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# Effects of acephate on the distribution of tarnished plant bugs within the cotton plant profile

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EFFECTS OF ACEPHATE ON THE DISTRIBUTION OF TARNISHED PLANT BUGS
WITHIN THE COTTON PLANT PROFILE

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In

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by
Kyle A. Fontenot
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thank you for your love and support every day. You inspire me to be the best man I can and I look forward to the many adventures that our long lives will bring us.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

## ABSTRACT

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is a primary pest of cotton in the mid-southern United States. Chemical control strategies are the primary integrated pest management tool used to manage this pest in cotton. A better understanding of tarnished plant bug behavior and distribution on cotton plants is needed to improve the scouting and monitoring protocols used to estimate population and crop injury levels needed to initiate treatments. This pest frequently re-infests fields after insecticide treatments. Sampling protocols should consider the sub-lethal effects of insecticides on migrating populations or on survivors that remain on insecticide-treated plants. Studies were performed during 2007-2008 to evaluate the effects of acephate on tarnished plant bug nymph age-classes, preference for selected fruiting structures, and vertical distribution within the cotton canopy. The test sites included flowering stage cotton plants that were infested with native populations of nymphs (>1 insect / row ft). Non-treated and acephate-treated (Orthene 90SP 0.8 lb AI/acre) cotton plants were evaluated at 0 (pre-treatment) to 120 hours after treatment (HAT). Numbers of small ($1^{st}$ – 3rd instars) nymphs were significantly greater than large ($\geq 4^{th}$ instars) on non-treated plants, but no differences between age-classes were detected on acephate-treated plants. Regardless of insecticide treatment, nymphs were significantly greater on flower buds (squares) compared to bolls or white flowers. Nymphs were greater on sympodial branches of plant main stem nodes 1-5 (top five) and 6-10 compared to those on main stem nodes 11-15 for both treatments. On non-treated plants, the numbers of nymphs found on nodes 1-5 compared to those on 6-10 were not significantly different. However, on acephate-treated plants from 24 to 72 HAT, more nymphs were found on sympodial branches 6-10 compared to sympodial branches 1-5. The results of this study showed that acephate influenced tarnished plant bug nymph age-class, short-term

vertical distribution on cotton plants, but did not change the preference for squares. Whole-plant sampling protocols that measure infestations throughout a cotton plant's entire profile or examination of squares for injury should provide the best estimate of tarnished plant bugs on non-treated and insecticide (acephate)-treated cotton plants.

# INTRODUCTION

Cotton, *Gossypium hirsutum* (L.), is one of Louisiana's major agronomic commodities. This crop is traditionally produced in over 20 parishes across three general regions of the state. The number of cotton producers and acreage has decreased in recent years for a variety of reasons including adverse weather, fluctuating commodity prices, and excessive input costs. In 2006, 1432 Louisiana cotton producers harvested 630,000 acres of cotton that yielded approximately 1.2 million (mill.) bales (Anonymous 2007, Williams 2007). In 2007, 926 Louisiana cotton producers produced over 325,000 acres that yielded over 667,000 bales. With fewer acres in 2007, cotton still contributed over $224 mill. to Louisiana's economy compared to sugar ($666 mill.), rice ($257 mill.) and soybeans ($222 mill.) (Anonymous 2007).

Cotton has numerous arthropod, disease, and weed problems capable of limiting optimum production and crop value. Historical research has shown that these pests not only reduce yield, but also influence cotton fiber quality. In Louisiana during 2007, cotton producers spent over $25 mill. for arthropod pest management (Williams 2008). In addition to the cost per acre of the actual insecticides, producers have to factor in additional control costs including: boll weevil eradication fees ($6.00/acre), Bt trait ($12.50 / acre), aerial application ($3.25/acre), ground application ($3.25/acre), and insect monitoring ($9.25/acre). In spite of these expenses, arthropod pests still accounted for a loss of greater than 38,000 cotton bales. The costs of arthropod pest management coupled with direct yield losses in cotton totaled more than $36 mill.

There are a wide variety of arthropods considered major cotton pests in Louisiana. The heliothine complex (tobacco budworm, *Heliothis virescens* (F.) and bollworm, *Helicoverpa zea* Boddie); thrips, *Frankliniella spp.*; and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), are some of the more important pests. Other pests such as cotton aphid, *Aphis*

*gossypii* (Glover), two-spotted spider mite, *Tetranychus urticae* (Koch), and stink bugs (Pentatomidae) are less common and generally considered occasional pests. Across the U.S., the heliothines have been ranked as the number one cotton pest for over a decade. However, in recent years, the tarnished plant bug has become the primary yield limiting cotton pest throughout the Mid-South (Louisiana, Arkansas, Mississippi, Tennessee) cotton producing states. The tarnished plant bug is the most common species in a Hemipteran "bug" complex that includes the clouded plant bug, *Neurocolpus nubilus* (Say) and the cotton fleahopper, *Pseudatomoscells seriatus* (Reuter) (Layton 2000). In 2007, tarnished plant bug was ranked as the number one pest infesting over 90 percent of Louisiana's cotton acres and was responsible for approximately a 3.6% yield loss as compared to a 0.35% loss from heliothines (Williams 2008).

In a review of pest significance by regions, the tarnished plant bug has had the most severe impact on Mid-South cotton. In these areas, specifically in the delta regions of Arkansas, Louisiana, and Mississippi, it has been ranked the number one most yield limiting pest over the last several years (Williams 2008). In 2007, control cost for tarnished plant bug in the Mid-South was at least three-fold greater than the national average control cost (Figure 1).
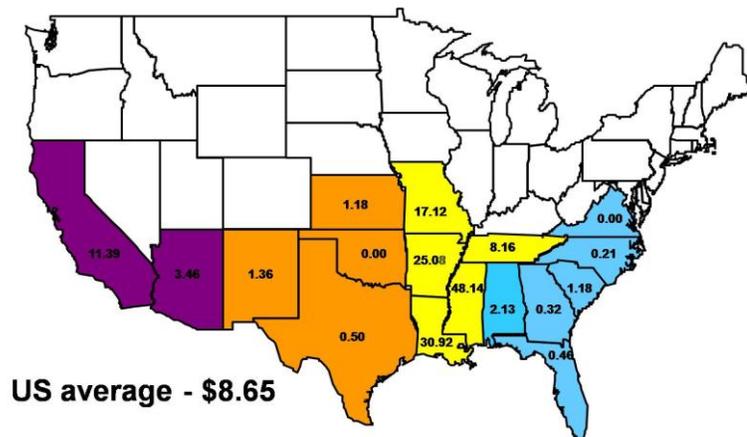


**Figure 1. Average control cost per acre for phytophagous plant bugs on cotton in each cotton producing state during 2007.**

Historically, tarnished plant bugs were inadvertently controlled by insecticides such as organochlorines, organophosphates, carbamates, and pyrethroids used for boll weevil, *Anthonomus grandis grandis* Boheman, and heliothine control (Leonard 2006). The insecticide application frequency used to control these pests has been reduced with the success of Louisiana's boll weevil eradication program and the wide-spread adoption of transgenic *Bacillus thuringiensis* Berliner *var. kurstaki* cotton cultivars (Roberts 1999, Leonard 2006). In addition, there has been an increase in the use of target-specific insecticides that do not provide satisfactory levels of efficacy against tarnished plant bug (Leonard 2006).

