyzing an internal transcribed spacer (ITS) region of the genome established that isolates 5 and 6 are actually *F. proliferatum* (Leite, et al., 2006). After 10 to 14 days, spores were rinsed from the surface using deionized water. A suspension of 90,000-330,000 spores per ml was created, and with refrigeration remained viable for 3 days. Thrips were exposed to inoculum by placing them on a plate of *F. verticillioides* for approximately one hour, and then 10 of these thrips were transferred into each available flower. Unlike flowers produced in a field setting, the petals in the greenhouse expanded further apart, yielding a more open flower. Treatments were spatially separated to prevent thrips from moving between treatments. Flowers were tagged with ribbons to indicate the treatment and date. After all bolls on a plant were open, they were

evaluated for hardlock. Locules displaying the characteristic failure of fiber to expand were deemed to be affected. The numbers of affected and total locules for each boll were recorded.

The first study was conducted from January to May of 2003, using variety DPL 555 BG/RR. Flowering occurred from March to April, and 15 plants were used in each of 4 treatments. *Frankliniella occidentalis* was raised in cages, using green beans and pollen as a food source. *Frankliniella occidentalis* frequently occurred prior to 2003, and was suspected to be a common species in cotton flowers. In the *Fusarium* inoculation treatment, approximately 5 ml of spore suspension was sprayed into each open flower. In the second study, flowering occurred from April-May 2005. The *Fusarium* inoculation procedure was modified by using a syringe to place approximately 1 ml of inoculum (same concentration as before) onto the stigma. Exposure to water results in lysing of cotton pollen, reducing the chance of successful pollination (Burke, 2002). This modification reduced the flower abortion rate and was maintained in subsequent studies. However, insufficient thrips were produced, resulting in too few treated bolls to evaluate the thrips treatments. The third study, flowering from July to August, substituted wild-captured *F. tritici* to more effectively model field conditions. Thrips were captured from the same plot as the *Fusarium*-isolation study, and sometimes kept in captivity for several days using tomatillo (*Physalis ixocarpa*) fruit as a food source. A second cotton variety, DPL 444 BG/RR, was added and 8 plants per variety were used in each of the 4 treatments. The DPL 444 plants required 1-2 extra applications of mepiquat chloride to keep them similar in height to DPL 555. DPL 444 also continued flowering for 4-5 weeks longer than DPL 555. The third study was replicated from October to November. The first and second studies were analyzed individually using the SAS GLM procedure, and means were separated using Duncan's multiple range test. The third study consisted of two replications separated by time.

Effect of Fungicides and Insecticides on Hardlock

Two field studies were performed, approximately 40 miles apart, at branches of the North Florida Research and Education Center in Quincy and Marianna, Florida. These were described in chapter 2. Variety DPL 555 Bt/RR was used, and plots were maintained according to the recommendations of the University of Florida extension service unless otherwise noted. Orthene (acephate) and Karate (lambda cyhalothrin) were used when needed to control the southern green stink bug (*Nezara viridula*) and the brown stink bug (*Euschistus servus*).

In Quincy, a large fungicide-insecticide study was utilized to evaluate thrips, hardlock, and yield for 2 years. It was a randomized complete block design, with 4 blocks. There were 28 treatments in 2004 and 10 treatments in 2005. Plots were 4 rows (0.9 m between rows) by 9 m long. Control and insecticide-treated plots (with or without fungicide, depending on the year) were sampled for thrips. Other treatments were varied rates and timings of fungicide applications, and were not sampled. In 2004, the insecticide treatment consisted of weekly applications of 0.10 kg a.i. (active ingredient)/ha of spinosad (Tracer) on Mondays and 0.56kg/ha acephate + 0.04 kg a.i./ha lambda cyhalothrin (Warrior) on Thursdays. In 2005, 0.02 kg a.i./ha Karate (lambda cyhalothrin) was substituted for Warrior, and 0.9 kg/ha of thiophanate-methyl (Topsin M) was applied every 2 weeks. In each of the 8 plots, 12 flowers were collected weekly, and stored for identification. Hardlock severity was assessed approximately 2 weeks after defoliation. Five plants were selected at random, and the number of hardlocked and total locules per boll was recorded for each plot. The two center rows of each plot were harvested with a spindle plot picker. The data were analyzed using the SAS GLM procedure, and means were separated using Duncan's multiple range test (SAS, 2003).

The Marianna study examined the effects of insecticides and fungicides on thrips, hardlock and yield for 3 years. The site was part of a *Paspalum notatum* (bahiagrass) rotation, and the

cotton was planted after peanuts each year. The plots were eight rows in width, with 0.9 m between rows, and 18 m in length. Rows were oriented north to south, and at each end a 6 m wide section of peanuts was planted. Peanuts support large numbers of *F. fusca*, and are often planted in proximity to cotton. It was suspected they could influence the species ratio found in cotton flowers. A randomized complete block design was used with 4 blocks and 4 treatments. The experiment included unsprayed control plots and three other treatments which were applied during the bloom period. The insecticide treatment consisted of spinosad at 0.07 kg a.i./ha alternated weekly with acephate at 0.9 kg a.i./ha. The fungicide treatment consisted of thiophanate-methyl at 1.1 kg a.i./ha applied weekly. A fourth treatment included a weekly application of both the insecticide and fungicide sprays listed above. Thrips were sampled from the two outer rows of each plot, while yield data was obtained from two of the inner rows. Flowers were sampled weekly, 8 from each plot, and stored until thrips could be counted. The data were analyzed using the SAS GLM procedure, and means were separated using Duncan's multiple range test.

Results

Isolation of Fusarium from Thrips

Between 7 and 13 percent of flowers contained thrips that were carrying *Fusarium* (Table 3.1). No significant differences were observed by year. The number of *Fusarium* isolations also varied by day, with several days contributing most of the isolations for the season. High inoculum days were randomly scattered throughout the season. Weather conditions were compared, but no similarities between high inoculum days were identified.

Influence of Thrips and Fusarium on Hardlock Incidence in Greenhouse

In 2003, the addition of thrips (*F. occidentalis*) previously exposed to *F. verticillioides* resulted in the most hardlock (Table 3.2). The *Fusarium*-inoculation treatment was also

significantly higher than the control. Thrips in the absence of *Fusarium* did not differ significantly from the control group. In the second study, the *Fusarium*-inoculated and control groups only differed at the p=0.10 level of significance.

In the third study, the results differed slightly by variety, although similar results were obtained in both replications. In DPL 555, the same pattern was observed as in the first study, despite using *F. tritici* instead of *F. occidentalis*. *Fusarium*-exposed thrips resulted in the highest levels of hardlock. *Fusarium*-inoculation and thrips each increased hardlock compared to the control treatment. In DPL 444, *Fusarium*-inoculation and thrips each resulted in significantly more hardlock than the control group, although *Fusarium*-exposed thrips did not differ significantly from the control.

In many cases, fewer bolls successfully reached maturity when they had been treated with *Fusarium*-carrying thrips. This was primarily due to more bolls aborting in their first 2 weeks of development, although flowers also aborted slightly more often. On several days during the experiments, flowers were not treated and the resulting bolls were not included in the experiment. However, the bolls which formed from those untreated flowers rarely aborted. If hardlock symptoms were a result of shortages in photosynthates, the reduction in boll numbers might have alleviated some of the problem. Alternatively, the aborted bolls could represent a more severe or advanced category of infection, although this is unlikely since *F. verticillioides* could not be re-isolated from aborted bolls.

Effect of Fungicides and Insecticides on Thrips, Hardlock, and Yield

In Quincy, the use of insecticide, both alone and with a fungicide, significantly reduced thrips numbers (Table 3.3) by 84% in 2005 and 92% in 2005. In 2005, spraying reduced hardlock from 32% to 18%, but was not significant in 2004. Yield was not significantly affected in either year.

In Marianna, insecticide provided significant reductions in thrips numbers for all years (Table 3.4), while fungicide predictably had no impact. The reduction was more modest than experienced in Quincy, ranging from 21 to 36%. Insecticide significantly reduced hardlock in all years, ranging from approximately 30 to 50%. Fungicide applications were significant in some years, but not all. Combining applications of insecticide and fungicide did not provide additional reductions in hardlock in any year. It should also be noted that the greater percentage reduction in thrips due to spraying in 2005 compared to previous years coincided with a greater reduction in hardlock. Yields were low in 2003, and spraying was not beneficial. Yields were higher in 2004, but only the combined sprays resulted in a significant improvement. In 2005, yields were higher than previous years, and insecticide applications provided significant increases in yield.

The relationship between thrips numbers and hardlock incidence in Marianna, FL is illustrated in Fig. 3.1. The mean number of thrips found within flowers of a plot during the entire season is compared to the hardlock incidence for that same plot. Clear patterns can be distinguished for 2003 and 2005, with R^2 values of 0.19 and 0.34. In 2004, removing the outlier with the highest hardlock incidence only increases the R^2 value to 0.06, so other factors may have proved more important in explaining hardlock incidence. When treatments are examined individually with all three years included (Fig. 3.2), the relationships are more clear. R^2 values range from 0.29 to 0.73. Removing one outlier each from the control and insecticide-only plots increased R^2 values to 0.54 and 0.77, respectively. When data is combined from all treatments and years (Fig. 3.3), the relationship is also apparent.

Although Duncan's multiple range test did not show consistant differences in hardlock severity in the Quincy study (Table 3.3), a regression showed a similar pattern to that observed in

Marianna. Combining all treatments and year resulted in an R^2 value of 0.25. The relationship was actually stronger in 2004 than 2005, with R^2 values of 0.63 and 0.39.

Discussion

The rate at which Fusarium was isolated from thrips in flowers was lower than the observed rate of hardlock in the field. Because thrips numbers within flowers increase during the day, sampling in the afternoon could have resulted in isolation rates more similar to observed rates of hardlock. Although hardlock incidence declined between 2003 and 2005, there was no change in the frequency with which *Fusarium* was isolated from thrips in the field. This suggests yearly fluctuations in inoculum quantity may not be an important determinant of the severity of hardlock. Sanders and Snow (1978) found the amount of airborne spores increased for several weeks after first bloom, possibly due to saprophytic growth on shed flowers and other tissue, before finally declining approximately 6 weeks later. That trend was not observed in this study, although taking more samples might have shown it to be the case. Ooka and Kommedahl (1977) observed a similar situation with *F. moniliforme* spores in corn fields. Spores numbers were lowest while the plant was actively growing , and increased as it reached maturity. They also observed wind-blown soil containing *F. moniliforme* spores which was likely to have traveled 300-400 km. However, Fernando et al. (2000) found more airborne spores within 1.5 m of an infected wheat field than at 5 m, suggesting local production of inoculum may be most important.

The thrips-*Fusarium* isolation study was undertaken with the assumption thrips were either transporting inoculum into the cotton flowers or their feeding damage to plant tissue made infection more likely to occur. The results show thrips are capable of transporting inoculum, and this appears realistic based on previous studies. Atakan (2001) observed thrips populations in red flowers were highest at 05:30, after which they began dispersing. Sanders and Snow (1978)

found the release of boll-rotting pathogen spores (including *Fusarium*) to be highest between 18:00 and 06:00. Fernando et al. (2000) found spore release of *F. moniliforme* to be highest in wheat fields between 16:00 and 08:00. This allows a time period between 05:30 and around 09:30 before thrips enter new flowers and during which they could come into contact with recently deposited spores from the previous night. This does not preclude the possibility that thrips damage allows more infections, and perhaps both pathways work synergistically.

The 3 isolates used in the greenhouse study had been identified in fall of 2002 as *F. verticillioides* based on spore types and shapes, as well as pigmentation in culture. It was not until summer of 2006 that further work revealed isolates 5 and 6 to be *F. proliferatum*. Previous inoculation studies showed no significant differences between the isolates in the ability to cause hardlock (Marois and Wright, 2004; Marois et al., 2005). This suggests at least two *Fusarium* species are capable of causing symptoms. Palmateer et al. (2004) found *F. moniliforme* to be relatively uncommon among *Fusarium* species isolated from living cotton plant tissue. *F. proliferatum* was also fairly uncommon. In contrast, Baird and Carling (1998) found *Fusarium* species to be present on 22 to 38% of dead cotton roots. Although *F. verticillioides* and *F. proliferatum* were not listed in the 6 most frequently isolated *Fusarium* species, 5 other unlisted species were isolated from 1 to 6% of samples. Bolkan et al. (1979) demonstrated *F. moniliforme* conidia to be short-lived (6 to 13 weeks) in the soil in the absence of host tissues. However, incorporating stem and leaf tissue from pineapple into the soil increased survival to at least 12 months. It should be noted cotton stems, roots, and fiber from the previous year are commonly found in cotton fields.

