# Experimental Treatment Threshold for the Cotton Aphid (Homoptera: Aphididae) Using Natural Enemies in Arkansas Cotton<sup>1</sup>

Hugh E. Conway,<sup>2</sup> Donald C. Steinkraus, John R. Ruberson,<sup>3</sup> and Timothy J. Kring<sup>4</sup>

Department of Entomology, University of Arkansas, Fayetteville, Arkansas 72701 USA

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Abstract The potential of an experimental threshold for reducing the number of insecticide applications for control of the cotton aphid, Aphis gossypii Glover, was demonstrated in cotton. A 3-yr field study at Clarkedale, AR, compared the current economic threshold for the cotton aphid to an experimental threshold that incorporates predaceous coccinellids, aphid parasitoids and the aphid fungus, Neozygites fresenii (Nowakowski) Batko. Treatments consisted of untreated plots, conventional treatment threshold plots, experimental threshold plots, and a fungicide treatment designed to disrupt aphid fungus epizootics. This fungicide treatment failed to influence fungal epizootics, so data for these plots were removed. Twice weekly, aphids were counted on 20 plants per plot and predators were counted using a dislodgment method. An application of 0.033 kg ai/ha of imidacloprid was made when aphids reached treatment level according to conventional or experimental threshold levels. Insecticide applications were triggered by the conventional threshold on 18 and 28 June 1999, on 28 June and 3 July 2000, and on 7 and 12 July 2001. The experimental threshold called for applications on 28 June 1999. 3 July 2000, and 19 July 2001. Aphid densities peaked in untreated plots at 142, 76, and 27 aphids per leaf and in treated plots at 49, 34, and 29 in 1999, 2000 and 2001, respectively. Larval coccinellid densities in untreated plots peaked at 9.0, 3.7, and 0.6 larvae per row-m and in treated plots 3.0, 1.4, and 0.5 in 1999, 2000 and 2001, respectively. Adult coccinellid densities peaked in untreated plots at 3.0, 2.7, and 0.5 adults/row-m and in treated plots at 0.8, 2.1, and 0.5 in 1999, 2000, and 2001, respectively. During each year, an epizootic of N. fresenii caused a rapid decrease in aphid numbers in mid to late July, eliminating the need for additional treatments. In 1999, significantly lower yield of lint occurred in the untreated plots than in conventional or experimental plots. No significant yield differences among treatments occurred in 2000 or 2001. Use of the experimental threshold delayed the initial insecticide application by 1 wk and eliminated one insecticide application on the experimental plots whereas maintaining cotton yields.

Key Words biological control, Aphis gossypii, Neozygites fresenii, coccinellid, threshold

The cotton aphid, *Aphis gossypii* Glover, has been an economic pest of cotton since the 1940s with recent outbreaks causing serious damage, resulting in delayed

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<sup>&</sup>lt;sup>2</sup>Department of Entomology, Clemson University, Clemson, South Carolina 29634.

<sup>&</sup>lt;sup>3</sup>Department of Entomology, University of Georgia, Tifton, Georgia 31793.

<sup>&</sup>lt;sup>4</sup>Address inquiries: (email: tkring@uark.edu).

maturity and annual yield losses in excess of 45.4 million kg of lint (Roberts et al. 1997). Texas alone reported the loss of more than 50,000 bales of cotton to aphids in 2000 (Williams 2001). In 2002, aphids were present in 70% or 3.8 million ha of U.S. cotton (Williams 2003). Use of insecticides to manage aphids may cause disruption of beneficial species and the development of insecticide resistance in many pest species, including the cotton aphid (O'Brien et al. 1992, Luttrell 1994). Two significant, recent changes in cotton production systems, i.e., the use of transgenic Bt cotton and the boll weevil eradication program, provide an opportunity to develop and implement a more biologically based management system that offers the potential to reduce foliar insecticide use whereas increasing profitability.