Several IPM strategies are recommended for controlling tarnished plant bug in cotton. Area wide control of non-crop alternate hosts and selected host plant resistance traits are being explored and developed. However, chemical control strategies remain the primary tool used to manage this pest. Presently, numerous insecticides are recommended against tarnished plant bug, but varying levels of resistance has been documented to nearly every class of these compounds among Mid-South populations of this insect.

**Table 1. Insecticides to which tarnished plant bug populations have expressed resistance.**

| Common Name | Class | Resistance Reference |
|---|---|---|
| acephate | organophosphate | Snodgrass 2006 |
| bifenthrin | pyrethroid | Snodgrass 1994 |
| permethrin | pyrethroid | Snodgrass 1994 |
| dicrotophos | organophosphate | McCaa and Schuster 1986 |
| dimethoate | organophosphate | Snodgrass and Scott 1988 |
| oxamyl | carbamate | Pankey et al. 1996 |
| malathion | organophosphate | Zhu et al. 2004 |

Resistance to pyrethroids and organophosphate is partly metabolic, but the resistance mechanisms for other common insecticides have not been well studied (Snodgrass and Gore

2007a). Cook et al. (2007) showed that standard insecticide use strategies can reduce tarnished plant bug numbers, but none are consistently effective and can maintain sub-economic injury levels for the season. In recent years, the tarnished plant bug control problem peaked in Mississippi where producers averaged approximately 7-10 insecticide applications during 2007 (Catchot 2007a). The highest insecticide application frequency prior to 2007 was 5.2 sprays per year and occurred during 2004. Current trends with insecticide resistance and lack of effective alternative technologies will likely serve to intensify problems in tarnished plant bug management in the Mid-South states.

Cotton yield losses and arthropod management control costs have increased in recent years and are likely to continue this trend. Therefore, protocols for efficiently monitoring insect populations and damage to cotton plants are vital to effectively time insecticide applications to assure maximum control and manage input costs. Effective sampling tools and procedures are a key component of a successful cotton IPM program (Layton 1995). The sweep net, shake-sheet, and plant-examination techniques are used to sample tarnished plant bug populations and trigger insecticide applications in cotton (Bagwell et al. 2008). However, most of these protocols were established with the goal of triggering the initial insecticide application during the season without consideration of the need for subsequent sprays. The sub-lethal effects on surviving insects as well as on immigrants from alternate hosts into an insecticide-treated cotton field could affect the accuracy and efficiency of post-treatment sampling. Ultimately, if these findings do not correctly reflect insecticide performance, the decision-making process could be impaired.

The sampling methods currently recommended may not be providing adequate estimates of population and/or damage levels in cotton fields receiving multiple insecticide applications. The sweep net, for example, is not as effective sampling tarnished plant bug nymphs as the

shake-sheet (Musser 2007). Also, the sweep net concentrates on the upper canopy, which may not be as accurate in detecting populations during mid-to-late season when plants are taller and maintain fruiting forms throughout the entire plant. The shake-sheet protocol has been the most efficient process during the flowering stages of cotton development because it samples the entire plant profile. However, the shake-sheet is relatively inconsistent in sampling adult populations in cotton due to their mobility. To more effectively monitor the effects of tarnished plant bug on cotton plants, sampling methods that evaluate injury to cotton fruit could be used in addition to monitoring insect population levels. However, some of these plant-based sampling methods are not as well-defined as some of our insect-based methods. Considerable research is underway to refine plant-based sampling protocols and action thresholds to trigger insecticide applications.

Insecticide resistance to recommended products and new insecticide modes of action are influencing the effectiveness of sampling protocols and action thresholds. Tarnished plant bugs are a full-season pest, frequently re-infesting fields, and requiring multiple insecticide applications for management. Collectively, these issues justify a re-evaluation of sampling protocols and action thresholds. To improve the insect and plant-based sampling methods, an understanding of the distribution of tarnished plant bug within cotton plants and how the distribution might change in response to insecticide sprays is needed. These results should provide insights into the limitations of the current sampling protocols and offer an opportunity to improve the existing recommendations for managing tarnished plant bugs in cotton.

## REVIEW OF LITERATURE

### Identification and Biology

The tarnished plant bug is in the family Miridae and the order Hemiptera (Borror and White 1970, Stewart 2004). Adults are soft-bodied, elongate in shape and are 5.0 to 6.0 mm in length (Borror and White 1970, Dietz et al. 1976, Borror et al. 1989). Adults vary in color from light to dark brown and are marked with yellow and black. A light yellow triangular mark is usually visible on the scutellum. The antennae and proboscis are four segmented (Borror et al. 1989). First and second instars have reddish-tipped antennae with a pale green body. The two thoracic segments behind the head exhibit two spots each and one dark spot on the dorsal side of the abdomen (Leigh et al. 1996). The egg is flask-shaped approximately 1.0 mm long and 0.25 mm wide, with the anterior end being curved and slightly compressed at the apex (Crosby and Leonard 1914). This insect has piercing-sucking mouthparts and feeds on plant liquids in flower buds and fruit of a broad range of plants. Populations have been recorded on 169 species of plant hosts in the Arkansas, Louisiana, and Mississippi Delta (Snodgrass et al. 1984). In agro-ecosystems that include cotton, tarnished plant bugs usually complete at least one generation on native hosts or other crops and then migrate into cotton fields when alternate host plants are no longer attractive (Layton 2000). Females will usually insert eggs into plant tissue of cotton squares, terminals, and leaf petioles (Fleisher and Gaylor 1988). According to Bariola (1969), one generation on cotton can be completed in 33 d at $80^o$ F. Eggs require approximately 8 d to hatch. Nymphs will molt five times in approximately 17 d to become an adult and the pre-oviposition period lasts about 8 d before females become reproductive. Adults will overwinter in decaying native vegetation, tree bark, ground trash, and other protected places (Cleveland 1982).

6

**Damage to Cotton Plants**

Tarnished plant bug is capable of damaging cotton plants in developmental stages throughout the entire production season. Early season feeding on tissue in the terminals of cotton seedlings can cause a loss in apical dominance and result in the development of multiple secondary terminals (Scales and Furr 1968, Hanney et al. 1977). This early season damage can reduce total fruiting forms per plant, delay maturity, and decrease yield (Scales and Furr 1968, Tugwell et al. 1976, Scott et al. 1985). Historically, most tarnished plant bug feeding occurs on cotton during the period of flower bud (square) initiation through early flowering stages of development (Layton 1995, Craig and Luttrell 1997). Historically, economic injury from tarnished plant bug feeding was most common during the early- to mid-season period of plant development (Black 1973, Tugwell et al. 1976). Feeding on small squares can result in abscission and lower fruit retention rates (Pack and Tugwell 1976, Cleveland 1982, Layton 1995). The target sites within a square or flower for feeding are anthers and pollen, causing necrotic anthers and atrophy of pollen sacs (Pack and Tugwell 1976). Digestive enzymes in the tarnished plant bug saliva injected into anther and pollen sac tissue are responsible for the necrosis and atrophy (Reid 1968, Agusti and Cohen 2000).