In the greenhouse inoculation studies, hardlock symptoms occurred in the control group, although at lower rates than the experimental treatments. Flowers were observed closely for

thrips to ensure *Fusarium* was not being transported to non-inoculated flowers. In addition, the air entering this greenhouse passed through an evaporative cooler, reducing the opportunity for airborne inoculum from outdoors to reach the flowers. It appears a certain amount of observed hardlock may result from unknown factors. The first and third greenhouse studies also suggest the particular species of thrips may not be important in hardlock, since similar results were obtained with both *F. occidentalis* and *F. tritici*. It also demonstrated cotton varieties do not respond identically to hardlock. However, the two varieties also showed large differences in response to growth regulators, flower production, and boll retention, so comparisons between them should not be extrapolated to field performance. The observation of more bolls aborting in their first 2 to 3 weeks of development may be related to the higher levels of gossypol observed at that stage in development (Bell, 1967).

Several observations from Figs. 3.1 to 3.4 are worth noting. In Fig. 3.1, the only treatment which is consistently above the trendline is the unsprayed control. Within a season, it is assumed factors other than thrips that might affect hardlock influence all plots equally. This suggests something other than the quantity of thrips is influencing hardlock. It may be that in addition to reducing their numbers, the insecticide applications reduce activities of thrips which contribute to hardlock. In Fig. 3.2, plots were grouped by treatment across years. This is useful in that it would reduce the possible complicating factor just described. However, because it is unknown why thrips numbers decreased each year of the study, or if that cause also reduced hardlock independently of the thrips numbers, the results should be viewed with caution. The same could be said of Fig. 3.3, although it includes more overlap between years. In Fig. 3.4, the 2004 control plots show the largest range of values along the trendline, making it most useful for confirming the thrips-hardlock connection. Each of the other 3 groups is tightly clustered, and

these clusters are considerable distances apart. Because of this, any comparison between these groups will yield a good R^2 value. While this makes them less useful for showing the connection between thrips and hardlock, they are very useful for demonstrating the benefits obtained from a spray program.

Applications of insecticide to field plots were effective at reducing thrips numbers, and this effect was observed in both Quincy and Marianna. This was expected based on *F. tritici* being the dominant species and the particular insecticides chosen to control thrips without severely affecting *Orius*. By preserving the natural predator, the mixed results sometimes associated with spraying (Funderburk et al., 2000) were avoided. The connection between thrips numbers and hardlock was clear in Marianna, but only obvious in one of two years in Quincy. This suggests that while thrips appear to be the most significant factor, they are not the only determinant of hardlock incidence. Although it can be concluded that reducing thrips numbers within a season reduced the incidence of hardlock, it is not clear whether declining thrips populations led to reduced hardlock, or if both were being influenced by a third factor. Yield can be adversely affected by many factors, and in some cases hardlock may not be a significant contributor to low yields. At the same time, reducing thrips numbers, regardless of their starting point, was usually beneficial, suggesting it may be a useful management strategy for controlling hardlock.

Table 3.1. Proportion of flowers containing thrips-carried Fusarium.

Year	Percent Fusarium	N
2003	7.0 a	329
2004	13 a	23
2005	6.6 a	273

Numbers followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 3.2. Results of greenhouse hardlock experiments.

		2003		2005			
		March-April		April-May		Jul-Aug	Oct-Nov
		Percent Hardlock	N	Percent Hardlock	N	Percent Hardlock	N
DP 444	Fusarium+thrips	---	--	---	--	37 a b	37
	Fusarium	---	--	---	--	44 a	51
	Thrips	---	--	---	--	45 a	58
	Control	---	--	---	--	27 b	41
DP 555	Fusarium+thrips	66 a	25	---	--	57 a	26
	Fusarium	56 a b	58	20 a	30	37 b	80
	Thrips	39 c b	36	---	--	40 b	85
	Control	32 c	50	12 a	67	23 c	76

Numbers in a column followed by the same letter between horizontal lines are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 3.3. Effect of fungicides and insecticides on thrips, hardlock, and yield in Quincy, FL.

Treatment	thrips		hardlock		yield	
	2004	2005	2004	2005	2004	2005
Control	6.10 a	4.18 a	0.48 a	0.32 a	1369 a	1547 a
Insecticide +fungicide	---	0.32 b	0.42 a	0.18 b	1530 a	1590 a
Insecticide	0.95 b	---	0.42 a	---	1280 a	---

Numbers followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 3.4. Effect of fungicide and insecticide treatments on thrips, hardlock, and yield in Marianna, FL.

Treatment	thrips		
	2003	2004	2005
Control	4.05 a	3.28 a	1.68 a
Fungicide	3.84 a b	2.96 a b	1.79 a
Insecticide +fungicide	3.14 c b	2.38 b	1.16 b
Insecticide	2.83 c	2.59 b	1.08 b

	hardlock			yield		
	2003	2004	2005	2003	2004	2005
Control	0.72 a	0.41 a	0.41 a	528 a b	911 b	1276 b
Fungicide	0.69 a	0.31 b	0.33 b	533 a	1003 a b	1237 b
Insecticide +fungicide	0.52 b	0.28 b	0.23 c	417 b	1178 a	1688 a
Insecticide	0.47 b	0.29 b	0.19 c	499 a b	1110 a b	1602 a

Numbers followed by the same letter in a column are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

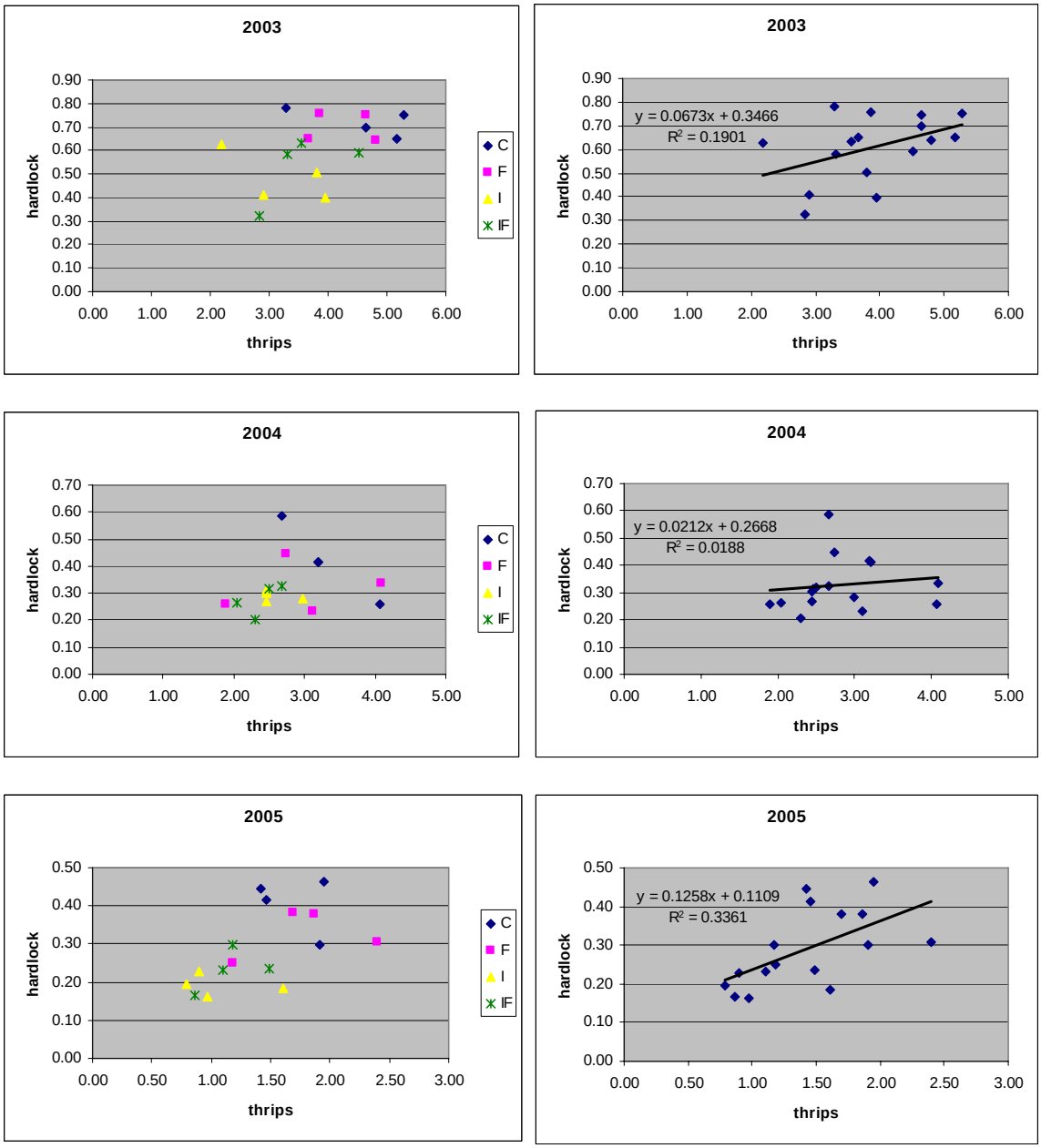


Figure 3.1. Regression of thrips numbers and hardlock incidence by year in Marianna, FL.

C = control, unsprayed
F = fungicide treatment, weekly application of Topsin M at 1.25 lb/acre
I = insecticide treatment, weekly alternating application of Tracer at 2 oz/acre or Orthene at 1 lb/acre
IF= insecticide with fungicide treatment, simultaneous application of insecticide and fungicide regimes listed above

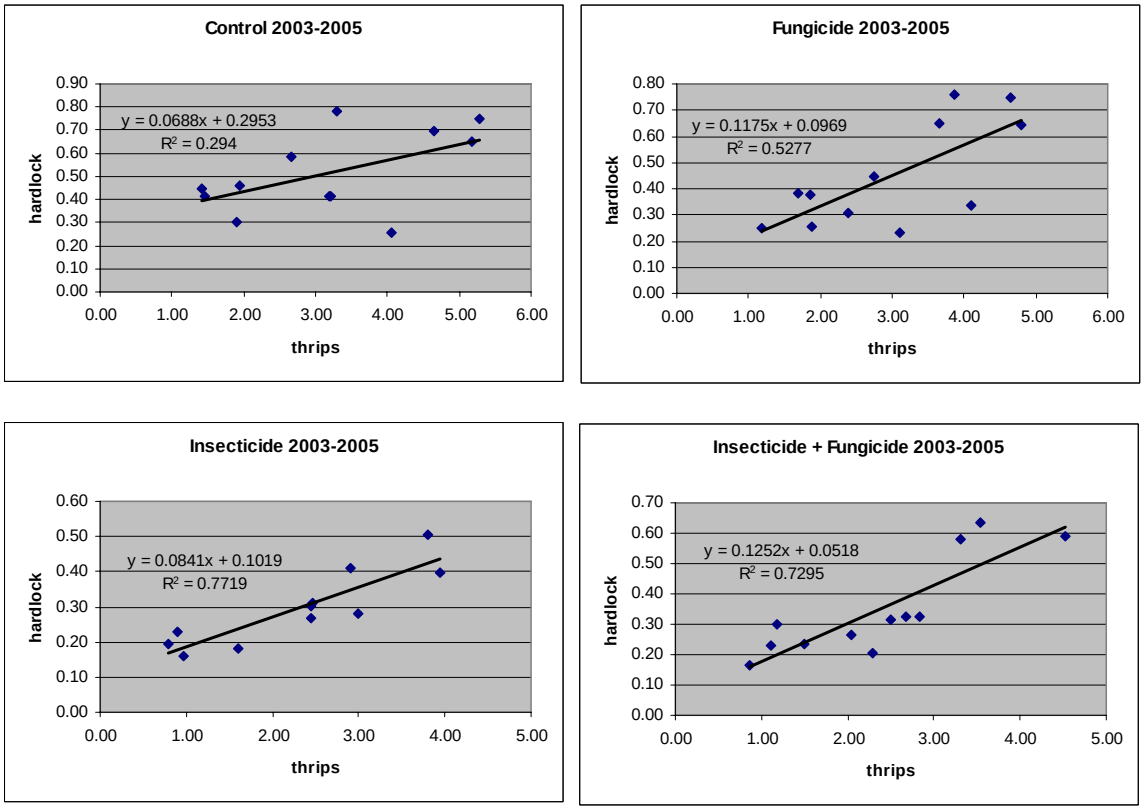


Figure 3.2. Regression of thrips numbers and hardlock incidence by treatment in Marianna, FL.

