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Impact of Early Infestation of Two-Spotted Spider Mites (*Tetranychus urticae*) on Cotton Growth and Yield Impact of Early Infestation of Two-Spotted Spider Mites (*Tetranychus urticae*) on Cotton Growth and Yield

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology

By

Luis Orellana Jimenez Zamorano University Bachelor of Agricultural Science, 2001

> May 2014 University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

Two-spotted spider mites (Tetranychus urticae Koch, 1836) are pests of vegetables, ornamentals, and row crops around the world. Two-spotted spider mites have become an important long-season pests of cotton, causing injury to cotton from an early vegetative stage. In the past eight years, Arkansas cotton acreage treated for spider mites has more than doubled and most of the increase has been attributed to early season infestations. Yield losses of up to 30% have been observed in other studies where spider mite infestation started at third true leaf. Because of the apparent change in this pest's population dynamics, particularly at early stages of crop development, the objective of the present study was to understand the impact of two spotted spider mites on cotton growth and yield. This project focuses on the impact of the timing and duration of infestations. Cotton plots were artificially infested at fourth, sixth true leaf in 2012, and at cotyledon and fourth true leaf during 2013. Both years included three infestation durations (short, medium, and long) at each infestation time. Two-spotted spider mites remaining on cotton at damaging densities for two weeks or more regardless of infestation time, caused significant yield loss. However, spider mites did not cause significant yield loss when environmental conditions did not favor spider mite development for extended periods.

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DEDICATION

To my grandfather Ricardo Jiménez, my wife Kristie Orellana and Baby O., they have been my major sources of motivation and inspiration.

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CHAPTER 1

INTRODUCTION

In the United States, cotton is produced in 17 southern states. During the 1970's and 1980's, cotton acreage was estimated at 5 million hectares and increasing to almost 6 million hectares by 2005 (Meyer et al. 2012). However, due to market prices, growers have favored planting alternative crops like soybean and corn, and cotton acreage declined to just over 4 million hectares in 2013 (Meyer and MacDonald 2013). During 2011, cotton acreage in Arkansas was estimated at 275,000 hectares with production totaling over 278,000 tons of cotton fiber and 437,000 tons of cotton seed. This yield resulted in ~ \$694,489,000, making Arkansas the 3rd largest cotton producer in the USA (NASS 2011).

After the success of the boll weevil eradication program and the introduction of transgenic *Bt* cotton varieties targeting lepidopteran pests, insect pests previously considered secondary insect pests have become key pests. Important pest in the US include: tarnished plant bugs, *Lygus lineolaris* (Palisot), western plant bug, *L. hepersus* Knight, clouded plant bug, *Neurocolpus nubilus* (Say), green stink bugs, *Acrosternum hilare* (Say), southern green stink bugs, *Nezara viridula* (Linnaeus), and the brown stink bug, *Euschistus servus* (Say), the bollworm/budworm complex, *Helicoverpa virescens* (Fabricius) and *Helicoverpa zea* (Boddie), armyworm complex, *Spodoptera exigua* (Hubner), *Spodoptera frugiperda* (J. E. Smith), and *Spodoptera ornithogalli* (Guenee), the tobacco thrips, *Frankliniella fusca* (Hinds), the western flower thrips, *Frankliniella occidentalis* (Pergrande), the flower thrips, *Frankliniella tritici* (Fitch), the soybean thrips, *Neohydatothrips variabilis* (Beach), the onion thrips, *Thrips tabaci* Lindeman, and cotton aphid, *Aphis gossypii* Glover (Greene et al. 2001, Reed et al. 2006, Naranjo 2011, Williams 2011).

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Spider mites (Tetranychidae) are pests in a wide range of crops around the world and cause considerable annual losses (Migeon et al. 2010). Thirty-two tetranychid species are known to cause damage to cotton, 19 of which are present in the United States. However, the two-spotted spider mite green strain (*Tetranychus urticae* Koch, 1836) and the red strain (previously the carmine spider mite *Tetranychus cinnabarinus* Boisduval, 1867) is the predominant species in cotton fields of the Mid-South (Dupont 1979, Kerns et al. 2009). In 2011, producers in the United States lost over 12,000 tons of cotton due to spider mites (Williams 2012), which represented a total loss of more than \$24 million (America 2013). That same year, Arkansas producers lost over \$1 million due to spider mite infestations.

Changes in pesticide practices resulted in early season spider mite outbreaks in Arkansas cotton fields during the 50's, when chlorinated hydrocarbon first became commonly used (Lincoln and Leigh 1957, Boyer and Bell 1961). However, until recently, two-spotted spider mites were considered a late season pests of cotton in Mid-South U. S. Two-spotted spider mites have become a long-season problem, damaging cotton even at a very early vegetative stages (Catchot et al. 2006, Williams 2006). Early season outbreaks have been partially attributed to the replacement of aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) with newer neonicotinoid seed treatments (Gore et al. 2013, Smith et al. 2013). Also, high rate applications of wide spectrum insecticides to control thrips or tarnished plant bugs, can result in disruption of natural enemy balance and consequently outbreaks of spider mites (Gerson and Cohen 1989, Gore et al. 2013, Smith et al. 2013). Changes in mite dynamics necessitate the study of the impact of early spider mites infestations under present crop production conditions. Particularly, it is important to evaluate the initial timing of infestations as well as the duration of

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the infestation impact cotton growth and yield. This information can be useful to cotton producers whenever spider mites outbreaks occur in their fields.

