

## ARTHROPOD MANAGEMENT

### Impact of Twospotted Spider Mite (Acari: Tetranychidae) Infestation Timing on Cotton Yields

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#### ABSTRACT

**Twospotted spider mite, *Tetranychus urticae* (Koch), has become a significant early season pest of cotton, *Gossypium hirsutum* L., in the midsouthern U.S. Sixteen experiments were conducted across the midsouthern U. S. to determine the impact of twospotted spider mite infestation timing on cotton injury, stunting, and yields. Twospotted spider mites from a greenhouse colony were used to initiate infestations at the three-leaf stage, at first flower, and at 200 heat unit intervals after first flower. Twospotted spider mite injury on a scale of zero to five (0 = no injury, 5 = severe injury), plant stunting, and final cotton yields were measured. In general, all infestation timings had higher injury ratings compared to the uninfested control. The highest injury ratings were observed for the three-leaf and first flower infestations. Additionally, infestations at the three-leaf stage caused more plant stunting than later infestation timings. In most of the experiments, the three-leaf infestation resulted in significant**

**stunting of cotton plants. For cotton yields, early infestations caused the greatest yield losses. Significant yield losses compared to the untreated control were observed for infestations initiated up to first flower plus 800 heat units. These results suggest that cotton should be protected from twospotted spider mite infestations beyond that point in the growing season. Results from this experiment will be used to improve integrated pest management of twospotted spider mite in cotton.**

The pest status of twospotted spider mite, *Tetranychus urticae* (Koch), in cotton, *Gossypium hirsutum* L., has changed over the last decade across the midsouthern U. S. Historically, spider mites were considered a late season pest in this part of the U.S., and pesticide applications were rarely needed during the pre-flowering and early flowering stages of cotton development. Spider mites have become an increasing problem in recent years in the Midsouth. For example, the number of acres sprayed in Mississippi has more than doubled since 2005 (Williams, 2010). The majority of that increase can be attributed to applications made during the early- to mid-flowering stages that were not made prior to 2005. Several factors might have contributed to the increase in the importance of spider mite as a season-long pest. Insecticide, fungicide, and nematicide seed treatments replaced the use of aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) in most fields across the Midsouth. The neonicotinoids, imidacloprid (Gaucho Grande 5FS, Bayer CropScience, Research Triangle Park, NC), and thiamethoxam (Cruiser 5 FS, Syngenta Crop Protection, Greensboro, NC) used as the insecticidal component of these seed treatments has been shown to increase mite densities compared to aldicarb (Troxclair, 2007). Similar results have been observed with neonicotinoids in other crops applied as seed treatments and foliar sprays (Beers et al., 2005; Sclar et al. 1998).

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Additionally, the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has become a serious pest of cotton in the Midsouth (Musser et al., 2009). Multiple applications of broad spectrum insecticides are needed every year to minimize yield losses from this insect. Because of widespread resistance, high rates of organophosphates or neonicotinoids applied in a tank mix with pyrethroids are the most common treatments for tarnished plant bug. These applications disrupt beneficial arthropod populations and create an ideal environment for rapid population increases of secondary pests such as twospotted spider mite.

Currently, little information exists about the impact of twospotted spider mites on yields of cotton in the Midsouth with current transgenic varieties. In the Midsouth, previously reported yield losses ranged from 30 to 35% during the early fruiting period (Furr and Pfrimmer, 1968). In more arid environments such as California and Australia, greater than 90% yield losses have been observed from spider mites (Sadras and Wilson, 1997; Wilson, 1993; Wilson et al., 1987, 1991). Although no data exist that suggest cotton should respond differently to spider mite infestations in different environments, variability in environmental conditions might impact spider mite population dynamics. For instance, spider mite populations would be expected to increase more rapidly in arid environments such as California and Australia compared to the subtropical Midsouth where high humidity and rainfall frequently occur (Boudreaux, 1958). As a result, yield losses from spider mites would be expected to be lower in the Midsouth than in more arid environments. Given the lack of information about yield losses caused by spider mites in cotton in the Midsouth, thresholds in most states are vague and not reliable. For instance, the thresholds in Mississippi (<http://msucare.com/pubs/publications/p2471.pdf>), Arkansas ([http://www.uaex.edu/Other\\_Areas/publications/PDF/MP144/MP144.pdf](http://www.uaex.edu/Other_Areas/publications/PDF/MP144/MP144.pdf)), and Tennessee (<http://eppserver.ag.utk.edu/redbook/redbook.htm>) suggest that acaricides should be applied when 30 to 50% of plants are infested and populations are increasing.