C = control, unsprayed
F = fungicide treatment, weekly application of Topsin M at 1.25 lb/acre
I = insecticide treatment, weekly alternating application of Tracer at 2 oz/acre or Orthene at 1 lb/acre
IF= insecticide with fungicide treatment, simultaneous application of insecticide and fungicide regimes listed above

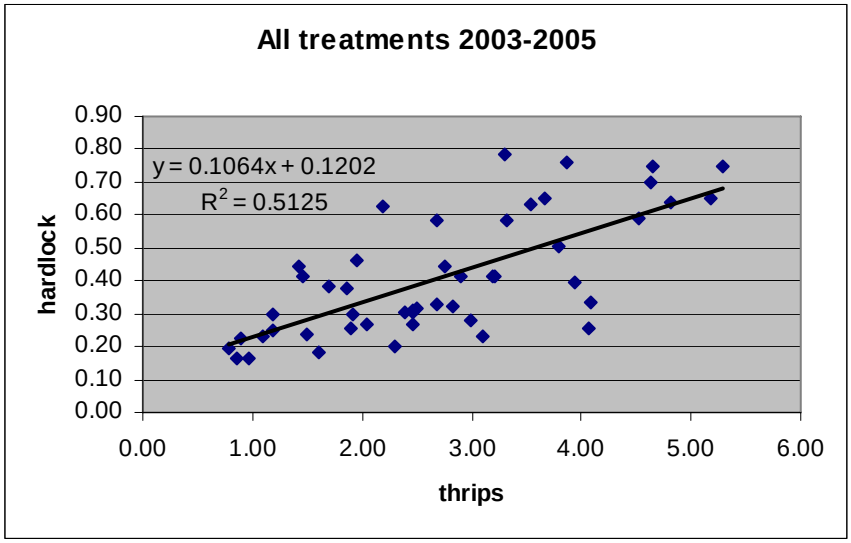


Figure 3.3. Regression of thrips numbers and hardlock for all treatments and years in Quincy, FL.

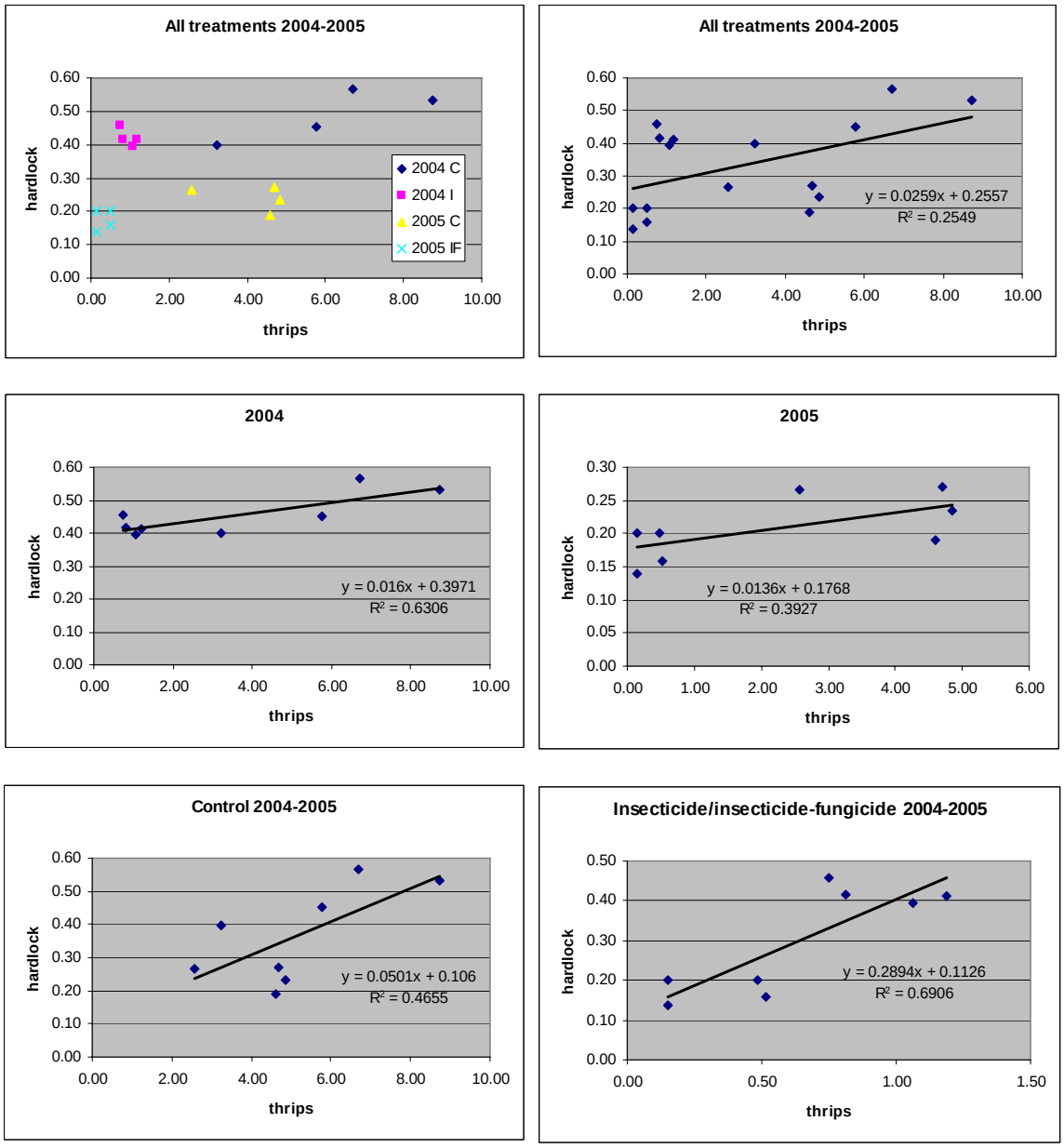


Figure 3.4. Regression of thrips numbers and hardlock in Quincy, FL.

2004 C = control, unsprayed
2004 I = insecticide treatment, 2.9 oz/acre of Tracer on Mondays and 8 oz/acre Orthene + 5 oz/acre Warrior on Thursdays
2005 C = control, unsprayed
2005 IF = insecticide with fungicide treatment, 2.9 oz/acre of Tracer on Mondays, 8 oz/acre Orthene + 2 oz/acre Karate on Thursdays and 1.0 lb/acre of Topsin M every 2 weeks

CHAPTER 4
RELATIONSHIP OF HARDLOCK TO WEATHER, THRIPS, AND YIELD

Introduction

Hardlock of cotton (*Gossypium hirsutum* L.) occurs when the fiber does not fluff out as the boll opens at maturity. Mature locules look like wedges of an orange when broken apart (Fig. 4.1). Although the quality of the cotton fiber may not be severely affected, conventional spindle harvesting equipment is not able to capture the fiber and bring it into the harvester as the hardlocked cotton is knocked from the plant and falls to the ground or is strung out of the boll giving the appearance of poor harvesting procedures. Attempts to "scrap" the field by running the picker a second time to get the hardlocked cotton often results in little lint increase, more trash and lower lint quality. The severity of hardlock in cotton has been associated with high nitrogen, high plant density, high temperature and humidity, insect damage, and seed rot. Because bolls affected by hardlock are not harvested by conventional pickers, yield losses of over 50% have occurred in states in the Southeast.

In 2002, hardlock was shown to be associated with *Fusarium verticillioides* (Marois et al., 2002). Before a publication by Michailieds and Morgan (1998), *F. verticillioides* was referred to as *Fusarium moniliforme*. *F. verticillioides* occurs frequently in cotton fields, growing saprophytically on crop residue. It has been isolated from seeds, peduncles, and mature fiber. It is hypothesized that *F. verticillioides* infects the flowers on the day of bloom and enters the immature boll before flower dehiscence. Flower inoculation using a suspension of *F. verticillioides* micro and macroconida has been shown to increase hardlock severity (Marois et al., 2005).

Hardlock can be differentiated from the traditional boll rots. Boll rots result from pathogen damage where the carpel turns brown or black and never opens or after the bolls have opened,

microorganisms destroy the cotton fibers. Batson (2001) identified 15 species of fungi and bacteria associated with boll rots, but he does not describe hardlock independently of boll rot. Boll rots occur during wet weather when the cotton boll or fiber is colonized by a number of microbes, although only a few fungi are responsible for the majority of infections (Pinkard and Chilton, 1966). These include *Alternaria gossypina* (Thuem.) Hopkins, *Curvularia* spp., *Diplodia gossypina* Cke., *Helminthosporium gossypii* Tucker, *Fusarium* spp., and *Phomopsis* spp. (Palmateer et al., 2003: Pinkard and Chilton, 1966). Sanders and Snow (1978) found a correlation between the numbers of airborne spores of these fungi and the incidence of boll rot caused by them. They also proposed that the likely source of the fungi was not from infected bolls but from spores being produced on shed plant parts, as there was a correlation between airborne spore numbers and the normal seasonal shedding of flowers, bolls, squares, and leaves (Sanders and Snow, 1977).

Flower thrips rapidly colonize cotton flowers on the morning they open, typically between 0900 and 0930. The most common species in north Florida is *Frankliniella tritici* (eastern flower thrips). *F. occidentalis* (western flower thrips) and *F. bispinosa* (Florida flower thrips) are also present (Chapter 2). Thrips continue entering the flowers for much of the day, typically reaching a peak of 10 to 40 per flower between 1400 and 1800. Thrips numbers can be reduced by predatory insects such as *Orius insidiosus*, the minute pirate bug (Ramachandran et al., 2001), but these predators can sometimes be harmed by insecticide applications used to control thrips (Studebaker and Kring, 2000). A connection between thrips numbers and temperature has been demonstrated. The average temperature between 0800 and 1000 is negatively correlated with the number of thrips in the flowers at 1000 (Ch.2).

In 2004, a model was proposed linking temperature and relative humidity on the day of bloom to hardlock incidence in those same bolls after opening (Marois et al., 2004). Temperature and relative humidity between 0700 and 1900 were shown to be positively associated with hardlock severity. This model was consistent with the geographic hypothesis of coastal areas having more hardlock due to their climate. It was also intuitive since *F. verticillioides* germinates and grows more readily in high temperature, high moisture conditions. Subbarao and Michailides (1995) determined the optimal temperature for *F. verticillioides (moniliforme)* infection on pollinator figs is 30°C. The temperatures observed in the model were usually below 30°C.

The objectives of this study were to explore the connection between weather conditions and hardlock, and to clarify the relationships between thrips, hardlock and yield.

Materials and Methods

Flowers were tagged using ribbons on a weekly basis. This occurred at 4 separate locations in Florida (Altha, Jay, Marianna, and Quincy) for 2 to 4 years. Temperature and relative humidity were recorded at 15-minute intervals using a CR10X data logger and 2 HMP 45 AC temperature-relative humidity probes. This allowed comparisons between each of the 2 temperature and 2 relative humidity measurements to improve accuracy. The weather measurement system was located within 100 m of the study flowers. After defoliation, all bolls from tagged flowers were removed from the plants and evaluated for hardlock severity. Hardlock severity was calculated as the number of hardlocked locules of the total present. Data were recorded separately for each boll. Bolls were categorized by date of bloom, and the average hardlock severity was calculated for each date.

The number of bolls recovered for each date varied considerably. Typically 20 to 40 bolls were recovered, and dates with fewer than 5 bolls were excluded from the analysis. The 2 temperature and 2 relative humidity values for each time interval were averaged to produce 1

temperature and 1 relative humidity value. After choosing the time interval of interest, the mean for each weather variable was determined using SAS for each day for which flowers had been tagged. The PROC REG command was used to perform regressions for each location and year.