Loss of fruiting forms from tarnished plant bug feeding can decrease yield because of fewer effective fruiting sites (Tugwell et al. 1976). In the past, tarnished plant bug feeding on bolls was not considered to be a significant problem in the presence of squares. The use of broad-spectrum insecticides for others pests such as boll weevil and heliothines during the flowering period of development concurrently controlled tarnished plant bug. The general overall reduction in insecticide applications in recent years has allowed tarnished plant bug to become a season-long pest. In a similar manner to that described for squares, tarnished plant bug also can cause

abscission of young bolls further reducing yield.  Boll injury can also affect seed and lint quality (Pack and Tugwell 1976, Russell et al. 1999, Layton 2000). In most environments, cotton yield is not susceptible to tarnished plant bug injury after crop cutout and the last harvestable bolls have accumulated >150 heat units (Teague et al. 2001).

## Management Strategies in Cotton

Although insecticides are the primary tool used to control tarnished plant bug, other methods such as host plant resistance in cotton cultivars are being evaluated and developed.  The glabrous and frego bract traits have been studied for many years but can actually increase plant susceptibility to tarnished plant bug injury (Bailey 1982, Milam et al. 1985). The nectariless trait can reduce tarnished plant bug numbers by reducing female fecundity, and provide a host non-preference to this insect (Milam et al. 1989, Bailey 1982, Bailey et al. 1984). This effect has translated into consistently lower populations of tarnished plant bug adults in nectariless cotton cultivars (Scott et al. 1986).  The selection of cultivars with early-season yielding ability and early maturity may reduce chances of tarnished plant bug damage by reaching physiological crop cutout before peak tarnished plant bug populations occur (Milam et al. 1989, Gore et al. 2007). Another management tactic is associated with area-wide tarnished plant bug plant host manipulation. Gore et al. (2007) showed spring herbicide application used to terminate wild host plants on borders of cotton fields reduced tarnished plant bug numbers and the frequency of insecticide applications for all cotton fields in that area. Biological control options to control this insect have been limited, but the entomopathogenic fungi, *Beauveria bassiana*, has shown potential in controlling tarnished plant bug in cotton and canola (Steinkraus and Tugwell 1997, Al Mazra'awi et al. 2006). A parasitic wasp, *Peristenus diageutis* Loan (Hymenoptera: Braconidae), was released by United States Department of Agriculture – Agricultural Research

Service as biological control agent against tarnished plant bug (Day et al. 2008). The parasitoid has been successfully dispersed across the Northeastern United States and Canada. An egg parasitoid of tarnished plant bug, Anaphis *iole* Girault (Hymenoptera: Mymiaridae) (Jackson and Graham 1983), is also being considered for mass release as a biological control agent across the southern United States (Jones and Jackson 1990).

Historically, numerous insecticides have been recommended against this pest, but the development of insecticide-resistant populations has limited the available options. Tarnished plant bug populations have shown resistance to methyl parathion (Cleveland and Furr 1979) and dimethoate (Snodgrass and Scott 1988). McCaa and Schuster (1986) observed resistance to dicrotophos and monocrotophos. Additional populations demonstrating resistance to organophosphates and cyclodiene insecticides were reported by Snodgrass (1996). Pyrethroid-resistance was confirmed in tarnished plant bug populations from Arkansas, Louisiana and Mississippi (Snodgrass 1994, Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000). Presently, the organophosphates, acephate and dicrotophos, are the most common insecticides used against this pest. However, Snodrass (2006) has reported a consistent increase in tolerance to acephate for several years, with some populations demonstrating >3-fold tolerance compared to a susceptible population. Snodgrass and Gore (2007b) recently have developed a bioassay to monitor the changes in resistance to the neonicotinoids and have documented considerable variability among populations in susceptibility to insecticides in this class (imadacloprid and thiamethoxam). Other insecticide options that have been recently registered and have yet to be evaluated in laboratory tests for susceptibility include an insect growth regulator (novaluron), and a pyridine carboxamide (flonicamid). These new chemistries have novel modes of action which typically are slower to kill insects, but have been shown to

rapidly halt feeding. A better understanding of their field activity and the effects on tarnished plant bug behavior is necessary before one can accurately estimate sustainable performance.

**Sampling Protocols for Cotton**

Insecticide application timing relies upon action thresholds that are recommended by a state's cooperative extension service (Snodgrass 1993, Layton 1995). Multiple sampling methods are used to estimate the relative infestation levels of these pests and trigger insecticide applications (Bagwell et al. 2008, Catchot 2007b). Absolute estimates of tarnished plant bug population levels are not used because of the time requirement and dispersal habit of the pest (Snodgrass 1993). The sweep net, shake-sheet and various whole-plant inspection techniques are among the most common methods used in sampling (Young and Tugwell 1975). The sweep net has been the most popular because of its ease of use by scouts (Snodgrass 1993). Compared to other sampling methods, the sweep net protocol is not as consistent in estimating tarnished plant bug numbers as other methods (Young and Tugwell 1975, Byerly et al. 1978, Wilson and Guiterrez 1980). Wilson and Guiterrez (1980) suggest that the accuracy of the sweep net depends on the cotton's phenology. The sweep net sample is concentrated from the upper parts of the plant. It is more effective during square initiation when plants are smaller and squares are concentrated in the upper nodes of the cotton plant. During the flowering stages, cotton plants can be much taller and fruiting forms are dispersed throughout the plant profile. Snodgrass (1993) showed that the shake-sheet was more accurate than the sweep net in estimating tarnished plant bug nymphs in fields of cotton during the flowering periods of development. The sweep net only samples the upper 10-12 inches whereas the shake-sheet samples the entire plant and provides a better estimate of insects throughout the plant (Young and Tugwell 1975).

Recently, a team of Mid-South cotton entomologists participated in a regional project to re-evaluate and validate tarnished plant bug sampling protocols and action thresholds to initiate insecticide sprays in cotton (Musser et al. 2007).   All common sampling protocols that have been recommended to estimate tarnished plant bug populations and their associated injury to cotton fruiting forms were compared for efficiency and precision. Several methods were successfully used and provided relatively consistent information across a range of environmental conditions.  Results obtained with direct sampling methods using the sweep net or shake-sheet were similar, but with different strengths and weaknesses. Indirect methods that sampled insect damage proved to be efficient and effective when surveying squares and white flowers for feeding evidence, while boll sampling proved not to be as efficient. There is concern when sampling white flowers that the damage may be 5 to 7 days old and therefore not giving an accurate measurement of current infestations.

In many crop IPM systems, sampling plants and/or plant parts targeted by pests provides a more sensitive and repeatable process of estimating economic infestations of insects.  Gore (2005) conducted further studies and suggested that the presence of frass-stained squares or squares with evidence of feeding (SFE) also may provide precise estimates of tarnished plant bug injury in cotton fields.  Studies in Louisiana supported a tarnished plant bug action threshold in cotton based on SFE which was effective in reducing insecticide applications without sacrificing cotton lint yield (Fontenot et al. 2008).

As in most crops and IPM systems, these protocols do not consider the need for additional treatments to manage season-long pest populations.  Louisiana cotton fields received an average of 2.4 and 3.8 applications during 2006 and 2007, respectively, for tarnished plant bug control (Williams 2007, 2008). These sampling methods currently recommended may not be

11

providing adequate estimates of population and/or damage levels in cotton fields receiving multiple insecticide applications. Understanding the distribution and behavior of tarnished plant bugs on insecticide-treated cotton plants could further refine sampling protocols for insects as well as plant inspections.