CHAPTER 2

LITERATURE REVIEW

Two-spotted spider mites (*Tetranychus urticae* Koch, 1836) belong to the superfamily Tetranychoidea. This superfamily is composed by Tetranychidae (spider mites) and Tenuipalpidae (flat mites). In this superfamily the chelicerae are fused at the base to form a retractable and extrudable stylophore. The stylophore is composed of two separate chambers that contains the styles that when extended forward are joined to form a hollow tube that is an adaptation to feed on plant tissues (Lindquist 1985).

Effects of Spider Mites Damage to Cotton Yield

The polyphagus nature and short generation time make two-spotted spider mites an ideal pest. Mites have potential to inflict yield losses when left unchecked under favorable environmental conditions (Wilson et al. 1987, and Scott et al. 2013). In Mississippi, a study with spider mite infestations started at 3rd true leaf resulted in 45% yield loss (Smith 2010). In California, cotton plants infested with T. urticae at squaring resulted in a yield loss of 21-27 % (Canerday and Arant 1964b). A study infesting cotton with T. urticae at different stages found that infestation closer to squaring had greater reductions in yield (78%) and fiber quality compared to later infestations (Wilson 1993). In a study infesting cotton during boll development with Tetranychus turkestani (Ugarov & Nikolskii 1937) (strawberry spider mite), yield was reduced between 13% to 22% (Canerday and Arant 1964a). In North Carolina, an experiment where cotton plants were infested with strawberry mites (*T. turkestani*) at three times, (1) bloom, (2) three weeks after bloom, and (3) eight weeks after bloom, resulted in yield losses of 63, 31, and 18%, respectively. A considerable number of studies agree that early infestation of mites result in increased crop damage and yield loss (Canerday and Arant 1964a, b, Mistric 1969, Wilson et al. 1987, Wilson 1993, Reddall et al. 2007).

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In general, infestations of spider mites on cotton have mainly focused on the plant's reproductive stage, namely from squaring through boll development and maturation (Canerday and Arant 1964a, b, Mistric 1969, Wilson et al. 1987, Wilson et al. 1991). However, since 2005 in the mid-south of the United States, spider mites have become a pest of concern throughout the season. Presumably, the replacement of Temik® with seed treatments and the use of broad spectrum insecticides applied to control early season pests (i.e., thrips and plant bugs) have disrupted the natural enemies that normally keep occasional pest such spider mites in check (Kerns et al. 2009, Gore et al. 2013, Scott et al. 2013).

Ecology of Spider Mites

Both abiotic and biotic factors influence spider mite populations (Kerns et al. 2009). Spider mite infestations are often reported in association with prolonged intervals of hot dry weather (Smith 2010). Rain and hail are known to negatively affect spider mite abundance (Canerday and Arant 1964b, and Wilson 1993). Additionally, two-spotted spider mite densities are higher in cotton fields adjacent to dusty roads than cotton fields adjacent to asphalt roads (Demirel and Cabuk 2008).

Wild hosts are an important reservoir for initial mite infestation (Smith 2010). In Arkansas palmer amaranth (*Amaranthus palmeri*, S. Watson), goose grass (*Eleusine indica* (L.) Gaerth), curled dock (*Rumex crispus* L.), hedge bindweed (*Convolvulus arvensis* L.), and entireleaf morningglory (*Ipomoea hederacea* Jaquin) all support mite populations (Steinkraus et al. 1999). In a study conducted from 2007 to 2009 in Mississippi, infestation of cotton seedlings initiated from henbit (*Lamium amplexicaule* L.) and a total of eight other dicotyledonous and three monocotyledonous plants were found to be major hosts of two-spotted spider mites during the spring months (Smith 2010). Spider mites overwinter as diapausing female adults (Van de Vrie et al. 1972), however recent studies reported winter activity of all spider mite stages on henbit, concluding that two-spotted spider mites can reproduce throughout the winter in Mississippi (Smith 2010).

Spider Mite Dispersal and Spread

A series of greenhouse studies on broad bean and carnation plants were used to understand two-spotted spider mite dispersal (Hussey and Parr 1963). After producing heavy infestations, two-spotted spider mites congregate on the upper leaflet apices of bean plants where they form a 'ball'. The weight of the mites direct the leaf downward and several mites fall, leaving a silk thread that is followed by multiple mites forming another smaller 'ball' at end the thread. Some mites just drop down from the ball, others continue using the threads in their migration downward. Occasionally the silken "rope" either swings like a pendulums until reaching a different plant or it lengthens until it reaches the ground. Upon hitting the ground, mites disperse towards the most illuminated areas. Mites in this study were not observed producing silken parachutes, even after exposure to light wind (0.1 m / sec) (Hussey and Parr 1963). Furthermore, damp air (100% relative humidity, RH) reduces spider mite migration by 86% when compared to air at 70% RH. Wind has a direct effect on the direction spider mites spread in greenhouses, even when mites are inside a greenhouse, as all motile stages and even some eggs are dispersed by wind (Escudero et al. 1999). Obviously, favorable conditions are necessary for an increase of mite populations to allow dispersal (Boykin and Campbell 1984, Smitley and Kennedy 1988, Escudero et al. 1999).