The relationship between thrips numbers, hardlock, and yield was evaluated in fungicide-insecticide studies at branch stations of the North Florida Research and Education Center in Marianna and Quincy, FL. These studies were reported in Ch.2 and Ch.3, but all 3 variables were not compared. In this analysis it is the thrips numbers, and not the treatments, that are of interest.

Results

Weather Models of Hardlock Severity

The model involving the temperature and relative humidity from 0700 to 1900, as reported in (Marois et al., 2004), was tested for each location and year of this study (Table 4.1). It was significant for the data set from which it was developed (Quincy, 2002), showing a connection between higher temperature and humidity to increased hardlock serverity. At that same location in 2003 it was also significant. However, the relationship between hardlock and the temperature component of the model was reversed from the previous year. In other location years the model was frequently insignificant, and the relationships between model components varied regularly. Grouping the data sets by year or location did not improve significance. Grouping all 63 days from the data sets showed little or no relationship. The temperature or relative humidity components were also analyzed separately, but their significance and adjusted R^2 values were generally low, and their relationships to hardlock varied from positive to negative (Tables 4.2 and 4.3).

In Chapter 2, the temperature between 0800 and 1000 was shown to be negatively associated with thrips numbers at 1000. To determine if weather conditions may influence hardlock indirectly, through its impact on thrips numbers in flowers, the mean temperature

between 0800 and 1000 was compared to hardlock incidence for that day (Table 4.4). When viewed by location-year, the relationships were mostly negative, and more significant than the first model. When examined in groupings by location or year, the models were typically better or equal to the individual-location year models. Grouping all days produced a significant ($p=0.07$), negative relationship with an extremely low R^2 value (0.05). The average relative humidity during this period was also examined (Table 4.5). The relationships between humidity and hardlock fluctuated between positive and negative, and the adjusted R^2 values were usually low. Constructing a model using both temperature and relative humidity from 0800 to 1000 was no better than using temperature alone, and the relative humidity component varied between a positive and negative association (Table 4.6).

Although the temperature from 0800 to 1000 predicted hardlock incidence better than the first model, it was unclear if other time periods might be a better predictor. To determine this, regression analyses were performed for each location-year comparing hardlock incidence to temperature (Table 4.7) for each hour from 1700 on the day prior to bloom to 2400 on the day of bloom. Hours showing a significant ($p < 0.10$) relationship were compared for consistency across each of the location years. Significant relationships were observed from 1700 (prior to bloom day) to 1000 and 1300 to 2400 on the day of bloom. These ranges varied considerably by location-year, and when analyzed together only the period from 0000 to 0600 was shown to be important, based on significance and adjusted R^2 values (>0.20). The peak significance time within this period varied by model-year, so an average for the period was calculated for each. The resulting model tested a linear relationship between temperature from 0000 to 0600 and hardlock incidence for that same day (Table 4.8). In all location-years, locations, years, and combined data, night time temperature was negatively associated with hardlock incidence. In 4 of 11 location-

years, the model was significant at $p < 0.20$ and adjusted R^2 values were >0.50. Grouped by location, $p < 0.05$, and adjusted R^2 values varied from 0.15 to 0.79. Examined by year the results were similar, with $p < 0.10$, and adjusted R^2 values varied from 0.12 to 0.28. The combined data set showed a significance of $p = <0.0001$ and an adjusted R^2 value of 0.27. To determine if this result was complicated by plant maturity or varying hardlock intensity within the growing seasons, the temperatures (Fig. 4.3) and hardlock severity (Fig. 4.4) were plotted against the date. These did not appear to influence the model. Adding the date (in the form of Julian day) as a model component did not increase the adjusted R^2 value (data not shown). A problem with this model was that the residuals between the predicted and actual hardlock did not fit a normal distribution, and were larger toward the extremes of the temperature range.

To further refine the model, days on which fewer than 10 bolls were recovered were removed from the analysis (Table 4.9). This improved the adjusted R^2 value from 0.26 to 0.30. Then days with temperatures below 21 or above 25°C were also excluded, leaving 49 of the 63 (77%) original days (Fig. 4.5). This improved the adjusted R^2 value to 0.40 (Table 4.9). When the resulting equation was used to predict hardlock in that same data, the residuals were normally distributed, with a mean of 0.00 and a standard deviation of 0.20 (Table 4.10). To further explore the data, days with 10 or more bolls were assigned to two temperature groups for separate regression analyses: 17.1 to 22.9°C or 23.0 to 26.0°C. Days between 17.1 and 22.9°C showed a gradual slope, significant ($p=0.06$) relationship, and low adjusted R^2 value (0.07). The second group (23.0 to 26.0°C) showed a more gradual slope, an insignificant relationship ($p=0.66$), and an adjusted R^2 value of –0.04. The mean hardlock severity for low and high temperature groups were 62 and 30%, respectively. The change in hardlock severity at 23°C is fairly abrupt. As an alternative model, days above 23°C were categorized as having 30% hardlock severity, while

those below 23°C were categorized as having 62% hardlock. The resulting residuals were similar to those achieved using the linear regression model (Table 4.10). For days within the 21 to 25°C range, the linear regression model had a slightly better residual mean and standard deviation than did the categorical model. When applying these models to the full temperature range (17 to 26°C), the categorical model performed better. This suggests the specific temperature of the day should determine which model to use.

To validate the model (linear regression, >10 bolls, 21 to 25°C), 25 individual days were randomly sampled from the data set. A regression was performed, and the resulting equation was used to predict hardlock in the other 24 days. The residuals between the predicted and actual hardlock severity for those days were calculated (Table 4.11). This procedure was performed a total of four times. The mean residual ranged from –0.007 to –0.090, and the standard deviations were between 0.16 and 0.23.

The association of flower thrips with hardlock for individual days was also tested. The flowers tagged in Marianna were from rows adjacent to the fungicide-insecticide hardlock study described in Ch.2 and Ch.3. The 2 adjacent plots were a control and an insecticide+fungicide treatment. The mean number of thrips from the 2 adjacent plots was used as an estimate of thrips numbers within the tagged rows, since they were not sampled. Thrips were not a good predictor of hardlock (Table 4.12). When both thrips numbers and temperature from 0000 to 0600 were included in the model, the results were better than using thrips alone but worse than using temperature alone. However, these models using thrips have several shortfalls. First, if thrips are important, they should be significant on their own. Thrips numbers vary throughout an area, and their numbers appear to fluctuate independently of the overall trend. It is possible the estimates derived from adjacent plots were not representative of the tagged flowers. A second problem is

the temperature model did not perform as well in Marianna as other locations, so combining it with the thrips numbers which themselves did not predict well does not seem likely to improve the accuracy. The models were based on a relatively small number of days, and adding additional variables to the models makes them less reliable.

Connections between Thrips, Hardlock, and Yield

The relationship between thrips numbers, hardlock, and yield on a per plot basis at the season-level was examined (Table 4.13). In Marianna, higher thrips numbers were associated with more hardlock. The model was significant in 2 of 3 years, and also when the data for all the years was merged. Both R^2 and adjusted R^2 values remained below 0.40. Hardlock was inversely associated with yield, and this was significant in all years except 2003. In 2003, the yields were very low in all treatments (Ch.3), and it is likely that factors other than hardlock were more important. When the data was merged across years, the result was highly significant ($p < 0.0001$), and R^2 and adjusted R^2 values were both 0.57. The association between thrips numbers and yield was less consistent, but negative overall. In only 1 of 3 years was the relationship significant ($p = 0.0129$), and in the non-significant years higher thrips numbers were associated with higher yielding plots. Combining the data from all 3 years improved the significance of the model ($p = <0.0001$), and boosted the R^2 and adjusted R^2 values to 0.48. Using thrips numbers and hardlock to predict yield proved highly significant in 2005 ($p = 0.0009$) and when data was combined across years ($p = <0.0001$). The highest adjusted R^2 value by year was 0.34, but reached 0.64 when the data was combined across years. Similar results were obtained in Quincy. Higher thrips numbers were associated with higher hardlock in 2004 ($p = 0.02$) and 2005 ($p = 0.08$). R^2 and adjusted R^2 values were higher than in Marianna for both years. Higher-yielding plots were generally associated with lower hardlock, although the relationship was weaker than in Marianna.

Thrips numbers were not directly related to yield. Using thrips numbers and hardlock as a model to predict yield did not prove significant in Quincy.

Discussion

In this study, the association of weather conditions and hardlock incidence was explored. The first reported hardlock-weather model (Marois et al., 2004) was tested, but did not prove adequate in all locations. A second model, previously used for predicting thrips numbers, was also evaluated. Although it was an improvement from the first model, its predictive ability was very limited. Examining the temperatures by hour revealed the time between 0000 and 0600 to be most important for predicting hardlock. A model was constructed using the average temperature for that time period. This model out-performed those tested previously. It was further refined by limiting it to days within the temperature range of 21 to 25°C. It was also determined that days can be assigned to a high or low hardlock severity group with some success based on whether they are below or above 23C.

The first two models tested had straightforward biological explanations. In the first model, temperature and relative humidity were positively associated with hardlock incidence. Such conditions would be favorable for the germination and growth of the causal agent, *F. verticillioides*. In the second model, temperature from 0800 to 1000 were negatively associated with hardlock. This temperature range had previously been shown to be negatively associated with thrips numbers at 1000. Since warm temperatures slow the movement of thrips into flowers, it is assumed their harmful effects would be diminished, resulting in less hardlock. However, neither model fit the data as effectively as did the third. It is possible that cooler night temperatures have a subtle effect on the developing flower making it more susceptible to infection. Oosterhuis and Jernstedt (1999) noted cool temperatures can delay anthesis (period when pollen is shed) by two to three hours. This would delay pollination, possibly increasing the

amount of time the stigma is receptive and susceptible to infection by *Fusarium*. Although the low temperatures from 0800 to 1000 that are associated with higher thrips numbers did not consistently predict hardlock, in some instances they might be important. It may be possible to develop a model to predict hardlock based on temperature and the number of thrips known to be inhabiting cotton flowers. At the Marianna site (Chapter 3), larger increases in the proportion of non-hardlocked cotton due to insecticide applications were obtained in the years with the highest hardlock severity. If temperatures from 0000 to 0600 are used to estimate hardlock severity, this could allow a more optimal use of insecticide applications. Models have been developed previously to optimize management of cotton. The GOSSYM/COMAX model has been used for estimating irrigation needs (Staggenborg et al, 1996) and exploring the result of various fertilizer and defoliation strategies on yield (McKinion et al., 1989)

The relationships between thrips numbers, hardlock severity, and yield were also explored. The models comparing thrips to hardlock were mostly significant. Among the significant ones, the adjusted R^2 values ranged from 0.09 to 0.54, suggesting thrips are an important, but not exclusive, factor in hardlock severity. This lends further support to the findings of Chapter 3, in which significant treatment effects were observed in the severity of hardlock. There are probably other factors influencing the probability of infection, progression of the disease, and expression of symptoms after boll opening. The relationship between hardlock and yield was significant in many cases, and those location-years in which it wasn't were characterized by below average yield. This suggests hardlock is one of many factors that can influence yields. It is also interesting to note that when viewed by year, there was no relationship between thrips numbers and yield. This suggests they have no direct impact on the cotton plants, and any effect is due to hardlock.

In conclusion, the hardlock severity for a given day can be predicted with an adjusted R^2 of 0.40 using the mean temperature between 0000 and 0600. Cooler temperatures are associated with higher hardlock severity, and these predictions are most valid for temperatures between 21 and 25ºC. Below 21ºC severity is typically above 50%, while above 25ºC severity is below 50%. This model could be used to optimize spraying, but needs to be tested further. The impact of thrips control at different temperatures is unknown, but this must be determined for the model to optimize spraying. This could be done by tagging flowers in control and insecticide-treated plots, and quantifying the number of thrips in each plot on that day. Night temperatures can vary considerably from day to day, and tagging flowers on consecutive days may reduce interference from any other sources. Each flower is at risk of infection for one day, and this occurs over an eight week period. Targeting protective strategies to those days with the highest risk allows the most judicious use of pesticides, thereby maximizing economic and environmental benefits.

Figure 4.1. Cotton boll exhibiting symptoms of hardlock.

