TIMED RELEASES WITH *Neoseiulus californicus* AS A BIOLOGICAL CONTROL AGENT FOR *Tetranychus urticae* Koch AND ITS ECOLOGICAL IMPACT ON NORTH FLORIDA STRAWBERRY FIELDS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2007

To my parents who gave me the gift of curiosity and a love of learning

ACKNOWLEDGMENTS

I would like to thank everyone who made this thesis possible. Most importantly I would like to thank my supervisory committee, Dr. Oscar E Liburd, Dr. Robert McSorley, and Mr. Stanley Latimer. I am also eternally grateful to Drs. Christian Russell and Matt Cohan for their help on the imagery analysis. I would also like to thank the staff at the Small Fruit and Vegetable IPM Laboratory for their moral support, and the staff at the Research and Education Center for their help with the strawberry maintenance. Finally, I would like to thank my family and friends for providing unending support through this whole process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

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August 2007

Chair: Oscar E. Liburd
Major: Interdisciplinary Ecology

Twospotted spider mite (TSSM), *Tetranychus urticae* Koch, is considered to be a key pest in north Florida strawberry fields. Management of TSSM is difficult because it can become resistant to miticides within a year of exposure, making chemical control difficult. As a result, biological control is becoming a popular alternative. *Neoseiulus californicus* McGregor is known to be an effective biological control agent for TSSM in north Florida strawberries. The objectives of this study were to determine the effect of timed releases with *N. californicus* to control TSSM throughout the season and also to evaluate the effect of predatory releases on the arthropod communities in the field. We found that *N. californicus* can control TSSM effectively if released early in the season at an appropriate predator: prey ratio and that *N. californicus* show a functional response to high densities of TSSM. The Shannon-Weaver index, evenness, and richness measures were used to evaluate the arthropod communities in plots treated with *N. californicus*. Results showed that *N. californicus* does not significantly alter the arthropod assemblage in the field. The generalist feeding habits of *N. californicus* and the natural diversity of the strawberry system may reduce the effect of *N. californicus* releases on the strawberry system. Finally, we conducted exploratory laboratory studies to correlate TSSM infestation with

spectral reflectance values of the leaves. A spectroradiometer was used to collect spectral data from individual leaflets that were infested with known levels of TSSM. Data obtained were used to construct categories of infestation levels at specific spectral regions. Results indicate that TSSM can be detected spectrally at very low levels of infestation. Information obtained from these studies suggests that biological control and spectral imagery could be integrated into a management program to develop an effective precision pest management program.

CHAPTER 1
INTRODUCTION

The strawberry industry began in early 15th century Europe as a collection of small localized enterprises. The wild fruit was originally used for medicinal purposes and was later propagated in household gardens. The first records of the modern strawberry trade date from the colonization of the New World, ca. 16th century (Wilhelm and Sagen 1974). Today, the United States is one of the top producers of strawberries worldwide. The average yield in the United States ranks highest in the world, and total harvested area is second only to Poland (Economic Research Service-USDA 2005). California is the center of strawberry production in the United States. However, Florida produces 100% of the domestic winter strawberry crop and ranks second in the country in overall production (Mossler and Nesheim 2002).

Strawberries are the most valuable berry crop in Florida. No other plant bears more fruit earlier, as soon after planting, nets more profit per acre in such a short period of time, or thrives in so many different environments (Wilhem and Sagen 1974). In 2003, Florida strawberries had a market value of $200 million and a production cost of $20,000 per acre (Brown 2003). By the year 2005, there were 7,300 acres in production comprising approximately three-quarters of the state's cultivated berry acreage (Economic Research Service-USDA 2005). Exports have been growing at an average rate of seven percent annually since 1990 (Economic Research Service-USDA 2006).

In northern Florida, strawberries are grown as an annual crop on a raised bed, or "hill system". The soil is injected with a soil fumigant such as methyl bromide to control soil pests and pathogens and the beds are covered with black plastic mulch. Drip irrigation is run under the beds to improve water and nutrient efficiency. This "plasticuture" system mitigates pest and disease transmission in the field (Mossler and Nesheim 2002).

Strawberries are susceptible to a number of fungal diseases and are host to many arthropod pests. The most economically important pre-harvest fungal diseases are Botrytis fruit rot (*Botrytis cinerea*) and anthracnose fruit rot (*Colletotrichum acutatum*) (Ellis and Legard 2003). Major arthropod pests include coleopterans such as the Strawberry Root Weevil, *Otiorhynchus ovatus* (Linnaeus), Black Vine Weevil, *Otiorhynchus sulcatus* (Fabricus), Rootworm, *Paria fragaria* Wilcox, and several species of sap beetles. Homopterous pests include potato leafhopper, *Empoasca fabae* (Harris), spittlebug, *Philaenus spumarius* (Linnaeus)**,** aphids, (Aphididae), and whiteflies, *Bemisia tabaci* and *Trialeurodes* spp. Armyworms, cutworms and strawberry fruitworms (Noctuidae), tarnished plant bugs, (*Lygus* spp.*)* and several species of thrips occur as well (Handly and Price 2003).

The principal pest on strawberry plants is the twospotted spider (TSSM), *Tetranychus urticae* Koch (Waite 2002, Oatman et al. 1985, Escudero and Ferragut 2005, Cakmak and Cobanoglu 2006, Rhodes and Liburd 2006). Twospotted spider mites feed on the leaves by piercing through the mesophyll layer with its needle-like chelicerae and sucking out the contents of the cells. This destroys the protective leaf surface, nutrient availability, and decreases photosynthetic activity, resulting in a speckled or bronzed appearance of the leaves. If severe, the damage may reduce yields (Sonnevelt et al. 1996, Huffaker et al. 1969, Colfer et al. 2004).

It is a common practice for growers to “scout” their fields to monitor the presence of TSSM, this includes taking a systematic or random sample of at least 60 leaflets from a field and inspecting each leaflet for the presence of at least one mite motile (nymph or adult) or egg. Fields must be monitored regularly at least once per week as TSSMs aggregate quickly into “hot spots”. Leaves can be examined with a 10X hand lens, or under a microscope in a laboratory. When 5%

of the leaves are infested it is generally recommended for growers to take some type of control action (Greco et al 2004, Handley and Price 2003).

Historically, miticides have been used to control TSSM infestations, but due to increased resistance, harmful effects to the environment and to non-target organisms, and high costs, many growers are looking at alternative methods of control (White 2003). There are several known natural enemies of TSSM, such as the sixspotted thrips, *Scolothrips sexmaculaus*, minute pirate bug, *Orius tristicolor* (White), and Bigeyed bug, *Geocoris punctipes* Stal, and brown lacewing (hemerobiid) (Oatman and Voth 1972). However, predatory mites from the family Phytoseiidae are known to be among the most effective control agents. Prior to 2006, *Phytoseiulus persimilis* Athias-Henriot was the most commonly utilized predatory mite in Florida strawberries (Rhodes and Liburd 2006). However, the cooler climate that exists in northern Florida during the production season does not support its viability in this region of the state. Research conducted by White (2003) found that *Neoseiulus californicus* (McGregor) is a better choice in north Florida strawberry fields because of the wide fluctuation in temperature, moisture and humidity.

Neoseiulus californicus is a commercially available phytoseiid mite that is a generalist type II feeder (McMurtry and Croft, Rhodes and Liburd 2005). It can survive without prey and for long periods at low temperatures (Hart et al. 2002). It has been used either alone or in conjunction with *P. persimilis* for TSSM control (Rhodes et al. 2006). However, the time when *N. californicus* should be released, as well as the frequency of applications (releases) has not been determined (Cakmak and Cobanoglu 2006, Hart et al. 2002). The objectives of this study are to determine the most appropriate time during the growing season for inoculative releases of *N. californicus* to control TSSM, to evaluate the effect of predatory releases on naturally occurring arthropods in the strawberry plant environment, and to establish a pest monitoring system for growers using geographic information technology (GIT) to detect early damage of TSSM in strawberries.

A strawberry pest management survey was also administered. The survey was distributed to growers across the state of Florida to gather information regarding specific pest management concerns. Questions addressed growers' perceptions of pest problems and their primary concerns when considering a management plan. The results were compiled and analyzed to help us develop appropriate management strategies that target growers'specific needs.

CHAPTER 2
LITERATURE REVIEW

Twospotted spider mite

The twospotted spider mite, *Tetranychus urticae* Koch, belongs to the family Tetranychidae. It feeds on strawberry (*Fargaria* spp.) leaves by piercing the photosynthetic sites of the mesophyll tissue with specialized chelicerae. It pierces the chloroplasts, ingests the chlorophyll, and collapses the interior structure of the leaf, altering the ability of the leaves to utilize solar radiant energy, reduces carbon dioxide assimilation and decreases transpiration (Jansen et al. 1997, Jensen 2005, Shanks and Doss 1989). The twospotted spider mite is a particularly harmful pest due to its high rate of fecundity and its short life cycle. Females lay an average of six eggs per day and for a total of 70-100 eggs during a lifetime (Williams 2000). In high temperatures (>33˚ C) a female can lay as many as 20 eggs a day and the life cycle decreases from the average 30 days to seven days (Shanks and Doss 1989, Grostal and Dicke 2000, White and Liburd 2005). Twospotted spider mites also thrive in environments with low soil moisture. White and Liburd (2005) reported two times as many motiles on plants exposed to low moisture regimes compared with those exposed to high soil moisture.

Twospotted spider mite develops from a pale yellow egg into a six-legged yellowish-white larva. It molts two more times becoming an eight-legged protonymph and then deutonymph before maturing into an adult. Depending on the temperature, it may take between 4-12 days for the mite to mature. Spider mites may live for three weeks as an adult. The female is about 0.5 mm in length and the male grows to be approximately 0.3 mm (Williams 2000). Spider mites produce silk from glands located in the palpi to spin fine webs on the underside of the leaves to protect the colonies from predators and facilitate movement across the leaf. The webs may also be used for courtship, to protect against weather and miticides, to conserve their eggs and reduce interguild predation (McMurtry et al 1907, Kranz, 1979, Roda et al. 2000).

During the summer months when TSSM is in a feeding reproductive stage it is a yellowish green color. The female is globular in shape and has two pronounced dark lateral “feeding spots” on the abdomen. The male has a more rectangular shape and has similar dark markings. These markings are lighter and less defined than on the females. During the winter months, low temperatures and short day length induce diapause in female mites. They cease feeding and reproduction and become dark red in color. The females over-winter in the soil (Bollard et al. 1999, Sugasawa et al. 2001). Sex determination is entirely haploid-diploid (Huffaker et al. 1969). Both male and female TSSM adults are brightly colored ranging from green to yellow to red. Due to broad morphological differences TSSM has been described with over 50 different names. The inability for some individuals to successfully mate suggests that *T. urticae* may be a species complex (Bollard et al. 1999).

Twospotted spider mite is an important pest on a broad range of agricultural crops. In strawberries, early season infestation can severely decrease yield (Sugasawa 2001, Rhodes 2005). A high infestation often results in defoliation and a significant reduction in fruit yield. Yield reduction has been recorded to be as high as 29%, but usually averages between 10-15% (Walsh 2002, Oatman et al. 1985). Because of its short life cycle, TSSM has the capacity to become highly resistant to most pesticides in a short period of time. Outbreaks have intensified over the last few decades due to increased use of pesticides and modern cultural practices (Huffaker and McMurtry, 1969 and Escudero and Ferragut, 2005). As traditional methods of chemical control are less effective, and marketability due to chemical residue has become a social concern, growers are beginning to rely on natural enemies as an alternative to chemical management systems (Escudero and Ferragut 2005).

Control Methods

Many commercial growers have developed the practice of applying multiple treatments of chemical pesticides, mainly acaricides, on a calendar basis throughout the season (Villanueva and Walgenbach 2005). Compounds such as hexythiazox, bifenatzate, abamectin and bifentrhrin have been heavily relied upon to control arthropod pest infestations. During the last half a century, concern over resistance of mites to specific miticides and of the deleterious effect that the miticides have on beneficial predators has increased (Escudero and Ferragut 2005).

Broad spectrum insecticides such as pyrethroids and organophosphates have been shown in laboratory tests to be harmful to beneficials. These chemicals decrease rates of oviposition, affect egg numbers and increase rates of mortality in adults. Even reduced-risk compounds have variable levels of toxicity and sub-lethal effects on reproduction in beneficials (Villanueva and Walgenbach 2005).

Many growers are investigating alternative methods of control due to the concerns over chemical tactics. A principal alternative to chemical tactics for management of key arthropod pests is inundative releases of predatory mites and insects. This practice has been used to manage TSSM populations. Approximately 40% of Florida's strawberry growers, particularly in the southern part of the state, practice a method of biocontrol, which has led to a significant decrease in chemical applications (Mossler and Nesheim 2002).

Natural Enemies

There are several natural enemies of TSSM. Oatman et al. (1985) identified ten insect species belonging to families Thripidae, Cecidomyiidae, Coccinellidae, Staphylinidae, Anthocoridae, Lygaediae, Chrysopodae and Hemerobiidae and nine phytoseiid mite species that are natural enemies of TSSM. Rondon et al. (2004) conducted laboratory studies measuring rates of consumption, efficacy and feeding preference by bigeyed bugs, *Geocoris punctipes* Say, minute pirate bugs, *Orius insidiosus* (Say), and the pink spotted lady beetle *Coleomegilla maculata* DeGeer. The experiments concluded that these insects do consume spider mites, but also show a preference for aphids and other phytophagous insects, reducing their effectiveness as target control agents for TSSM. The most successful predators in suppressing TSSM are the predaceous phytoseiid mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* (Blackwood and Shausberger 2001, McMurtry and Croft 1997, Jung and Croft 2001, Croft et al. 1998, Sabelis and Janssen 1994).

Predatory Mites: *Phytoseiulus persimilis* and *N. californicus* belong to the family Phytoseiidae. Phytoseiids are voracious predators of spider mites. They compose one of the most important families of predatory mites used in biological control systems (Blackwood and Shausberger 2001). Phytoseiid mites are approximately 0.5 to 0.8 mm and live in topsoil under leaf litter. They have a pair of needle-like chelicerae which are inserted into the prey and extract its internal fluids. They range from type I, highly specialized feeders that are genus specific with regard to prey preference, to type IV generalist feeders that are able to utilize mites, pollen, or honeydew as an energy source.

Phytoseiulus persimilis

Phytoseiulus persimilis is a type I obligate specialist that is genus specific in terms of its feeding preferences (McMurtry and Croft 1997). It feeds exclusively on *Tetranychus* spp.and has one of the highest rates of population increase and the shortest development time of Phytoseiid mites (McMurtry and Croft 1997). *Phytoseiulus persimilis* depends on TSSM to survive and reproduce. It has been observed to consume up to five times as many TSSM as *N. californicus*, and has a greater fecundity (Gilstrap and Friese 1985). With its long setae, it is well adapted to move over webbed spider mite colonies. As a type I specialist *P. persimilis* tends to aggregate in areas of high prey, has high walking activity and aerial dispersal, and is ephemeral and difficult to maintain as a reliable control agent (Jung and Croft 2001, Croft et al. 1998). It is effective in the short term, but it over exploits the prey and perishes when the food source is eliminated. *Phytoseiulus persimilis* is a more voracious and well adapted predator than *N. californicus*, but is unable to survive in temperate climates. (Escudero and Ferragut 2005, Easterbrook 1992).

Neoseiulus californicus

Neoseiulus californicus is a type II/III generalist predator, meaning that it can survive on a variety of live prey as well as on pollen (McMurtry and Croft 1997). It is innately adapted to strawberry plant structure (Castignoli et al. 1999). As a generalist, it moves further from a central release point than specialists such as *P. persimilis* and provides more stable and regulatory pest suppression (Croft et al.1998). It is prone to engage in intraguild predation because it is not dependent solely on TSSMs to survive (Rhodes et al. 2006). In trials combining *P. persimilis* and *N. californicus*, *N. californicus* continuously displaced *P. persimilis* (Rhodes et al 2006). Higher interspecific predation rates are common among generalist phytoseiid species. For *N. californicus,* heterospecific feeding may be beneficial in terms of nutrition, development and oviposition, whereas *P. persimilis* cannot develop on prey other than *Tetranychus* spp. and tends to be cannibalistic when prey densities are low (Walzer and Schausberger 1999).

Neoseiulus californicus has five developmental phases, egg, larva, protonymph, deutonymph, and adult. The life cycle is half as long as TSSM. It can be completed within four days, depending on temperature. *Neoseiulus californicus* lives for approximately 20 days. Females lay an average of three eggs a day and consume five adult TSSM a day (Krantz 1978, Escudero and Ferragut 2005). The population corresponds to the increases and decreases of TSSM (Oatman et al. 1985). While the development rate of phytoseiids is linear and TSSM is non-linear, high rates of development and consumption enable them to achieve and maintain control over the TSSM population under the right environmental conditions (Sabelis and Janssen 1994).

Neoseiulus californicus disperses earlier than *P. persimilis*, has a slower metabolism, a lower searching efficiency, but a high rate of spatial coincidence with TSSM and can tolerate starvation (Greco et al. 2004). *Neoseiulus californicus* has a broad diet range including sap, honeydew, and pollen and can reproduce on pollen at a comparable rate to a diet of prey (McMurtry and Croft 1997). *Neoseiulus californicus* is used widely in Mediterranean regions where *P. persimilis* has failed to establish. It is more tolerant to some pesticides and is adapted to the fluctuations of prey dynamics and a mild climate (Escudero and Ferragut 2005).

Temperature is the most important abiotic factor in predator establishment. It affects development, survival and reproduction of predatory mites. High temperature fluctuation is the primary limiting factor that restricts the use of *P. persimilis* as a biological control agent (Escudero and Ferragut 2005). The developmental threshold in *N. californicus* is estimated to be 9.9˚C. Adult females can survive throughout the winter, for over three months under sheltered conditions. Without entering diapause, or consuming prey, they continue to oviposit and develop. They have a short generation time and can complete six generations in summer and one during the winter (Hart et al. 2002).

Effect of Timing of Predatory Releases on TSSM Populations

At high levels of TSSM infestation, the introduction of predatory mites may not be able to control and maintain TSSM below the economic threshold, which in strawberries is considered to be between five and 20 motiles per leaf (Oatman 1972, Hardman 2005). Laboratory and field experiments show that for *N. californicus* to be an effective biological control agent, it must be released early in the season when there is a low incidence of spider mite infestation (Greco et al. 2005). If the predator is released too late in the season when TSSM population is high and the ratio of TSSM to predator is greater than 10:1, the released predator will not have the capacity to consume the pest in high enough numbers to control it to a level below the economic injury level (Greco et al. 1994).

Other studies have found no relationship between the time when predators are released and suppression of TSSM. Studies conducted in northern California of Persea mite (Acari: Tetranychidae) infestations on avacado trees have shown that timing of inundative releases of various species of predatory mites had no significant effect on the prey population density or on yield (Hoddle 1998). Another study done in cotton showed similar results, i.e.: there were no significant differences (P = 0.07) between the early release, late releases (P = 0.06) and the control (Colfer et al. 2004), nor did releases enhance the natural predator population. However, in both of these studies, there was a reduction in spider mite populations in all treatments. This reduction was not attributed to the treatment, but to ecological factors such as plant phylogeny, climate, and other naturally occurring enemies (Hoddle 1998, Colfer et al. 2004). Plant physiology has an impact on TSSM population dynamics due to decreasing nutrient availability from previous mite infestations or other physiological factors (Shanks and Doss 1989, Croft and Coop 1998). Twospotted spider mite populations naturally begin to decrease after harvest and with foliar aging (McFarlane and Hepworth 1994).