**Distribution of Insects on Cotton Plants**

Limited research in cotton has evaluated the distribution of insects within plants. Fye (1971) suggested that 75 to 100% of insect populations (boll weevils, heliothines, and soybean loopers, *Pseudoplusia includens* [Walker]) in cotton occur in the upper 2 ft of plants ranging in height from 3 to 6 ft. In Australian cotton fields, sampling the upper 30 to 40% of the main-stem nodes is recommended when sampling for the cotton harlequin bug, *Tetocoris diophtalmus* (Thunberg) (Wilson et al. 1983). The majority of *Lygus hesperus* (Knight) were detected on cotton fruiting forms, with twice as many nymphs on squares than bolls, but the opposite was observed for adults (Wilson et al. 1984). In addition, both adults and nymphs were most often recorded on plant structures located on the fifth through seventh main stem nodes below the terminal. Parajulee et al. (2006) divided cotton plants into three vertical strata and found populations of thrips (*Frankliniella* spp.) and cotton fleahoppers, *Pseudatomoscelis seriatus* (Reuter) were significantly higher in the top stratum compared to the bottom two strata. Snodgrass (1998) found that on non-treated cotton plants, 75% of tarnished plant bug adults and nymphs were located in the upper six main stem nodes. The majority of both stages were recorded on squares compared to other reproductive structures. In a free choice test, Pack and Tugwell (1976) demonstrated that tarnished plant bugs prefer to feed on pinhead squares rather than large squares or bolls.

12

**OBJECTIVE**

The objective of this study was to evaluate the within plant distribution of tarnished plant bug nymphs in non-treated and insecticide-treated flowering stage cotton plants.

**Hypothesis**

$H_0$: Tarnished plant bug distributions within insecticide-treated and non-treated flowering cotton plants is equal.

## MATERIALS AND METHODS

A series of field trials were performed at the Macon Ridge location of the Northeast Research Station (Louisiana State University Agricultural Center, Louisiana Agricultural Experimental Station) near Winnsboro, LA (Franklin Parish) during 2007 and 2008. Cotton cultivars including ST 4554 B2RF and ST 5599 B2RF (Stoneville Seed Company, Stoneville, MS), as well as DP 555 BGRR and DP 117 B2RF (Delta & Pine Land Company, Scott, MS) were used for this study.  All cultivars were herbicide-resistant and expressed Roundup Ready (glyphosate) or Roundup Ready Flex technologies (Monsanto Co., St. Louis, MO). These transgenic varieties also contained either Bollgard (BG) or Bollgard II (B2) (Monsanto Co., St. Louis, MO) technologies derived from the soil bacterium, *Bacillus thuringiensis* (Bt). Soils in the test areas were classified as Gigger-Gilbert silt loams with medium fertility levels. Cotton was planted across multiple dates during mid-May of both years to ensure the availability of cotton in the flowering stages of development that was suitable for natural infestations of tarnished plant bugs during the testing period.

All normal cultural practices and IPM strategies for cotton production recommended by Louisiana Cooperative Extension Service were used to optimize plant development across the test site. Cotton seed was planted in a hill-drop configuration at a rate of 52,000 seeds / acre with a John Deere planter. Cotton seed was commercially treated with multiple fungicides, an insecticide (thiamethoxam), and a nematicide (abamectin) to combat seedling diseases, early season insect pests, and nematodes. Aldicarb (Temik 15% Granular [G], 7.5 lb [form] / acre, Bayer CropScience, Raleigh, NC) was applied in the seed furrow at the time of planting for additional nematode and early season arthropod pest suppression. Weekly monitoring of arthropod populations was done throughout season and pesticide applications to all non-target

pests were made according to Louisiana Cooperative Extension Service recommendations. The Bt traits were used to control lepidopteran pests and reduce the need for mid-to-late season treatments of broad spectrum insecticides that may be toxic to tarnished plant bugs. When needed, lepidopteran-specific insecticides, spinosad (Tracer 4 flowable [F], 2.0 oz. [form] / acre Dow AgroSciences, Indianapolis, IN) and methoxyfenozide (Intrepid 2F, 8.0 oz. [form] / acre Dow AgroSciences, Indianapolis, IN) were used for supplemental control strategies. Full-season weed management was accomplished using pre-emergence, mid-season, and lay-by herbicide applications of recommended products. Glyphosate (Roundup WeatherMax, Monsanto Co., St. Louis, MO) was used on glyphosate-resistant varieties as needed according to the product label. Crop nutrient requirements were managed with a complete fertilizer (nitrogen, phosphorus and potassium) program. Those treatments were applied based upon soil test results to maximize plant vigor and yield potential. In several tests, adequate soil moisture was maintained across test areas with an over-head irrigation system to ensure healthy plants that were attractive to tarnished plant bug. A general cotton harvest aid strategy was applied at crop maturity, but no yield data was collected as a component of this study due to the destructive sampling procedures in each plot.

Multiple field sites were used within each year and were selected using three criteria: (1) Cotton plants across the test area had begun to flower. The first week of flowering was defined as that period when >50% of the plants had one flower or boll. The presence of flowers and main stem nodes above white flower were used to measure the growth stage of cotton plants during the test; (2) the test area was large enough to encompass the desired experimental design. Each test site consisted of a series of plots (8 to16 rows centered on 40 in. and extending >300 row-ft in length) with two to four buffer rows between treatments. A new test site was used for each

replication of treatments and post-treatment sampling; and 3) the native tarnished plant bug infestation exceeded a mean of one nymph / row ft in random black shake-sheet (2.5 ft x 2.5 ft) samples in border (non-treated) plots across the test area.  Sampling with the shake-sheet was accomplished by vigorously shaking adjacent plants (on 10 row ft.) to dislodge tarnished plant bugs.

The two treatments in this test included (1) non-insecticide-treated and (2) insecticide-treated cotton plants. Insecticide-treated cotton plants were sprayed with an organophosphate, acephate (Orthene 90% soluble powder [S], 0.8 lb [AI] / acre of product, Valent USA Corporation, Walnut Creek, CA) This product is a standard insecticide recommended by the Louisiana Cooperative Extension for tarnished plant bug control, but its efficacy has been declining across Louisiana in recent years (Copes et al. 2008).  Therefore, tarnished plant bug nymph survivorship following this insecticide was sufficient to detect insects throughout the plant.  All insecticides were applied using a John Deere 6000 high clearance sprayer calibrated to deliver 11.5 gallons per acre with two Teejet TX-6 hollow cone nozzles / row at 48 psi. Acephate applications were initiated on 10, 29 Jul; 5 Aug during 2007 and on 13 Jul; 4 Aug during 2008.