Table 4.1. Relationship between average temperature and relative humidity from 0700 to 1900 on the day of bloom and hardlock severity for that day.

location	year	N days	equation	p	R^2	adj.-R^2
Altha	2003	6	H = 3.38485 -T719*0.03388 -RH719*0.02245	0.6703	0.2341	-0.2765
Altha	2004	4	H = 1.49718 +T719*0.10730 -RH719*0.06072	0.3958	0.8433	0.5300
Jay	2003	8	H = 1.61997 -T719*0.03756 -RH719*0.00376	0.5768	0.1976	-0.1234
Jay	2004	8	H = 8.06744 -T719*0.23627 -RH719*0.01480	0.1048	0.5943	0.4320
Jay	2005	4	H = 0.86125 +T719*0.01482 -RH719*0.01169	0.2698	0.9272	0.7816
Marianna	2003	4	H = -12.52706 +T719*0.28892 +RH719*0.06857	0.6429	0.5867	-0.2400
Marianna	2004	4	H = 0.12447 +T719*0.02388 -RH719*0.00769	0.7222	0.4784	-0.5648
Marianna	2005	5	H = 31.72419 -T719*0.70494 -RH719*0.14080	0.5165	0.4835	-0.0330
Quincy	2002	8	H = -5.53090 +T719*0.13061 +RH719*0.03539	0.0232	0.7780	0.6892
Quincy	2003	8	H = 1.62170 -T719*0.05959 +RH719*0.00662	0.0240	0.7749	0.6849
Quincy	2004	4	H = 5.07364 -T719*0.10155 -RH719*0.02803	0.1916	0.9633	0.8899
Altha		10	H = 6.32756 -T719*0.11106 -RH719*0.03409	0.5888	0.1404	-0.1052
Jay		20	H = 3.64325 -T719*0.08808 -RH719*0.01061	0.0316	0.3341	0.2557
Marianna		13	H = 2.78049 -T719*0.08754 +RH719*0.00429	0.1003	0.3687	0.2424
Quincy		20	H = -2.14070 +T719*0.05701 +RH719*0.01397	0.7066	0.0400	-0.0729
	2003	26	H = -1.15743 +T719*0.02203 +RH719*0.01389	0.4931	0.0596	-0.0221
	2004	20	H = 4.36586 -T719*0.10977 -RH719*0.01140	0.0602	0.2816	0.1970
	2005	9	H = 1.83714 -T719*0.03499 -RH719*0.00510	0.6282	0.1435	-0.1419
all	all	63	H = 1.46886 -T719*0.02710 -RH719*0.00259	0.5649	0.0189	-0.0138

Table 4.2. Relationship between average temperature from 0700 to 1900 on the day of bloom and hardlock severity for that day.

location	year	N days	Temp. ºC	Percent hardlock	equation	p	R^2	adj.-R^2
Altha	2003	6	25.5 - 30.6	54 - 97	H = -0.52745 +T719*0.0446	0.3620	0.2090	0.0113
Altha	2004	4	29.2 - 30.6	6 - 80	H = -7.53823 +T719*0.27067	0.5045	0.2455	-0.1317
Jay	2003	8	26.2 - 31.1	4 - 44	H = 1.00990 -T719*0.02586	0.2811	0.1894	0.0543
Jay	2004	8	25.7 - 28.9	12 - 94	H = 5.88195 -T719*0.19692	0.0669	0.4541	0.3631
Jay	2005	4	26.6 - 29.2	37 - 59	H = 2.30545 -T719*0.06512	0.3206	0.4616	0.1925
Marianna	2003	4	26.1 - 29.9	72 - 91	H = 1.52993 -T719*0.02607	0.4803	0.2701	-0.0949
Marianna	2004	4	28.5 - 30.5	26 - 39	H = 1.29241 -T719*0.03191	0.5291	0.2217	-0.1674
Marianna	2005	5	28.1 - 31.4	19 - 80	H = 1.82540 -T719*0.04629	0.6963	0.0580	-0.2560
Quincy	2002	8	27.6 - 33.0	40 - 96	H = 0.05871 +T719*0.02353	0.6928	0.0279	-0.1342
Quincy	2003	8	26.1 - 30.5	29 - 70	H = 2.88915 -T719*0.08660	0.0043	0.7684	0.7298
Quincy	2004	4	29.1 - 31.0	2 - 34	H = 3.06464 -T719*0.09699	0.4876	0.2626	-0.1061
Altha		10			H = 0.21026 +T719*0.01483	0.7937	0.0091	-0.1148
Jay		20			H = 2.18815 -T719*0.06390	0.0360	0.2219	0.1787
Marianna		13			H = 3.48033 -T719*0.10098	0.0316	0.3553	0.2967
Quincy		20			H = 0.47849 +T719*0.00140	0.9729	0.0001	-0.0555
	2003	26			H = 1.28771 -T719*0.02744	0.3221	0.0408	0.0009
	2004	20			H = 2.77654 -T719*0.08238	0.0459	0.2035	0.1593
	2005	9			H = 1.59363 -T719*0.03912	0.4173	0.0959	-0.0332
all	all	63			H = 1.05481 -T719*0.01921	0.3283	0.0157	-0.0005

Table 4.3. Relationship between average relative humidity from 0700 to 1900 on the day of bloom and hardlock severity for that day.

location	year	N days	RH %	Percent hardlock	equation	p	R^2	adj.-R^2
Altha	2003	6	68 - 86	54 - 97	H = 1.72035 -RH719*0.01310	0.3363	0.2296	0.0370
Altha	2004	4	65 - 73	6 - 80	H = 5.04400 -RH719*0.06591	0.1001	0.8098	0.7146
Jay	2003	8	62 - 87	4 - 44	H = -0.18292 +RH719*0.00621	0.3837	0.1282	-0.0170
Jay	2004	8	62 - 85	12 - 94	H = 0.84503 -RH719*0.00462	0.7695	0.0154	-0.1487
Jay	2005	4	56 - 79	37 - 59	H = 1.18929 -RH719*0.01039	0.0421	0.9176	0.8764
Marianna	2003	4	68 - 86	72 - 91	H = 0.32869 +RH719*0.00617	0.4322	0.3224	-0.0164
Marianna	2004	4	54 - 71	26 - 39	H = 0.68295 -RH719*0.00530	0.3335	0.4443	0.1664
Marianna	2005	5	66 - 81	19 - 80	H = 0.10734 +RH719*0.00463	0.8544	0.0131	-0.3158
Quincy	2002	8	60 - 79	40 - 96	H = -0.33560 +RH719*0.01663	0.1660	0.2929	0.1750
Quincy	2003	8	67 - 85	29 - 70	H = -1.08503 +RH719*0.02004	0.0057	0.7457	0.7033
Quincy	2004	4	60 - 70	2 - 34	H = 1.98995 -RH719*0.02752	0.1780	0.6757	0.5135
Altha		10			H = 1.29985 -RH719*0.00906	0.5130	0.0553	-0.0628
Jay		20			H = 0.55598 -RH719*0.00208	0.7558	0.0055	-0.0497
Marianna		13			H = -0.42226 +RH719*0.01323	0.1398	0.1872	0.1133
Quincy		20			H = 0.26393 +RH719*0.00362	0.6800	0.0097	-0.0453
	2003	26			H = -0.12656 +RH719*0.00847	0.2429	0.0564	0.0170
	2004	20			H = 0.48189 -RH719*0.00118	0.8911	0.0011	-0.0544
	2005	9			H = 0.88559 -RH719*0.00605	0.4965	0.0684	-0.0646
all	all	63			H = 0.39754 +RH719*0.00144	0.7409	0.0018	-0.0146

Table 4.4. Relationship between average temperature from 0800 to 1000 on the day of bloom and hardlock severity for that day.

location	year	N days	Temp. ºC	Percent hardlock	equation	p	R^2	adj.-R^2
Altha	2003	6	24.2 - 28.4	54 - 97	H = -0.86394 +T810*0.06303	0.2939	0.2670	0.0838
Altha	2004	4	25.5 - 27.6	6 - 80	H = 9.68415 -T810*0.34434	0.0738	0.8579	0.7868
Jay	2003	8	23.8 - 27.4	4 - 44	H = 1.47193 -T810*0.04634	0.3596	0.1409	-0.0023
Jay	2004	8	22.2 - 27.1	12 - 94	H = 2.94612 -T810*0.09854	0.1263	0.3442	0.2349
Jay	2005	4	21.7 - 26.8	37 - 59	H = 1.56850 -T810*0.04426	0.0266	0.9475	0.9212
Marianna	2003	4	25.5 - 30.3	72 - 91	H = 1.87963 -T810*0.03817	0.0061	0.9878	0.9817
Marianna	2004	4	24.8 - 27.3	26 - 39	H = 1.43672 -T810*0.04154	0.2332	0.5879	0.3819
Marianna	2005	5	25.1 - 28.6	19 - 80	H = 1.97583 -T810*0.05644	0.5042	0.1603	-0.1196
Quincy	2002	8	23.4 - 27.8	40 - 96	H = 0.18795 +T810*0.02307	0.7143	0.0240	-0.1387
Quincy	2003	8	23.5 - 27.2	29 - 70	H = 2.45497 -T810*0.07822	0.0967	0.3921	0.2908
Quincy	2004	4	26.0 - 27.4	2 - 34	H = 2.37327 -T810*0.08271	0.6656	0.1118	-0.3323
Altha		10			H = 1.70746 -T810*0.04154	0.5209	0.0533	-0.0650
Jay		20			H = 2.47235 -T810*0.08200	0.0061	0.3488	0.3127
Marianna		13			H = 0.06460 +T810*0.01672	0.6983	0.0142	-0.0754
Quincy		20			H = 3.00672 -T810*0.09678	0.0536	0.1916	0.1467
	2003	26			H = 0.08964 +T810*0.01632	0.5871	0.0125	-0.0287
	2004	20			H = 3.18640 -T810*0.10754	0.0048	0.3651	0.3292
	2005	9			H = 1.43866 -T810*0.03766	0.1939	0.2278	0.1174
all	all	63			H = 1.44105 -T810*0.03629	0.0692	0.0531	0.0376

Table 4.5. Relationship between average relative humidity from 0800 to 1000 on the day of bloom and hardlock severity for that day.

location	year	N days	RH %	Percent hardlock	equation	p	R^2	adj.-R^2
Altha	2003	6	77 - 94	54 - 97	H = 2.50525 -RH810*0.02010	0.1750	0.4040	0.2550
Altha	2004	4	77 - 81	6 - 80	H = 4.96424 -RH810*0.05535	0.6117	0.1508	-0.2739
Jay	2003	8	76 - 92	4 - 44	H = 0.33838 -RH810*0.00072459	0.9559	0.0006	-0.1660
Jay	2004	8	70 - 99	12 - 94	H = 0.59850 -RH810*0.00121	0.9366	0.0011	-0.1653
Jay	2005	4	71 - 89	37 - 59	H = 1.12832 -RH810*0.00798	0.4064	0.3524	0.0285
Marianna	2003	4	70 - 87	72 - 91	H = 0.00280 +RH810*0.01027	0.0266	0.9476	0.9214
Marianna	2004	4	74 - 83	26 - 39	H = 1.34000 -RH810*0.01254	0.2326	0.5889	0.3833
Marianna	2005	5	77 - 94	19 - 80	H = -0.61222 +RH810*0.01245	0.5012	0.1624	-0.1168
Quincy	2002	8	76 - 94	40 - 96	H = -1.16870 +RH810*0.02271	0.0214	0.6140	0.5496
Quincy	2003	8	79 - 96	29 - 70	H = -0.94665 -RH810*0.01597	0.1952	0.2615	0.1384
Quincy	2004	4	76 - 85	2 - 34	H = 2.17161 -RH810*0.02457	0.2680	0.5358	0.3037
Altha		10			H = 0.78812 -RH810*0.00177	0.9095	0.0017	-0.1231
Jay		20			H = 0.92170 -RH810*0.00618	0.4426	0.0331	-0.0206
Marianna		13			H = 0.60392 -RH810*0.00103	0.9243	0.0009	-0.0900
Quincy		20			H = -1.13329 +RH810*0.01930	0.0838	0.1569	0.1101
	2003	26			H = 1.00986 -RH810*0.00579	0.4669	0.0223	-0.0185
	2004	20			H = 0.83577 -RH810*0.00535	0.6368	0.0127	-0.0422
	2005	9			H = 0.25670 +RH810*0.00236	0.8013	0.0097	-0.1318
all	all	63			H = 0.24149 +RH810*0.00309	0.5502	0.0059	-0.0104