No information exists about when to terminate acaricide applications during the cotton growing season. In an experiment that investigated two spider mite infestation timings, plant productivity increased as time of infestation after planting increased (Wilson et al., 1987). Infestations were not initiated be-

yond the early flowering period in that experiment. In the Midsouth, infestations do not generally begin until the late squaring period and extend through the flowering period. Additional research is needed to improve the thresholds for spider mites and to determine the time during the season when cotton is no longer susceptible to yield losses from twospotted spider mite. The objective of the current experiment was to determine the impact of twospotted spider mite infestation timing during the flowering period on cotton injury, stunting, and yields.

## METHODS

Experiments were conducted in Arkansas, Louisiana, Mississippi, Missouri, and Tennessee from 2009 to 2011 to determine how the timing of twospotted spider mite infestations affected cotton yields. Infestation timings were artificially established from greenhouse colonies reared on bush-type green beans, *Phaseolus vulgaris* L. Colonies were collected each year from henbit, *Lamium amplexicaule* L., during March and maintained in the greenhouse until the time of infestation. At the time of infestation, twospotted spider mite densities averaged approximately 150 to 300 mites per bean plant. The infested green beans were clipped near the soil surface and placed directly over the top of cotton plants, ensuring direct contact between cotton foliage and green bean foliage. Green bean plants were laid from end to end over the entire length of the center two rows of each plot.

Treatments included twospotted spider mite infestations initiated at the third true leaf stage, first flower, and at various intervals after first flower, resulting in a total of seven infestation timing treatments plus an uninfested control. The intervals of infestation after first flower were based on heat unit accumulation from the degree day model for cotton development (Mauney, 1986). Daily heat unit totals were derived by subtracting 60 °F (15.5 °C) from the sum of the daily high and low temperatures divided by 2, where 60 °F is the minimum temperature for cotton growth. The intervals included infestations every 200 heat units until 1000 heat units after first flower. Daily heat unit accumulations ranged from 22 to 28 during the time of these experiments. Infestation timings were derived by adding the daily heat units from the date of infestation, beginning at the first flower infestation, until the next infestation date when at least 200 heat units had been accumulated.

Plot size was four rows (96.5-101.6 cm centers) by 6.1 m long. The experiment was arranged as a randomized complete block with four to five replications at each location. Plots were separated by two unplanted rows and 3.0-m alleys consisting of unplanted bare ground to reduce migration of mites between plots. Only the two center rows of each plot were infested with twospotted spider mites. Once infested, every effort was made to maintain damaging densities of mites on the cotton until defoliation. If densities began to decline, plants were re-infested and/or treated with acephate to reduce predatory arthropods. Specific cotton varieties varied among locations and years, but a dual gene Bt cotton variety (Bollgard II<sup>®</sup>, Monsanto Company, St. Louis, MO, or Widestrike<sup>™</sup> Dow AgroSciences, Indianapolis, IN) was planted at each location to minimize the impact of lepidopteran pests on final cotton yields. Additionally, prophylactic applications were made with neonicotinoid, pyrethroid, and organophosphate insecticides applied as tank mixtures to manage other insect pests and reduce beneficial arthropod populations to assist with the establishment of mite populations. If twospotted spider mites began migrating into plots before they were designated to be infested, an application of an appropriate acaricide was made to minimize their impact until the time of infestation. Acaricides were chosen based on their relative residual activity and activity on different life stages. If plots were within 10 d of infestation, bifenthrin (Brigade 2EC, FMC Corporation, Philadelphia, PA) was used because it does not have ovicidal activity and has relatively short residual activity. If twospotted spider mites began migrating into plots more than 10 d before infestation, abamectin (Agri-Mek 0.15EC, Syngenta Crop Protection, Greensboro, NC), spiromesifen (Oberon 4SC, Bayer CropScience, Research Triangle Park, NC), or etoxazole (Zéal 72WSP, Valent U.S.A. Corporation, Walnut Creek, CA) was applied because they provide longer residual control and have ovicidal activity. Uninfested plots were sprayed as needed with one or more of these acaricides.