Both the non-treated and insecticide-treated plants were evaluated 24 hours after treatment (HAT), 48 HAT, 72 HAT, 96 HAT, and 120 HAT for tarnished plant bug nymph survivors.  Sampling occurred from 10:00 A.M. – 12:00 P.M. to eliminate time of day as a factor. Independent blocks (8-16 rows x 50 row-ft sections) were used for each sampling period within a replicate because of destructive plant sampling during the process of locating and mapping the insects (Figure 1).
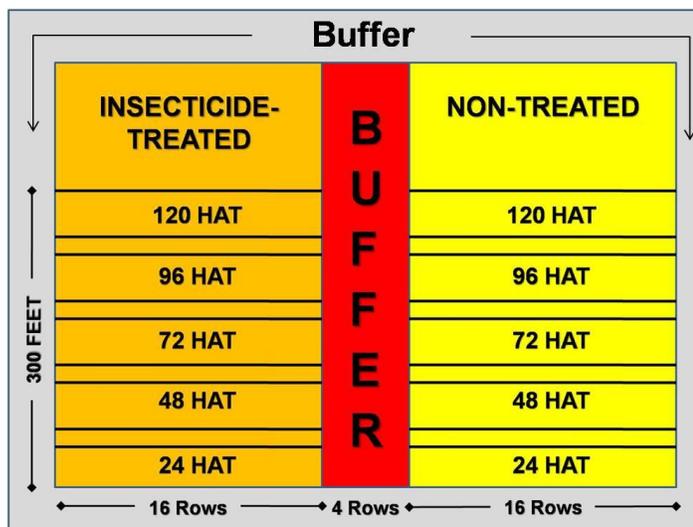
**Figure 2. Field test design for each replication of a study evaluating tarnished plant bug survivors on non-treated and insecticide-treated cotton plants.**

Numbers of tarnished plant bug nymphs were recorded using the black shake-sheet sampling protocol previously described for pre-treatment surveys. Two samples (10 row feet) were used to estimate the nymph population within test area. To determine the distribution of these insects on plants, a team of four to five scouts searched randomly selected plants within each plot until the locations of 20 tarnished plant bug nymphs were mapped. Each plant was examined in its entirety beginning at the top (terminal=sympodial node 1) and proceeding down the main stem to the lowest sympodial or vegetative branch. All fruiting forms on each branch were inspected for the presence of tarnished plant bug nymphs. This process continued until the locations of 20 nymphs was completed. The total number of plants sampled within each treatment was also recorded; however, the number of branches or fruiting forms which were searched was not counted. Tarnished plant bug nymphs were classified in two categories: large ($\geq 4^{th}$ instars; presence of wing pads) or small ($1^{st} - 3^{rd}$ instars; wing pads not present) (Figure 3).

17

**Figure 3.  Describing  large (≥4th instars; presence of wing pads) or small ($1^{st}$ – $3^{rd}$ instars; wing pads not present) tarnished plant bug nymphs.**

The within-plant location of nymphs was described by fruiting form classification (square, white flower, or capsule [boll]), and main stem sympodial (or vegetative) node below the plant terminal (Figure 4).
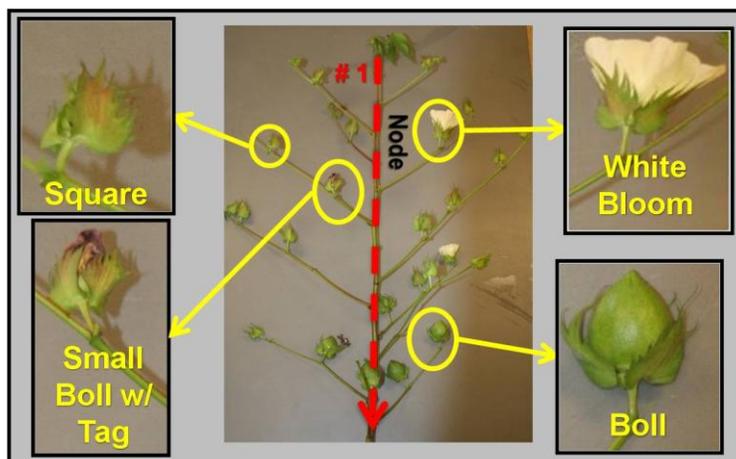


**Figure 4.  Classification of fruiting structures and an illustration of whole-plant sampling along the main-stem for tarnished plant bug nymphs.**

To analyze the vertical distribution of tarnished plant bug nymphs, the cotton plant's main stem nodes were divided into three vertical strata: (1) upper stratum (nodes 1-5), (2) middle stratum (nodes 6-10), and (3) bottom stratum (nodes 11-15) beginning with the terminal region.

The results were summarized and used to graphically describe tarnished plant bug nymph distribution on cotton plants.  All data were subjected to an ANOVA to determine significant treatment effects using PROC MIXED (SAS Institute 2003). When necessary, treatment means were separated using Tukey's Studentized Range Test (*P = 0.10*).

## RESULTS

**Nymph Populations in Test Area**

Native populations of tarnished plant bug nymphs averaged 1.4 nymphs / row ft (4.6 nymphs / row meter) across all test areas prior to application of the acephate treatment. On non-treated plants, the population remained between 1.4 (4.6 nymphs / row meter) and 0.7 nymphs / row ft (2.3 nymphs / row meter) throughout the duration of the post-treatment sampling periods. The acephate treatment significantly reduced the number of tarnished plant bug nymphs below that on non-treated plants at 48 to 120 HAT ($P < 0.07$). On acephate-treated plants, the nymph population ranged between 0.8 nymphs / row ft (2.6 nymphs / row meter) at 24 HAT and 0.3 nymphs / row ft (1 nymph / row meter) at 120 HAT.
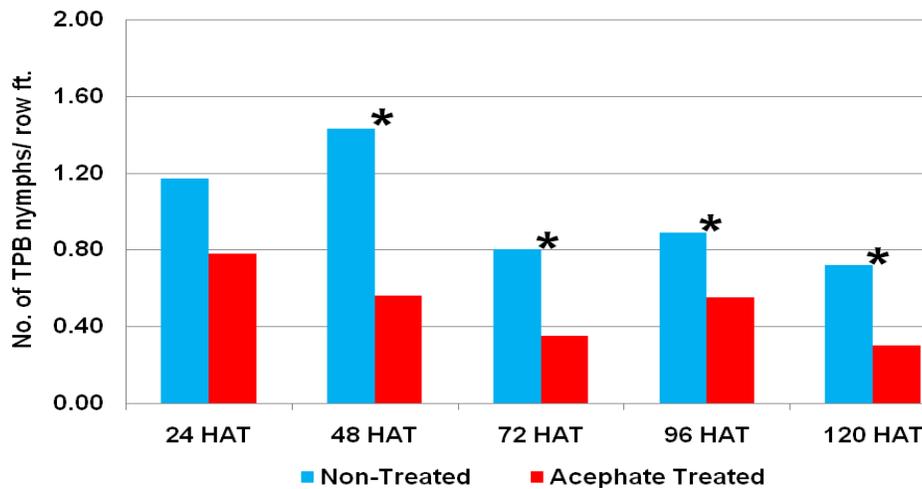


**Figure 5. Number of tarnished plant bug nymphs per row ft in non-treated and acephate-treated cotton plants within each sampling period. Sampling periods with an (\*) are significantly different.**

During the pre-insecticide application samples, between 9 and 39 plants were sampled to find 20 tarnished plant bug nymphs. To maintain a consistent sample size (20 nymphs/treatment) on non-treated and acephate-treated cotton plants, more plants (7 - 20) had to be examined in the

insecticide-treated plots. Averaged across all sampling periods, 58% more acephate-treated plants were sampled compared to non-treated plants.