Table 4.6. Relationship between average temperature and relative humidity from 0800 to 1000 on the day of bloom to hardlock severity for that day.

location	year	N days	equation	p	R^2	adj.-R^2
Altha	2003	6	H = 5.03406 -T810*0.05457 -RH810*0.03313	0.4252	0.4345	0.0576
Altha	2004	4	H = 7.38611 -T810*0.47509 +RH810*0.07181	0.1097	0.9880	0.9639
Jay	2003	8	H = 2.00074 -T810*0.05174 -RH810*0.00454	0.6452	0.1608	-0.1749
Jay	2004	8	H = 3.38390 -T810*0.10157 -RH810*0.00439	0.3290	0.3590	0.1026
Jay	2005	4	H = 1.47966 -T810*0.05652 +RH810*0.00476	0.0091	0.9999	0.9998
Marianna	2003	4	H = 3.25879 -T810*0.06587 -RH810*0.00768	0.0584	0.9966	0.9898
Marianna	2004	4	H = 1.41568 -T810*0.02101 -RH810*0.00654	0.6292	0.6042	-0.1875
Marianna	2005	5	H = -0.23261 -T810*0.00833 +RH810*0.01064	0.8375	0.1625	-0.6751
Quincy	2002	8	H = -3.70374 +T810*0.08013 +RH810*0.02873	0.0073	0.8599	0.8038
Quincy	2003	8	H = 2.44961 -T810*0.07812 -RH810*0.00003128	0.2881	0.3921	0.1490
Quincy	2004	4	H = 6.73137 -T810*0.15094 -RH810*0.03074	0.3545	0.8743	0.6230
Altha		10	H = 6.22996 -T810*0.12984 -RH810*0.02631	0.4737	0.1922	-0.0386
Jay		20	H = 2.69654 -T810*0.08001 -RH810*0.00327	0.0231	0.3579	0.2824
Marianna		13	H = -0.45543 -T810*0.02583 -RH810*0.00335	0.9079	0.0191	-0.1770
Quincy		20	H = 1.37903 -T810*0.06973 -RH810*0.01089	0.1126	0.2266	0.1356
	2003	26	H = 1.42445 -T810*0.00911 -RH810*0.00786	0.7626	0.0233	-0.6160
	2004	20	H = 3.85551 -T810*0.10964 -RH810*0.00754	0.0150	0.3900	0.3183
	2005	9	H = 1.19601 -T810*0.03831 -RH810*0.00309	0.4315	0.2443	-0.0075
all	all	63	H = 1.53183 -T810*0.03742 -RH810*0.00072999	0.1928	0.0534	0.0218

Table 4.7. Association of temperature and hardlock severity by hour for each location.

	Altha		Jay		Marianna		Quincy		all locations			
hour	p	relat.	p	relat.	p	relat.	p	relat.	p	relat.	R^2	adj-R^2
17b	0.63	+	0.28	-	0.00	-	0.99	+	0.18	-	0.02	0.01
18b	0.66	-	0.29	-	0.00	-	0.32	+	0.35	-	0.01	0.00
19b	0.72	-	0.34	-	0.00	-	0.50	+	0.25	-	0.02	0.00
20b	0.55	-	0.22	-	0.00	-	0.59	-	0.02	-	0.08	0.06
21b	0.10	-	0.13	-	0.00	-	0.02	-	0.00	-	0.22	0.21
22b	0.04	-	0.10	-	0.00	-	0.13	-	0.00	-	0.17	0.16
23b	0.02	-	0.13	-	0.00	-	0.27	-	0.00	-	0.17	0.15
24b	0.01	-	0.16	-	0.01	-	0.18	-	0.00	-	0.16	0.14
1	0.00	-	0.11	-	0.02	-	0.00	-	0.00	-	0.23	0.22
2	0.00	-	0.07	-	0.01	-	0.00	-	0.00	-	0.27	0.26
3	0.00	-	0.05	-	0.02	-	0.00	-	0.00	-	0.30	0.29
4	0.00	-	0.03	-	0.03	-	0.00	-	0.00	-	0.29	0.27
5	0.00	-	0.04	-	0.13	-	0.00	-	0.00	-	0.26	0.24
6	0.00	-	0.03	-	0.38	-	0.01	-	0.00	-	0.22	0.21
7	0.00	-	0.04	-	0.43	+	0.04	-	0.00	-	0.12	0.10
8	0.00	-	0.04	-	0.52	+	0.03	-	0.01	-	0.08	0.07
9	0.23	-	0.01	-	0.76	+	0.00	-	0.02	-	0.07	0.06
10	0.92	+	0.00	-	0.92	+	0.07	-	0.06	-	0.05	0.03
11	0.99	-	0.06	-	0.65	-	0.94	-	0.61	-	0.00	-0.01
12	0.89	+	0.23	-	0.15	-	0.97	-	0.49	-	0.00	0.00
13	0.92	-	0.34	-	0.04	-	0.77	+	0.41	-	0.01	0.00
14	0.90	+	0.23	-	0.03	-	0.59	-	0.16	-	0.03	0.01
15	0.64	+	0.29	-	0.03	-	0.51	-	0.24	-	0.02	0.00
16	0.04	+	0.47	-	0.03	-	0.83	-	0.82	-	0.00	-0.01
17	0.07	+	0.88	-	0.06	-	0.83	+	0.77	+	0.00	-0.01
18	0.81	+	0.99	+	0.04	-	0.85	+	0.91	-	0.00	-0.01
19	0.58	-	0.42	-	0.01	-	0.66	-	0.25	-	0.02	0.00
20	0.29	-	0.42	-	0.01	-	0.08	-	0.03	-	0.07	0.05
21	0.20	+	0.07	-	0.12	-	0.00	-	0.05	-	0.05	0.04
22	0.04	+	0.00	-	0.13	-	0.00	-	0.00	-	0.11	0.09
23	0.05	+	0.00	-	0.17	-	0.00	-	0.00	-	0.15	0.14
24	0.09	+	0.00	-	0.11	-	0.00	-	0.00	-	0.14	0.13

Table 4.8. Relationship between average temperature from 0000 to 0600 on the day of bloom to hardlock severity for all days.

location	year	N days	Temp. ºC	Percent hardlock	equation	p	R^2	adj.-R^2
Altha	2003	6	21.8 - 22.9	54 - 97	H = 7.90058 -T0106*0.32403	0.0253	0.7520	0.6901
Altha	2004	4	20.6 - 24.8	6 - 80	H = 4.61249 -T0106*0.18074	0.0385	0.9244	0.8866
Jay	2003	8	21.9 - 24.6	4 - 44	H = 1.38649 -T0106*0.04749	0.4719	0.0894	-0.0624
Jay	2004	8	18.7 - 25.2	12 - 94	H = 1.45101 -T0106*0.04274	0.4591	0.0944	-0.0565
Jay	2005	4	19.7 - 24.5	37 - 59	H = 1.57428 -T0106*0.04930	0.0259	0.9489	0.9233
Marianna	2003	4	22.2 -23.5	72 - 91	H = 1.08427 -T0106*0.01260	0.9087	0.0083	-0.4875
Marianna	2004	4	22.6 - 26.0	26 - 39	H = 0.42002 -T0106*0.00327	0.9182	0.0067	-0.4900
Marianna	2005	5	21.8 - 24.3	19 - 80	H = 3.10125 -T0106*0.11400	0.2816	0.3637	0.1517
Quincy	2002	8	17.1 - 22.3	40 - 96	H = 1.65947 -T0106*0.04368	0.2845	0.1870	0.0515
Quincy	2003	8	21.7 - 23.3	29 - 70	H = 1.09049 -T0106*0.02837	0.8331	0.0080	-0.1573
Quincy	2004	4	19.7 - 23.9	2 - 34	H = 1.64555 -T0106*0.06688	0.1646	0.6979	0.5468
Altha		10			H = 5.30923 -T0106*0.20881	0.0004	0.8136	0.7903
Jay		20			H = 1.75501 -T0106*0.05940	0.0495	0.1976	0.1531
Marianna		13			H = 2.99630 -T0106*0.10534	0.0426	0.3233	0.2617
Quincy		20			H = 2.60580 -T0106*0.09657	0.0051	0.3614	0.3259
	2003	26			H = 4.27822 -T0106*0.16566	0.0066	0.2689	0.2385
	2004	20			H = 1.72184 -T0106*0.05796	0.0682	0.1729	0.1270
	2005	9			H = 1.94613 -T0106*0.06506	0.0809	0.3726	0.2829
all	all	63			H = 2.33353 -T0106*0.08148	<0.0001	0.2770	0.2652

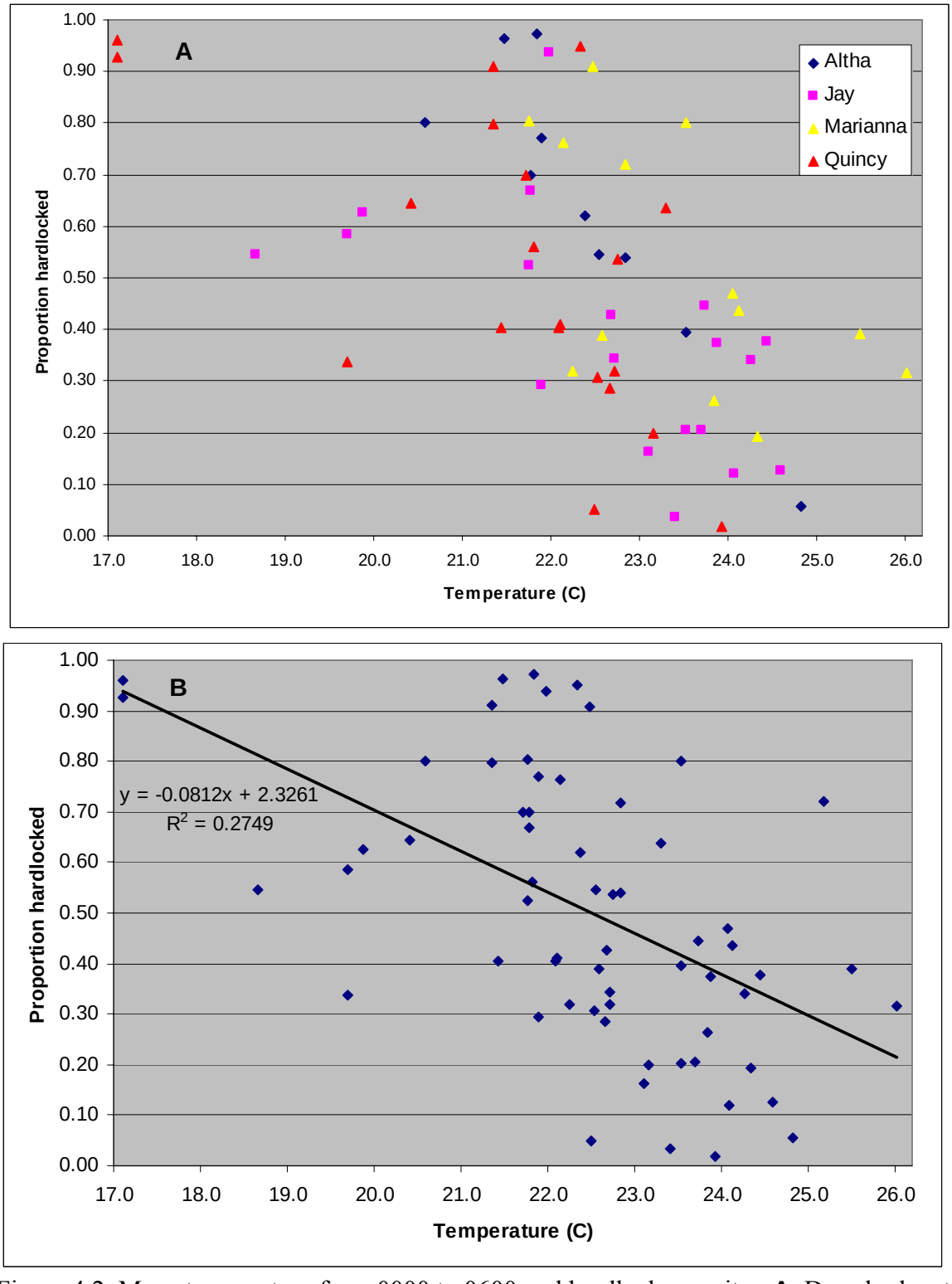


Figure 4.2. Mean temperature from 0000 to 0600 and hardlock severity. **A**, Days by location. **B**, Regression line.