The beneficial insect fauna in cotton is extremely diverse (Whitcomb and Bell 1964, van den Bosch and Hagen 1966, Wells et al. 2001), but the diversity is reduced by insecticidal disruptions (Goodenough et al. 1986). Aphids are attacked by a large number of parasitoids and generalist predators, encompassing eight insect orders (Frazer 1988). The groups commonly credited with aphid control are parasitic braconids, and predaceous coccinellids, chrysopids, and syrphids. The parasitoids *Lysi-phlebus testaceipes* (Cresson) and, to a lesser extent, *Diaeretiella rapae* (M'Intosh) are important parasitoids of the cotton aphid in the southeastern and midsouthern U.S. (Weathersbee and Hardee 1994, Whitcomb and Bell 1964).

Entomophagous insects are an important means of aphid control in early and midseason cotton, and adult and larval coccinellids as a group appear to be the most important of these (Knutson and Ruberson 1996). Coccinellids have been associated with biological control more often than any other insect taxa (Obrycki and Kring 1998). *Hippodamia convergens* Guerin, *Coleomegilla maculata* (Degeer), *Coccinella septempunctata* L., *Harmonia axyridis* (Pallas), *Cycloneda munda* (Say), *Scymnus* spp., and *Diomus* spp. are all commonly collected from cotton fields (Knutson and Ruberson 1996).

Cotton aphid populations are subject to epizootics caused by the naturallyoccurring entomopathogenic aphid fungus *Neozygites fresenii* (Nowakowski) Batko usually in early to mid-July in Arkansas (Steinkraus et al. 1992). After an epizootic starts, an aphid population is rapidly reduced (Steinkraus et al. 1996) from its peak densities to numbers below economic thresholds (Steinkraus and Hollingsworth 1994). When aphid populations are highest, the majority of aphids are usually distributed in the middle and lower canopy where a more favorable microenvironment exists for aphid fungal epizootics (O'Brien et al. 1993). During an epizootic event, cotton fields are saturated with fungal spores yet infections in arthropods other than aphids have not been reported. At a prevalence of 15% infection with *N. fresenii*, cotton aphid densities often decline below economic thresholds within a week (Hollingsworth et al. 1995).

Improved aphid management that reduces the number of insecticide applications will allow natural enemies to survive, multiply and disperse. This reduction in insecticides is especially important during the early cotton season when aphids present in the field attract generalist predators (Knutson and Ruberson 1996, Wells et al. 1999). Currently, treatment threshold in Arkansas rely on repeated samples for detection of increasing aphid populations that reach 50% infested plants (Greene 2004). The current threshold does not take aphid densities into account nor does it look at the presence of natural enemies.

A 3-yr field study at Clarkedale, AR, compared the current economic threshold for

the cotton aphid to a tentative threshold incorporating beneficial insects and the aphid fungus, *N. fresenii*, into the decision-making process for insecticide applications. Seasonal fluctuations in aphid densities (aphids per leaf), natural enemy densities (percentage parasitism, coccinellid per row-m), and cotton lint yields were compared in plots using conventional treatment, experimental treatment, and untreated control. This study is the first step in designing a management method that incorporates the action of natural enemies into a threshold for the cotton aphid.

## Materials and Methods

A 3-yr (1999-2001) field study was conducted at the University of Arkansas Delta Branch Research Station in Clarkedale, AR. Sixteen plots, each 0.3 ha (50 rows × 55 m), were planted with *Gossypium hirsutum* L. (NuCott 33B, Delta and Pine Land Company, Scott, MS 38,772) on 4 May 1999, 2 May 2000, and 28 April 2001 in 1-m wide rows. NuCotn 33B is a transgenic Bt cotton that limits feeding damage from lepidopteran pests. Cotton was grown under standard cultural practices with respect to weed control, irrigation, fertilization and insect management (other than aphids). Cotton lint yields were measured in each plot at the conclusion of the season using a 2-row picker.