Research conducted by Waite (2002) in strawberries supports the “Pest in First” (PIF) theory, which suggests that pests can be controlled by artificially introducing them into the field and allowing them to establish high population densities for several weeks. Predators are then introduced at the appropriate ratio. As the pests disperse and spread throughout the field, the predators are already established and maintain adequate control and pest: predator ratio throughout the season.

Rhodes and Liburd (2006) found that releasing predators one time early in the season alone and in combination with chemical or another biological control could maintain control of TSSM throughout the season. There is not yet adequate evidence regarding the affect and appropriate date of release for predatory mites on strawberries. Biocontrol companies differ in recommendations regarding general release dates. Koppert Biological systems™ (Netherlands) recommend that growers apply *N. californicus* preventively at 10,000 mites/ha at 21 day intervals. Biobest Biological Systems ™ (Westerlo, Belgium) and Biocontrol Network™ (Brentwood, TN) recommend bi-weekly releases throughout the season, and Green Method Biocontrols™ (Nottingham, NH) recommends "as needed" monthly releases (2006). Most growers release biocontrol agents on a standard calendar basis (Greco et al. 2004).

Geographic Information Systems in Pest Monitoring

Mite damage begins at a cellular level in the mesophyll; therefore, it is difficult to observe early mite damage with visual inspection and field scouting. Precision insect pest management (PIPM) is a developing technique that would enable growers to detect and analyze spatial and temporal distribution of agricultural pest damage before it is visible in their fields. This enables growers to employ preventative tactics before the pests reach economic threshold. As *N. californicus* is able to survive in fields with low mite density (Greco et al. 1994), predators released before damage is visible may have the potential to prevent crop damage by TSSM populations, consequently, reducing costs and increasing production (Dayang and Kamaruzaman 1999).

Geospatial Information Technology (GIT) is a set of tools associated with site-specific precision agriculture. The three main elements consist of Global Positioning System (GPS), imagery systems, and Geographic Information Systems (GIS). These tools can be used to collect and monitor information about a pest population that can be illustrated as a density or topographical map referred to as a “bug map”. A variety of biological, chemical, and spectral data from a field can be integrated and analyzed to create maps of vegetative condition in relation to pest damage (Brewster 1999, Dayang and Kamaruzaman 1999).

The Global Positioning System (GPS) component is essential in constructing an accurate field map. This system uses a constellation of satellites that provides accurate geographic position coordinates anywhere in the world. Signals are transmitted from satellites and compared with the time of transmition to the time that they are received by the GPS unit on the ground. Using triangulation the GPS calculates the user’s exact location. Global positioning systems have been used to make initial field measurements and monitor and manage field operations by using application maps.

Vegetation maps commonly use spectral reflectance images. The reflectance values are correlated with pest infestations. Instruments such as spectroradiometers or other hyperspectral imaging systems such as a liquid crystal tunable filter (Cambridge Research Instruments, Woburn, MA) have been used to obtain reflectance images of physiological stress in a plant. The values of these spectral responses, or signatures, must be discriminated between by constructing a spectral library. The library is constructed by isolating distinct areas of the electromagnetic spectrum that are reflected from the leaves. This response is read as a wavelength (nm). The wavelengths correspond to different energy reflectance that is related to transpiration and photosynthetic activity and used to assess the health of a plant (Barnes et al. 1996).

Healthy plants reflect in the green (~550 nm) and absorb in the red spectra (~650 nm). When plants are damaged, they often lose their ability to synthesize carbon compounds and the ability for chlorophyll to capture and absorb specific wavelengths of light. They have a much higher reflectance in the blue and red chlorophyll *a* and *b* absorption regions of the spectra and may appear "chlorotic" or yellowish. The reflectance of near infrared energy is also reduced in a damaged plant. These physiological changes are the most consistent leaf response to environmental stress. The optimum ranges of sensing a decrease in chlorophyll production and absorption are between 535-640 nm and 685-700 nm, blue and red spectra, respectively (Jensen 2005). The relationship between plant stress and pest population enables an imagery system such as infrared photography or a spectrometer to analyze correlated vegetation spectral reflectance using indices such as NDVI and infrared: red ratioing (Fitzgerald 2004).

The GPS points may be used to georeference the field imagery data when it is input into a geographic information system such as ArcGIS or ENVI (Fitzgerald 2004). Images can be positioned within a geographically corrected map and viewed spatially to observe the distribution of pests and related vegetation stress. The GIS could be used to analyze the pest interaction in the field by using spectral imagery or be integrated into a model used to predict the risk of further infestation. A user could then query the predictive model and receive a map of projected pest occurrences (Brewster 1999). Geographic information technology has been used extensively in agricultural crops to determine levels of hydration and nutrient content in the soil. Fitzgerald (2004) used hyperspectrometry to determine presence/absence of the strawberry spider mite, *Tetranychus turkestani,* on cotton.

This technology has potential to reduce information collecting and use of pesticides, but due to a lack of research on the field level, particularly in crops such as strawberries, little application has been attempted. Although GIT has been used to assess mite damage on cotton and apple trees (Dayang and Kamaruzaman 1999), cost and time constraints are still limitations to wide use of this technology (Swinton 2003).

CHAPTER 3
BIOLOGICAL CONTROL OF TWOSPOTTED SPIDER MITE, *Tetranychus urticae Koch*, WITH PREDATORY MITE, *Neoseiulus californicus* IN GREENHOUSE AND FIELD EXPERIMENTS

Twospotted spider mite (TSSM), *Tetranychus urticae* Koch, is a major pest of strawberries (*Fragaria* spp.) throughout the world (Oatman et al. 1985, Cloyd et al. 2006, Wynam et al. 1979, Walsh et al. 2002, Stonneveld et al. 1996, Huffakker et al. 1969, Sanches et al. 1979). Its management is of particular concern for growers because it has a rapid lifecycle that can be less than two weeks and sex determination is exclusively arrenotokous (males are haploid, females are diploid). Genes that are resistant to a miticide will be directly passed to offspring. Resistance can occur within a year of exposure to pesticides, making chemical control difficult (Huffaker et al. 1969, Cross et al. 2001).

Biological control is becoming a popular alternative for many strawberry growers in north Florida (Rhodes et al. 2006, Rhodes and Liburd 2006). *Phytoseiulus persimilis* is a phytoseiid mite that has been commonly used to control TSSM in north Florida strawberry fields. It is an extremely effective predator, but is classified as a type I specialist, meaning that it is genus-specific with regard to prey preference (McMurtry and Croft 1997). *Phytoseiulus persimilis* exploits its prey and perishes when the food source is eliminated. It is highly sensitive to chemical miticides and fungicides and is unable to survive in temperate climates (Escudero and Ferragut 2005, Easterbrook 1992).

Neoseiulus californicus (McGregor) is another phytoseiid that is highly effective in controlling TSSM. Its use is becoming increasingly more important in north-central Florida (Liburd et al. 2003, Rhodes and Liburd 2006). As a type II generalist, *N. californicus* provides stable and regulatory pest suppression (Croft et al.1998). Studies by Oatman et al. (1981), Escudero and Ferragut (2005), Easterbrook (1992), and Croft et al.(1998), have demonstrated that *N. californicus* is resistant to many chemical pesticides, and is able to remain viable at variable temperatures (Hart et al. 2002). Studies conducted in California and in Belgium evaluating two different rates and times *of N. californicus* releases found that TSSM populations were significantly reduced if *N. californicus* was present early in the season and if the plots had low TSSM populations (Oatman et al. 1977). Rhodes et al. (2006) found that *N. californicus* is able to maintain more consistent control of TSSM populations compared with *P. persimilis* throughout the season in north Florida strawberry fields. In that study, *N. californicus* displaced *P. persimilis* in both greenhouse and field experiments.

Biocontrol companies differ in their recommendations for release of *N. californicus*. For preventative measures, commercial distributors recommend releasing *N. californicus* at a rate of 10,000-20,000 mites/ha at 21-day intervals, whereas bi-weekly curative releases of 60,000 mites/ha are recommended for continued suppression of TSSM throughout the season. Most growers release biocontrol agents on a standard calendar basis (Greco et al. 2004).

In this study, both greenhouse and field experiments were conducted to assess the effectiveness of *N. californicus* when released at different times throughout the season in north Florida strawberries. Greenhouse trials were conducted under controlled conditions to isolate the effect of the predatory releases from environmental effects. Field experiments were conducted to validate the results from the greenhouse in a larger strawberry ecosystem. The goal of the study is to determine the phenological stage in the strawberry system when *N. californicus* is most effective in controlling TSSM infestations.

Materials and Methods

Colony

A TSSM colony was maintained in the Small Fruit and Vegetable IPM Laboratory at the University of Florida, Gainesville, FL .The colony was reared on strawberry transplants contained in one gallon polyethylene pots. Plants were kept under two 60-watt bulbs, 14L:10D photoperiod, at approximately 32°C (day) and 24°C (night) at 35% relative humidity. Plants were provided with ~250 ml of water three times per week (Figure 3-1).

Greenhouse experiment

To assess the most appropriate time to release predatory mites, four treatments were evaluated in a completely randomized design with 5 replicates. The treatments included the release of *N. californicus* at 5-day intervals: 1) early release on day 0; 2) a middle release on day 5; 3) late release on day 10; and 4) a control with no predatory releases.

The experiment was conducted in a fiberglass greenhouse at the University of Florida. Twenty strawberry plants (var. Festival) ~20 cm in height were taken from the shade house at the Entomology and Nematology Department of the University of Florida. Plants were visually inspected and hand-cleaned to ensure that there were no initial insects or mites on the leaves. Each plant was trimmed to four trifoliates. Forty TSSM motiles from the laboratory colony were distributed evenly (10 mites/trifoliate) on each plant using a probe constructed from a 0.020 stainless steel morpho minutien insect pin (Bioqip, Rancho Dominquez, CA) attached to the stem of a medical cotton swab (Figure 3-2). Each plant was contained within a mesh cage to reduce cross contamination. The cages were constructed of galvanized hardware cloth (Garden Plus, North Wilkesboro, NC) of 0.6-cm mesh, 23 gauge, which was bent into a cylinder (30.5 cm in height, 14.0 cm in diameter) and covered in no-thrips insect screen, mesh size 81µ X 81µ (Bioquip, Rancho Dominguez, CA). The mesh was attached to the cylinder with a hot glue gun (Surebonder glue gun, FPC Corp., Wauconda, IL) (Figure 3-3). The greenhouse had natural light, with no artificial light source added. The temperature averaged between 28° C (day) and 15°C (night). Plants were hand-watered with 250 ml of water every five days. Predators were purchased from Koppert Biological Systems, Romulus, MI. Their viability was tested by observing them in a Petri dish for 15 minutes to assess level of activity. They were used within 48 hours of observation. The ratio of predator to TSSM was 1:10 respectively for each release. The ratio was determined by calculating the average number of TSSM motiles from the sample leaflets in each treatment. The number of motiles found on the leaflets of each treatment were averaged, multiplied by the total number of leaflets on the plant and divided by 10. The laboratory trials were conducted three times, March 2006, December 2006, and January 2007. Each trial lasted approximately 30 days.

Sampling

Plants were sampled every five days by detaching one leaflet from each plant and counting the number of TSSM and *N. californicus* motiles and eggs under a dissecting microscope to determine the effect of the timed releases on TSSM populations.

Statistical Analysis. The data were subjected to statistical analysis using analysis of variance (ANOVA) and LSD means separation ($P < 0.05$) using the general linear model (GLM) procedure of the SAS statistical software package (SAS Institute, 2002). The average number of TSSM motiles and eggs at five-day intervals were compared across treatments. Twospotted spider mite motiles and eggs were log transformed to comply with the assumptions of the ANOVA (SAS Institute, 2002).

Field Experiment

A field experiment was established to validate our greenhouse findings. The field was located at the University of Florida Plant Science Research and Education Unit near Citra, Florida (82.17°W, 29.41°N). The areas had not been cultivated previously. Prior to planting, the field was treated with a granulated fertilizer (10-10-10) (N-P_2O_5-K_2O) at a rate of 653.3 kg/ha. Beds with black polyethylene mulch (1.6 mm thick) were laid using a Kennco™ power bedder (Ruskin, FL) and the soil was injected with methyl bromide:chloropicrin (80:20) at a rate of

326.4 kg/ha. Devrinol (napropamide) was applied between row middles at the rate of 4.32 kg/ha as a pre-emergent herbicide. Drip irrigation tape was laid under the polyethylene sheets with emitters every 20.3 cm. Strawberries, variety ‘Festival’, were planted the first week of October 2005 and 2006 on raised beds. There were six 7.3 m long rows per plot, each bed contained double rows of transplants 0.35 m apart within row and 0.35 m between row (24 plants per row). For the first two weeks overhead irrigation was run one time a day for two hours between the hours of 10:00 AM and 2:00 PM to keep the young plants cool. After establishment, the strawberry plants were irrigated by the drip tape on a timer 3 times a day for a half hour at the rate of 8.7 liters per 100 m (0.65 gal/100 ft). The strawberry plants were fertilized through the drip irrigation line once weekly with 18.5 kg of ammonium nitrate and 32.7 kg of muriate of potash per ha. In February, the nitrogen was increased to 27.1 kg/ha. A fungicide was applied throughout the season 3 times per week in a rotation of several different products (Abound® [azoxystrobin], Topsin® [thiophanate], Alliette® [aluminum tris], and Serenade® [*Bacillus subtilis*]). No insecticides were applied to the research plots. Weeds were controlled by hoeing between rows and using an s-tine around the border of the plot. Strawberries were harvested one time per week beginning in January and increased to two times per week in February to reduce opportunity for damage by birds and other vertebrates.

The experimental design in the 2005-2006 season was a randomized complete block with six replications of four treatments. In the 2006-2007 season, there were four replications of the four treatments. Each plot was 7.3 m² with an 11 m buffer zone between plots. The treatments were assigned based on plant phenology and included 1) an early release of *N. californicus* (Koppert Biological systems, Romulus, MI) 4 weeks after planting (WAP); 2) a middle release at eight WAP; 3) a late release at 12-16 WAP; and 4) a control with no releases. The late application was moved up four weeks in the 2006-2007 season due to high temperatures and faster accumulation of degree days (DD), resulting in high mite populations. In both seasons, the late release was applied at approximately 1380 DD at a 10°C threshold) (Figure 3-4a,b). During the first season (2005-2006) TSSM was introduced at a rate of 100 TSSM per plot on 27 February, because numbers present initially were extremely low.

Sampling

Systematic random samples were taken once weekly throughout the season. Each week, eight trifoliate leaves were taken from each plot, four old and four young leaves. The young leaves were taken from the upper strata of the crown and the old leaves were taken from the lower strata. We analyzed the old and young leaves separately since previous research by Croft and Coop (1998) and Sances et al. (1981) indicated that TSSM occurrence differs with foliar age. The samples were transported back to the laboratory in Zipper Seal Storage Bags© (American Value, Goodlettsville, TN) for analysis. *Neoseiulus californicus* and TSSM motiles and eggs were counted and recorded using a dissecting binocular microscope (10-20X) (Leica MZ12.5, McBain Instruments, Chatsworth, CA).

Statistical Analysis

Twospotted spider mite motiles and eggs were log transformed to standardize the variances and then subjected to an Analysis of Variance (ANOVA) and Least Significant Differences (LSD) test for mean separation ($P < 0.05$). Twospotted spider mite infestations did not occur until late in the 2005-2006 season, so the total yield data in each treatment was used to analyze differences. However, during 2006-2007, TSSM was present throughout the season and yield was calculated for each month of the harvest period. The yield for each treatment was compared using an ANOVA followed by an LSD test to separate the means. All statistical analyses were performed using the SAS system (SAS Institute Inc. 2002).

Results

Laboratory Experiments

Releases of *N. californicus* resulted in significantly lower numbers of TSSM motiles compared with the control treatment ($F = 28.69$; df = 4, 335; $P < 0.0001$) (Figure 3-5 a). By day 10, the number of TSSM motiles in the early (day 0) and the middle (day 5) *N. californicus* release were significantly lower compared with the control treatments ($F = 5.80$; df = 4, 55; $P = 0.002$). On days 15 and 25, the late treatment (day 10 release) resulted in significantly fewer TSSM motiles compared with the early and middle releases ($F = 29.83$; df = 4, 55; $P < 0.0001$) (Figure 3-5 b). The number of TSSM eggs showed a similar trend to the motiles. Significant differences between the treatments and the control occurred in total eggs accumulated throughout the trial ($F = 39.79$; df = 4, 55; $P < 0.0001$) (Figure 3-6 a). By day 25, significantly fewer eggs were found in the late treatment compared with the early and middle treatments ($F = 6.82$; df = 4, 55; $P = 0.0005$) (Figure 3-6 b).

2005-2006 Field Season

During the first 15 weeks of the season, TSSM population was extremely low. Data were insufficient to conduct a statistical analysis and to report results. Twospotted spider mite numbers increased from the time of TSSM introduction on 27 February. The release of *N. californicus* resulted in significantly fewer TSSM motiles and eggs on the old leaves in the early (16 November release) and middle (14 December release) treatments compared with the late (22 March release) and control treatments (Figure 3-7). Releases of *N. californicus* in the early and middle season maintained significantly lower populations of TSSM throughout the season while the populations in the late and control treatments continued to increase (motiles: $F = 22.84$; df = 8, 135; $P < 0.0001$, eggs: $F = 44.62$; df = 3,135; $P < 0.0001$) (Figure 3-8). The young leaves also contained significantly fewer TSSM motiles and eggs in the early and middle releases of *N.*

californicus compared with the control (motiles: $F = 20.12$, df = 8, 135, $P < 0.0001$; eggs: $F = 29.37$; df = 3, 135; $P < 0.0001$) (Figure 3-9). Similar to the results on the old leaves, the *N. californicus* releases early in the season significantly suppressed TSSM throughout the season compared with the late and control treatments (Figure 13-10). The age of the leaves did not affect TSSM eggs and motiles throughout the season ($F = 1.30$; df = 1,280; $P = 0.3$ (Figure 3-11).

2006-2007 Field Season

Significant differences in TSSM populations did occur between leaf age classes in the second season (2006-2007). There were no significant differences during the pretreatment period between any of the treatments in the old leaves (motiles: $F = 1.53$; df = 3,12; $P = 0.3$ eggs: $F = 1.05$; df = 3,12; $P = 0.4$) or in the young leaves (motiles: $F = 0.41$; df = 3,12; $P = 0.7$ eggs: $F = 1.58$; df = 3,12; $P = 0.2$). Significant differences began to appear between the treatments in the old leaves (motiles: $F = 5.79$ df = 3,12; $P = 0.01$ eggs: $F = 5.46$ df = 3,12; $P = 0.01$) and young leaves (motiles : $F = 3.43$; df =3,12; $P = 0.05$ eggs: $F = 3.95$ df = 3,12; $P = 0.04$) on 26 December. The numbers of TSSM motiles and eggs continued to decrease in plots treated with *N. californicus* throughout the season and differences between treated and untreated plots became more statistically significant on 30 January in the old leaves (motiles: $F = 20.62$ df = 3,12; $P < 0.0001$ eggs: $F = 6.03$ df = 3,12; $P = 0.01$). However, the young leaves no longer showed a significant difference between treated and untreated (motiles: $F = 1.33$ df = 3,12; $P = 0.05$ eggs: $F = 3.95$ df = 3,12; $P = 0.3$). These trends persisted throughout the remainder of the season. The old leaves contained significantly more TSSM motiles and eggs than the young leaves in the 2006-2007 season (Figure 3-12).