**Acephate Efficacy against Selected (Large and Small) Instars**.

In the pre-insecticide application samples across the test area, significantly (*t-value = 3.64, d.f .= 4, P = 0.0217*) more small nymphs ($\leq 3^{rd}$ instars; 72%) were found compared to large nymphs ($\geq 4^{th}$ instars; 28%) (Figure 6). This relative 3:1 ratio of small nymphs to large nymphs was consistent throughout each of the sampling periods at 24 to 120 HAT on non-treated cotton plants (Table 2). The frequency of small nymphs represented in the tarnished plant bug population on non-treated cotton plants ranged from 61% at 48 HAT to 83% at 120 HAT. On acephate-treated cotton plants, there were no significant differences (*P > 0.15*) between the frequencies of small and large nymphs for all sampling periods (24 to 120 HAT). The frequency of small nymphs represented in the tarnished plant bug population on acephate-treated cotton plants ranged from 54% at 72 HAT to 62% at 48 HAT.
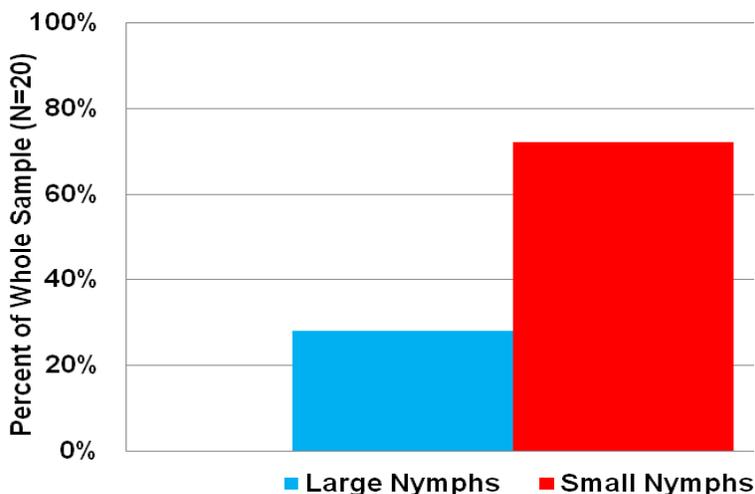


**Figure 6. Tarnished plant bug nymph size distribution, pre-treatment sample. There were significantly more small nymphs compared large nymphs (*t-value = 3.64, d.f. = 4, P = 0.0217*). Large Nymphs ($\geq 4^{th}$ instars; presence of wing pads) or Small Nymphs ($1^{st} – 3^{rd}$ instars; wing pads not present)**

**Table 2. Tarnished plant bug nymph size proportions for non-treated and acephate-treated cotton plants from 24 to 120 hours after treatment (HAT). Means listed are percentages of the whole sample (N=20). Sampling periods with *P > 0.10* were considered significantly different.**

| Treatment | Nymph Size | 24 HAT | 48 HAT | 72 HAT | 96 HAT | 120 HAT |
|---|---|---|---|---|---|---|
| Non-Treated | Large[1] | 32 | 38 | 26 | 35 | 17 |
| | Small | 68 | 61 | 74 | 65 | 83 |
| | *P > t* | *0.04* | *0.02* | *0.03* | *<0.01* | *0.06* |
| Acephate-Treated | Large | 39 | 38 | 46 | 40 | 45 |
| | Small | 61 | 62 | 54 | 60 | 55 |
| | *P > t* | *0.3* | *0.15* | *0.69* | *0.16* | *0.58* |

[1] **Large Nymph (≥4th instars; presence of wing pads) or Small Nymph (1st – 3rd instars; wing pads not present).**

**Nymph Distribution on Cotton Fruiting Forms.**

The pre-treatment sample found significantly (*P > 0.001*) more nymphs on squares (93%) compared to white flowers (4%) and bolls (3%). There was no difference (*P = 0.8597*) in the number of nymphs on white flowers compared to those on bolls. At each post-application sampling period (24 to 120 HAT), nymphs were significantly higher on squares compared to that on white flowers (*P > 0.009*) and bolls (*P < 0.0026*) of non-treated plants (Figure 7). The frequencies of nymphs on squares ranged from 74% at 48 HAT to 92% at 24 HAT. Regardless of sampling period, there were no differences (*P > 0.26*) in percentages of nymphs between white flowers and bolls. Ratios on white flowers ranged between 1% to 8%, whereas on bolls, ratios ranged from 7% to 18%.
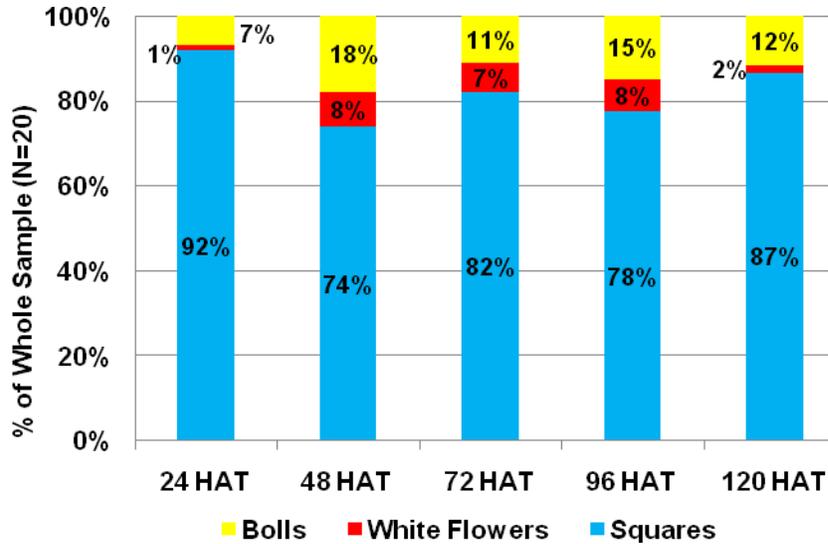
**Figure 7. Distribution of tarnished plant bug nymphs on fruiting forms in non-treated cotton plants from 24 to 120 hours after treatment (HAT).**

Nymph distribution on fruiting forms of acephate-treated cotton plants was similar to that on non-treated cotton plants (Figure 8). Significantly more nymphs were recorded on squares compared to that on white flowers (*P < 0.0038*) and bolls *(P < 0.0038)* at each sampling period. Percentages of nymphs on squares ranged from 77% at 120 HAT to 86% at 24 and 96 HAT. There were no significant differences in percentages found on white flowers compared to that on bolls (*P > 0.52*) at each sampling period. Ratios on bolls ranged from 6% to 15% and ratios on white flowers ranged between 5% to 15%.
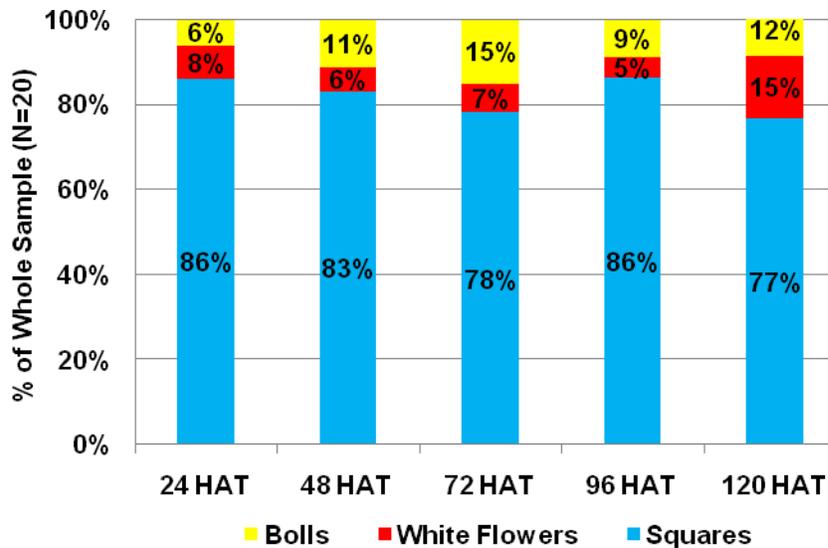
**Figure 8. Distribution of tarnished plant bug nymphs on fruiting forms in acephate-treated cotton plants from 24 to 120 hours after treatment (HAT).**