Mites orient themselves to the wind when exposed to light and winds of about 2.58 m/sec (Osakabe et al. 2008). Under those conditions, mites position themselves facing the opposite

direction of the light and raise their forelegs and bodies. Mites that face the wind while exhibiting the aerial posture are 25 times more likely to be carried by the wind than mites situated perpendicular to the direction of the wind (Smitley and Kennedy 1985, and Osakabe et al. 2008). Even though *T. urticae* has a complex behavior that allows for aerial dispersal, it appears to not use silk thread to become airborne (Fleschner et al. 1956, Hussey and Parr 1963, Smitley and Kennedy 1985, Bell et al. 2005).

Damage to Plant Tissue

Two-spotted spider mites are a common pest of many crops around the world (Van Leeuwen et al. 2010). Spider mites species have stylet-like sucking mouth parts composed of opposing movable digits that when everted, connect to form a hollow piercing probe (Lindquist 1985). The length of the everted section of the two-spotted spider mites stylet is $132 \pm 27 \,\mu m$ (Sances et al. 1979). Spider mites feed mostly on the underside of leaves, damaging important photosynthetic sites (Reddall et al. 2004). Initial damage of leaf surfaces by spider mites involves minute light punctures. After prolonged feeding, damage is extended into irregular light yellow or grayish spots. The color of the lesions can vary from yellow to bronze. Damage can also turn in necrotic areas on leaves and stems and can even cause defoliation (Jeppson et al. 1975, and Tomczyk and Kropczynska 1985). The depth of injury on leaves can range from 85.1 $(\pm 18.4) \,\mu\text{m}$ to $117.5 \pm (24.9) \,\mu\text{m}$. This variation is due to time and duration of infestation, mite density, and host type (Sances et al. 1979). When spider mites pierce the leaf tissue, they suck out cell contents and destroy the mesophyll, causing dehydration of the remaining cells (Jeppson et al. 1975). Consequently, guard cell turgor is reduced, resulting in closing of stomata situated along lower surface of the leaf. Mite-free plants have an almost even distribution among three classes of stomata (a) turgid and open, (b) intermediate turgid but not fully open, and (c) flaccid

and closed. Among damaged leaves, 61.4% of the stomata are flaccid and closed (Sances et al. 1979). Multiple studies of spider mite damage on leaves of almond, apple, cotton, peach, peppermint, strawberry, and soybean have revealed that spider mites reduce stomatal conductance, transpiration, chlorophyll content, and photosynthesis (Hall and Ferree 1975, Hislop and Jeppson 1976, Sances et al. 1979, DeAngelis et al. 1983, Tomczyk and Kropczynska 1985, Brito et al. 1986, Bondada et al. 1995, Haile and Higley 2003, Reddall et al. 2004). The physiological sequence of events of cotton leaves that are decreased after spider mite damage is 1) stomatal conductance; 2) transpiration; 3) photosynthetic rate; and 4) transpiration efficiency (Reddall et al. 2004). In addition to mechanical damage, spider mites may inject proteolytic enzymes into the plants through their saliva (Storms 1971, and Tomczyk and Kropczynska 1985).

Spider Mites and Pesticides

Hormoligosis has been used to describe an event where sub-lethal amounts of any stressor, when exposed to organisms in suboptimal environments, may result in beneficial effects to the exposed organism. The stressor could be any kind of chemical, temperature, radiation, or non-lethal injury (Luckey 1968). However, the term hormesis was re-defined to describe more specifically a dose-response situation where an organism that is exposed to a sub-lethal amount of a stressor will experience a stimulatory or beneficial effect which results in a more fit individual. Exposure to a higher amount of the same stressor results in an inhibitory or lethal effect (Calabrese and Baldwin 2003). The term hormesis only applies when a stressor affects its intended targeted organism, e.g., when a sub-lethal dose of an insecticide results in a benefit to (not eliminating) its targeted insect. The term should not be used to describe the effects of insecticides on non-target organisms like spider mites. In this case, the term should be pesticideinduced homeostatic modulation (PIHM) (Cohen 2006).

Several pyrethroids insecticides have PIHM effects on spider mites and detrimental effects on their natural enemies. *Tetranychus urticae* obtained from permethrin and fenvalerate-treated beans exhibit increased fecundity in female adults and reduced the developmental period of 1-2 days in immatures (Gerson and Cohen 1989). The PIHM effects of exposing spider mites to imidacloprid, whether by directly spraying them or by feeding them previously-sprayed plant material, are increased fecundity and longevity of spider mites, the latter only occurring when imidacloprid is ingested through plant tissue (James and Price 2002).