Overall, old leaves contained significantly fewer TSSM motiles and eggs among the early, middle and late treatments compared with the control (motiles; $F = 13.13$; df = 3, 268; $P <$

0.0001, eggs: F = 6.81; df = 3,268; P = 0.0002) (Figure 3-13). The average weekly number of TSSM motiles shows that within ca. two weeks after each release, *N. californicus* was able to reduce TSSM for the remainder of the season (Figure 3-14). No treatment differences were observed on the young leaves (motiles: F = 2.26; df = 3, 12; P = 0.08, eggs: F = 0.59; df = 3,12; P = 0.6) (Figure 3-15). The weekly average number of TSSM on the young leaves was not significantly different in any of the treatments (Figure 3-16).

Yield: In the 2005-2006 season the yield was not significantly different among treatments (F = 1.60; df =3,15; P = 0.231). The total yield for the control averaged 74.8± 7 kg/plot; early treatment averaged 62.1±4 kg/plot, middle treatment averaged 64.4± 4 kg/plot and late treatment averaged 59.4±4 kg/plot.

The average total yield through the 2006-2007 season was not significantly difference among any of the treatments (F = 0.09; df = 3,12; P = 0.97). The same pattern occurred for yield collected in January (F = 1.47; df = 6,41; P = 0.2), February (F = 4.84; df = 6,41; P = 0.5), and March (F = 1.43; df = 6,41; P = 0.98) (Table 3-1).

Discussion

Greenhouse Experiment

The greenhouse experiment demonstrated that one early season release of *N. californicus* was able to maintain low numbers of TSSM. Within five days of each release, *N. californicus* significantly reduced numbers of TSSM in the treated plants compared with the untreated plants. The early and middle treatments had low initial TSSM populations that remained low with the introduction of *N. californicus*. *Neoseiulus californicus* has also been observed in previous studies to maintain TSSM populations at low densities (McMurtry and Croft 1997). Twospotted spider mite populations naturally increased in the untreated plants to very high levels. Within five days of *N. californicus* release, the TSSM populations in the late-treated plants fell sharply and were significantly ($P = 0.05$) lower than in the early and middle treatments. The sharp decline in the late treatments, along with stable control in the early and middle treatments, indicates that *N. californicus* demonstrate a functional response with variations in prey density (Hassell et al. 1976).

Field Experiments.

The field experiments in both seasons validate our greenhouse trial that indicates the ability for *N. californicus* to control TSSM populations. The number of TSSM recorded during the 2005-2006 field season was lower than the 2006-2007 season. Several environmental factors differed between the two field seasons. The 2005-2006 season was the first time the field was used to cultivate strawberries. The lack of host plants prior to the growing season may have contributed to the absence of TSSM population. Throughout the season there were extreme temperature fluctuations. Twospotted spider mite populations are sensitive to ambient temperature. They reproduce rapidly in warm temperatures and populations have been observed to decrease at cooler temperatures (Hart et al. 2002, White and Liburd 2005). In the 2005-2006 season, there were frequent recurrent freezes throughout the season. The daily low temperature fell below 0°C for a total of 15 days in the 2005-2006 season and only for 2 days in the 2006-2007 season. The maximum daily temperature ranged from 7°C to 32°C in 2005-2006 and the range was 12°C to 32°C in 2006-2007. (Florida Automated Weather Network, 2007). The colder temperatures throughout the 2005-2006 season may have helped to suppress the TSSM population.

The 2005-2006 Season

The 2005-2006 season had low initial TSSM populations in all plots. Within one week of introduction of TSSM on 27 February, the population in the late-release treatment plots and control plots rapidly increased throughout the rest of the season. However, *N. californicus* which had been released on 16 November (early treatment) and on 14 December (middle treatment) had been able to establish in strawberry plots without TSSM prey and when TSSM populations developed, *N. californicus* was able to maintain consistent control of TSSM to a level significantly lower compared with the late-release and control treatments. We found no difference in TSSM occurrence among the age classes of leaves, most likely due to the low TSSM populations on all leaves throughout the season. The number of TSSM in the early-release and middle-release treated plots of both old and young trifoliates never exceeded 5 motiles per trifoliate. The late treatment contained higher numbers of TSSM when the late application of *N. californicus* was released. As a result of low initial predator: prey ratio and the warm temperatures late in the season, *N. californicus* never achieved control in the late treatment plots (Greco 2005). The lack of treatment effect on yield may have been due to the late season infestation. Plants are more vulnerable to yield loss due to TSSM damage during the critical vegetative growth period early in the season than they are later in the growing season (Rhodes et al. 2006, Oatman and Voth 1972 and Sances et al. 1981). Results by English-Loeb and Hestler (2004) suggest that June bearing varieties like Festival are very tolerant of TSSM damage in their first year of planting and do not show signs of vegetation or fruit loss.

The 2006-2007 Season

The second season (2006-2007) introduced several new variables. Temperatures were milder than in the previous year, and the natural vegetative cover that was left unmanaged between seasons provided abundant habitat for TSSM development. Despite the ecological challenges of the 2006-2007 season, *N. californicus* was able to achieve control of TSSM populations in all the treatments throughout the season with the early and middle release treatments being significantly lower than the late treatment in the older leaves. We did not see treatment differences in the young leaves, which may be because of the low numbers of TSSM found on the younger leaves. Twospotted spider mite aggregated on the older leaves and *N. californicus* dispersed to the areas of higher prey density. The lack of treatment effect on the young leaves is consistent with our greenhouse findings of the tendency for *N. californicus* to demonstrate a density-dependent response. However, plots of all treatments contained higher numbers of TSSM throughout the 2006-2007 season than the 2005-2006 season. Results indicate that when released at the appropriate ratio (1: 5 to 1: 10 predator: prey), one early release of *N. californicus* is able to maintain significant control of TSSM throughout a growing season. However, if initial TSSM populations are too high to achieve an appropriate ratio, as we had in the 2006-2007 season, a grower may need to apply a miticide to reduce TSSM numbers prior to an early-season release of *N. californicus* to maintain adequate season long control (Rhodes et al. 2006).

Yield was substantially lower in the 2006-2007 season than in the 2005-2006 season. This may be due to a combination of factors including a much higher TSSM population in the field early in the second season, severe bird and squirrel infestation and mid-season nutrient deficiency. No yield differences in either season could be directly attributed to TSSM damage. English-Loeb and Hestler (2004) found that reduction in strawberry yield from TSSM varies with maturity of plant and timing of TSSM infestation. Oatman and Voth (1972) also found that TSSM presence was not directly correlated with yield loss. However, Walsh (2002) and Oatman et al. (1985) found that under certain conditions, potential yield reduction due to TSSM infestation can range from 10%-29%. The relationship between TSSM and berry varies widely which suggests that it is an ecologically dynamic process and is difficult to test under field conditions.

Figure 3-1. Twospotted spider mite colony maintained in the laboratory for greenhouse experiments.

Figure 3-2. Transferring TSSM from the laboratory colony onto strawberry plants for the greenhouse trials.

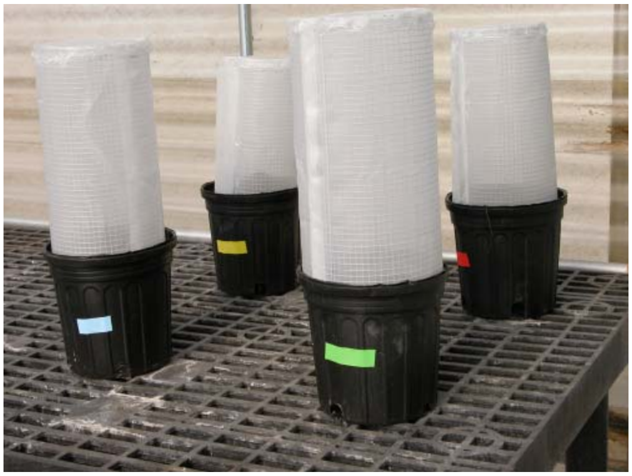
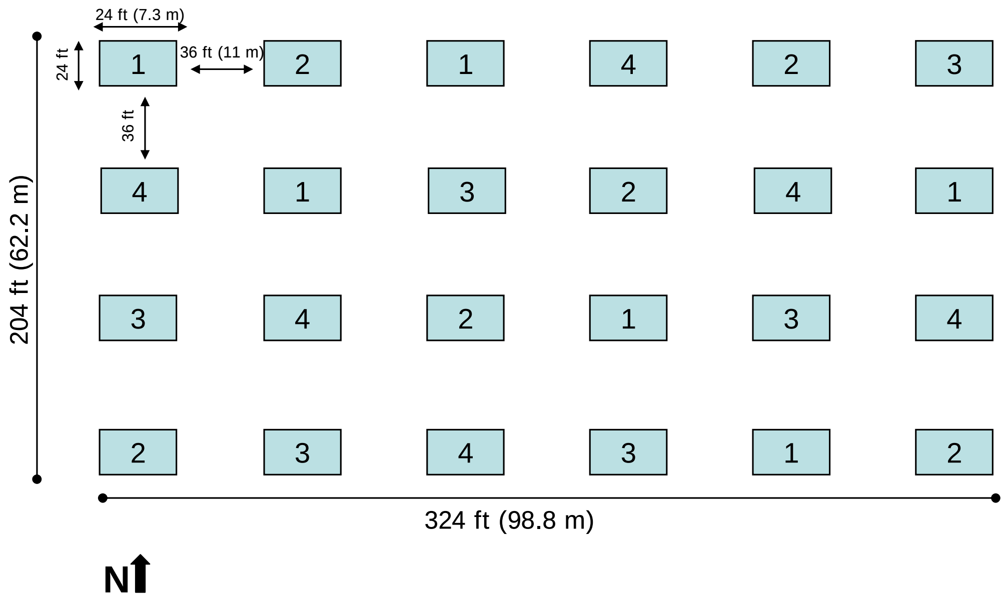
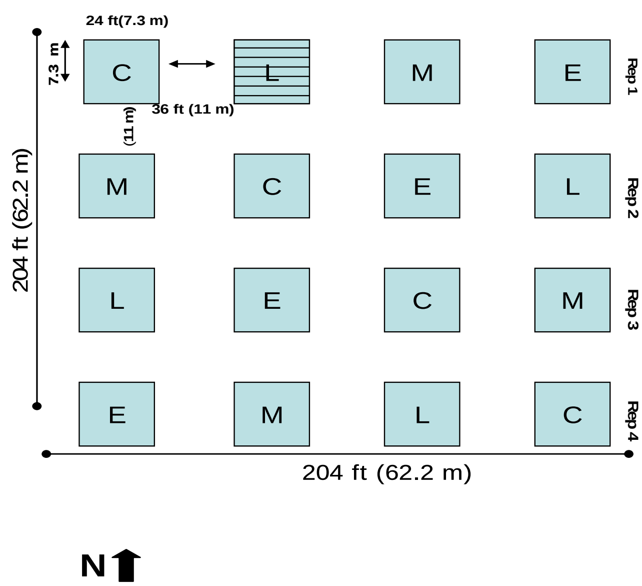


Figure 3-3. Mesh cages constructed to contain TSSM and *N. californicus* during the greenhouse trials.



A



B

Figure 3-4. Maps of the layout of the treatments in the field A) Map of strawberry field 2005-2006 field season B) Map of strawberry field 2006-2007 field season.

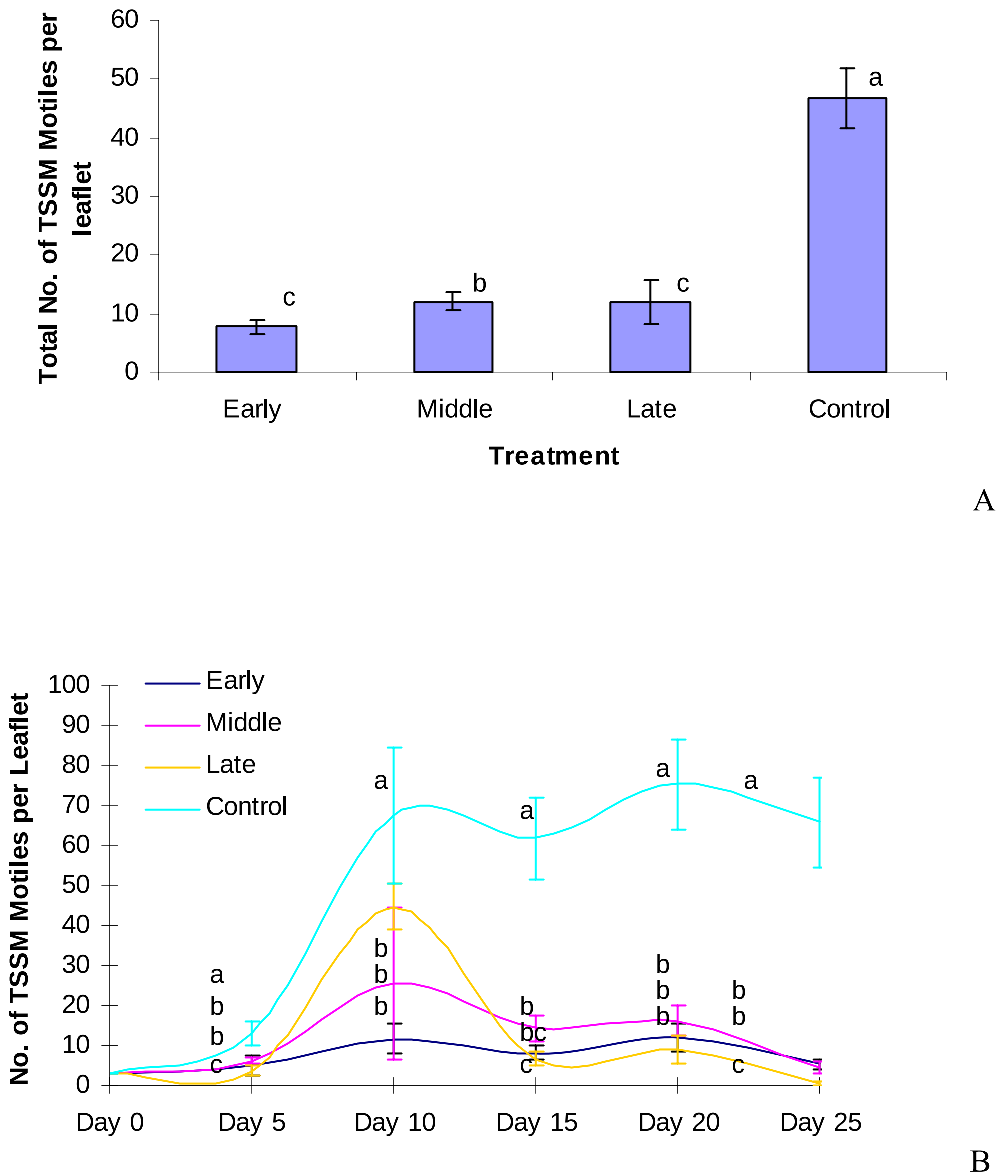
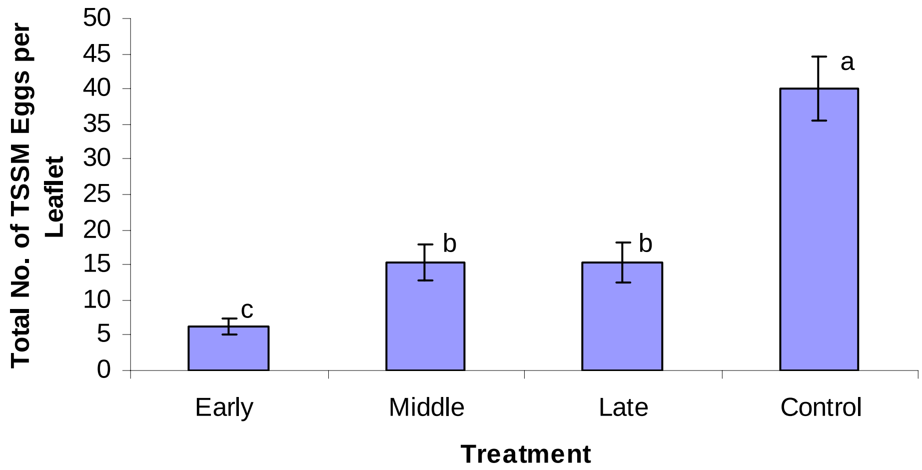
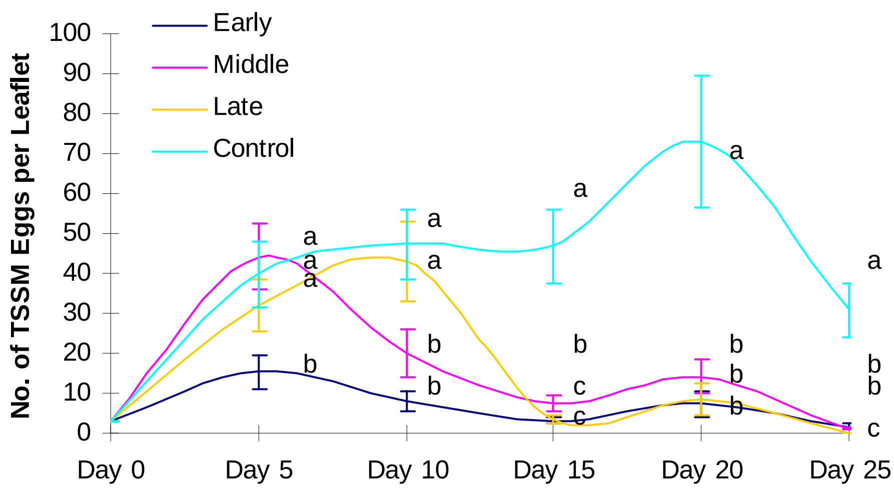


Figure 3-5. Twospotted spider mite motiles in greenhouse trials. A) Overall average number of TSSM motiles. B) Five day interval observations of TSSM motiles per leaflet for each treatment. Treatments with the same letters are not significantly different from each other at $P < 0.05$, according to LSD test performed on log-transformed data.



A



B

Figure 3-6. Twospotted spider mite eggs in the greenhouse trials A) Overall average number of TSSM eggs. B) Five day interval observations of TSSM eggs per leaflet for each treatment. Treatments with the same letters are not significantly different from each other at $P < 0.05$, according to LSD test performed on log-transformed data.