Visual damage ratings were taken to capture and describe the physical damage caused by twospotted spider mites throughout the growing season. Because individual rating times varied significantly among locations and years, a single rating was used from each location where twospotted spider mites were successfully established for analysis. The rating used for the combined analy-

sis was chosen based on when the average mite ratings were at their maximum for the selected locations and was within 1 wk of the first flower plus 1000 heat unit infestation timing. Twospotted spider mite injury was rated based on a leaf reddening index scale of zero to five where: 0 equals no damage, 1 equals light stippling occurring on sporadic leaves, 2 equals stippling and reddening present on 15 to 20% of leaves, 3 equals 50% of leaves have apparent reddening on basal portions, 4 equals greater than 50% of leaves contain extensive reddening and leaves begin to excise, and 5 equals nearly complete reddening and/or defoliation on all leaves. In addition to visual damage ratings, plant stunting was recorded on a scale of zero to 100% based on relative plant heights in the infested plots compared to mean plant heights in the uninfested control.

At the end of the season, the two center rows of each plot were mechanically harvested. A sample of seed cotton from each plot was ginned to determine lint percentage. The lint percentage was used to calculate lint yield in kilograms per hectare for each plot.

Response variables were analyzed with a mixed model analysis of variance (PROC MIXED, Littell et al., 1996). Test (location\*year) and infestation timing were treated as fixed effects in the model. Replication nested within year was the random factor and served as the error term for test, infestation timing, and the test by infestation timing interaction. Degrees of freedom were estimated using the Kenwood-Rodgers method. Means were estimated using the lsmeans statement and separated using Fisher's Protected LSD.

## RESULTS

A total of 16 experiments were conducted from 2009 to 2011. Only seven experiments were able to establish successfully and maintain spider mite infestations at a level to analyze their impact on cotton yields. For the final analyses, only those seven experiments were included in the model. One of the locations (Mississippi, 2010) used in the final analysis did not have ratings for twospotted spider mite injury at a similar timing to the other six locations and was excluded from the final analysis for injury. For twospotted spider mite injury ratings, there was a significant test by treatment interaction ( $F = 5.13$ ;  $df = 25, 114$ ;  $P < 0.01$ ). Therefore, data were analyzed by test (year\*location) for spider mite injury ratings.

Injury ratings were significantly impacted by twospotted spider mite infestation timings in all tests (Table 1). In general, the earlier infestation timings had higher injury ratings than the later infestation timings and twospotted spider mite infestations initiated at third leaf, first flower, and first flower plus 200 heat units had injury ratings higher than the uninfested control. Infestations initiated at first flower plus 400 heat units and 600 heat units had higher injury ratings in four and three tests, respectively.

Similar to twospotted spider mite injury ratings, one of the locations (Mississippi, 2010) used in the final analysis did not have ratings for stunting at a similar timing to the other six locations and was excluded from the final analysis for those factors. For percent stunting caused by twospotted spider mite, there was a significant test by treatment interaction ( $F = 4.44$ ;  $df = 20, 95$ ;  $P < 0.01$ ). Therefore, data were analyzed by test (year\*location) for percent stunting. Significant stunting was observed in four of the six tests (Table 2). In those tests, infestations that were

initiated at third leaf had significant levels of stunting. Significant stunting for infestations initiated at first flower was observed in only one test.