In a direct comparison of percentages of nymphs on non-treated squares to those treated with acephate at each time interval, only the results for the 24 HAT sample were significantly different (t-*value = 4, d.f. = 4, P = 0.0161*) (Figure 9). For the remainder of the 48 to 120 HAT sampling periods, ratios of nymphs on non-treated squares and acephate-treated squares were not significantly different (*P > 0.22*). In addition, there were no differences in frequencies of nymphs on non-treated and acephate-treated white flowers (*P > 0.3206*) (Figure 10) and bolls (*P > 0.1942*) (Figure 11) within all sampling periods.
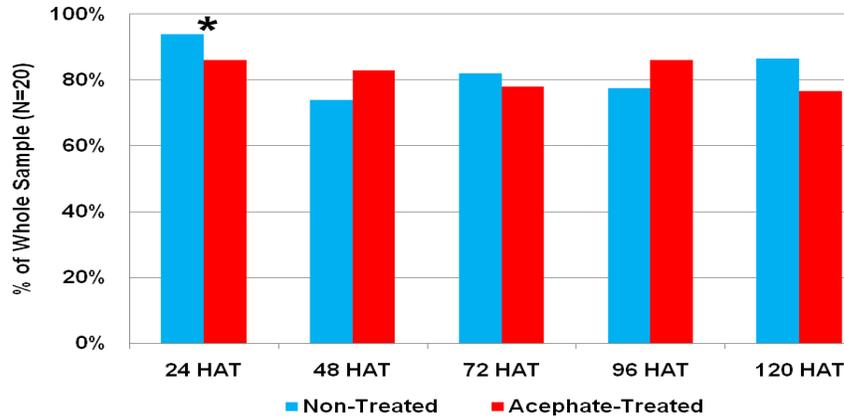
**Figure 9. Comparison of nymphs on non-treated and acephate-treated squares from 24 to 120 hours after treatment (HAT). Sampling periods marked with (\*) are signifcantly different. (Tukey's; P> 0.10).**
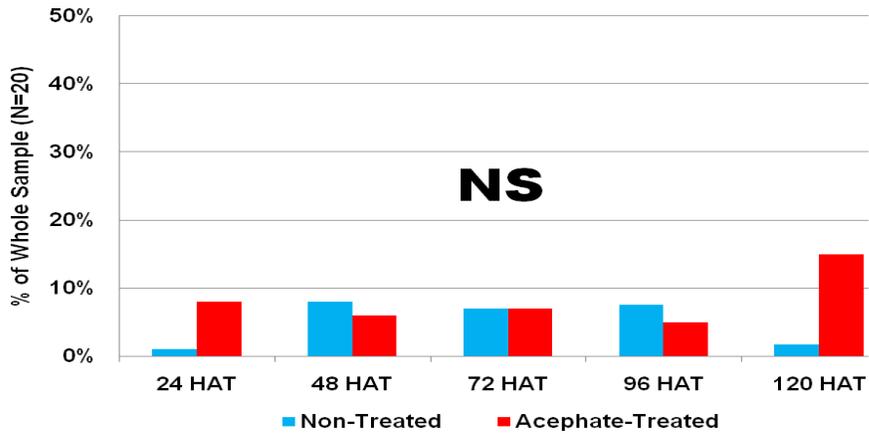


**Figure 10. Comparison of nymphs on non-treated and acephate-treated white flowers from 24 to 120 hours after treatment (HAT). (Tukey's; P> 0.10).**
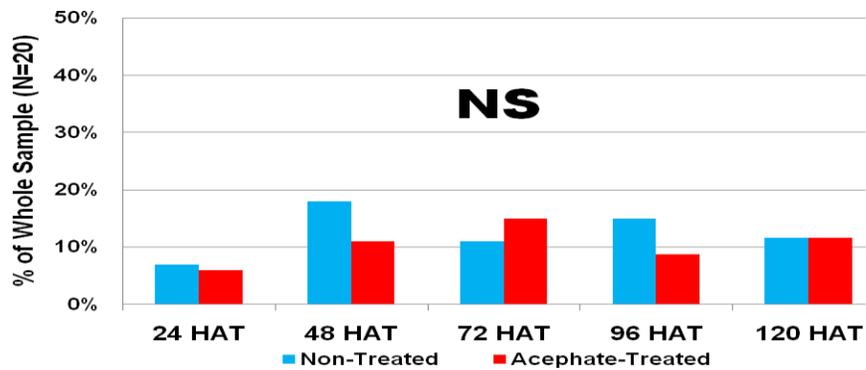


**Figure 11. Comparison of nymphs on non-treated and acephate-treated bolls from 24 to 120 hours after treatment (HAT). (Tukey's; P> 0.10).**

**Vertical Distribution of Nymphs along Main Stem Nodes.**

Tarnished plant bug nymphs were recorded throughout the cotton plant on nearly all of the 15 sympodial branches and vegetative main stem branches. However, the locations of insects were not equally distributed throughout the plant profile during the pre-treatment and post-treatment samples. Attempting to define location by individual main stem sympodial branch was not successful due to limited (n=20 nymphs) sample size. Establishing three strata (upper, middle, lower) that defined three plant main stem zones (nodes 1-5, 6-10, and 11-15) allowed for sufficient numbers of insects to statistically compare vertical distribution. In the pre-treatment sample, significantly ($P > 0.01$) more nymphs were found in the upper strata (38%) and middle strata (59%) compared to that the lower strata (3%).

In all post-treatment sampling periods of non-treated plants, the upper and middle strata had a significantly ($P < 0.093$) greater proportion of nymphs compared to that in the lower strata (Table 3). Percentages in the upper, middle, and lower strata ranged from 35-55%, 44-64%, and 1-6%, respectively, across all sample periods. At 24 HAT and at 72 HAT through 120 HAT, the ratios of nymphs in the upper and middle strata were not significantly different ($P > 0.6015$). However, the proportions of nymphs between the upper strata (35%) and the middle strata (64%) on non-treated plants were significantly different (*t-value = 3.11, d.f. = 4, P = 0.0346*) at 48 HAT.

On acephate-treated plants at all post-treatment sampling periods, the upper and middle strata had significantly higher proportions of nymphs compared to that in lower strata (Table 2). Percentages in the upper, middle, and lower strata ranged from 27-60%, 35-68%, and 5-6%, respectively, across all sample periods. In contrast to the results recorded on non-treated plants,

the proportion of nymphs in the middle strata were significantly higher ($P<0.0356$) than that in

the upper strata at 24 HAT to 72 HAT. Although the proportion of nymphs in the middle strata

was nearly two-fold that in the upper strata, the difference was not significant ($P > 0.267$) at 96

HAT. By 120 HAT, there were significantly ($P > 0.0079$) more nymphs in the upper strata

(60%) compared to the middle strata (35%).