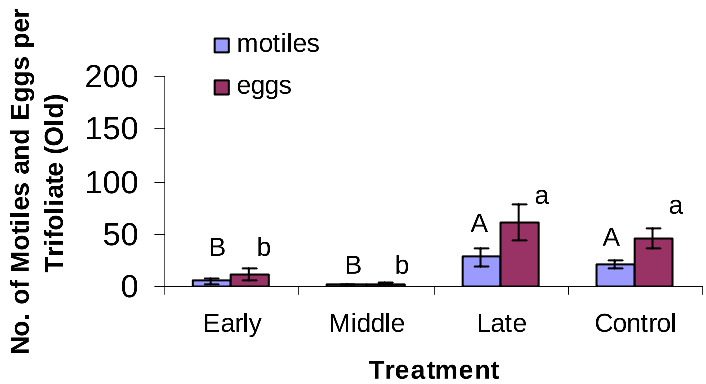


Figure 3-7. Average number of TSSM motiles and eggs per trifoliate on the old strawberry leaves 2005-2006 field season. Treatments with the same letters (A, B for motiles and a, b for eggs) are not significantly different from each other at $P < 0.05$, according to LSD test preformed on log-transformed data.

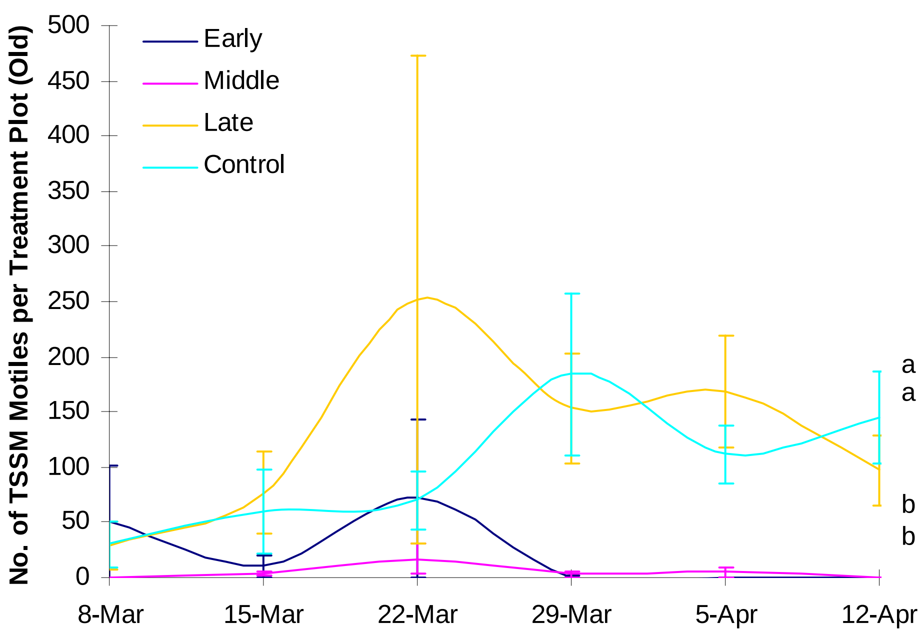


Figure 3-8. Weekly average of TSSM motiles per plot on old leaves in 2005-2006 field season. Treatments with the same letters are not significantly different from each other at $P < 0.05$, according to LSD test preformed on log-transformed data. No letters indicate no significant differences on a given date.

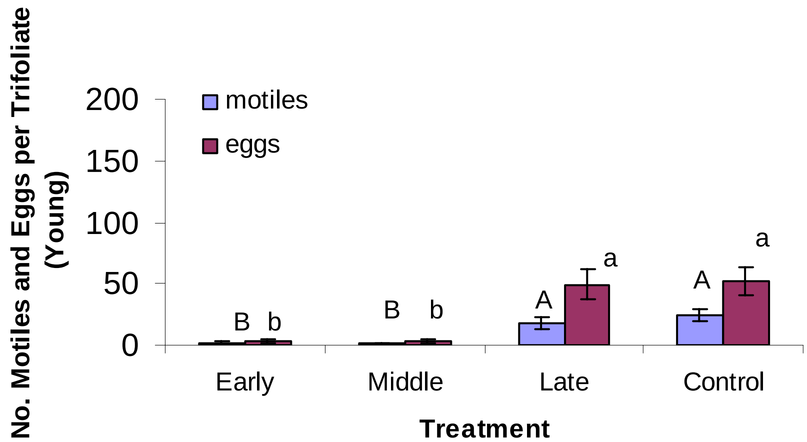


Figure 3-9. Average number of TSSM motiles and eggs per trifoliate on the young strawberry leaves 2005-2006 field season. Treatments with the same letters (A, B for motiles and a, b for eggs) are not significantly different from each other at $P < 0.05$, according to LSD test preformed on log-transformed data.

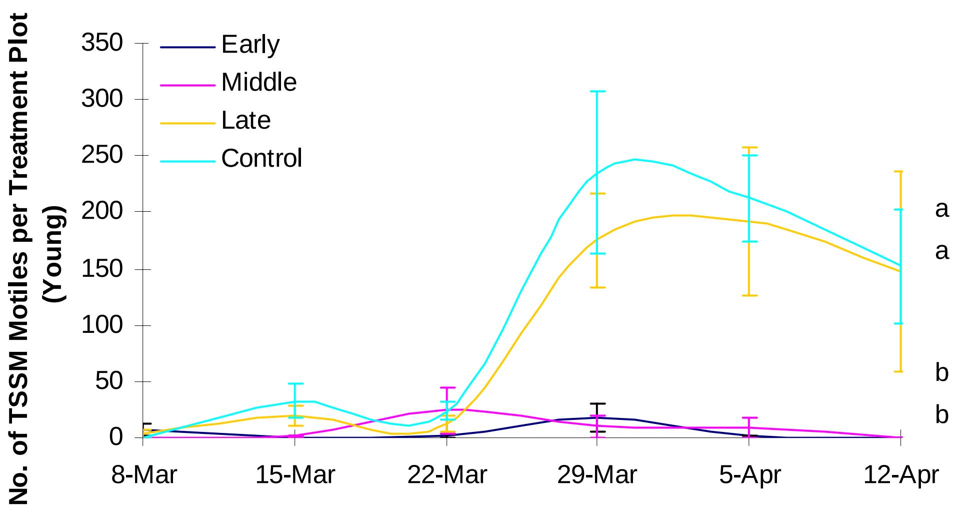


Figure 3-10. Weekly average of TSSM motiles per plot on young leaves in 2005-2006 field season. Treatments with the same letters are not significantly different from each other at $P < 0.05$, according to LSD test preformed on log-transformed data. No letters indicate no significant differences on a given data.

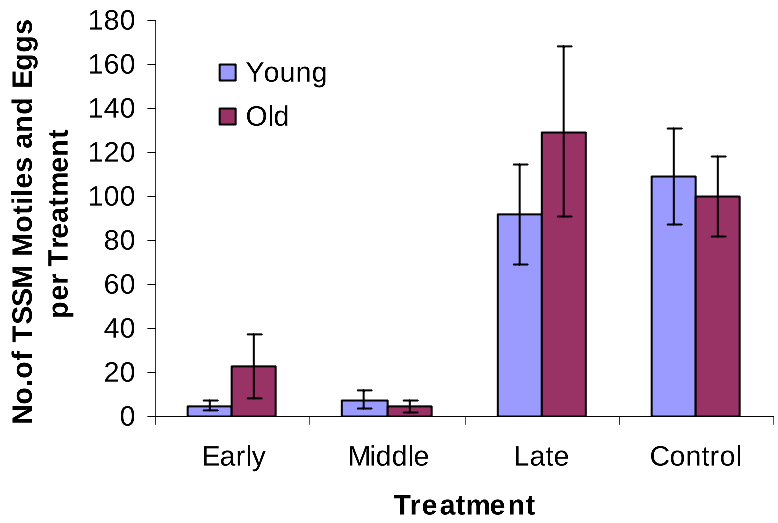


Figure 3-11. Comparison of young and old leaves 2005-2006 season. No significant ($P < 0.05$) differences between young and old leaves for any treatment.

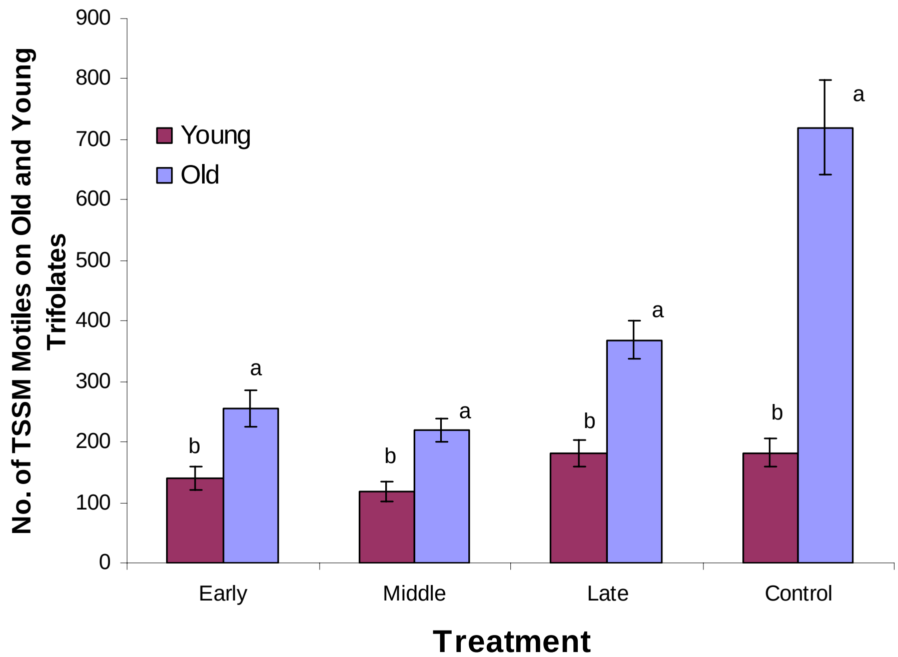


Figure 3-12. Comparison of young and old leaves 2006-2007 season within each treatment. Letters represent difference between young and old leaves at $P < 0.05$.

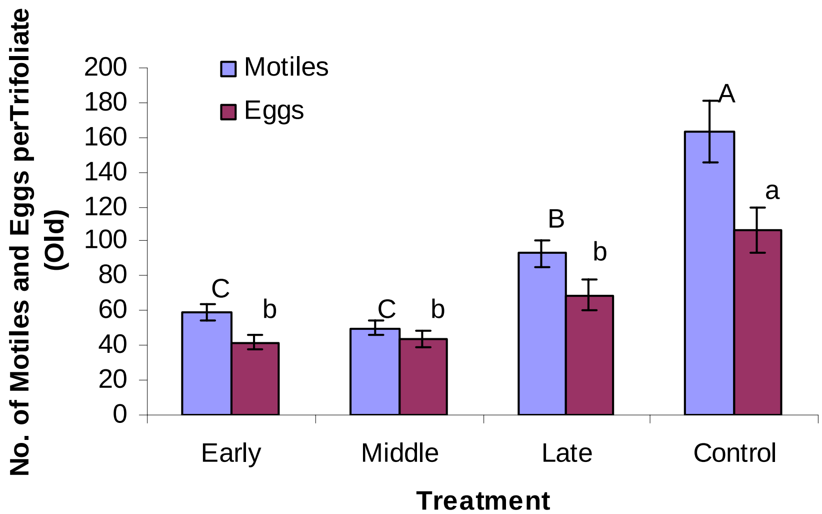


Figure 3-13. Average number of TSSM motiles and eggs on old trifoliates during the 2006-2007 season. Treatments with the same letters (A, B, C for motiles, and a, b) are not significantly different from each other at $P < 0.05$, according to LSD test performed on log-transformed data.

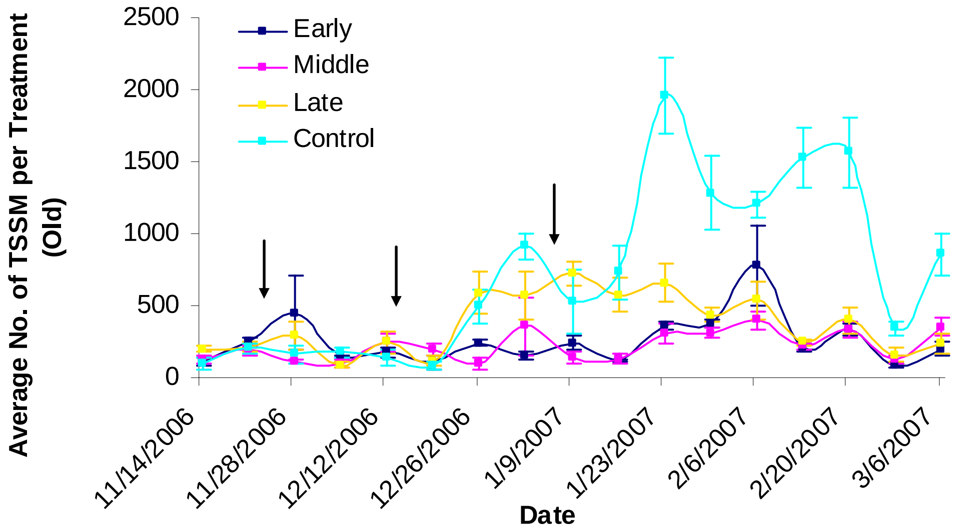


Figure 3-14. Average weekly number of TSSM motiles on the old leaves in each treatment. Arrows indicate the release dates of *N. californicus.*

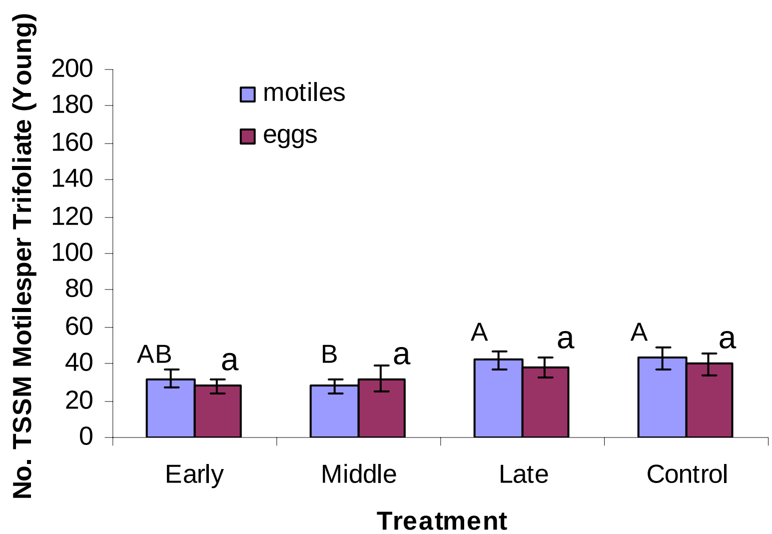


Figure 3-15. Average number of TSSM motiles and eggs on young trifoliates during the 2006-2007 field season.Treatments with the same letters (A, B for motiles and a for eggs) are not significantly different from each other at $P < 0.05$, according to LSD test performed on log-transformed data.

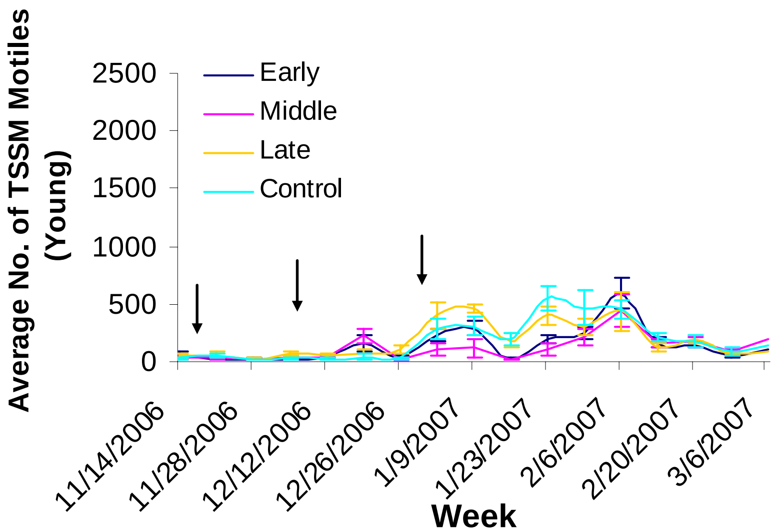


Figure 3-16 Average weekly number of TSSM motiles on the young leaves in each treatment. Arrows indicate the release dates of *N. californicus*.

Table 3-1. Strawberry yield for January, February, and March 2007, with total yield in kg/plot.

Treatment	January	February	March	Total Yield
	Mean SE			
Early	12.7 ± 1.4	10.1 ± 1.8	10.4 ± 1.4	33.0.± 0.86
Middle	8.2 ± 1.4	8.3 ± 1.8	10.0 ± 3.1	26.1± 0.63
Late	11.8 ± 10	9.8 ± 2.3	8.2 ± 3.1	29.7± 1.08
Control	12.2± 0.0	10.0 ±1.4	10.0 ± 2.7	32.2 ± 0.72

No significant (P < 0.05) differences occurred between treatment means.

CHAPTER 4
EFFECT OF *Neoseiulus californicus* RELEASES ON ARTHROPOD COMMUNITIES IN NORTH FLORIDA STRAWBERRY FIELDS

Twospotted spider mite (TSSM), *Tetranychus urticae* Koch, is a key pest on strawberries (*Fragaria x ananassa* Duchesne) in north Florida. High populations of TSSM can lead to a significant reduction in foliar and flower development, decreasing the quality and quantity of mature fruit (Rhodes et al. 2006). Because of its short life cycle and high fecundity, TSSM can increase rapidly and is able to become highly resistant to most pesticides in a short period of time (Williams 2000). Outbreaks have intensified over the last few decades due to increased use of pesticides and modern cultural practices (Huffaker and McMurtry, 1969 and Escudero and Ferragut, 2005). As traditional methods of chemical control are becoming less effective, and marketability due to chemical residue has become a social concern, growers are beginning to rely on natural enemies as an alternative to chemical management systems (Escudero and Ferragut 2005 and Rhodes et al. 2006). Approximately 40% of Florida's strawberry growers practice inundative biological control methods (Mossler and Nesheim 2002).

Field studies conducted between 1964-1980 in southern California identified ten insect species belonging to families Thripidae, Cecidomyiidae, Coccinellidae, Staphylinidae, Anthocoridae, Lygaediae, Chrysopidae, and Hemerobiidae and nine phytoseiid mite species to be natural enemies of TSSM (Oatman et al. 1985). Rondon et al. (2004) conducted laboratory studies with bigeyed bugs, *Geocoris punctipes* Say, minute pirate bugs, *Orius insidiosus* (Say), and the pink spotted lady beetle, *Coleomegilla maculata* DeGeer, to assess their effectiveness as predators for TSSM and found that many of these insects prey on TSSM, but also show a preference for other phytophagous insects.

Predatory mites in the phytoseiid family have been found to be the most effective predators in controlling TSSM and have been used successfully in glasshouses since 1968 (Kozlova et al.