Two-spotted spider mites are among the pests most resistance to insecticides, showing tolerance to a considerable number of compounds (92 active ingredients) across several classes, e.g., organophosphates, carbamates, pyrethroids, pyrazoles, pyridazinones, and ketoenols (e.g. spirodiclofen). A short life cycle resulting in many generations per year, arrhenotokous reproduction, and prolific fecundity, all help spider mites achieve high levels of pesticide resistance (Van Leeuwen et al. 2010).

CHAPTER 3

EFFECT OF TWO-SPOTTED SPIDER MITES, TIMING AND DURATIOIN OF

INFESTATION ON COTTON GROWTH AND YIELD

Introduction

Changes in pesticide practices can result in early season spider mite outbreaks as evidenced by numerous spider mite outbreaks in Arkansas cotton fields during the 50's, after chlorinated hydrocarbon pesticides became widely used (Lincoln and Leigh 1957, Boyer and Bell 1961). Until recently, two-spotted spider mites were known as occasional post-flowering pests in cotton production areas of the mid-south region of the U.S. In recent years, two-spotted spider mites have become more of a long-season problem, causing injury to cotton in early vegetative stages (Catchot et al. 2006, Williams 2006). This situation seems to have coincided with the replacement of in-furrow applied aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) with neonicotinoid seed treatments. Aldicarb suppresses early season spider mite outbreaks, adding 205 kg/ha of lint largely due to early season control of spider mites (Roberts et al. 1990, Gore et al. 2013, Smith et al. 2013). Additionally, management of the tarnished plant bug, Lygus lineolaris (Palisot 1818), requires multiple applications of organophosphates and/or neonicotinoids combined with pyrethroids. These applications can disrupt the natural enemy balance, alter the reproductive physiology of mites, and in the case of pyrethroids, can induce premature spread of mites in a field (Gerson and Cohen 1989, Roberts et al. 1990, James and Price 2002, Studebaker and Kring 2003, Gore et al. 2013, Smith et al. 2013). Arkansas cotton acreage treated for spider mites has more than doubled in recent years compared to the previous ten years (Williams 1999, 2006, 2012, 2013), and most of the increase can be attributed to early season infestations (Gore et al. 2013, Scott et al. 2013). Spider mite infestation studies starting as early as third true leaf results in yield losses of up to 30% (Roberts et al. 1990, Gore et al. 2013, Smith et al. 2013). The duration of a spider mite infestation is important in the damage caused. Infestation durations of twenty one and twenty eight days result in significant yield reduction (Scott et al. 2013). With increasing significance of spider mites on cotton in the mid-south, it is necessary to study the impact of early spider mite infestations and duration of infestation in order to advise producers on timing of pesticide use to avoid economic damage. The objective of this study was to evaluate the impact of infestation timing and exposure interval of two-spotted spider mites on cotton growth and yield. Scott et al. (2013)

Materials and Methods

Research plots were located in Lee County, Arkansas. Trials were established the 2nd and 25th of May 2012; and the 13th and 29th of May 2003. Each year, the first and second planting dates were considered early and late planting, respectively. In 2012, early and late planted cotton were cotton plants were infested during three plant growth stages: fourth true leaf, sixth true leaf and at ninth true leaf. In 2013 early planted cotton was infested at cotyledon, fourth true leaf and ninth true leaf. The late planted plots were infested at cotyledon, fourth true leaf, and sixth true leaf. Within each plant stage, two-spotted spider mites (Tetranychus urticae) were left on cotton for three infestation durations: short (3-6 d), medium (9-10 d) and long (14-36 d). The combination of plant stage and duration of infestation resulted in 12 treatments. Plots had mites only for the duration of infestation, as foliarly-applied miticides were used to keep plants mitefree before and after prescribed infestation times (Figures 1 and 2). A control plot which was kept mite free for the duration of the study was maintained each year. Each treatment time was replicated four to six times. Plots consisted of two 0.96 m rows, 4.5 m long with one empty row between plots and 3 m empty space between replicates. Early-maturity cotton varieties used were DP 0912 B2RF and ST 4946 GLB2 during 2012 and 2013, respectively.

Mites were reared in a greenhouse on green beans (*Phaseolus vulgaris* L.) at the University of Arkansas Extension and Research Center in Lonoke. One bean mite-infested plant was used to inoculate ≈ 36 cm of a cotton row with between 2-3 mites/cm². Cotton plants were infested by cutting bean plants at the base of the stem and interweaving them through the cotton plants. Mite density (mites/cm²) counts were made using a lens (linen tester) with an area of 2.25 cm². Mites located on one leaf in the upper third main stem node were counted on five randomly-selected plants per plot (Smith 2010). Leaf damage was assessed on a plot basis using a visual standard scale: 0 = no damage and 5 = total reddening (Gore et al. 2012). Mite counts and leaf damage were assessed between three and five days after infestation, and once a week thereafter until mites were eliminated. All measurements, except for nodes above white flower, were made on five randomly-selected plants per plot. Match head squaring was considered when squares (i.e., flower buds) had a diameter of 3 mm, bloom was considered when cotton flowers started opening, and cotton plants were considered physiologically mature when cotton plants had five nodes above white flower (NAWF) (Oosterhuis 1990, Bourland et al. 2001). Plant heights were measured from the base of the plant to the terminal. Main stem nodes were counted starting with cotyledon (main stem node zero) and then counting upward until the top unfurled leaf. For nodes to first square counts, first the cotyledon node was located (considered main stem node zero) and then nodes were counted upward until finding the node with a square in the first position. NAWF were counted by starting at the top node with an unfurled leaf and counting downward until finding the node with a white flower in the first position. NAWF counts were based on ten plants per plot. In 2012, number of nodes to first square, plant heights and plant nodes were measure only at match head squaring. During the 2013 growing season, plant heights were measured at two weeks after emergence, squaring, bloom, and cutout (Table 1). Plant nodes were counted at match head square, bloom and cutout. Both years, the numbers of nodes to first square were measure at match head square. Maturity was assessed when control