The test was a Latin square design with four replicates of four treatments: (1) aphids treated under the conventional threshold, (2) aphids treated using the experimental threshold, (3) untreated control, and (4) fungicide treated. The conventional threshold plots were treated with the insecticide imidacloprid (0.033 kg ai/ha) (Provado®1.6F, Bayer CropScience, KS City, MO 64,120) when >50% of the plants were infested and aphid populations were increasing (Greene 2004). Experimental plots were treated with imidacloprid (0.033 kg ai/ha) when aphid numbers were increasing, aphids were present on >50% of cotton plants, and aphid densities exceeded: (1) 15 aphids/leaf if "no" fungus (i.e., *N. fresenii*), parasitoids or coccinellid adults/row-m, or 0.6 coccinellid larvae/row-m were observed, (3) 50 aphids/leaf if <10% visible fungus, 10% mummies, 0.9 coccinellid adults/row-m, or 0.6 coccinellid larvae/row-m were observed, or (4) 70 aphids/leaf if <10% visible fungus, 10% mummies, 0.9 coccinellid adults/row-m, or 0.6 coccinellid larvae/row-m were observed, or (4) 70 aphids/leaf if <10% visible fungus, 10% mummies, 0.9 coccinellid adults/row-m, or 0.6 coccinellid larvae/row-m were observed, or (4) 70 aphids/leaf if <10% visible fungus, 10% mummies, 0.9 coccinellid adults/row-m, or 0.6 coc-

The treatment and replicate combinations were randomized yearly. The fungicide treatments involved prophylactic multiple applications of fungicide. Plots were treated in 1999 (3×) with 280 g ai/ha benomyl (Benelate®, DuPont Wilmington, DE 19,898) and in 2000 (3×) and 2001(4×) with 210 g ai/ha azoxystrobin (Quadris®, Syngenta Crop Protection Greensboro, NC 27,419). This treatment regimen was an attempt to disrupt the action of the aphid fungus in an attempt to partition its effect, based on previous research (Wilding 1982, Smith and Hardee 1993). Because these treatments failed to disrupt epizootics, data from the fungicide treatment are not included here.

Beginning in early June, aphid counts were taken twice weekly from one fullyexpanded terminal and one middle leaf of 20 randomly sampled plants per plot. Aphids were counted and classified as small (first or second stage), large (third stage or larger), winged, and mummified (parasitized). Sampling continued until aphid populations dropped to near zero in late July 1999 and 2000 and early August 2001. When aphid densities were sufficient, five aphid-infested terminal and five aphidinfested middle leaves per plot were collected weekly and placed in labeled vials with 70% ethanol. These aphids were diagnosed under a microscope for the presence and percentage infection by the fungus *N. fresenii* using established laboratory techniques (Steinkraus and Boys 1997).

Samples of predators were taken twice weekly by a dislodgement method in which plants were struck onto a hardware mesh (0.079 cells per cm) covering a plastic wash basin (355 mm × 285 mm × 135 mm) (Elkassabany 1994). Density levels of predators were obtained by sampling 8 row-m per plot (8 samples per plot with each sample 1 row-m long). Predator sampling began in early June and continued until late July in 1999 and 2000, and early August 2001.

Lint yields among treatments were compared by standard analysis of variance procedures with means separated by LSD when appropriate (SAS Institute Inc. 1999). The daily means of aphid populations and larval and adult populations of coccinellids were plotted, as were overall relative densities of the most common beneficial insects on a seasonal basis. Combined seasonal aphid densities were compared by treatment using analysis of variance, and significantly different means were separated using LSD (SAS Institute Inc. 1997). Population dynamics between aphids, coccinellid larvae, and coccinellid adults were compared across the years for 1999 and 2000.

#### Results

Aphid densities varied from year to year with overall densities decreasing from 1999-2001 (Fig. 1). Aphid densities in untreated plots increased rapidly, reaching population peaks in early July 1999 and 2000 and late July 2001 until a fungal epizootic caused the population to rapidly decline. Each year in imidacloprid-treated plots, the aphid densities increased rapidly to a treatment threshold, then after treatment declined. Aphids infected with *N. fresenii* were first observed in samples during the first week of July 1999, last week of June 2000, and the second week of July 2001. The fungus spread across the field reaching infection rates above 15% (Fig. 1) from 5-16 d after initial detection. Within a week of reaching the 15% infection rate, aphid densities fell below threshold levels and rapidly declined in early July 1999 and 2000 and late July 2001 (Fig. 1).