2005). Two of the most commonly used phytoseiid mites are *Phytoseiulus persimilis* and *Neoseiulus californicus* (McMurtry and Croft 1997 and Cloyd et al. 2006). Oatman et al. (1972) and Kozlova et al. (2005) found that while *P. persimilis* is effective in controlling TSSM, as a Type I specialist predator, it is genus specific with regard to prey preference and tends to decimate TSSM populations altering the ecosystem. Its introduction leads to significant intraspecific competition and a disruption of natural predation on TSSM. However, Colfer et al. (2004) found that releases of generalist species of phytoseiid mites did not enhance or decrease the diversity or abundance of natural enemy populations. Studies have not been attempted to assess the effects of *N. californicus* on the diversity of native arthropods in the field.

Indices to measure diversity in ecological systems have been available for many years (Magurran 2004). These are numerically expressed to indicate the relative abundance of taxa in an ecosystem. The most commonly used indices are richness (*s*), Shannon-Weaver index (*H*') (Shannon and Weaver 1949) and evenness index (*J*) (Pielou 1966). Richness is calculated by the sum of the taxa present in a system. The Shannon index is the most often used index of diversity. This index is the negative sum of the proportional abundance of taxa multiplied by the natural logarithm of the proportional abundance of each taxon:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

Where P_i = the proportional abundance of taxon.

The Shannon Index weighs all taxa proportionately to their abundance in the sample therefore, reducing bias (Powers and McSorley 2000). Many additional indices have been developed (Magurran 2004) but the original Shannon index remains widely used, facilitating comparisons among various studies. The evenness index compares and standardizes the natural log of richness (s) and ranges from 0-1.

$$\boldsymbol{J} = \frac{\underline{H'}}{\ln s}$$

Our hypothesis is that inundative releases of *N. californicus* to control TSSM in strawberries will not negatively impact other key natural enemies and arthropod diversity in the system. The objective of this study is to identify and document common arthropods in the strawberry system and determine the impact of *N. californicus* releases on the presence and abundance of these taxa. In addition, we will measure evenness and richness of organisms in the system.

Materials and Methods

An experiment was conducted at the University of Florida Plant Science Research and Education Unit, near Citra, FL (82.17°W, 29.41°N). The experimental design was a randomized complete block with four replications and four treatments. Treatments were assigned based on plant phenology and included: 1) an early release *of N. californicus* at four weeks after planting (WAP), 2) a middle release of *N. californicus* at eight WAP, 3) a late release of *N. californicus* at 12 WAP and 4) a control (no releases). The site was prepared in beds with black polyethylene mulch (1.6mm) using a Kennco™ power bedder (Ruskin, FL). The soil was injected with methyl bromide: chloropicrin (80:20) at a rate of 326.4 kg/ha per acre two weeks prior to planting. Strawberries, variety 'Festival', were planted the first week of October on raised beds. There were 6 rows per plot, each bed contained double rows of transplants 0.35 m apart within row and 0.35m between row (24 plants per row). Strawberry plants were fertilized, weeded and sprayed with fungicides using standard commercial practices (Brown 2003). No insecticides were applied to the plots.

Preliminary study. A preliminary study was conducted one year prior to the main study to determine appropriate sample size and to evaluate the indices. Data were collected during the late season of the 2005-2006 field season at 12 WAP, to assess the season-long effect of *N. californicus* released in the early-season, mid-season, and late-season. One yellow sticky Pherocon® AM Trap (YST) (Trece, Inc., Adair, OK) constructed from a 28 cm X 23 cm yellow board with 59 one cm squares forming a grid on the board was hung on a garden stake 0.3 meters above the plants. Each trap was placed in the center row of each treatment plot. Traps were collected weekly and placed into Zipper Seal Storage Bags© (American Value, Dolgencorp, Inc, Goodlettsville, TN) and brought back to the laboratory to be examined under a dissecting microscope. Three YSTs were randomly chosen from the samples and each of the 59 one-centimeter squares on each trap was examined to determine the number of unique families found on each square. The families that were observed on each square of the YST were counted and compiled into a comprehensive list. The number of unique families represented on each square was recorded. The percentage of unique families found on each square was plotted for each square to create a cumulative frequency distribution. The optimal number of squares was determined from this distribution to be 28 squares. We used these data to create a sub-sampling for data analysis in the main study.

Main study. Based on the homogeneity of the preliminary results, field observations, and previous research by Garcia-Mari and Gonzalez-Samora (1999) indicating that *N. californicus* takes approximately two weeks to establish in a field we decided to conduct sampling at one-month intervals two weeks after each release date to obtain more complete data on the effect of

N. californicus presence in the strawberry field throughout the season. Samples were taken in the 2006-2007 field season during the early-season (1-2 months after planting), middle-season (3 to 4 months after planting) and late-season (4-5 months after planting). Individuals were identified to family or genus and counted and recorded using the same method as described in the preliminary sampling. Data were collected throughout the season and compared to determine the effect of *N. californicus* releases on interspecific insects and mites in the field with respect to plant phenology. The treatments were categorized into "treated" and "untreated" in order to isolate the effect of the *N. californicus* releases.

In order to avoid bias in the sampling techniques used to assess the diversity of arthropods present in the field, we employed four sampling methods. These methods included 1) *In situ* (visual inspection) 2) foliar sampling 3) pitfall traps and 4) yellow sticky traps.

***In situ* sampling**. Twenty-four strawberry plants from the center of the inner rows of each plot were visually inspected once weekly for two weeks during each sample period. The visual inspection consisted of a scan for 30-45 seconds per plant. This enabled us to sample the larger arthropods occurring in the field including hymenopterans and some hemipterans and coleopterans.

Foliar sampling. Four young and four old trifoliates were randomly taken from each treatment plot once weekly for two weeks between each of the three predatory releases and were placed into Zipper Seal Storage Bags© and brought back to the laboratory. The leaflets were visually inspected under the dissecting binocular microscope for leaf dwelling and minute arthropods such as the thysanopterans and hemipterans.

Pitfall traps. Traps were constructed of a white polypropylene deli containers 14 cm deep and 10.5 cm in diameter (Fabri-Kal corp. Kalamazoo, MI) filled with two cm of 10% dish soap and water solution. The traps were placed in the soil and under the black plastic mulch in one of the two center rows of each treatment plot to capture cursorial soil arthropods and soil dwellers, such as collembola, arachnids, coleopterans, and some hymenopterans (Southwood 1966). The traps were left in the field for 48 hours each week for a 2-week period between each of the three predatory releases.

Yellow sticky traps. Traps were placed in one of the two center rows of each plot at foliar height, approximately 30 cm above ground to capture hymenopteran, dipteran, and other winged arthropods. The YST were left in the field for 48 hours each week for a two-week period among each of the three predatory releases. The 28 squares (47% of the trap area) were observed for analysis.

Statistical analysis

Families of arthropods with the highest relative abundance among treatments were compared with an analysis of variance (ANOVA) and an LSD means separation to determine differences between treatments (SAS Institutes, 2002). Families that were present in numbers too low to conduct meaningful statistical analysis were recorded in a species list for each treatment, but were included in diversity calculations (Tables 4-1 to 4-4).

Shannon diversity index, evenness, and richness were calculated for each plot and for each sampling period. The results were subjected to an ANOVA and means were separated with LSD (α=0.05) to determine significant differences between the treatments.

Results

Preliminary Study. Twenty-eight 1-cm squares included 90% of the arthropod families found on the YST (Figure 4-1). Therefore, 28 squares could be analyzed to assess the diversity on the YSTs.There were no significant differences in level of diversity between any of the treatments. All measures, Shannon (F = 0.49; df = 3, 12; P = 0.7, evenness (F = 1.31; df = 3,12; P = 0.3), and richness (F = 1.05; df = 3, 12; P = 0.4) indicated that the families found in each treatment were consistent throughout the trial period and among treatments.

Main Study. In the early season (December 5-16) we compared the plots treated with *N. californicus* and untreated plots (controls). On the YST, we recorded insects from seven families: Aphididae, Cecidomyiidae, Dolichopodidae, Sciaridae, Phoridae, Thripidae, and Chalcidodoidea. However, no significant differences were found between the treated and untreated plots in five of the seven families (Figure 4-2), Aphididae (F = 0.07; df = 1, 30; P = 0.8), Cecidomyiidae (F = 0.24; df = 1, 30; P = 0.6), Dolichopodidae (F = 3.21; df = 1, 30; P = 0.9), Sciaridae (F = 0.07; df = 1, 30; P = 0.8), Muscidae (F = 0.07; df = 1, 30; P = 0.8) and Phoridae (F = 0.22; d f= 1, 30; P = 0.6). Numbers of Thripidae (F = 4.81; df = 1, 30; P = 0.04) and Chalcidodoidea (F = 8.44; df = 1, 30; P = 0.01), were higher in the plots treated with *N. californicus* than those that were untreated. In the pitfall traps, no significant differences occurred between the treated and untreated plots for the most abundant taxa, which included Collembola (F = 0.0; df = 1, 30; P = 1), Fomicidae (F = 0.95; df = 1, 30; P = 0.3), Aphididae (F = 0.29; df = 1, 30; P = 0.6) and Cecidomyiidae (F = 2.81; df = 1, 30; P = 0.1) (Figure 4-3). Data collected from YST indicate that the Shannon index (F = 0.24; df = 1, 14; P = 0.6), evenness (F = 0.0; df = 1, 14; P = 1.0), and richness (F = 1.52; df = 1, 14; P = 0.2) were not significantly different (P = 0.05) between treatments (Table 4-5). Similarly, data collected from the pitfall traps also indicated no differences in Shannon index (F = 0.0; df = 1, 14; P = 1.0), evenness (F = 0.20; df = 1, 14; P= 0.7), and richness (F = 0.01; df = 1, 14; P = 0.9) (Table 4-6).

During the mid-season (January 5-16) we recorded no significant ($P = 0.05$) differences among plot treatments on the YST for the most abundant families: Chalcidodoidea ($F = 0.41$; df = 2,29; $P = 0.7$), Muscidae ($F = 2.69$; df = 2, 29; $P = 0.1$), Aphididae ($F = 0.15$; df = 2, 29; $P = 0.9$), Sciaridae ($F = 0.73$; df = 2, 29; $P = 0.5$), Thripidae ($F = 0.78$, df = 2, 29, $P = 0.5$), Phoridae ($F = 0.33$; df = 2, 29; P = 0.7) and Cecidomyiidae($F = 0.38$; df = 2, 29; $P = 0.7$) (Figure 4-4). In the pitfall traps no significant differences among the treatment plots occurred for Collembola, ($F = 1.26$; df = 2, 29; $P = 0.3$,) Lygeaidae (*Pachybrachyus* spp.), ($F = 1.06$; df = 2, 29; $P = 0.4$) or spiders ($F = 0.46$; df = 2, 29; $P = 0.6$). Numbers of Formicidae were greater ($F = 3.39$; df = 2, 29; $P = 0.05$) in plots treated "early" with *N. californicus* than in untreated plots (Figure 4-5).

On the YSTs, the Shannon index ($F = 1.68$; df = 2, 13; $P = 0.2$), evenness ($F = 0.10$; df = 2, 13; $P = 9$), and richness ($F = 2.28$; df = 2, 13; $P = 0.1$) showed no significant ($P = 0.05$) difference among the early, middle releases, and the untreated plots (Table 4-7). Likewise, for the pitfall traps, the Shannon index ($F = 2.28$; df = 2, 13; $P = 0.1$), evenness ($F = 0.19$; df = 2, 13; $P = 0.8$) and richness ($F = 0.68$; df = 2, 13; $p = 0.5$) were not-significantly ($P = 0.05$) different among treatments (Table 4-8).

On YSTs measured late in the season (February 2-12), no significant differences among treatments occurred in the following families: Chalcidodoidea ($F = 0.31$; df = 3, 12; $P = 0.8$), Sciaridae ($F = 0.90$; df = 3, 12; $P = 0.5$), Muscidae ($F = 0.24$; df = 3, 12; $P = 0.9$), Cecidomyiidae, ($F = 1.95$; df = 3, 12; $P = 0.2$), Dolichopodidae ($F = 1.22$, df = 3, 12; $P = 0.3$), and Cicadellidae ($F = 2.02$, df = 3, 12, $P = 0.2$. (Figure 4-6). In the pitfall traps, there were no significant differences among treatments for Collembola, ($F = 0.35$, df = 3, 12, $P = 0.8$), Lygeaidae (*Pachybrachyus* spp.) ($F = 0.52$, df = 3, 12, $P = 0.7$), spiders ($F = 1.33$, df = 3, 12, $P = 0.3$), or Sciaridae ($F = 0.04$, df = 3, 12, $P = 0.99$). (Figure 4-7).

The diversity indices for the YST and the pitfall traps showed no significant differences among treatments. No differences ($P < 0.05$) among treatments were demonstrated for YST in the Shannon index ($F = 2.38$, df = 2, 13, $P = 0.1$), evenness ($F = 1.51$, df = 2, 13, $P = 0.3$), richness ($F = 2.34$, df = 2, 13, $P = 0.1$) (Table 4-9) nor for pitfall traps in the Shannon index ($F = 0.56$, df = 2, 13, $P = 0.7$), evenness ($F = 1.09$, df = 2, 13, $P = 0.4$) and richness ($F = 0.19$, df = 2, 13, $P = 0.9$) (Table 4-10).

Overall, the visual and foliar samples did not produce sufficient numbers of arthropods to conduct robust statistical analysis. However, they should not be dismissed since the foliar and visual counts revealed the presence of phenological trends and important natural predators of TSSM. Visual inspection during the mid-season revealed high numbers of *Pachybrachius* spp. and a dramatic decline in aphid population directly following an increase in syrphid fly abundance (Table 4-3). Foliar sampling indicated that as the season progressed the abundance of Coccinellids increased in all treatment plots (personal observation), as did Sixspotted thrips (*Scolothrips sexmaculats*) and Geocorid bugs (*Geocoridae* spp.) Late in the season, numbers of taxa decreased, and an increase in Sciaridae was observed (Table 4-4).

Discussion

As hypothesized, the release of *N. californicus* did not have a statistically significant effect on arthropod diversity in the strawberry system. The major insect families, which included Thripidae, Cecidomyiidae, Coccinellidae, Staphylinidae, and Lygaediae, as cited by Oatman et al. (1985) and Rondon et al. (2004), were observed. We did observe a period in the early season, which was unseasonably warm, when thrips (*Frankliniella* spp.) and Chalcidoidea were high in all plots and significantly higher in the treated plots compared with the untreated plots.

Adult thrips migrate into flowering strawberry crops during warm humid periods of the growing season. *Neoseiulus californicus* is a known predator of thrips in some crops, but *N.*

californicus cannot access them once they take shelter within the styles of the strawberry flowers (Cross et al, 2001). However, *N. californicus* may consume natural enemies, allowing thrips to flourish and indirectly increasing the number of thrips in the system (Cross et al. 2001). Increased levels of the superfamily Chalcidoidea, all of which are parasitoids of thrips, were found in significantly higher numbers in the plots with high numbers of thrips indicating a density dependent correlation.

High numbers of aphids were also found in all treatments during the early-season. Many species of aphids are known to be pests of strawberry early in the season when the climate is warm, but populations decrease rapidly as natural predators establish in the system (Jones 1976). Our observations of decreasing numbers of aphids in conjunction with increased syrphid fly populations are consistent with studies conducted in north Wales showing that syrphid flies are effective predators of aphids and can cause considerable reduction in aphid numbers (Cross et al. 2001). Foliar sampling indicated that as the season progressed the abundance of sixspotted thrips (*Scolothrips sexmaculats*) and *Geocoridae* spp. increased in all treatment plots. These findings are consistent with previous research by Jones (1976) indicating that predation tends to increase gradually throughout the season. At the time of the late-season, sampling, arthropod numbers had declined in all treatments and Sciaridae dominated in all plots. During the mid to late-season considerable damage to the fruits from bird and squirrel feeding was observed which may have been a factor in the increase of Sciaridae. Sciaridae have been shown to be attracted to damaged plant tissue as sources of food and habitat (Jones 1976).

Although we found very few statistically significant differences between treatments during the early sampling periods, we recorded differences in the arthropod assemblage throughout the season among all treatments. The high level of richness and insect diversity in the strawberry system may be a key factor that reduces the effect of *N. californicus* releases on the structure of the strawberry system (Jones 1976, Powers and McSorley 2000). One of the natural "services" provided by ecosystems is the natural control of pest and invasive species (Klein et al. 2006). The majority of arthropods in the strawberry system are generalist feeders. Therefore, abundance of alternate food sources may mitigate disruption by an introduced predator (Cross et al. 2001). The dynamics of the arthropod assemblage seem to be more highly related to plant phenology and ambient weather than to interspecific competition.

Table4-1. Cumulative number of each taxa found in each treatment in the three sampling periods in yellow sticky traps.

December

	E	M	L	C
Acanalonidae	2	1	2	1
Aleyrodidae	2	16	26	14
Aphididae	383	643	579	539
Bethylidae	—	—	1	—
Bibionidae	—	5	5	—
Cecidomyiidae	11	10	6	9
Chalcidoidae	34	59	83	91
Chrysomelidae	—	—	—	1
Cicadellidae	2	11	17	5
Coccinellidae	—	—	1	—
Cucujidae	—	1	11	—
Dolichopodidae	7	17	4	3
Drosophilidae	3	—	—	—
Ichneumonidae	—	—	4	4
Lepidoptera	1	1	—	1
Muscidae	8	7	9	30
Nitulidae	—	—	2	—
Phloeothripidae	3	2	4	3
Phoridae	3	16	7	7
Platygatroidae	1	—	—	—
Psychodidae	1	3	10	—
Sciaridae	5	5	50	10
Staphylinidae	—	2	—	—
Thripidae	39	39	—	23

January

	E	M	L	C
Acanalonidae	4	1	3	3
Aleyrodidae	2	17	3	1
Aphididae	12	10	5	11
Bethylidae	—	2	—	—
Bibionidae	3	1	—	1
Braconidae	2	1	3	5
Cecidomyiidae	10	8	14	4
Chalcidoidae	61	66	78	53
Chrysomelidae (Alticinae spp)	1	1	—	1
Cicadellidae	3	3	4	2
Coccinellidae	4	3	—	2
Dolichopodidae	2	4	4	5
Drosophilidae	4	1	2	1
Ichneumonidae	4	—	3	—
Leiodidae	—	1	1	1
Lepidoptera	—	—	—	1
Lygaeidae (Pachybrachius spp)	3	6	—	1
Muscidae	23	13	15	15
Nitulidae	1	—	1	—
Phloeothripidae	1	—	—	—
Phoridae	10	14	40	9
Psychodidae	5	2	—	1
Sciaridae	23	17	24	8
Staphylinidae	—	2	1	—
Syrphidae	—	—	1	1
Tachinidae	5	8	2	3
Thripidae	16	17	9	10

February

	E	M	L	C
Aleyrodidae	—	4	—	—
Aphididae	6	6	1	3
Cecidomyiidae	4	6	8	1
Chalcidoidae	33	26	33	31
Chrysomella	2	2	2	—
Cicadellidae	7	2	1	7
Coccinella	3	—	1	6
Dolichopodidae	10	7	3	4
Drosophilidae	1	—	—	—
Ichneumonoidae	3	8	4	4
Ichneumonoidea (Braconidae spp)	—	1	—	—
Lepidoperera spp.	1	—	—	—
Muscidae	17	14	14	19
Nitulidae	2	1	2	—
Lygaeidae (Pachybrachius spp)	1	4	5	2
Phoridae	2	6	1	2
Psychodidae	—	—	2	1
Sciaridae	52	48	94	48
Araneida	1	1	1	3
Staphylinidae	2	1	1	1
Syrphidae	3	2	1	1
Tachinidae	2	4	4	2
Thripidae	2	8	5	2

E = early treatment, M = middle treatment, L = late treatment and C = control.