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plots reached NAWF 5. Seed cotton yield was measured at harvest with a mechanized plot picker. Results of plant response measurements were analyzed as a factorial design both years. In 2012, the two factors analyzed were time of infestation and length of infestation were. Time of planting was added for analysis in 2013. Response variables were analyzed using ANOVA in JMP 11 (SAS Institute Inc., Cary, NC, USA), means calculated using the LS-means statement and separated using the Fisher's protected least significant differences (LSD) (α =0.05). For 2012, contrasts were used to estimate differences between control and infestation time, based on a simpler ANOVA model, where treatments resulted from the combination of both infestation variables, time and duration.

Results

At the research station in Marianna, Arkansas, rain accumulation was 39.6 mm between May 25th and July 16th 2012 and 64.2 mm between May 28th and July 18th 2013. In 2012, low and high monthly average temperatures (°C) for May, June, and July were: 17.8 and 30.4; 19.4 and 31.8; and 22.9 and 34.3, respectively (Table 2). In 2013, average low and high temperatures during the same months were: 19.4 and 25.9; 20.6 and 31.9; and 20.5 and 31.0, respectively.

In 2012, early planted cotton plots experienced adverse weather (Table 2) preventing successful establishments of spider mites, thus no data are reported. The 2012 late planted cotton infested at sixth true leaf and with medium infestation interval were not eliminated on time, hence no medium infestation data are presented. In 2013 data, the third infestation time during early planted cotton started at ninth true leaf while the third infestation time for late planted cotton started at sixth true leaf, because this mismatch in infestation time, these data was not used for analysis.

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In 2012, average mite densities at the end of each infestation interval for infestations started at fourth true leaf (Table 3) were 4.39 and 1.73 mites/cm² for short and long infestations, respectively. Average mite densities for sixth true leaf (Table 3) infestations were 4.06 and 1.37 mites/ cm² for short and long infestations, respectively. Mean mite densities for ninth true leaf infestation (Table 3) were 0.61 and 0.35 for short and long infestations, respectively. Mites in the treatments with long infestations at fourth and sixth true leaf reached peak densities 20 and 26 days after infestation, respectively. The highest mite densities for these treatments were 12.68 and 3.39 mites/cm², respectively, however between peak density and elimination (i.e. 5 d) the area received 25.4 mm of rain, which brought the mean density to 1.73 and 1.37 mites/cm², respectively.

In 2012, mean leaf damage ratings for short and long infestations started at fourth true leaf (Table 4) were 1.08 and 2.25, respectively, and leaf damage ratings for infestations started at sixth true leaf (Table 4) were 0.91 and 1.00 for short and long infestations, respectively. Mean leaf damage ratings for short and long infestations started at ninth true leaf (Table 4) were, 0.40 and 0.70, respectively.

In 2012, no differences were observed from plant response measurements (Table 7); nodes to first square (df=3, F=0.6587, P= 0.5849), plant heights (df=3, F=1.5336, P= 0.2294) and total plant nodes at squaring (df=3, F=1.6398, P= 0.2045), and number of nodes above white flower (df=3, F=2.0035, P= 0.1016) at cutout. There were differences in seed cotton yield (df=3, F=2.7574, P= 0.0329) infestation length (df=1, F=8.0708, P= 0.0074) (Table 8). Means for seed cotton yield for infestation with long duration are reported in table 9. Contrasts were used to determine differences in yield between control and each one of the long infestation durations at fourth, sixth and ninth true leaf (Table 10). Yield contrast between control and all long infestation duration combined resulted in a yield difference of 10.6 % (df= 1, F=4.7300, P= 0.0340). More specifically, the contrasts between control and long infestation intervals at fourth true leaf (df= 1, F=2.1009, P= 0.0404), sixth true leaf (df= 1, F=2.2093, P= 0.0315), and ninth true leaf (df= 1, F=0.5718, P= 0.5698) were, 15.1, 12.5 and 4.1 %, respectively. The contrast between ninth true leaf long infestations and the control treatment was not statistically different. There were no differences observed for time of infestation (df=2, F=1.6406, P= 0.2080).