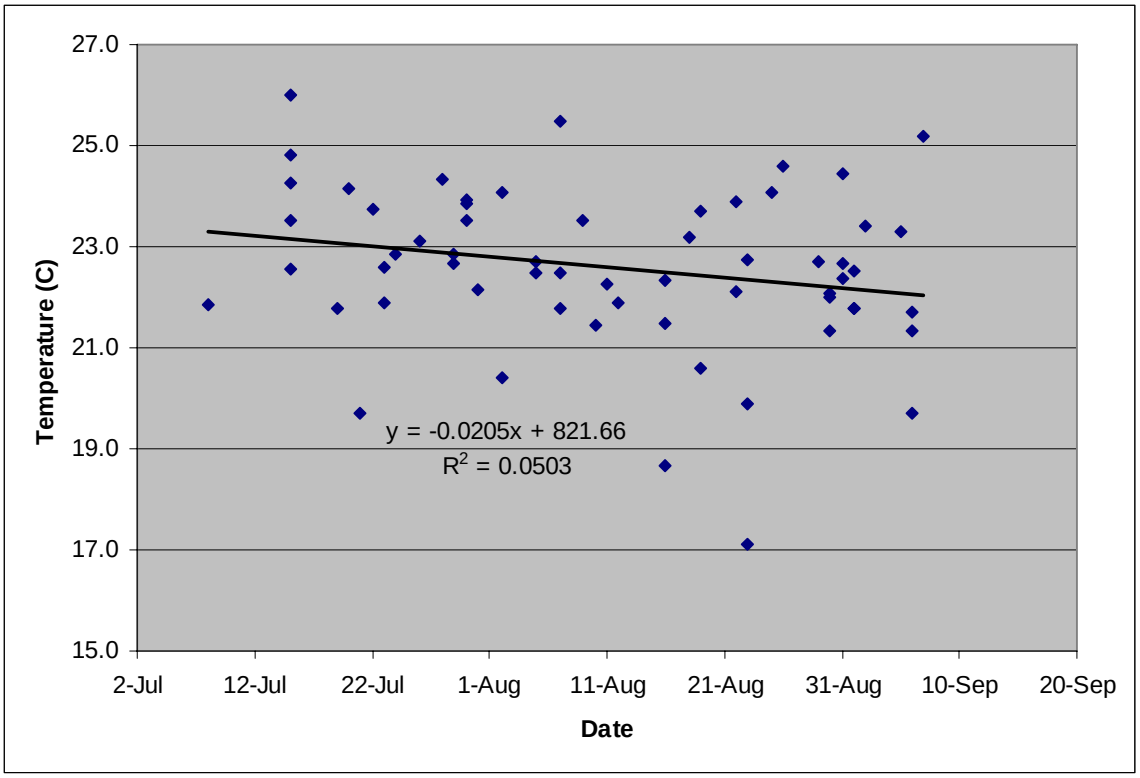


Figure 4.3. Distribution of mean temperatures from 0000 to 0600 for days in the climate model.

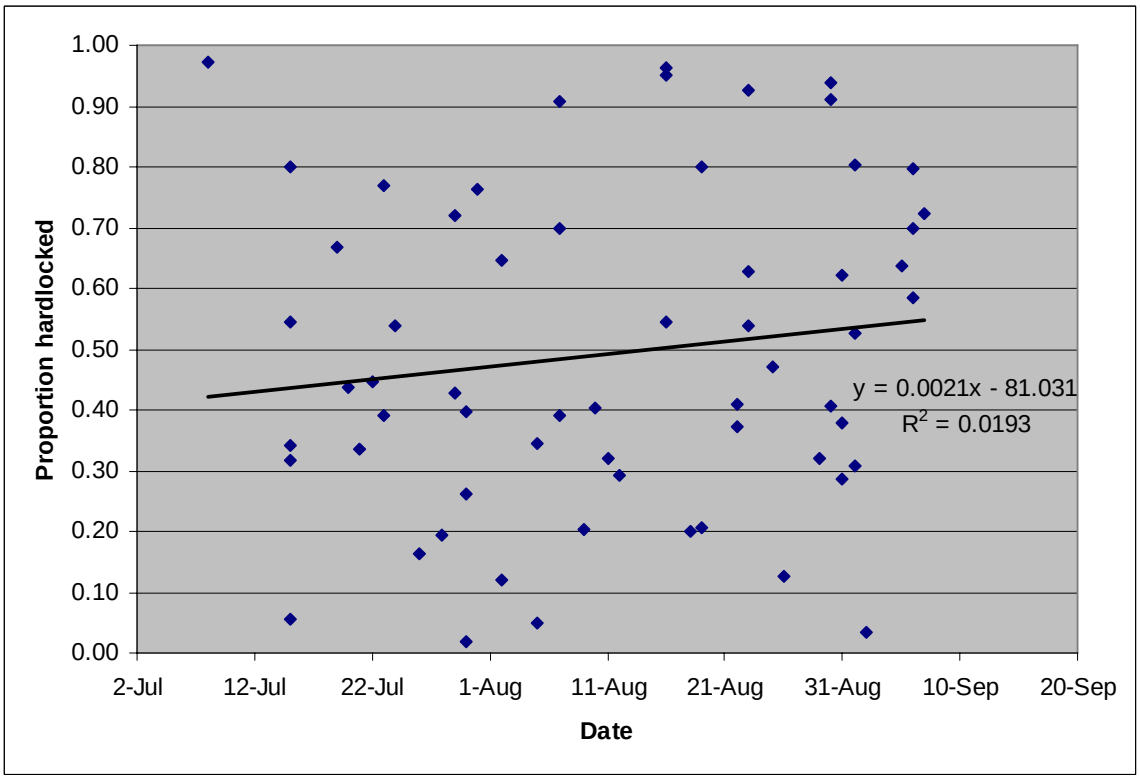


Figure 4.4. Incidence of hardlock during the growing season for days evaluated.

Table 4.9. Relationship between average temperature from 0000 to 0600 on the day of bloom to hardlock severity for temperatures between 21 and 25°C.

N days	Temp.	hardlock	bolls recovered	equation	p	R^2	adj.-R^2
63	17.1 - 26.0	0.02 - 0.97	>5	H = 2.32467 -T*0.08106	<0.0001	0.2731	0.2611
58	17.1 - 26.0	0.02 - 0.97	>10	H = 2.42539 -T*0.08543	<0.0001	0.3145	0.3022
49	21.4 - 24.8	0.02 - 0.97	>10	H = 4.57773 -T*0.17898	<0.0001	0.4173	0.4049
37	17.1 - 22.9	0.29 - 0.97	>10	H = 1.66745 -T*0.04858	0.0602	0.0973	0.0715
21	23.1 - 26.0	0.02 - 0.80	>10	H = 0.97826 -T*0.02803	0.6627	0.0102	-0.0419

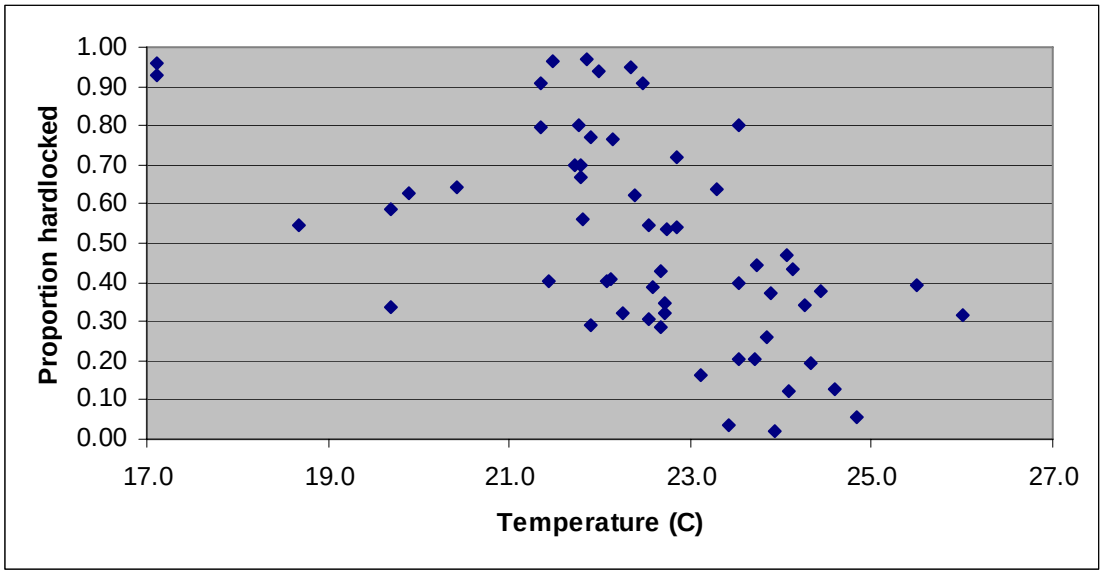


Figure 4.5. Days in model with >10 bolls recovered.

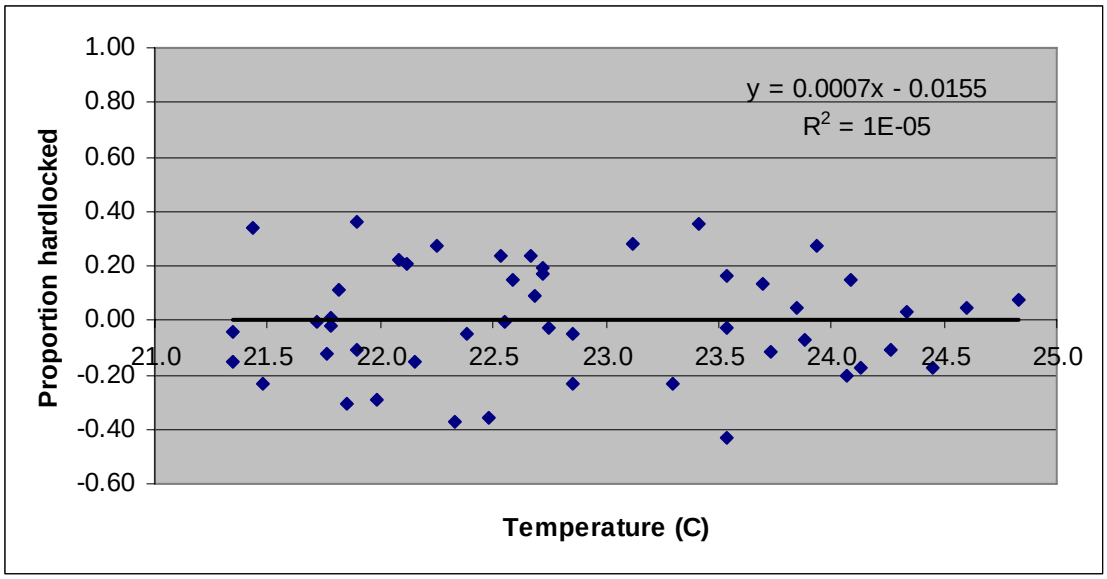


Figure 4.6. Prediction residuals for days with >10 bolls, 21 to 25°C (N=49).