Table 4-2. Cumulative number of each taxa found in each treatment in the three sampling periods of the pitfall traps.

December	E	M	L	C
Chrysomelidae (Alticinae spp.)	—	1	1	—
Aphididae	19	9	5	9
Apoidea	—	—	1	1
Bibionidae	2	—	—	—
Cecidomyiidae	8	7	7	17
Chalcidodea	—	4	3	2
Chironomidae	—	2	—	—
Chrysomelidae	1	—	—	—
Cicadellidae	1	—	1	—
Coccinelidae	1	—	—	—
Collembola	41	21	23	31
Cucujidae	1	—	—	—
Drosophilidea	—	—	—	2
Eliteridae	—	—	1	—
Formicidae	35	26	6	42
Ichnueumonidae	—	1	—	—
Lepidoptera	—	—	—	2
Miridae	1	—	—	—
Muscidae	2	2	—	—
Mutillidae	1	—	—	—
Nematode	—	2	—	—
Phloeothripidae	—	1	1	—
Phoridae	2	3	2	2
Scariaridae	—	1	1	2
Araneida	1	2	5	2
Staphylinidae	3	1	1	1
Tettigoniidae	—	2	—	—
Thripidae	1	5	3	1

January	E	M	L	C
Aphididae	11	2	6	5
Cecidomyiidae	4	—	6	2
Chalcidiodea	1	2	1	—
Cicadelidae	—	1	—	—
Collembola	50	85	132	65
Gryllidae	1	—	—	—
Cucjidae	1	—	1	—
Drosophilidae	—	2	—	1
Formicidae	44	9	2	2
Lygaeidae (Pachybrachius spp.)	18	21	47	29
Miridae	1	—	—	—
Muscidae	—	—	1	—
Nitulidae	—	—	1	—
Phoridae	—	—	1	—
Scariaridae	4	2	1	2
Scollidae	—	—	1	—
Araneida	4	3	2	2
Staphylinidae	1	—	—	—
Tettigoniidae	1	—	—	—
Thripidae	2	2	2	4

February	E	M	L	C
Aphididae	1	—	1	1
Ichneumonoidea (braconidae)	—	—	1	—
Cecidomyiidae	—	—	—	1
Chalcidoidae	1	1	—	—
Chrysomellidae	—	—	1	—
Collembola	10	24	13	39
Formicidae	1	1	11	2
Lygaeidae	1	1	—	3
Lygaeidae(immature)	3	—	—	5
Muscidae	—	—	2	—
Scariaridae	9	7	14	15
Araneida	3	1	1	—
Staphalenidae	—	—	1	2
Thripidae	1	—	—	—
Vespidae	—	1	—	—

E = early treatment, M = middle treatment, L = late; C = control.

Table 4-3. Cumulative numbers of each taxa in the three visual sampling periods

	December				January				February			
	E	M	L	C	E	M	L	C	E	M	L	C
Tettigoniidae	3	1	3	3	—	—	—	—	4	7	2	4
Apoidea	6	9	5	3	—	—	—	—	0	1	3	2
Syrphidae	3	3	1	4	16	13	6	8	2	1	—	1
Araneida	—	—	1	1	2	1	8	4	2	—	—	—
Lepidoptera	1	1	1	1	—	—	—	—	—	—	1	—
Muscidae	1	2	2	2	—	—	—	—	1	—	1	2
Chrysomelidae	1	—	—	—	—	—	—	—	1	—	—	1
Coccinelidae	1	—	—	—	1	2	—	—	1	—	—	—
Lygaeidae												
(Tristicolor spp)	—	—	—	—	—	—	—	—	—	—	—	1
(pachybrachius spp)	—	—	—	—	8	2	6	12	1	—	—	1
Sciaridae	—	—	—	—	—	—	—	—	52	28	39	14
Cicadellidae	—	—	—	—	—	—	—	—	—	1	—	—

E = early treatment, M = middle treatment, L = late treatment and C = control.

Table 4-4. Cumulative numbers of each taxa in the three foliar sampling periods

	December				January				February			
	E	M	L	C	E	M	L	C	E	M	L	C
S.sexmaculatus	—	1	2	2	6	3	10	10	4	1	3	2
Aphididae	43	64	28	40	—	1	4	4	1	1	—	—
Aleyrodidae	29	22	22	2	2	2	2	2	—	—	—	3
Thripidae	7	2	1	1	14	4	14	14	—	3	—	3
Chalcidoidae	—	—	1	—	—	—	—	—	—	—	—	—
Lepidoptera	1	—	—	—	—	—	—	—	6	6	2	9
Syrphidae (eggs)	—	—	—	—	9	8	9	9	4	5	10	15
Lygaeidae (Geocoridae spp)	—	—	—	—	—	—	—	—	3	—	—	9

E= early treatment, M=middle treatment, L=late treatment and C=control.

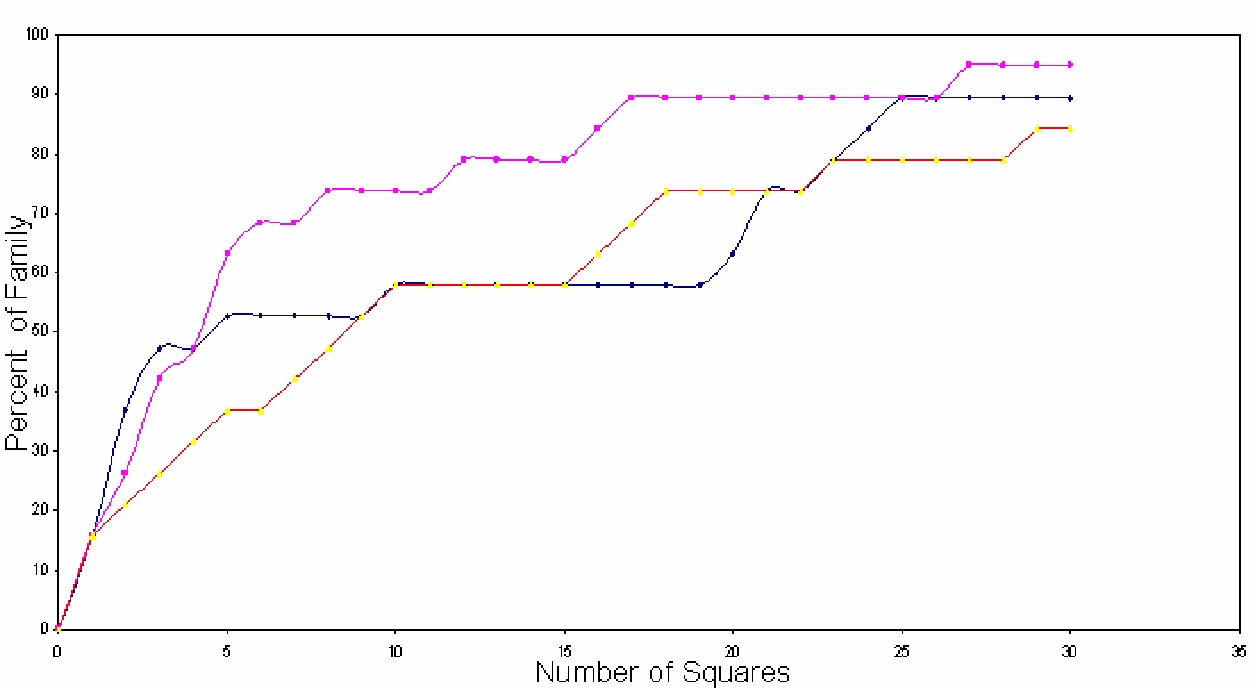


Figure 4-1. Cumulative percent of families found on yellow sticky trap 1-cm squares.

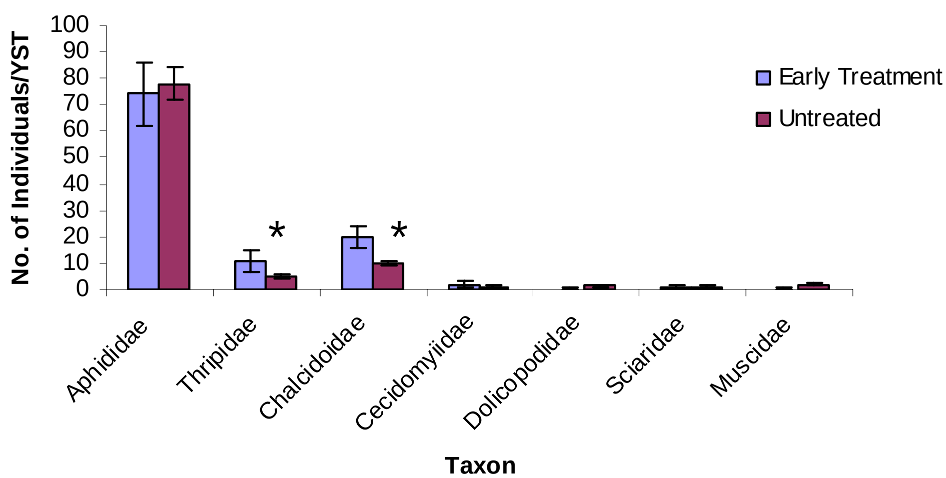


Figure 4-2.The most abundant families found on early-season yellow sticky traps between the treated and untreated plots. * indicates significant differences ($P < 0.05$) between treated and untreated.

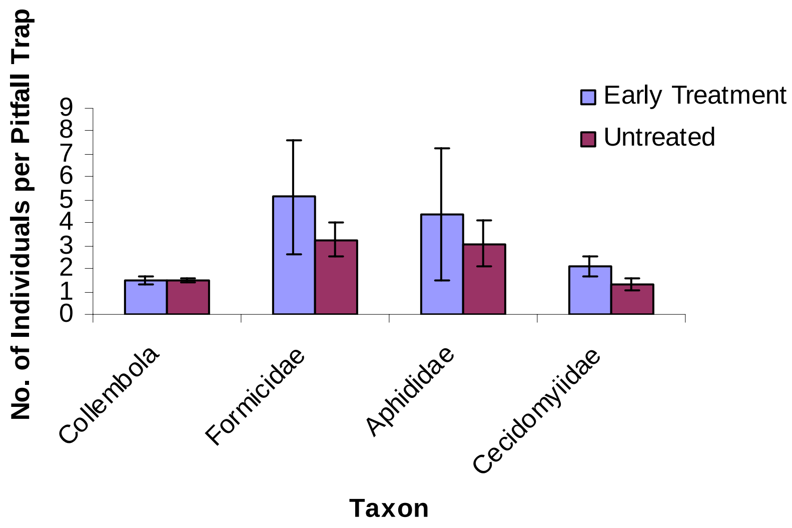


Figure 4-3. The most abundant families found in the early-season pitfall traps in the treated and untreated plots. No significant ($P < 0.05$) differences were found among treatments.

Table 4-5. Mean values of diversity indices in plots in early-season yellow sticky traps.

Treatment	**Shannon**	**Richness**	**Evenness**
		Mean± SE	
Early	1.06±0.50	11.5±1.0	0.44±0.05
Untreated	1.13±0.07	13.3±1.0	0.44±0.03

No significant ($P < 0.05$) differences with treatment.

Table 4-6. Mean value of diversity indices in plots in early-season pitfall traps

Treatment	**Shannon**	**Richness**	**Evenness**
		Mean± SE	
Early	1.7±0.15	8.0±0.40	0.8±0.06
Untreated	1.7±0.06	7.9±0.65	0.8±0.04

No significant ($P < 0.05$) differences with treatment.

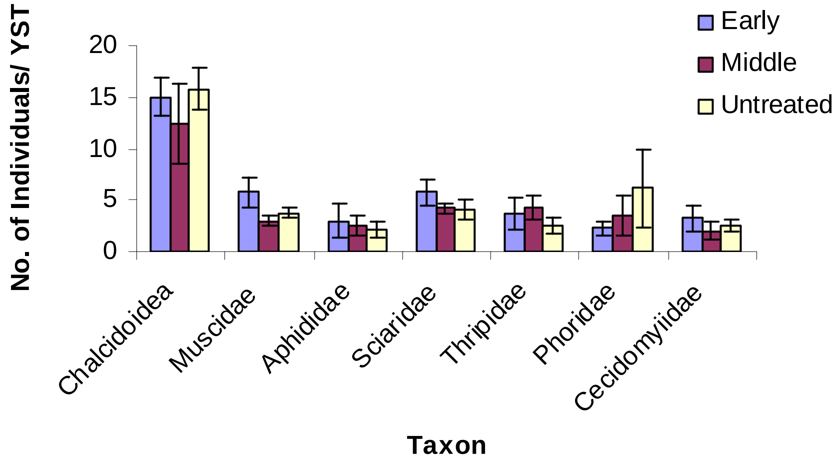


Figure 4-4. The most abundant families found in the mid-season yellow sticky traps among the early, middle and untreated plots. No significant ($P < 0.05$) differences among treatments.

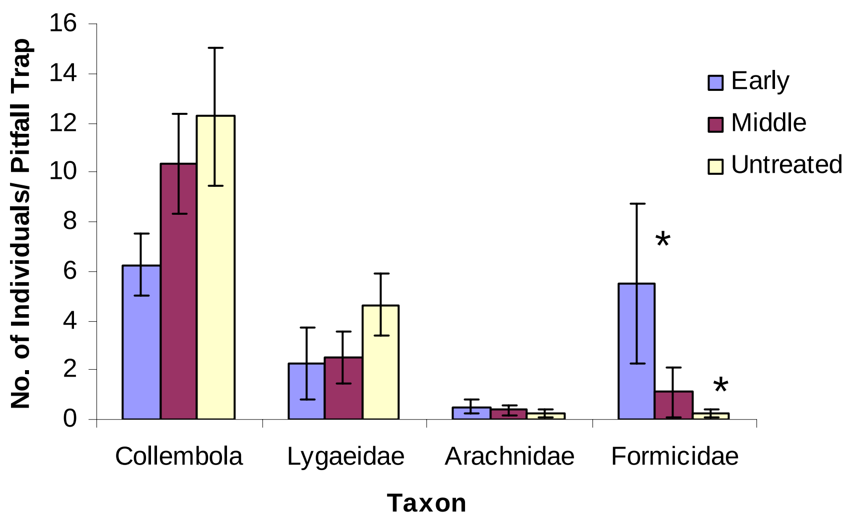


Figure 4-5. The most abundant taxa found in the mid-season pitfall traps between the early, middle, and untreated plots. * indicates significantly ($P < 0.05$) higher numbers of Formicidae in the early-release treated plots than the untreated plots.

Table 4-7. Mean values of diversity indices in plots in mid-season yellow sticky traps.

Treatment	Shannon	Richness	Evenness
		Mean± SE	
Early	2.2±0.10	15.5±1.3	0.8±0.02
Middle	2.2±0.03	14.8±0.9	0.8±0.02
Untreated	2.1±0.05	13.3±0.5	0.8±0.02

No significant ($P < 0.05$) differences with treatment.

Table 4-8. Mean values of diversity indices in plots in mid-season pitfall traps.

Treatment	Shannon	Richness	Evenness
		Mean± SE	
Early	1.5±0.20	8.0±1.2	0.7±0.05
Middle	1.3±0.12	7.5±1.3	0.7±0.08
Untreated	1.3±0.09	6.6±0.5	0.7±0.04

No significant ($P < 0.05$) differences with treatment.

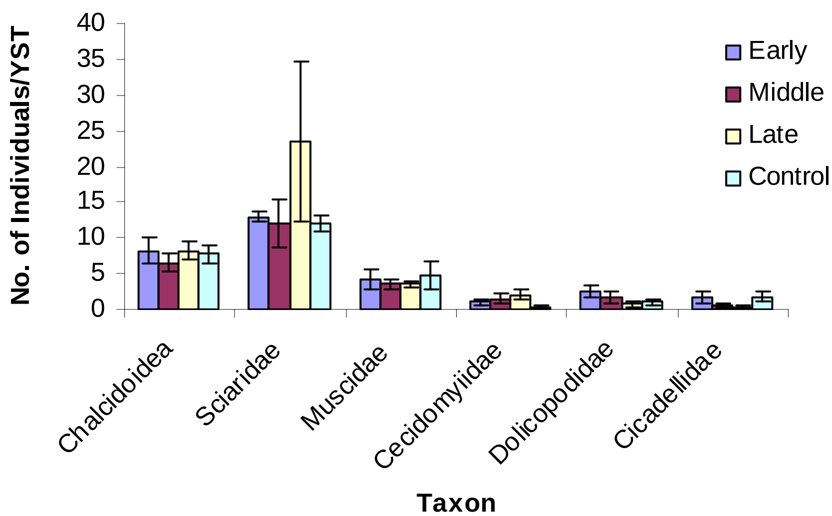


Figure 4-6. The most abundant families found in the late-season yellow sticky traps in early, middle, late, and control plots. No significant ($P < 0.05$) difference among treatments.

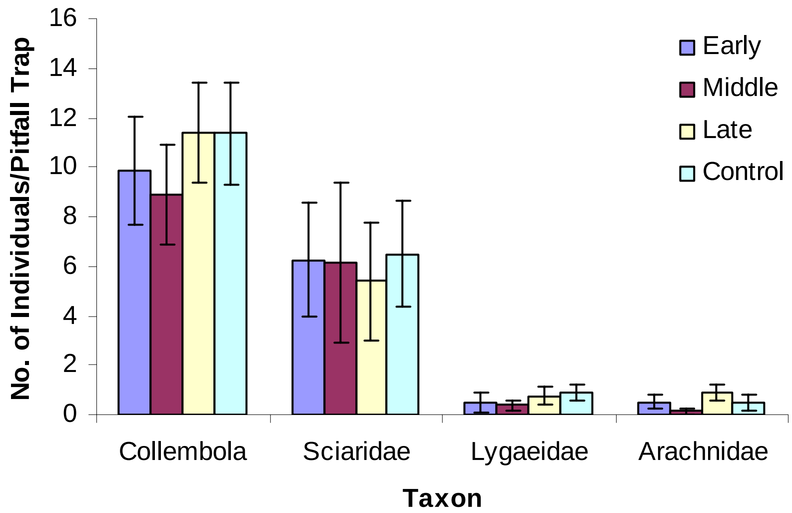


Figure 4-7. The most abundant families found in the late-season pitfall traps in the early, middle, late, and control plots. No significant ($P < 0.05$) difference among treatments.

Table 4-9. Mean value of diversity indices in plots in late-season yellow sticky traps.

Treatment	Shannon	Richness	Evenness
		Mean± SE	
Early	1.95±0.10	0.75±0.03	12.8±0.69
Middle	2.16±0.12	0.85±0.06	12.8±0.48
Late	1.68±0.20	0.69±0.10	11.3±0.25
Control	1.83±0.73	0.77±0.91	11.0±0.10

No significant ($P < 0.05$) differences with treatment.

Table 4-10. Mean value of diversity indices in plots in late-season pitfall traps.