During 2013 average mite densities for infestations started at cotyledon (Table 5) were 0.30, 0.53, and 1.86 mites/ cm² for short, medium, and long infestations, respectively. Mean mite densities for infestations started at fourth true leaf (Table 5) were 1.08, 0.47, and 1.41 for short, medium, and long infestations, respectively. Peak mite densities for long infestations starting at cotyledon and fourth true leaf were 1.86 and 1.41 at 36 and 23 days after infestation, respectively.

During 2013, mean leaf damage ratings for short, medium, and long interval infestations at cotyledon (Table 6) were 0, 0.72, and 1.27, respectively. For infestations at fourth true leaf (Table 6), mean leaf damage ratings for short, medium and long intervals were 0.27, 0.72, and 0.91, respectively.

During 2013, early-planted cotton was significantly taller and had more nodes throughout the three stages assessed than did late-planted cotton ($P \le 0.5$) (Table 11). Plant height differences between early- and late-planted cotton were: 27.77 cm (df=1, F=641.4241, P= 0.0001) at squaring, 17.77 cm (df=1, F=223.9869, P= 0.0001) at bloom, and 28.30 cm (df=1, F=641.4241, P= 0.0001) at cutout. Differences in plant nodes counts between early and late planted cotton were: 3.49 nodes (df=1, F=292.2233, P= 0.0001) at squaring, 1.71 nodes (df=1, F=82.9613, P= 0.0001) at bloom, and 3.12 (df=1, F=72.1837, P= 0.0001) at bloom. Nodes to first square (df=11, F=0.4947, P= 0.8983), nodes above white flower (df=11, F=1.2185, P= 0.2977), and seed cotton yield (df=11, F=1.1451, P= 0.3465) were not different between early and late planting dates (Table 12).

Discussion

The higher mite densities recorded during 2012 compared to 2013 can be partially explained due to environmental conditions being more favorable in 2012 than 2013 for spider mite development (i.e., warmer temperatures, dryer weather) (Table 2). During 2012, higher mite densities led to significant yield loss, this occurred in the treatments with long infestations at fourth (28d) and sixth true leaf (14 d), where yield loss was estimated to be 15.1 and 12.5 %, respectively. These results are similar to those obtained by Scott et al. (2013) where 14 and 20% yield loss occurred in cotton plants that were infested at the third true leaf stage and mites were left for 21 and 28 days reaching densities of 0.34 and 0.64 mites/cm², respectively. Yield loss from pre-flowering mite infestations irrigated fields have been documented in Louisiana (38.2%) and Mississippi (42.8%) (Smith 2010). Spider mite infestations in that study started at third true leaf and infestations persisted for ~ 4 weeks, but mite densities were not reported. Although, our yield loss was similar, spider mite densities reported by Scott et al. (2013) were considerably lower. The primary reason for not having as much yield loss in 2012 as in Scott et al. (2013) may be related with our control plots having some yield loss cross infestation with spider mites from surrounding plots (Tables 3 and 4). Smith (2010) found that only cotton plants inside nonirrigated fields experienced defoliation and significant yield loss (48%), but spider mite damage was moderate in the irrigated plots, resulting only in reddening necrotic patches of leaf tissue. The different damage and yield loss between irrigated plots and non-irrigated plots from Smith (2010) may also help to explain in part why greater spider mites densities in our study did not

result in greater yield loss than 10.6%. Differences in irrigation practices may also explain differences in mite densities and yield loss between the two studies. In the same study, Scott et al. (2013) reported damage ratings for 14 and 28 day infestations of 1 and 3.5, which were similar to the ratings in our study of 1.6 and 2.25, respectively. Based on these results, leaf damage ratings may be a better way to associate damage with cotton yield loss than mite density, as was suggested in the study by Smith (2010).

Although infestation time was not significant in our study, studies by Gore et al. (2013) reported infestations that started at third true leaf, first flower and thereafter every 200 heat units (HU) until first flower + 800 HU had significantly lower yield than did the uninfested control. In essence, yield loss increased from 7% for infestations started at first flower +600 HU to 30% for infestations started at the third true leaf.

In 2013, late planted cotton was significantly taller (17.77 -28.30 cm) and had more nodes (2-3 nodes) than early planted cotton. This cannot be explained as an effect of mite infestations since nodes of first square, nodes above white flower, and yield were not statistically significant between planting dates. Cotton is known to be sensitive to fluctuations in temperature and light intensity (Baker 1965, Gipson 1986, Reddy et al. 1991), hence it is presumed that environmental conditions favored faster growth in the late planted cotton.

Conclusions

Spider mites can reduce yield when environmental conditions favor sustained densities for intervals greater than 14 days. Conversely, spider mites will not cause significant yield loss if environmental conditions do not favor spider mite development for extended periods of time.

Continued research is needed to understand how outbreaks of spider mites at different stages of cotton development and the duration of these infestation will affect yield. These

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studies should determine the relative **importance** of the duration of infestation and timing of infestation on cotton growth, development, and yield.

Relating mite density to yield loss remains challenging as our results indicate that depending on environmental conditions, cotton may tolerate higher spider mite densities without suffering yield loss, especially when weather conditions do not induce any stress on cotton plants. Overall, rating leaf damage is a more practical way of developing economic thresholds and implementing monitoring programs with producers instead of mite densities counts.