Aphid densities were significantly higher (F = 5.2, df = 8, P < 0.05) in untreated plots relative to treated plots with the overall number of aphids in untreated plots having a seasonal mean of 29.9 ± 9.2 aphids/leaf per day, experimental plots with 8.0 ± 2.0 aphids/leaf per day, and conventional plots with 7.0 ± 2.7 aphids/leaf per day (±SE). No significant difference in aphid numbers occurred between conventional and experimental plots.

Common beneficial insects collected and identified over the 3-year study included adult and larval coccinellids (*Hippodamia convergens* Guerin, *Scymnus* spp., *Diomus* spp., *Coleomegilla maculata* (Degeer), *Harmonia axyridis* (Pallas), *Coccinella septempuncta* L.); adult and larval lacewings (*Chrysopa* spp., *Chrysoperla* spp., *Hemerobius* spp.); adult and larval predaceous Heteroptera (*Geocoris punctipes* (Say), *Geocoris uliginosus* (Say), *Orius insidiosus* (Say), *Nabis* spp.); and spiders. The percentage of collected beneficial insects was based on a total capture of 3,634 insects in 1999, 3,562 in 2000, and 1644 in 2001. In this study, the most abundant aphid predators were adult and larval coccinellids, which together composed 79% of the captured beneficial insects in 1999, 68% in 2000, and 53% in 2001 (Fig. 2). There was a decrease in overall numbers of coccinellid larvae over the 3-yr study period



Fig. 1. Aphids per leaf in untreated plots and those using conventional and experimental treatment thresholds. Imidacloprid applications are indicated by arrows.



Fig. 2. Predaceous insects collected from cotton in Clarkedale, AR, by year.

(Fig. 3), likely due to the similar yearly decrease in aphid densities (Fig. 1). The number of coccinellid adults fluctuated less predictably among years with 750, 1100, and 500 sampled in the years 1999, 2000, and 2001, respectively. Predaceous Heteroptera, Chrysopidae, Hemerobiidae, and other insects fluctuated with little relationship to aphid densities (data not shown) in number from year to year (Fig. 2).

**1999 season.** Data collection of insects began on 16 June and ended on 19 July 1999. Aphid densities in untreated plots were 3× higher than in treated plots. Aphid densities in untreated plots peaked at 142 aphids per leaf on 4 July then dropped to near 0 on 14 July. Aphid densities reached the conventional threshold levels in midJune, resulting in treatments on 18 and 25 June (Fig. 1). The densities in experimental plots reached threshold approximately 1 wk later than the conventional treatment and were treated only once on 25 June.

The aphidophagous fungus was first detected on 1 July from samples taken in the northwest section of the field. The fungal epizootic spread across the field reaching infection for *N. fresenii* above 15% in all samples by 6 July.

A delayed density-dependent relationship was observed as the cotton aphid population increase was followed in 5 d by a larval coccinellid increase (Fig. 3). Adult coccinellid densities subsequently increased as larvae pupated and new adults emerged (Fig. 4). Aphid densities had already started to decline from the fungus outbreak when coccinellid densities were highest. The number of larvae in untreated control plots contained almost 5× the larvae number per row-m as did treated plots (Fig. 3). Larval coccinellid densities in the experimental and conventional treatments fluctuated near 2 larvae per row-m from 24 June to 12 July, before dropping to 0 on 19 July. Untreated plots contained almost 4× the number of adult coccinellids per row-m as did the treated plots, peaking on 14 July. Adult coccinellid densities in conventional and experimental plots gradually increased up to 0.8 adult per row-m (Fig. 4).

**2000 season.** Sampling began 6 June and continued until 24 July 2000. Overall, aphid densities in 2000 were lower than in 1999. Aphid densities in the untreated plots



Fig. 3. Larval coccinellids per row-m by year at Clarkedale, AR, in untreated plots and those using conventional and experimental treatment thresholds.