When averaged across the seven experiments where infestations were established, there was a significant effect of twospotted spider mites on cotton yields ( $F = 15.49$ ;  $df = 7, 206$ ;  $P < 0.01$ ) (Fig. 1). Twospotted spider mites reduced yields below that observed in the uninfested plots for infestations that were established up to first flower plus 800 heat units. The lowest yields were observed when infestations were initiated at the three-leaf stage. When twospotted spider mite infestations were initiated at first flower and first flower plus 200 heat units, yields were lower than when infestations were initiated at first flower plus 400 heat units to first flower plus 1000 heat units. Overall, mean (SEM) yields ranged from 878.8 (50.7) kg lint per hectare when twospotted spider mite infestations were initiated at the three-leaf stage to 1256.8 (42.5) kg lint per hectare in the uninfested control, representing an average maximum yield loss of 30%.

**Table 1. Impact of twospotted spider mite infestation timing on injury ratings during the late flowering period of cotton**

Timing	Mean (SEM) Injury Rating at Each Location <sup>z</sup>					
	1	2	3	4	5	6
Third Leaf	2.9 (0.21)a	0.5 (0.29)bc	3.6 (0.14)a	4.0 (0.00)a	2.6 (0.24)ab	3.7 (0.20)a
First Flower	2.3 (0.29)a	2.5 (0.50)a	2.4 (0.31)b	4.0 (0.00)a	3.0 (0.32)a	2.3 (0.18)b
FF + 200 HU	1.5 (0.18)b	1.3 (0.48)b	1.3 (0.27)c	3.0 (0.58)b	2.6 (0.24)ab	1.6 (0.18)c
FF + 400 HU	1.4 (0.21)b	0.8 (0.25)bc	0.9 (0.33)cd	1.5 (0.29)c	3.0 (0.32)a	1.1 (0.29)cd
FF + 600 HU	1.4 (0.26)b	0.3 (0.25)c	0.3 (0.08)de	1.0 (0.41)c	1.8 (0.37)b	0.6 (0.23)de
Uninfested	0.6 (0.30)c	0.3 (0.25)c	0.2 (0.03)e	0.8 (0.25)c	0.4 (0.24)c	0.2 (0.07)e
F	9.2	7.3	35.1	20.4	11.5	39.2
df	5, 17	5, 17	5, 18	5, 18	5, 24	5, 18
P>F	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

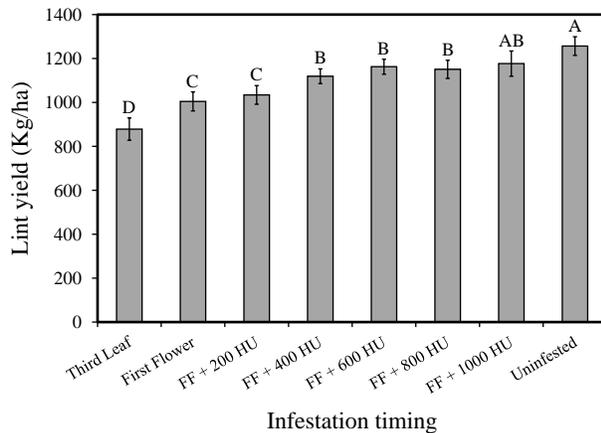
<sup>z</sup> Means within a column followed by a common letter are not significantly different according to Fisher's Protected LSD ( $\alpha = 0.05$ ).

**Table 2. Impact of twospotted spider mite infestation timing on percent stunting of cotton plants during the late flowering period**

Timing	Mean (SEM) Percent Stunting at Each Location <sup>z</sup>					
	1	2	3	4	5	6
Third Leaf	10.3 (1.18)a	20.0 (4.08)a	10.0 (2.04)a	32.5 (4.79)a	24.0 (3.67)a	2.5 (1.44)a
First Flower	6.3 (1.25)a	13.8 (4.73)ab	9.5 (3.69)a	2.5 (2.50)b	6.0 (2.45)b	1.3 (1.25)a
FF + 200 HU	4.5 (1.32)a	3.8 (1.25)b	2.8 (1.70)b	2.5 (2.50)b	6.0 (4.00)b	1.3 (1.25)a
FF + 400 HU	4.5 (2.60)a	0 <sup>y</sup>	1.0 (1.00)b	2.5 (2.50)b	2.0 (2.00)b	0 <sup>y</sup>
FF + 600 HU	4.3 (1.49)a	0 <sup>y</sup>	0.3 (0.25)b	2.5 (2.50)b	2.0 (2.00)b	0 <sup>y</sup>
F	2.6	5.0	9.8	19.2	9.7	0.3
df	4, 14	2, 9	4, 14	4, 14	4, 20	2, 9
P>F	0.08	0.04	< 0.01	< 0.01	< 0.01	0.75