Table 4.10. Mean and standard deviation of models when predictions are applied to data.

		linear	categorical
21-25°C	mean	0.00	0.01
	st. dev.	0.20	0.22
17-26°C	mean	0.05	0.00
	st. dev.	0.27	0.22

Table 4.11. Validation of the model by random sampling and applying predictions to data.

	models established from random sampling of days				prediction residuals		
N	equation	p	R^2	adj.-R^2	N	mean	SD
25	H = 3.55426 -T*0.13543	0.0071	0.2752	0.2437	24	-0.0581	0.2218
25	H = 4.77974 -T*0.18862	0.0034	0.316	0.2863	24	-0.0372	0.1644
25	H = 3.33086 -T*0.12455	0.0053	0.2921	0.2613	24	-0.0068	0.2324
25	H = 4.37491 -T*0.17204	0.0003	0.4392	0.4149	24	-0.0904	0.1928

Table 4.12. Relationship of temperature from 0000 to 0600 and estimated thrips numbers to hardlock in Marianna, FL.

location	year	N days	thrips	equation	p	R^2	adj.-R^2
Marianna	2003	4	2.3 - 4.4	H = 0.62834 +TH*0.05242	0.3332	0.4446	0.1669
Marianna	2004	4	0.4 - 4.7	H = 0.32735 +TH*0.00524	0.8473	0.0233	-0.4650
Marianna	2005	5	1.4 - 2.5	H = 0.71081 -TH*0.13613	0.6866	0.0619	-0.2509
Marianna	all	13		H = 0.35030 +TH*0.06850	0.2534	0.1166	0.0363
Marianna	2003	4	2.3 - 4.4	H = 3.17168 -T*0.11778 +TH*0.09484	0.3430	0.8823	0.6470
Marianna	2004	4	0.4 - 4.7	H = 0.30124 +T*0.00101 +TH*0.00580	0.9881	0.0237	-1.9289
Marianna	2005	5	1.4 - 2.5	H = 3.09767 -T*0.10907 -TH*0.05674	0.6262	0.3738	-0.2524
Marianna	all	13		H = 2.65757 -T*0.09486 +TH*0.03723	0.1121	0.3545	0.2254

Table 4.13. Relationships between thrips, hardlock and yield on a per plot , seasonal basis.

location	year	N	thrips	hardlock	yield	equation	p	R^2	adj.-R^2
Marianna	2003	32	x	x		hardlock = 0.41165 +thrips*0.05216	0.0460	0.1263	0.0971
Marianna	2004	32	x	x		hardlock = 0.34980 -thrips*0.00825	0.7560	0.0033	-0.0300
Marianna	2005	32	x	x		hardlock = 0.11918 +thrips*0.11944	0.0007	0.3219	0.2993
Marianna	all	96	x	x		hardlock = 0.15831 +thrips*0.09310	0.0001	0.3902	0.3837
Marianna	2003	32		x	x	yield = 516.60053 -hardlock*36.06410	0.7810	0.0026	-0.0306
Marianna	2004	32		x	x	yield = 1366.33802 -hardlock*967.06381	0.0026	0.2648	0.2403
Marianna	2005	32		x	x	yield = 1902.35967 -hardlock*1560.96214	0.0002	0.3735	0.3526
Marianna	all	96		x	x	yield = 1711.36408 -hardlock*1740.30903	<0.0001	0.5664	0.5618
Marianna	2003	32	x		x	yield = 474.83374 +thrips*5.09984	0.7888	0.0024	-0.0308
Marianna	2004	32	x		x	yield = 955.19283 +thrips*33.86048	0.4962	0.0156	-0.0172
Marianna	2005	32	x		x	yield = 1783.89191 -thrips*233.84959	0.0129	0.1892	0.1621
Marianna	all	96	x		x	yield = 1645.94504 -thrips*239.86385	<0.0001	0.4844	0.4790
Marianna	2003	32	x	x	x	yield = 497.64089 +thrips*7.98992 -hardlock*55.40358	0.8924	0.0078	-0.0606
Marianna	2004	32	x	x	x	yield = 1289.87769 +thrips*25.96324 -hardlock*956.78225	0.0096	0.2739	0.2238
Marianna	2005	32	x	x	x	yield = 1947.46192 -thrips*69.92373 -hardlock*1372.49023	0.0009	0.3849	0.3425
Marianna	all	96	x	x	x	yield = 1836.74673 -thrips*127.65422 -hardlock*1205.25120	<0.0001	0.6501	0.6426
Quincy	2004	8	x	x		hardlock = 0.39907 +thrips*0.01581	0.0230	0.6052	0.5394
Quincy	2005	8	x	x		hardlock = 0.17458 +thrips*0.03057	0.0751	0.4352	0.3411
Quincy	all	16	x	x		hardlock = 0.26420 +thrips*0.02937	0.0189	0.3347	0.2872
Quincy	2004	8		x	x	yield = 468.27660 +hardlock*1860.10638	0.2863	0.1858	0.0501
Quincy	2005	8		x	x	yield = 1817.62244 -hardlock*1021.01514	0.2288	0.1841	0.0481
Quincy	all	16		x	x	yield = 1731.20696 -hardlock*814.36697	0.1271	0.1582	0.0980
Quincy	2004	8	x		x	yield = 1219.43516 +thrips*29.73564	0.4113	0.1150	-0.0325
Quincy	2005	8	x		x	yield = 1599.82076 -thrips*13.73293	0.7689	0.0155	-0.1486
Quincy	all	16	x		x	yield = 1437.60645 +thrips*3.13140	0.9118	0.0009	-0.0705
Quincy	2004	8	x	x	x	yield = 489.77366 +thrips*0.82832 +hardlock*1828.39815	0.5981	0.1859	-0.1398
Quincy	2005	8	x	x	x	yield = 1854.99692 +thrips*30.95703 -hardlock*1461.69112	0.5227	0.2286	-0.0800
Quincy	all	16	x	x	x	yield = 1775.20536 +thrips*40.65899 -hardlock*1277.79295	0.1413	0.2600	0.1461

CHAPTER 5
SUMMARY AND CONCLUSIONS

The most common (>99%) flower thrips species observed was *F. tritici* (eastern flower thrips), and flowers contained an average of 1.4 to 4.2 individuals. Females outnumbered males, typically by a 2:1 to 5:1 ratio. *F. occidentalis* (western flower thrips) was rarely found in 2003 and 2004 (2 to 3 thrips per 1000 cotton flowers), and was not present in 2005. Although slightly more common (3 to 10 thrips per 1000 cotton flowers), the same pattern of diminishing numbers was observed in *F. bispinosa* (Florida flower thrips). These three species are all flower feeders, and likely competitors for resources. *F. fusca* was observed at low levels (3 to 16 per 1000 flowers) in all years. It is a foliar-feeding species, and could have been found in the flowers as a result of dispersion in search of suitable host plants. Immature thrips were also present, and their numbers ranged from 0.05 to 0.10 per flower. *Orius* species, predators of thrips, were also present at a rate of 0.03 to 0.14 per flower. Observed ratios of *Orius* to thrips ranged from 1:35 to 1:220 depending on location and year. *Orius* probably reduced thrips numbers, but this did not lead to localized extinctions as observed on other crops. *Aphis sp*. (aphids) were observed at levels of 1.2 to 5.3 per flower.

Samples from Louisiana, Alabama, and South Carolina were also examined to see if they were similar to those in Florida. In all locations, *F. tritici* was most common. Their numbers ranged from 0.42 to 14.8 per flower. Louisiana in 2003 contained the highest number of *F. occidentalis* recorded (0.31 per flower). One individual of *F. bispinosa* was found in Alabama, although they are most common to peninsular Florida. The sex ratios varied considerably, with the highest being 1 male per 48 females. In a third of the location/years, males were more common than females. The large range of thrips numbers and sex ratios observed may have been influenced by crop management practices. The number of immature thrips also varied

considerably, from 0.01 to 0.96 per flower. *Orius* was generally uncommon, except in two instances where 0.15 and 0.21 per flower were found.

The number of thrips observed weekly fluctuated during the growing season. In 2 years at Marianna, a rapid rise of 55 to 170% in thrips numbers was observed for the last sampling dates. This was associated with a decline in the number of white flowers, and possible crowding of thrips. The rate at which thrips accumulate in flowers was also examined. Cotton flowers open between 0900 and 0930. By 1000, there were typically 4 or fewer thrips per flower. Thrips numbers increased until 1400, at which point 4 to 35 thrips were present. The rate at which thrips arrived in flowers varied substantially by day and it was hypothesized this was due to weather conditions. Cool temperatures and low humidity from 0800 to 1000 was associated with more thrips at 1000.

The ability of insecticide treatment to reduce thrips numbers was also evaluated. In Marianna, FL, acephate was alternated weekly with spinosad reduced thrips numbers by 20 to 35%. In Quincy, FL, lambda cyhalothrin was alternated weekly with spinosad, and reduced by 84 to 92%.

To demonstrate the association of flower thrips with hardlock, a series of experiments was performed. Live thrips were captured from cotton flowers in research plots and placed on acidified potato dextrose agar to isolate *Fusarium*. It was determined that between 7 and 13% of flowers contained thrips that were carrying *Fusarium*. In another experiment, cotton flowers inside a greenhouse were inoculated with *Fusarium* spores, thrips, or thrips that had been exposed to *Fusarium* cultures. The result varied by cultivar, but the combination of thrips and *Fusarium* typically resulted in the most severe hardlock symptoms, while each of them alone increased severity compared to the control group. The combination of thrips and *Fusarium* also

resulted in bolls aborting at a higher rate. In field plots, fungicide and/or insecticides were applied to determine the impact on thrips, hardlock and yield. In Quincy, insecticides reduced thrips numbers and sometimes reduced hardlock, but did not improve yield. In Marianna, fungicides did not affect thrips and usually reduced hardlock, but did not improve yield. Insecticides reduced thrips numbers, reduced hardlock more consistently and further than fungicides, and improved yield in 1 of 3 years. Combining fungicide and insecticide applications had the same impact on thrips and hardlock as did insecticide alone, but increased yield in 2 of 3 years.

Models comparing weather conditions on the day of bloom to hardlock severity in resulting bolls were evaluated. The first reported hardlock-weather model (Marois et al., 2004) was based on a positive association between the average temperature and relative humidity from 0700 to 1900 and hardlock severity. Although it fit the early data set well, it did not prove adequate in all locations. A second model, previously used for predicting thrips numbers, was also evaluated. Although it was an improvement from the first model, its predictive ability was still very limited. Examining the temperatures by hour revealed the time between midnight and 0600 to be most important for predicting hardlock. A model was constructed using the average temperature for that time period. This model out-performed those tested previously. It was further refined by limiting it to days within the temperature range of 21 to 24°C. Below that range, it was more accurate to assume hardlock would be above 50%, and above that range assume it would be less than 50%.

The first 2 models tested had straightforward biological explanations. In the first model, temperature and relative humidity were positively associated with hardlock incidence. Such conditions would be favorable for the germination and growth of the causal agent, *F.*

verticillioides. In the second model, temperature from 0800 to 1000 was negatively associated with hardlock. This temperature range had previously been shown to be negatively associated with thrips numbers at 1000. By slowing the movement of thrips into flowers, it is assumed their harmful effects would be diminished, resulting in less hardlock. However, neither model fit the data as effectively as did the third. It is possible that cooler night temperatures have a subtle effect on the developing flower making it less susceptible to infection. Although the impact of temperature on thrips numbers does not appear to be the primary determinant of hardlock severity, it is possible the cool night temperatures have a residual effect on thrips that supplements any direct effect on the plant.

The relationships between thrips numbers, hardlock severity, and yield were also explored. Regression analyses comparing thrips to hardlock were mostly significant. Among the significant ones, the adjusted R^2 values ranged from 0.09 to 0.54, suggesting thrips are an important, but not exclusive, factor in hardlock severity. There are probably other factors influencing the probability of infection, progression of the disease, and expression of symptoms after boll opening. The relationship between hardlock and yield was significant in many cases, and those location-years in which it wasn't were characterized by below average yield. This suggests hardlock is one of the many factors that can influence yields. Comparing thrips numbers to yield on a yearly basis showed no relationship. This suggests they have no direct impact on the cotton plants, and any effect is due to hardlock.

These experiments have shown thrips numbers can be reduced in a field setting with insecticide applications. This reduced hardlock severity, and sometimes improved yield. Thrips in flowers were shown to be carrying *Fusarium*, and the greenhouse study showed this to increase hardlock severity compared to flowers that were untreated or contained thrips without

Fusarium. Temperature was shown to influence both thrips numbers and hardlock severity. Flower thrips and weather conditions are important contributors to hardlock.

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BIOGRAPHICAL SKETCH

Daniel Joseph Mailhot was born in Tallahassee, FL in 1981. After graduating from Leon High School in 1999, he attended the University of Florida. Daniel graduated with a Bachelor of Science degree in plant science in the summer of 2002. He accepted an assistantship at the University of Florida under the supervision of James Marois in the Plant Pathology Department, and started graduate school in fall of 2002. His research has focused on hardlock of cotton and its relationship to flower thrips and weather conditions. Daniel will graduate in spring of 2007.