Treatment	Shannon	Richness	Evenness
		Mean± SE	
Early	1.3±0.10	0.6±0.03	9.3±0.85
Middle	1.3±0.02	0.6±0.01	8.8±0.30
Late	1.5±0.90	0.7±0.90	9.0±1.10
Control	1.4±0.13	0.6±0.10	8.5±0.50

No significant ($P < 0.05$) differences with treatment.

CHAPTER 5
HYPERSPECTRAL IMAGERY DETECTION FOR TWOSPOTTED SPIDER MITE DAMAGE IN STRAWBERRIES

Twospotted spider mite (TSSM) *Tetranychus urticae* Koch is one of the most economically important pests in strawberries (*Fragaria* spp.) High infestations of TSSM have shown to cause leaf chlorosis, cessation or stimulation of plant growth, and reduction in yield (Oatman et al. 1985, Cloyd et al. 2006, Wynam et al. 1979, Walsh et al 2002, Stonneveld et al. 1996, Huffakker et al. 1969, Sanches et al.1979). As TSSM feed on the underside of the leaf, they pierce the chloroplast containing palisade and spongy parenchyma cells in the mesophyll layer at a rate of 18-22 cells/minute (Jeppson et al. 1975, Sanches et al 1979). Chlorotic symptoms appear when TSSM consume the chloroplasts, which contain chlorophyll, an essential pigment that absorbs solar radiation for photosynthesis (Smith and Smith 2003, Kiekliewicz 1985). A decrease in radiant energy use (REU) due to the consumption of the chloroplasts eventually leads to reduction in vegetative growth and yield (Reddall et al. 2004, Sances et al. 1981, Kielkiewicz 1985).

The chloroplasts are organelles within the mesophyll cells that contain pigments and are the primary catalyst for the light reaction of photosynthesis (Meyer et al. 1973, Smith and Smith, 2003). Each family of pigments (carotenoids, phycobilins, and chlorophylls) absorb strongly in specific wavelengths (µm) of the radiant light spectrum. Chlorophyll *a* and *b* are the predominant pigments located in the chloroplasts. They produce the green reflectance of healthy vegetation and absorb blue (0.43 µm and 0.45 µm) and red (0.66 µm and 0.65 µm) wavelengths (Meyer et al. 1973). The light absorbed and stored by the pigments in the chloroplasts is the principal source of energy for photosynthesis. Twospotted spider mite penetration and salivary injection into these cells dissolves and digests the structures and inhibits the function of the chloroplasts (Kielkiewicz 1985). Cellular injury disrupts the ability for these pigments to absorb the specific wavelengths in the radiant energy spectrum, making the leaves appear discolored and chlorotic (Meyer 1973, Jensen 2005). The resulting spectral variation in the visual wavelengths is one of the most consistent indicators of vegetation health. Leaf stress is evident in variations of the 0.55-0.64 µm and ~0.7 µm wavelengths (Jensen 2005).

Twospotted spider mite damage also affects the ability of a plant to absorb and reflect near infrared (NIR) wavebands. Plants have evolved to reflect highly in the infrared wavebands (0.7-1.1 µm) to protect the leaf tissue from absorbing too much radiant heat that may lead to the denaturing of essential proteins. The structure of the mesophyll layer regulates the reflectance of NIR energy by the internal scattering at the cell wall-air interface (Jensen 2005). Variations in the reflectance in the region between the red and NIR, known as the “red edge” (~ 0.7 µm), indicates plant stress that is often due to dehydration and cellular damage (Jensen 2005, Lillisand at al. 2003).

The correlation between TSSM damage and reduced photosynthetic function of strawberry plants has been documented extensively (Reddall et al. 2004, Iatrou et al. 2004, Kielkiewicz 1985, Sanches et al. 1979, Sanches et al. 1981). However, the effect of physiological inhibitions on spectral response of strawberry leaves has not been investigated. Studies by Fitzgerald (2004) and Landeros et al. (2004) have demonstrated that it is possible to detect spectral changes regarding the presence/absence of pest damage in agricultural fields in larger field crops like cotton.

Our goal is to identify specific regions of the reflectance spectrum that are affected by TSSM on strawberry leaves and develop a relationship between quantifiable levels of TSSM infestation and spectral foliar response. This study will provide a foundation for the development of models to better understand TSSM interactions with strawberry plants. The information can be integrated with other technologies and tactics into a set of tools known as geographic information technology (GIT), and used to identify and monitor spectral data from a field that can be analyzed to create maps of vegetative condition in relation to pest damage (Brewster 1999, Dayang and Kamaruzaman 1999). Geographic information technology is associated with site-specific Precision Insect Pest Management (PIPM) programs. Early identification of TSSM distribution before damage is visible in the field is the initial step to develop a management program that will have the potential to reduce economic and ecological costs and increase production in strawberries (Dayang and Kamaruzaman 1999).

Materials and Methods

Field Plots

Our experimental plots were located at the University of Florida Plant Science Research and Education Unit, near Citra, FL (82.17°W, 29.41°N). There were 16 plots of varying levels of TSSM infestation. Each plot was 7.3 m^2 with 11 m buffer between each plot. Within each plot there were 6 rows of strawberries. Each row had a double row of transplants 0.35 m apart within row and 0.35m between row. Strawberries were planted the first week of October, on raised beds over black plastic mulch and were fertilized, weeded and sprayed with fungicides using standard commercial practices (Brown 2003).

Sampling

Twenty mature leaflets of TSSM-infested strawberry plants were taken randomly one time per week from each of the 16 plots. We collected samples for four weeks between December 2006 and January 2007. Leaflets were taken back to the Small Fruit and Vegetable IPM Laboratory at the University of Florida, Gainesville, FL and analyzed under a dissecting microscope to determine the number of TSSM per leaflet. Each individual leaflet was then put into a Zipper Seal Storage Bag © (American Value, Dolgencorp, Inc, Goodlettsville, TN) and labeled with the approximate number of mites found on the leaflet. The leaflets were then scanned with a Fieldspec® 3 spectroradiometer (Analytic Spectral Devices, Inc., Boulder, CO) at the Soil Sciences Laboratory at the University of Florida. The samples were scanned within two hours after collection to reduce dehydration and foliar damage.

Spectral Scanning

During the scanning process, the spectrometer was re-calibrated with a white reference every 10 minutes to ensure an accurate spectral reading. Two separate areas of 3.2 cm^2 were scanned on the adaxial (top) side of each leaflet with a spectral lens that was 2 cm diameter. Each of the selected areas was rotated 90° and rescanned to increase spectral accuracy. The spectral signature of each wave band between 360 nm and 2480 nm from each reading was downloaded to a CSV (Comma Separated Values) file. The spectral response was "re-sampled", and every 10 nm were averaged to condense the 2000 wavebands into 200 wavebands to facilitate statistical analysis. The data were then derivative transformed, using first derivative transformation with second order smoothing to finalize the data (Statsoft, Inc. 2005). This procedure facilitated the detection of variations in the slope of the spectral curves between wavelengths. The data were exported into an Excel spreadsheet displaying the reflectance value of each sample in each of the 200 wavebands.

Data Analysis

Raw data were subjected to a linear regression analysis to assess the correlation and prediction accuracy of mite numbers with respect to reflectance values. The results were cross-validated by separating the data into groups of test data and validation data. The test data were subjected to the regression analysis and then the validation data were applied to the test model to assess the strength of the model (SAS Institute, Inc. 2002). The data were then subjected to a Principal Component Analysis (PCA) to condense the data using SPSS software (SPSS Inc.

2004). Due to high variability in the data revealed by the PCA, we chose to focus on regions of the spectrum that are directly related with TSSM damage rather than utilizing the entire radiant spectrum.

Determining Categories of TSSM Infestation. To better describe the correlation between mite density and leaflet reflectance in a practical representation for growers, we divided the leaves into three categories: no mites (0 mites); low/moderate infestation (10-60 mites/leaflet); and high infestation (≥ 70 mites/leaflet). A Linear Discriminate Analysis (LDA) of the first 5 principal components was performed. Discriminate Analysis is a multivariate statistical technique that is commonly used to build predictive models of group discrimination based on observed predictor variables and classify each observation into one of these groups:

$$d_{ik} = a + b_{1k}X_{i1} + b_{2k}X_{i2} \ldots b_{kn}X_{in} + e_i$$

d_{ik} is the value of the *k*th discriminate function for the *i*th case
a constant
b_{ik} is the value of the *i*th coefficient of the *k*th function
x_{i1} is the value of the *i*th case of the *j*th predictor
e_i is an error term

The objectives of LDA are to investigate differences between groups and create categories maximizing the variance between groups and minimizing the variance within groups (McCune and Grace 2004). Data were then cross-validated using the PROC DISCIM CROSSVALIDATE function in the SAS statistical program for the three categories to determine if the variation of the spectral differences will significantly discriminate among the levels of mite infestation (SAS Institute, 2002).

The data were first subjected to a one-way ANOVA (SPSS Inc. 2004) to reveal regions of the spectrum with the greatest statistical differences ($P > 0.0000001$). Discriminate Analysis was then conducted on the average of near infrared (NIR) wavelengths (700nm-1000nm), and in the green wavelengths (560nm-580nm). Finally, to test the application of the spectral analysis to commercially available satellite systems, the LDA was performed on NIR and green wavelengths at the spectral resolution of SPOT 5 (SPOT Image Corporation, Chantilly, VA) and Quickbird (GeoVAR, Katy, Texas) satellites.

The results of the LDA were evaluated in an error matrix to assess the level of accuracy (Jensen 2005). We used four measurements of accuracy to determine the reliability and appropriateness our classification: 1) total number of samples that were correctly categorized divided by the total number of remaining samples were calculated to test how accurately the data were classified (producer's accuracy) 2) the total number of samples from a category that were classified in the appropriate category was calculated as a measure of reliability (user's accuracy) 3) the overall accuracy was determined by dividing the total number of correctly categorized samples by the total number of samples and 4) kappa analysis (K) was calculated, which is a measure of agreement or accuracy between predicted classification data and reference data. A Kappa value of 80% or higher represents high level of accuracy, kappa values between 40% and 80% represent moderate accuracy and a value of less than 40% represents poor accuracy (Jensen 2005).

GIS Integration

To obtain geographic information, we used a Trimble® XRS GPS unit (Sunnyvale, CA) with Trimble TerraSync® software (Sunnyvale, CA). We recorded the subplots and field border as area features by averaging the vertices of each corner of the plots using real time differential corrections to improve position accuracy. Nine sample points from each plot were recorded as point features. The features collected with the GPS unit were transferred to Pathfinder Office 3.10 software (Trimble, Sunnyvale, CA) and differentially corrected in the Utilities/Differential Correction window using the Differential Correction Wizard to subject the data further geographic correction and increase accuracy. The Palatka base station (81.64°W, 29.65°N) was chosen as the base provider to use for reference position. The map was converted into an ESRI shapefile through the utilities/export dialog box >> new set up >> ESRI shapefile. The GPS map was then imported into ArcGIS 9.1 (ESRI Redwoods, CA). The map was re-projected into NAD_1927_UTM_zone 17 through ArcToolbox using the data management toolbox>>projection tool.

To obtain spectral information, we collected leaflets taken from the sample points previously marked with the GPS map. We transported the leaflets back to the laboratory in Zipper Seal Storage Bags and counted the number of TSSM on each leaflet using a dissecting microscope. Using a handheld Fieldspec®spectrometer (Analytical Spectral Devices, Boulder, CO) we recorded the reflectance values of each leaflet.

The mite number and reflectance values of each sample leaflet were stored as Microsoft Excel files. The spread sheet was imported into an Access file and then imported into ArcMap (ESRI, Redwoods, CA) as a layer using the 'add layer' dialogue box. These data contained field sample point position, mite number and reflectance value. The attribute table of the GPS sample points and the reflectance samples were joined through the layer properties dialogue box. The plots were unioned through the analysis>> overlay>> union function in ArcToolbox. The data were then converted to raster through the conversion tools>> to raster>> "feature to raster" function using ArcToolbox.

In the spatial analyst dropdown box in ArcMap, we used interpolate to raster>> inverse distance weighted (IDW) to create an interpolated image of the mite numbers as they are distributed on a field level. We then used the raster calculator in the spatial analyst dropdown to build the expression (Setnull([union]=1, [union]) to set a mask, excluding all the data except those in the plots. This defined the map extent to only those areas of interest within the plot. Finally, we used the raster multiplication function in raster calculator to build the expression ([setnull_plots]+1)*rast5, which overlaid the plot area with the interpolated raster layer. The result was an image displaying the mite distribution only in the plots (Figure 5-1).

Results

Spectral Analysis

The spectral signature of the samples displayed highest variability at the “red edge” (area between the red and NIR bands) and the green visible region (520nm-580nm). There was a close relationship between mite infestation and NIR reflectance (Figure 5-2). The TSSM numbers correlated with reflectance values showed a strong relationship between the variables. The validation data of mite numbers predicted by wavelength had an $R^2 = 0.7469$ between the expected and observed responses (Figure 5-3). The principal component analysis (PCA) condensed the data into 5 principal factors encompassing 89.8% of the variation in the data (Figure 5-4).

Accuracy of Categories of Mite Infestation

The NIR bands (700nm-1000nm) resulted in 90% of the total samples classified correctly with a 90% reliability score for the “Hi” category. The “Lo/Mod”category had 94% classified correctly with 92.5% reliability. The “No” category had 87.5% classified correctly with 87.5% reliability. The overall accuracy was 92.5% and K = 84.9% . The green bands (560nm-580nm) resulted in 95% correct classification in the “Hi” category with 79.1% reliability. The “Lo/Mod” category had 90.39% correct classification with 98% reliability. The “No” category had 100% correct classification with 100% reliability. The overall accuracy was 92.5% and K = 92.4% (Table 5-1).

The green and NIR bands in commercial satellite platforms, SPOT 5, which has a 500 nm-590 nm green band width and 780 nm - 890 nm NIR band width and Quickbird which has a green band width of 520 nm - 600 nm and NIR band width of 760 nm - 900 nm were compared. The green band produced an overall accuracy of 52% and 56%, respectively. The NIR for both platforms had 96% accuracy (Table 5-2).

The results of the accuracy matrix indicate that the three TSSM classifications selected in this study are highly accurate in discriminating spectral response in strawberries. The green bands preformed the best at high resolution and the NIR was highly reliable with lower resolution sensors.

Spectral Map

The joined raster and GPS layer created a visual representation of the spatial distribution of TSSM damage in the field plots. The model indicates that spectral pest detection is possible at a field level. The data suggest that mite distribution could be identified early through the reflectance correlation and precise preventative management could be preformed. Figure 5-4 shows the predicted distribution of TSSM within our experimental field. In a GIS, precise areas can be identified by pointing to a specific area of the map and the geographic coordinates of that area will be displayed.

Discussion

Analysis

There is a strong correlation between specific levels of mite infestation on strawberry leaves and alteration in leaf reflectance. However, the PCA analysis revealed that strawberry leaves reflect a high variation along the radiant spectrum, suggesting that the breadth of variance in the reflectance is a result of a complex physiological process within the leaf, not all of which is related to TSSM. The objective of the PCA is to condense the data into the smallest number of components (axes) to represent the strongest covariance among the variables, not necessarily varying with respect to the experimental variable (McCune and Grace 2002).

Due to the spectrum wide variability we chose to apply the LDA to the green and NIR regions, which indicated that the highest level of significance and corresponds spatially within the leaf to TSSM feeding sites. Both regions performed well under laboratory conditions. Spectral results indicate that a high spectral resolution sensor (~0.3μm band widths) is highly effective in discriminating between levels of TSSM infestation based on the variation in both the green and NIR regions. Fitzgerald (2004) noted that the strawberry spider mite (*Tetranychus turkestani* Ugarov and Nikolsk) damage in cotton is observable in the 850 nm wavelength, and subsequent work by Fitzgerald (2005) demonstrated that when using a commercial satellite platform, spectral variations in the green bands were difficult to detect in the field without subjecting the data to spectral unmixing. We found that at the lower spatial resolutions of commercial satellite platforms, the NIR is a good predictor of TSSM damage in strawberries, but finer resolution sensors are able to detect TSSM more acutely with the green bands.

GIT Application

The ultimate goal of this study is to detect TSSM numbers in a strawberry field before physiological damage is visible to the human eye. This may allow the application of a preventative strategy to reduce TSSM population before it becomes uncontrollable for growers. Large scale technology in conjunction with infrared sensory systems is already in popular use to detect presence/absence of agricultural pests. In addition to detecting TSSM, our goal is to apply this technology to monitor the level of TSSM infestation relative to radiant reflective response to early cellular damage of the strawberry leaves. The use of spectral maps of TSSM distribution in the field could aid in precision pest management programs.

Future Directions

There are several options of integrating this technology. A practical option is for a growers association to buy a field spectrometer such as a field-based Fieldspec ® 3 JR spectroradiometer (350nm-2500nm) (Analytical Spectral Devices Inc. Boulder, Co). The instrument can be mounted on a tractor or any field equipment and hooked directly to a GPS. The foreoptics on the spectrometer has a conical field of view which is 25° so the image is enlarged with respect to the height of the sensor. The high sensitivity of the sensor allows for up to 20 meters of height above the target with no detectable effect of signal to noise ratio. The spectral files are stored as binary data and are directly downloadable to the ENVI imagery processing system (ITT Visual Information Solutions, Boulder,CO) which can operate on a desktop computer. The binary files store the data as digital numbers in ASD (Microsoft Advanced Streaming Format description file). In ENVI these files can be exported directly into a "spectral library" and resampled to the spectral resolution of the image. A raster image is built from each of the spectra specified by a user. The fieldspec software also includes a post processor call a Viewspec Pro that can view and convert the binary files into other formats.

Once the image is built in the program it can be analyzed in ENVI or in ArcGIS. In ENVI, the image can be analyzed through the "basic tools" menu. By choosing regions of interest, the user can create training samples to conduct a supervised classification and use the maximum likelihood classification to classify the entire area (field) and then be able to identify regions of the field at different levels of mite damaged based on the reflectance values defined in this study. The image can also be analyzed in ArcGIS by importing the image constructed in ENVI with the bands of interest. The digital number will have been calculated in ENVI and imported into ArcGIS. In the attribute table in ArcGIS, the user could then use the "query builder" function to locate each pixel value of known mite infestation. The software will then be able to identify the pixels of interests in each mite level and enable the user to see the distribution of mite levels in the field based on the digital number. This technique with the fieldspec® is field based and the instruments can be easily mounted on field equipment. Similar technology has been used extensively in soil and nutrient assessment in agricultural fields. It provides high spectral resolution images that can be georeferenced when used in conjunction with a GPS and has been shown to have the spectral capabilities to discriminate between levels of TSSM.