<sup>z</sup> Means within a column followed by a common letter are not significantly different according to Fisher's Protected LSD ( $\alpha = 0.05$ ).

<sup>y</sup> Individual treatments with a mean of zero were excluded from the final analysis.



**Figure 1. Mean (SEM) lint yield across all locations and years as a result of twospotted spider mite, *T. urticae*, infestation timing (FF = first flower).**

## DISCUSSION

These experiments demonstrate the difficulty of using artificial infestation procedures to quantify the impact of twospotted spider mite on plant injury, stunting, and yield losses in cotton. Of the 16 experiments that were attempted in five states over a three-year period, spider mites were established at levels sufficient to quantify their impact on yields in only seven experiments. Multiple factors might have impacted the establishment of twospotted spider mites, but the most common factor was rainfall soon after the time of infestation.

Infestation timing of twospotted spider mites had a significant impact on injury, stunting, and yield in these experiments. Few experiments utilizing timed infestations of laboratory colonies to quantify the impacts of twospotted spider mites on cotton yields have been conducted. In a recent experiment conducted in Mississippi, up to a 45% yield loss was observed for infestations initiated at the three leaf stage of cotton development when those infestations persisted for four weeks (Smith, 2010). In a similar experiment, a 35% yield reduction was observed from infestations that were initiated during mid-season (Furr and Pfrimmer, 1968). Significant yield reductions were not observed for early-season or late-season infestations in that experiment. In similar experiments in Australia, significant yield reductions ranged from 13 to 48% for early-season infestations and 7 to 34% for mid-season infestations (Wilson, 1993). In the current experiment, yield reductions ranged from 20 (first flower) to 30% (three leaf) for infestations that

were initiated early-season. For infestations that were initiated mid-season, yield reductions ranged from 7% at first flower plus 600 heat units to 18% at first flower plus 200 heat units.

Although the methods and results are similar to previous experiments, the current experiment more accurately defined infestation timings based on plant development stages, as opposed to vague infestation timings of early, mid, and late. Additionally, the current experiment accurately defines when cotton is no longer susceptible to yield losses from twospotted spider mites. Significant yield reductions were not observed when twospotted spider mite infestations were initiated at first flower plus 1000 heat units. This indicates that management strategies targeting twospotted spider mites in cotton can be terminated at this point. Based on historic weather data obtained from the Mississippi State University weather center (<http://ext.msstate.edu/anr/drec/weather.cgi>), 1000 heat units would be accumulated in approximately 35 to 45 d during the normal flowering window of cotton development (June-July). These figures are based on mean daily heat unit accumulations of 28 and 22 per d, respectively. Therefore, growers should terminate acaricide applications approximately 35 to 45 d after first flower in the midsouthern U.S.

All of the locations in the current experiment were irrigated and heavily managed for optimum plant growth and yield. These recommendations provide a rough guide that might need to be adjusted under different conditions. For instance, cotton grown under dry land conditions might be more susceptible to twospotted spider mite for a longer period of time and might need additional protection beyond that described in the current experiment. For the majority of cotton fields in the midsouthern U.S., these recommendations will provide a good guide for managing twospotted spider mites. These results will be used in the midsouthern U.S. to improve integrated pest management of twospotted spider mites in cotton.

## ACKNOWLEDGMENT

The authors thank Cotton Incorporated for funding this research under agreement #09-604. This manuscript has been approved for publication as Journal Article No. J-12220 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

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