Finally, studying spider mites in the field has to be an organized effort, since our rate of establishing good infestations was low (25%) and Gore et al. (2013) had only 44 % success because environmental factors likely prevent the establishment of infestations. Therefore, spider mite field experiments should be replicated in multiple locations to increase the probability of successful establishing of infestations, thus improving the likelihood of generating results that can help improve management of this pest.

Table 1. Sequence of plant response parameters measured during four successive plant stages(2013).

	Square	Bloom	Cutout	Harvest
Plant Height	×*	×	×	_
Total Plant Nodes	×*	×	×	–
Node of First Square	×*	_	_	_
NAWF	_	_	×*	_
Yield	_	_	_	×*

 \times = measured, - = did not measure, * = measurements assessed in 2012 only.

Table 2. Monthly averages of high/low temperatures (C°) and precipitation (mm) during 2012, 2013, and 30 year average at Lon Mann Cotton Research Station, Marianna, Arkansas.

	2012				2013			30 Year Average		
	Avg	Avg	Total	Avg	Avg	Total	Avg	Avg	Total	
Month	high	low	Precipn	high	low	Precipn	high	low	Precipn	
January	13.4	1.9	48.8	10.9	1.9	216.2	8.9	-1.0	99.1	
February	13.6	4.0	100.6	12.0	1.9	122.7	11.6	1.2	108.5	
March	23.6	11.9	138.2	21.0	9.9	142.5	16.6	5.6	122.9	
April	25.1	12.4	28.4	21.0	9.9	142.5	22.1	10.4	127.8	
May	30.4	17.8	38.1	25.9	15.4	188.5	26.9	15.8	129.5	
June	31.8	19.4	19.8	32.0	20.6	18.8	31.2	20.1	100.1	
July	34.3	22.9	64.8	31.0	20.5	70.9	32.7	21.7	95.3	
August	34.3	22.9	64.8	31.7	20.9	47.8	32.6	20.7	67.1	
September	29.6	17.7	123.4	31.2	18.2	111.3	29.2	16.5	64.0	
October	21.8	9.9	114.6	23.0	12.0	68.3	23.3	10.3	104.6	
November	16.9	4.4	101.3	14.9	3.6	99.6	16.5	5.6	125.2	
December	13.3	4.6	90.4	9.8	1.3	180.1	10.3	0.6	140.2	

	Days After Initial Infestation						
Treatments	5	10	21	28	34	41	
Control	0.1 ± 0	0.6 ± 0	0	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	
Fourth true leaf short	4.4 ± 2.0	1.9 ± 0.4	0	0	0	-	
Fourth true leaf long	3.2 ± 0.9	2.8 ± 0.7	12.7 ± 5.7	1.7 ± 0.3	0	-	
Sixth true leaf short	0	0	4.1 ± 1.0	0	0	-	
Sixth true leaf long	0	0	3.4 ± 0.8	1.4 ± 0.2	0	-	
Ninth true leaf short	0	0	0	0	0.6 ± 0.3	0.3 ± 0.1	
Ninth true leaf long	0	0	0	0	0.6 ± 0.1	0.4 ± 0.1	

Table 3. Mean mite density (mites/cm² \pm SE) by treatments (infestation timing and infestation duration) in 2012, initial assessment started five days after first infestation time at fourth true leaf.

DAII= days after initial infestation

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Table 4. Mite leaf damage scores (score mean \pm SE) by treatment (infestation timing and infestation duration) during 2012, initial assessment started five days after first infestation time at fourth true leaf.

	Days After Initial Infestation							
Treatments	5	10	21	28	34	41		
Control	0.1 ± 0.1	0.1 ± 0.2	$0.2\pm~0.1$	03 ± 0.1	$0.5\pm~0.1$	2.0 ± 0.1		
Fourth true leaf short	1.1 ± 0.1	1.8 ± 0.2	0.3 ± 0.2	0.5 ± 0	0.3 ± 0.2			
Fourth true leaf long	1.4 ± 0.2	2.1 ± 0.2	2.0 ± 0.1	$2.3 (\pm 0.1)$	1.9 ± 0.2	-		
Sixth true leaf short	0.0	0.0	0.9 ± 0.2	1.3 (±0.1)	0.3 ± 0.1	-		
Sixth true leaf long	0.0	0.0	1.1 ± 0.1	1.6 (±0.1)	1.0 ± 0.1	-		
Ninth true leaf short	0.0	0.0	0.0	0.0	0.4 ± 0.2	0.4 ± 0.1		
Ninth true leaf long	0.0	0.0	0.0	0.0	0.7 ± 0.2	0.7 ± 0.1		

	Days After Initial Infestation				
Treatments	3	10	18	25	32
Control	0.0	0.0	0.0	0.0	0.0
Cotyledon short	0.3 ± 0.2	0.2 ± 0.1	0.0	0.0	0.0
Cotyledon medium	0.3 ± 0.2	0.2 ± 0.5	0.0	0.0	0.0
Cotyledon Long	0.5 ± 0.2	0.5 ± 0.1	1.0 ± 0.4	0.7 ± 0.3	1.9 ± 0.4
Fourth true leaf short	0.0	0.0	1.1 ± 0.2	0.1 ± 0	0.0
Fourth true leaf medium	0.0	0.0	0.8 ± 0.3	0.5 0.1	0.0
Fourth true leaf long	0.0	0.0	0.9 ± 0.3	0.6 ± 0.2	1.4 ± 0.3

Table 5. Mean mite density (mites/cm² \pm SE) by treatments (infestation timing and infestation duration) during 2013, initial assessment started five days after the first infestation time at cotyledon.