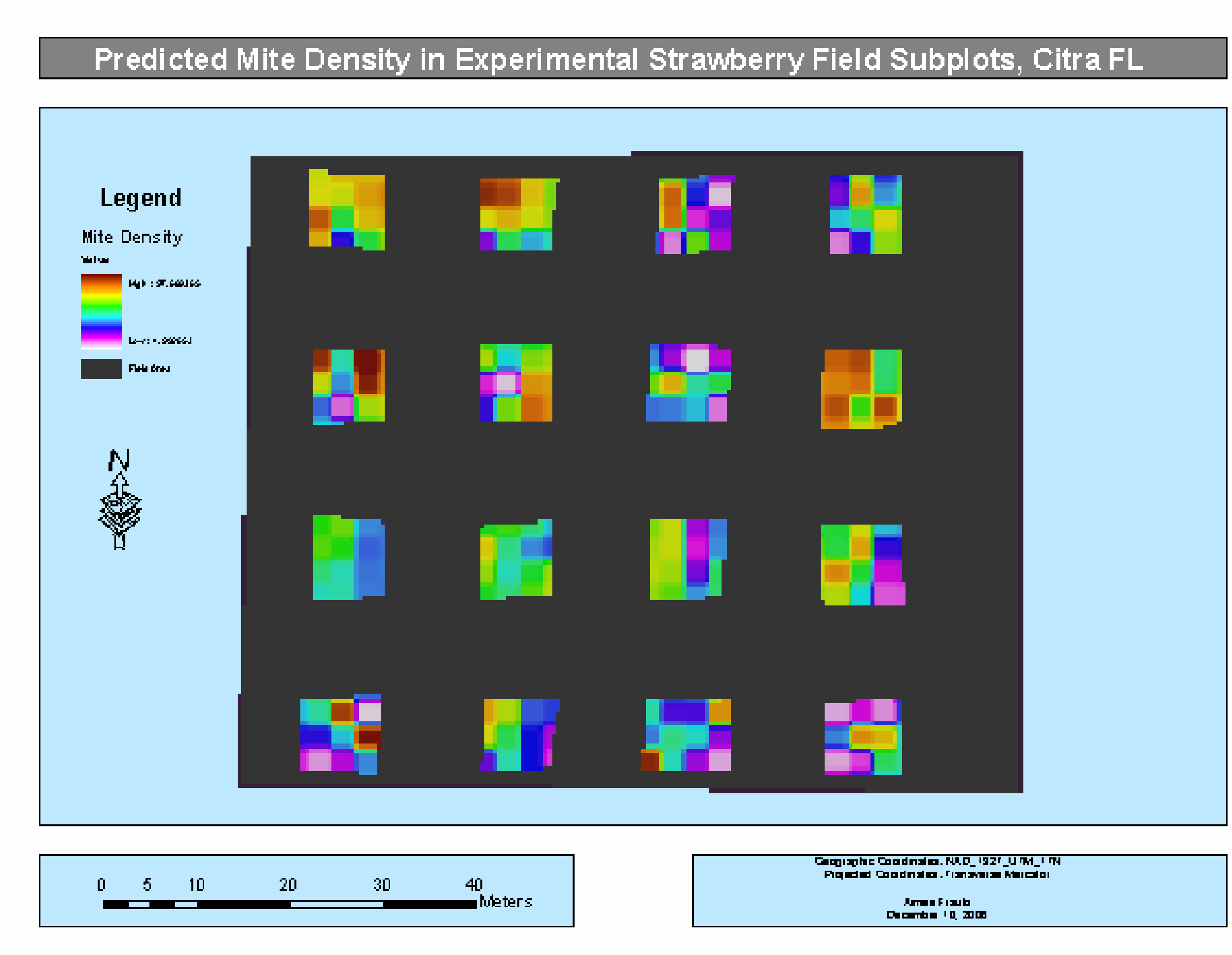


Figure 5-1. Reflectance map of TSSM distribution of the experimental strawberry field, Citra, FL.

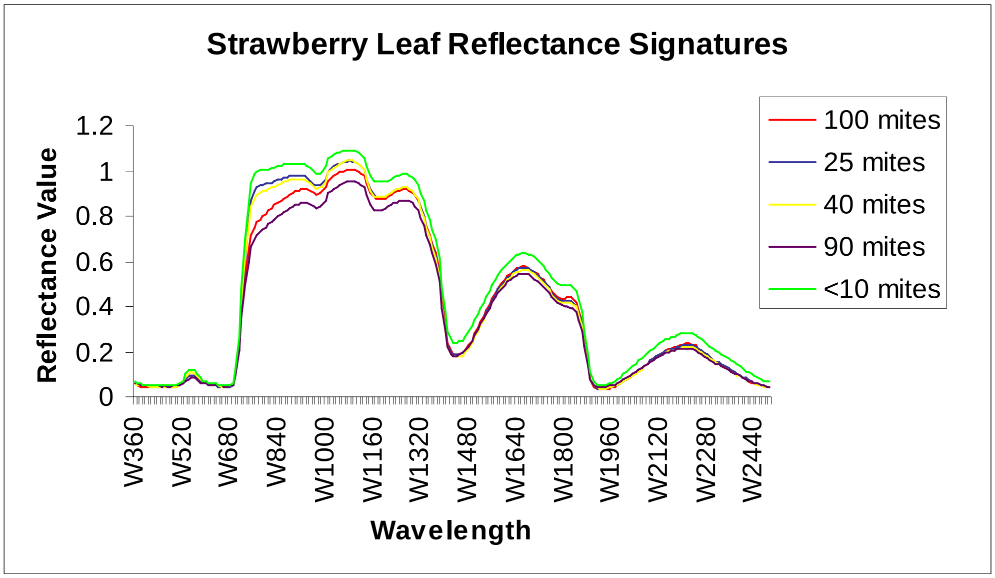


Figure 5-2. Variation of the spectral signatures of strawberry leaves at different levels of TSSM infestation.

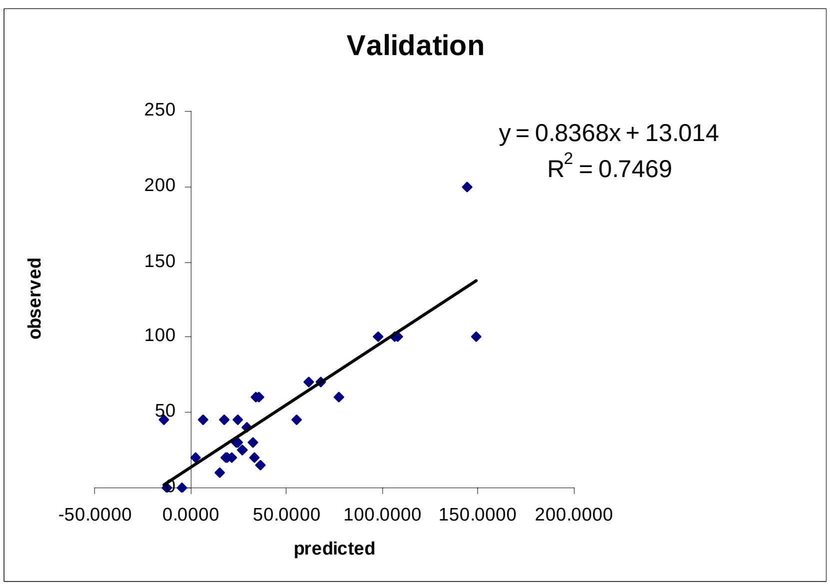


Figure 5-3. Regression of predicted versus observed raw TSSM numbers/leaflet.

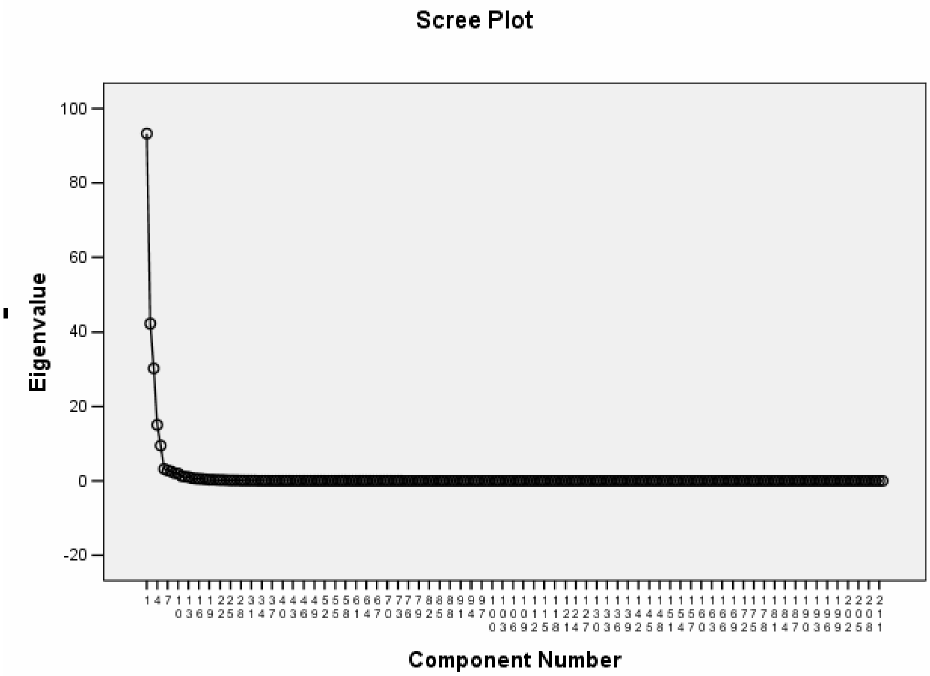


Figure. 5-4.Scree Plot of PCA. Each point on the graph indicates the cumulative percent of the data for which each factor accounts. The first five factors contain 89.9% of the data.

Table 5-1. Classification summary of LDA cross-validation of PCA. Highlighted percentages on the diagonal indicate the percent of observations classified correctly.

Expected

Observed	No	Lo/Mod	Hi	Total
No				
Observations classified	4	4	0	8
Percent classified	**50.00%**	50.00%	0%	100%
Lo/Mod				
Observations classified	0	50	2	52
Percent classified		**96.15%**	3.85%	100%
Hi				
Observations classified	0	2	18	20
Percent classified	0%	10.00%	**90%**	100%
Total	4	58	20	80
Total percent of classified	5%	70%	25%	100%

Table 5-2. Accuracy scores for the LDA

Green Bands (560nm-580nm)				
	Producers Accuracy	Users Accuracy	Overall Accuracy:	K coefficient
No mites	100%	100%	92.50%	92.40%
Low/Moderate	90.39%	98%		
High	95%	79.19%		
NIR (700-1000)				
No mites	87.50%	87.50%	92.50%	85%
Low/Moderate	94.23%	92.50%		
High	90%	90%		

Table 5-3. Accuracy scores for LDA of Quickbird and SPOT5

Quickbird (760nm-900nm)/Spot5 NIR (780nm-890nm)				
	Producers Accuracy	Users Accuracy	Overall Accuracy:	K Coefficient
No mites	100%	89%	93%	86%
Low/Moderate	90.38%	90.38%		
High	95%	95%		
Quickbird Green band(520nm-600nm)				
No mites	87%	22%	56%	31.00%
Low/Moderate	61%	89%		
High	30%	50%		
SPOT5 Green (500nm-590nm)				
No mites	63%	16%	53%	25%
Low/Moderate	60%	89%		
High	30%	43%		

CHAPTER 6
CONCLUSIONS

The principal management concern of strawberry growers in north Florida is the increasing incidence of twospotted spider mite (TSSM) and its effect on berry production. Managing TSSM with miticides is particularly difficult since TSSM has a short lifecycle and resistance can occur within a year of exposure to chemical treatments. However, many growers are skeptical about the efficacy of biological control. Growers have indicated that their primary concerns when considering pest management plans are economic viability and competitiveness in the industry. According to Shawn Crocker, Executive Director of the Florida Strawberry Growers Association, fruit and vegetable production is becoming more dynamic and highly mechanized. Growers are interested in managing their fields to maximize their production, while minimizing their inputs. The results obtained in our experiments regarding biological control, ecological studies on the sustainability of this method, and geospatial technology have proven that TSSM management can be both economically and ecologically efficient.

Our study shows that with minimal inputs from one early season release of the predatory mite, *N. californicus,* effective season-long management of TSSM can be achieved. The results of our first season (2005-2006) indicate that with low initial TSSM densities, *N. californicus* is able to provide consistent suppression of TSSM throughout the season. In treated plots, TSSM never exceeded five motiles per trifoliate. However, there was a higher initial TSSM population in the 2006-2007 season resulting in a higher ratio of predator to prey than the first season, reducing the level of effectiveness. The higher season-long average populations of TSSM in the 2006-2007 season indicate that initial prey populations and appropriate prey: predator ratio are critical factors in establishing control of TSSM. Greco et al. (2005) demonstrated that effective control seems to be limited by high initial TSSM density. An ideal predator: prey ratio is considered to be between 1:5 and 1:10 (Greco et al. 2005). Occasional freezes also seem to reduce TSSM populations. Hart et al. (2001) observed that cool temperatures suppress TSSM populations while not adversely affecting populations of predatory thripidae, staphiylinidae, lygaediae, and chalcidioidae. We observed this tendency in our experiments during the 2005-2006 season.

In the event of high initial TSSM infestation, as we had in the second field season (2006-2007), *N. californicus* must be released at extremely high rates to reach the appropriate predator: prey ratio to achieve adequate control of TSSM. Sabelis and Janssen (1994) confirmed that at low numbers, *N. californicus* is not able to reproduce quickly enough to decimate a high population, as would a more voracious predator such as *P. persimilis*. When TSSM populations are high, the number of *N. californium* needed may be cost prohibitive for many growers. An alternative, as discussed by Rhodes et al. (2006), is to use an initial reduced-risk miticide, or a biopesticide to reduce TSSM populations prior to the release of *N. californicus.*

Neoseiulus californicus do not affect the abundance of beneficial insects or disrupt the arthropod assemblage in the field. Plots in which *N. californicus* were released tended to have a similar arthropod richness and evenness compared with the control plots. The natural diversity in the strawberry system combined with the generalist feeding habits of *N. californicus* may contribute to the ecological stability of the ecosystem (Croft et al. 1998, Klein et al. 2006). The results indicate that a grower need not worry about the effects of releasing *N. californicus* on non-target organisms when this predator is introduced into the field.

The importance of maintaining a healthy ecosystem has led to the development of precision pest management. Many growers already utilize GPS to manage nutrients, soil, and moisture (Shawn Crocker, personal communication). The development and application of geographically referenced imagery analysis regarding TSSM damage is a natural addition to these strategies. Minimal additions to software capabilities on conventional field equipment could enable growers to precisely locate spot areas in the field that are developing populations of TSSM before visible by the human eye. Growers can then respond by treating spot infestations appropriately, conserving both economic and natural resources.

The concern about resource management and conservation has encouraged the development of strategies including biological control, precision agriculture, and precision pest management. The studies presented provide evidence that it is possible to reduce both economic and chemical inputs in strawberry fields, maintaining a healthy ecosystem and responding to both consumer and grower concerns regarding the health and marketability of the most valuable small fruit crop in Florida.

APPENDIX A
STRAWBERRY PEST MANAGEMENT SURVEY

Name: ____
Size of cultivated strawberry area: _________________________

1) What specific pest problems to you encounter (Insect, Weed, Diseases)

Pest	**Control Method**
______________________	____________________
______________________	____________________
______________________	____________________

2) Are you concerned about twospotted spider mites (TSSM)? Yes ___ No ___

3) Have you experience yield loss due to TSSM damage, if so what percentage/economic value of your production was lost?

____
____

4) How do you monitor for TSSM?

____
____
____

5) What is your current TSSM management program?

____
____
____

6) Would you be willing to use predators (biological control)? yes___ No____
If not why?

____
____
____

7) What factors do you consider when deciding on a management program?
(please circle all that apply)

a) economics
b) environmental concerns
c) customer concerns
d) time/labor requirments

This pest management survey was conducted with the help of Florida Strawberry Growers Association (N = 12). The results are as follows:

1) What pest problems do you encounter?

The commonest pest problems:
30% fungus
50% fungus and birds
20% TSSM

2) How do you monitor for Twospotted spider mite (TSSM)?

Frequency of scouting varies between once a day and once a week and the action threshold varies:
20% 1 TSSM per leaftlet,
20% 5 TSSM per field of view (FOV) of hand lens
20% 10 TSSM per FOV of hand lens
10% wait until leaves begin to yellow
20% spay regularly so do not have TSSM
10% no formal scouting

3) What is your current management program

90% use chemicals, only the organic growers rely on natural enemies (they do not have a problem with TSSM)

4) Would you be willing to use biological control?

100% said they would be willing to use biocontrol but the concerns are **cost** and **compatibility** with fungicides and other chemicals used in the field.

5) What factors do you consider when deciding on a management program?

100% are primarily concerned with economics.

APPENDIX B
FUTURE WORK

Objective 1: To determine the appropriate time for inoculative releases of N. californicus. Our work provides compelling evidence that releasing N. californicus early in the season can provide stable season-long control of TSSM. However, there are still several questions to be answered: 1) Greco (2004, 2005) determined that for control of TSSM, N. californicus needed to be released at a ratio of 1:5 to 1:10 (predator: prey). Further work on determining the optimal predator: prey ratio in the north Florida strawberry system would be helpful to verify this assertion; 2) investigating varying levels of TSSM densities on the efficacy of N. californicus in the field should be conducted. In our greenhouse trials, N. californicus demonstrated a functional response with prey density. Hassell et al. (1976) support this finding in laboratory studies. However, this response has not been validated in the field. Releasing N. californicus at a constant predator: prey ratio in areas of high and low TSSM densities to assess efficacy of N. californicus would contribute to our understanding of the predator: prey interaction; 3) future exploration is needed to understand the effect that the timing of TSSM infestation and plant phenology has on berry yield.

Objective 2: To evaluate the effect of predatory releases on naturally occurring arthropods. The findings in this study indicate that N. californicus does not have a significant impact on the arthropod assemblage in the strawberry system. However, the studies conducted captured the general distribution of taxa in the system. We found that plant phenology and ambient weather had an impact on arthropod assemblage in the field, which may affect the impact of N. californicus in the system. Repeated trials across seasons in different climactic conditions should be conducted. In addition, investigating the effect of N. californicus releases on taxonomic composition within arthropod families would contribute to a deeper understanding of the system.

Objective 3. To establish a pest monitoring program using geographic information technology (GIT). Our laboratory studies have shown that there is a strong correlation between specific levels of mite infestation on strawberry leaves and alteration in leaf reflectance in the green and NIR wavebands. However, the application of the laboratory analysis needs to be verified in the field, and an efficient data collection and analysis procedure needs to be established. Testing the laboratory results in the field under ambient conditions is important to make this technique practical. To test the results in the field, a field spectrometer should be acquired and connected to a GPS. The unit should be mounted onto a tractor, or other field equipment, to collect data from the green and NIR wavebands and input into an imagery analysis system as outlined in Chapter 5. Finally, the process must be validated by applying it to a number of fields and conditions to ensure reliability.

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BIOGRAPHICAL SKETCH

Aimee graduated with her BA in sociology with a concentration in environmental sociology from Indiana University in 1999. Before entering graduate school, she worked on and managed several experimental organic farms in both the United States and South Africa. She also taught marine ecology with the Chesapeake Bay Foundation in Maryland and Save the Sound in Connecticut. She earned a teaching degree in Montessori elementary education in 2003. During her master's work she held a position as a research assistant in the Small Fruit and Vegetable IPM lab in the Entomology Department at the University of Florida. Her study focused on biological control of twospotted spider mite in strawberry, an exploration of arthropod diversity, and geospatial imagery systems as a component of a precision pest management program.