Fig. 4. Adult coccinellids per row-m by year at Clarkedale, AR, in untreated plots and those using conventional and experimental treatment thresholds.

in 2000 were less than 1/3 of those in 1999 (Fig. 1). However, aphid dynamics were generally similar, with density increases in mid-June and the infestation continuing until mid-July. The aphid densities in imidacloprid-treated plots were also much lower in 2000 than in 1999. Cotton aphid populations increased in late June, reaching the conventional threshold level resulting in treatments on 28 June and 3 July. The experimental plots reached threshold level and were treated once on 3 July.

The aphid fungus was first detected on 27 June in aphid samples taken from the northwest and southwest sections of the field. The fungal epizootic spread across the field reaching infection above 15% positive for *N. fresenii* by 4 July.

A delayed density-dependent response of coccinellids to cotton aphid densities was again evident in 2000 (Figs. 3, 4). There were twice as many coccinellid larvae in the untreated plots than in the treated plots (Fig. 3), although there were only about half the number observed in 1999. This reduction from year to year correlates to the decrease in the number of aphids from 1999-2000. Although the number of adult coccinellids per row-m found in the untreated plots was much higher than in the treated plots and were similar in both years, the adult coccinellid populations for conventional and experimental plots were higher in 2000 than in 1999 (Fig. 4).

**2001 season.** Samples were taken from 4 June through 6 August and aphid dynamics were similar to previous years. Overall, aphid populations were much lower than in either 1999 or 2000 (Fig. 1). However, aphid increase in 2001 occurred approximately 1 month later, with the aphids increasing in midJuly and the infestation continuing until early August. Aphid densities reached threshold and required treatments on 7 and 12 July for conventional and on 19 July for the experimental plots.

The aphid fungus was first detected on 9 July in aphid samples taken from the western sections of the field. The fungal epizootic spread across the field, reaching levels exceeding 15% for *N. fresenii* by 25 July.

In 2001, there were treatments of malathion for the boll weevil eradication program in Clarkedale, AR, on 5 and 15 June, and on 3, 11, 18, and 24 July. The malathion treatments occurred between the 2 weekly beneficial insect counts, and although the plots themselves were not treated, coccinellid densities were clearly affected. Coccinellid larval and adult numbers in all treatments fluctuated below 0.6 larvae per row-m all summer (Figs. 3, 4).

**Population dynamics.** Comparison between years and across years indicated similar dynamics between populations of aphids, coccinellid larvae, and coccinellid adult with decreasing magnitude from 1999-2000. For both years, there was a two to three day lag period between increasing aphid populations and increasing coccinellid larvae densities. As cotton aphid populations peaked, *N. fresenii* produced mortality increased. During both years, larval populations increased for four days after the cotton aphid maximum per leaf count before beginning to decline. Adult coccinellid populations lagged behind the coccinellid larvae peaking nearly a week after the highest larval count. There was a demonstrated delayed density dependent relationship between coccinellids and the cotton aphid. Numerous malathion teatments for the boll weevil eradication program disrupted the population dynamics of coccinellid larvae and adults in 2001 making it impracticable to include this data in the comparisons.

**Study yields.** In 1999, lint yields were significantly higher (t = 2.2, df = 28, P < 0.05) in treated plots than in untreated plots. No significant difference in lint yields was observed between experimental and conventional plots (Table 1). There was no

Table 1.	Mean cotton lint yields (±SEM) in plots using conventional and experi-
	mental thresholds for cotton aphid treatment decisions relative to un-
	treated control plots

Lint yield (kg/ha)		
1999	2000	2001
635 ± 44 b	649 ± 40 a	997 ± 60 a
721 ± 20 a	596 ± 57 a	1001 ± 51 a
757 ± 35 a	644 ± 36 a	1062 ± 40 a
	1999 635 ± 44 b 721 ± 20 a 757 ± 35 a	Lint yield (kg/ha)19992000 $635 \pm 44$ b $649 \pm 40$ a $721 \pm 20$ a $596 \pm 57$ a $757 \pm 35$ a $644 \pm 36$ a

Means within a year in a column followed by the same letter are not significantly different (LSD, P = 0.05)

significant difference in the lint yields among treatments in 2000 or 2001. Lint yields were variable from year to year with the highest yields occurring in 2001 (Table 1).