Table 6. Mite leaf damage scores (mean \pm SE) by treatment (infestation timing and infestation duration) in 2013, initial assessment performed starting five days after the first infestation time at cotyledon.

	Days After Initial Infestation				
Treatments	3	10	18	25	32
Control	0.0	0.0	0.0	0.0	0.0
Cotyledon short	0.0	0.2 ± 0.1	0.0	0.0	0.0
Cotyledon medium	0.0	0.7 ± 0.2	0.0	0.0	0.0
Cotyledon Long	0.0	0.5 ± 0.7	0.6 ± 0.2	0.5 ± 0.2	1.3 ± 0.2
Fourth true leaf short	0.0	0.0	0.3 ± 0.1	0.5 ± 0.1	0.0
Fourth true leaf medium	0.0	0.0	0.2 ± 0.1	0.7 ± 0.1	0.2 ± 0.1
Fourth true leaf long	0.0	0.0	0.1 ± 0.1	0.5 ± 0.2	0.9 ± 0.2

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Measurements	df	F Ratio	Prob > F
Nodes to first square	3	0.6587	0.5849
Plant heights at squaring	3	1.5336	0.2294
Plant nodes at squaring	3	1.6398	0.2045
Number of nodes above white flower	3	2.0035	0.1016
Yield	3	2.7574	0.0329*

Table 7. Plant response ANOVA parameters (2012).

*= significant, α = 0.05, df =degrees of freedom

Table 8. Yield main factors (infestation time and infestation duration) and their interaction.

Measurements	df	F Ratio	Prob > F		
Infestation time (IT)	2	1.6406	0.208		
Infestation length (IL)	1	8.0708	0.0074*		
Interaction IT× IL	2	0.9107	0.4113		
*= significant, α = 0.05, df= degrees of freedom					

Table 9. 2012 yield means \pm SEM results by treatment (infestation timing and infestation duration) during 2012.

Treatment	Yield Means ± SEM
Control	3197.85 ± 114.84
Fourth true leaf short	3040.81 ± 198.91
Sixth true leaf short	3571.88 ± 198.91
Ninth true leaf short	3383.41 ± 198.91
Fourth true leaf Long	2715.31 ± 198.91
Sixth true leaf Long	2796.69 ± 140.65
Ninth true leaf Long	3066.51 ± 198.91

Table 10. Yield contrasts between control and infestation duration at fourth true leaf, sixth true leaf, ninth true leaf, and all infestation times during 2012.

Contrast Between Control and	df	F Ratio	Prob > F	Yield ± SEM Kg/Ha
Fourth true leaf	1	4.4137	0.0404*	482.53 ± 229.68
Sixth true leaf	1	4.8809	0.03155*	401.16 ± 181.58
Ninth true leaf	1	0.3270	0.5698	131.34 ± 229.68
All long Durations	3	4.7346	0.0340*	338.34 ± 155.49

*= significant, α = 0.05, df= degrees of freedom

Table 11. LS-means comparison of plant response early planted vs. late planted differences for plant nodes and plant heights during 2013.

Measurements	F Ratio	Prob > F	Early Planted (SE)	Late Planted (SE)
Plant Nodes				
Squaring	292.22	<.0001*	$8.05\pm0.14b$	$11.54 \pm 0.15a$
Bloom	82.96	<.0001*	$10.83\pm0.13b$	$12.54 \pm 0.14a$
Cutout	72.18	<.0001*	$15.98\pm0.25b$	$19.10\pm0.27a$
Plant Heights				
Squaring	641.42	<.0001*	$32.78\pm0.74b$	$60.55\pm0.81a$
Bloom	223.99	<.0001*	$71.00\pm\ 0.80b$	$84.54\pm\ 0.87a$
Cutout	326.7437	<.0001*	$91.51 \pm 1.06b$	$119.90 \pm 1.16a$

*= significant, means follow by same letter are not significantly different

 Table 12.
 Plant response ANOVA parameters (2013).

Source	df	Mean Square	F Ratio	Prob > F
Nodes to first square	11	0.106744	0.4947	0.8983
Numbers of nodes above white flower	11	0.471289	1.2185	0.2977
Yield	11	4.47927	1.1451	0.3465

df= degrees of freedom

Figure 1. Pre-flowering treatments in 2012.



4=fourth true leaf, 6=sixth true leaf, L=long, M=medium, S=short

Figure 2. Pre-flowering treatments in 2013.



C= cotyledon, 4=fourth true leaf, L=long, M=medium, S=short

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