#### Discussion

This study supports previous contentions that arthropod natural enemies are important to the suppression of cotton aphid populations, especially in early and midseason cotton (Kerns and Gaylor 1993, Knutson and Ruberson 1996, Wells et al. 1999). In 1999 and 2000, there was a consistent delayed density-dependent relationship between coccinellids and aphid density, suggesting that cotton aphids were their primary food source. Coccinellids were present in all field plots but were always highest in the untreated plots. During 2000, aphid populations were 50% of those in 1999, yet similar predator densities and dynamics occurred both years. During 2001, aphid populations were 20% of those in 1999 but with a large decrease in predator population likely due to the negative effect of almost weekly boll weevil eradication sprays.

Application of the experimental threshold delayed initial insecticide application by 5-10 d and eliminated one insecticide spray on the experimental plots whereas cotton yields remained similar. The delay in treatment in the experimental plots was due to the presence of coccinellids, as the potential experimental threshold did not require treatment until aphids exceeded 30 per leaf. Reducing the number of insecticide applications allows natural enemies to survive, multiply, and disperse which is especially important during the early cotton season when aphids are present in the field.

In an identical field study in Georgia, coccinellids (especially *H. convergens* and *Scymnus* spp.) were considered key factors in providing cotton aphid suppression (Wells et al. 2001). In these Georgia studies, an epizootic of the entomopathogenic fungus, *N. fresenii*, initially suppressed the cotton aphid, and the remaining aphids were kept below treatment thresholds by parasitoids and predators, especially the small coccinellid, *Scymnus* spp. (Wells et al. 1999). The conservation of insect natural enemies is also important as predators and parasitoids may play an important role in preventing recurrences of aphid outbreaks after fungal epizootics (Abney et al. 2002). In Arkansas, a similar epizootic occurred all 3 yrs, but natural enemies, especially the coccinellids, were responsible for the initial slowing of the aphid population growth during the early cotton season, prior to detection of *N. fresenii*.

*Neozygites fresenii* is considered the most important biological control agent of *A. gossypii* in midsouth and southeastern cotton fields. In our studies the fungus was most effective in reducing aphid population only after the population densities were high. When an epizootic begins, the aphid populations are reduced by 90% (Stein-kraus et al. 1996) from their peak numbers to very low numbers in only 5-10 d and seldom return to their previous high numbers (Steinkraus and Hollingsworth 1994). When the prevalence of *N. fresenii* reached 15% in this study, aphid densities dropped below threshold levels within 1 wk as the epizootic killed the majority of aphids. Similar to the results in the Georgia study (Wells et al. 1999), aphid densities remained below treatment thresholds after fungal epizootics occurred.

When aphid densities were highest (1999), there was a significant increase in cotton lint yield in plots treated relative to untreated plots (Table 1). Aphid density increases started earlier in 1999 on younger cotton which may have affected cotton lint yield. Aphid populations do not increase as rapidly in older cotton and damage is more consequential on the preferred younger cotton (Slosser et al. 1989). Lint production was highest in 2001, when overall aphid densities were lowest, although this difference was likely due to a combination of many other factors.

These data suggest that inclusion of natural enemies, particularly coccinellids and the aphid fungus, into the decision-making process has the potential for delaying the initial insecticide application, as well as preventing unnecessary applications when an epizootic is imminent (Steinkraus et al. 1999). Delays in insecticide application may oppose typical conventional wisdom of current practices, but these studies demonstrated the potential of maintaining cotton lint yields whereas decreasing the number of insecticide applications. Because such intensive sampling is too costly for traditional crop consultants, the experimental threshold evaluated in this study will be modified into a more practical threshold for consultants and growers that will not require the frequent estimates of aphid densities per leaf.

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