**Table 3. Tarnished plant bug nymph distribution within vertical strata for non-treated and acephate-treated plants from 24 to 120 hours after treatment (HAT). Means listed are percentages of the whole sample (N=20). Means followed by the same letter are not significantly different (Tukey's *P>0.10*) within a treatment and column.**

| Treatment | Strata | 24 HAT | 48 HAT | 72 HAT | 96 HAT | 120 HAT |
|---|---|---|---|---|---|---|
| **Non-Treated** | **Upper** | 42 a | 35 b | 42 a | 43 a | 55 a |
| | **Middle** | 52 a | 64 a | 52 a | 55 a | 44 a |
| | **Lower** | 6 b | 1 c | 6 b | 2 b | 1 b |
| | *P > F* | *0.02* | *<0.01* | *<0.01* | *<0.02* | *<0.01* |
| **Acephate-Treated** | **Upper** | 27 b | 34 b | 32 b | 35 a | 60 a |
| | **Middle** | 68 a | 60 a | 63 a | 59 a | 35 b |
| | **Lower** | 5 c | 6 c | 5 c | 6 b | 5 c |
| | *P > F* | *<0.01* | *<0.01* | *<0.01* | *<0.04* | *<0.01* |

## DISCUSSION

Total tarnished plant bugs (adults and nymphs) exceeded the action threshold to initiate insecticide applications on flowering stage cotton plants (two – three insects / five row ft) in all pre-treatment samples of individual replicates (Bagwell et al. 2008). This cohort of the population represented by nymphs also exceeded our prescribed threshold (one nymph / row ft) to initiate the treatments in our experiments. This nymph population level was critical in order to physically locate the sample size of 20 nymphs in a timely manner in the insecticide- treated plots. In most instances, the populations of tarnished plant bugs across the test sites were in the first stages of a generation and overall numbers of nymphs had begun to increase at the time acephate was applied.

The acephate treatment did not completely eliminate (100% control) the adult or nymph population. The population was reduced by approximately 50% (33 – 61% range of control) in the acephate-treated plots compared to that in the non-treated plots. The performance of acephate in this study is similar to that in reported in current research evaluating acephate efficacy and resistance in Louisiana populations of tarnished plant bugs (Copes et al. 2008). Although organophosphates such as acephate and dicrotophos have been the most common primary insecticides recommended against tarnished plant bug due to cost, efficacy has been declining for several years. During 2007-2008 in Louisiana, acephate (0.075 - 1.25 lb AI/acre) efficacy against field infestations generally did not exceed 50% control (Copes et al 2008). Numerous Louisiana populations collected during the previous two years expressed acephate resistance levels that could have resulted in measurable field control failures if high levels of tarnished plant bugs persisted in cotton fields (Copes et al 2008). A recent summary by Snodgrass et al. (2009) found an increasing number of tarnished plant bug populations

throughout Arkansas, Mississippi, and Louisiana with elevated acephate resistance levels that could limit satisfactory control.

To reduce the frequency of tarnished plant bug control failures in recent years, producers have increased the practice of rotating among insecticide classes (carbamates, neonicotinoids, organophosphates, and pyrethroids) to control tarnished plant bugs (Snodgrass 2009). This approach has been successful in maintaining satisfactory control of tarnished plants bugs in some areas, but none of the available insecticides are completely eliminating this pest as a season-long problem (Cook et al. 2007, Snodgrass et al. 2009). Even with insecticide rotation efforts, some tarnished plant bug populations are expressing resistance to three insecticide classes, carbamates, pyrethroids and organophosphates (Snodgrass 1994, Pankey et al 1996, Snodgrass 2006). The neonicotinoids are the only available options to which resistance has not been documented, but these insecticides have not performed as well as the organophosphates. Many producers across the Mid-South currently are using co-applications of insecticides from different classes to achieve satisfactory levels of control. Regardless of the insecticide use strategy, tarnished plant bug infestations can be found on insecticide-treated cotton plants during post-treatment evaluations.

Results of the current study indicated a significant difference in the age structure of tarnished plant bug nymphs on non-treated cotton plants. The proportion of the population of comprised of $\leq 3^{rd}$ (small nymphs) instars appeared to be greater than that for nymphs $\geq 4^{th}$ (large nymphs) instars at all sampling periods. This would be an expected result because of poor nymph survival on cotton (Fleischer and Gaylor 1988). On insecticide-treated cotton plants, there were no significant differences between the frequencies of small and large instars at all sampling periods. These results suggest that acephate was more efficacious against small nymphs. Allen

et al. (2008) found in laboratory bioassays that small nymphs ($1^{st}$ – $3^{rd}$ instars) were more susceptible to methamidaphos (organophosphate, acephate metabolite) compared to larger nymphs ($4^{th}$ – $5^{th}$ instars). The differential in insecticide toxicity reduced the differences between the ratios of instar sizes between the acephate-treated and non-treated plots.

In all sampling periods for both acephate-treated and non-treated plants, tarnished plant bugs nymphs showed an overwhelming preference for squares. During the flowering stage of cotton, multiple feeding sites are available for nymphs including meristems, squares, white flowers and bolls. These results are in direct agreement with those from previous research (Pack and Tugwell 1976, Snodgrass 1998) showing tarnished plant bug nymphs prefer squares over other feeding sites on non-treated cotton plants. However, the present study is unique in that it examined the feeding site preference of nymphs on acephate-treated plants. These results clearly show that an insecticide such as acephate does not affect the tarnished plant bug distribution on fruiting forms within cotton plants during flowering stages of development. These results are important to support the action threshold protocols for sampling fruiting forms before and after an insecticide application. This becomes even more significant due to the fact Louisiana cotton fields are treated multiple times for tarnished plant bugs during a season (Williams 2008).

Cotton's indeterminate growth habit allows fruiting structures to mature from the base of the plant to the terminal. During the early–mid flowering periods, there are more squares on the sympodial nodes of the plant and with bolls in the lower portions of the plant. Tarnished plant bug preference for squares should result in higher numbers in the upper plant canopy, especially when squares outnumber bolls on cotton plants. At five of the six sampling periods on non-treated cotton plants, the frequencies of nymphs in the upper (nodes 1-5) and middle (nodes 6-10) strata were not significantly different. In all sampling periods on non-treated cotton plants,

the upper and middle strata had more nymphs compared to that in the lower (nodes 11-15) stratum in which bolls were the dominant fruiting form. This observation further supports nymph preference for squares and suggests that nymphs will more likely be located in close proximity to squares when they are present on the plant. In the presence of multiple fruiting types, the majority of nymphs are found on squares compared to white flowers and bolls (Snodgrass 1998). In the absence of squares, tarnished plant bugs (adults and nymphs) will readily feed on other fruiting forms including white flowers and small bolls (personal communication, B. R. Leonard, LSU AgCenter).

On acephate-treated cotton plants, the results indicated more nymphs on fruiting forms within the upper two strata compared to that in the lower stratum throughout each sampling period which was similar to that for non-treated plants. However, acephate significantly affected the main stem vertical distribution of nymphs on cotton plants when the frequencies of nymphs are compared between the upper two strata. Tarnished plant bug nymphs were more common in the middle stratum compared to the upper stratum from 24 HAT to 72 HAT. This distribution became similar at 96 HAT, but reversed at 120 HAT with significantly more nymphs in the upper stratum compared to that in the middle stratum. Insecticide spray deposition studies have shown that the greatest insecticide coverage is deposited on plant tissue in the upper plant stratum with coverage decreasing proportionally when moving down plant main stem (B. R. Leonard unpublished, Dept. of Entomology, LSU AgCenter). These results suggest that immediately after the acephate application, the highest mortality of nymphs is occurring in the upper plant stratum. As the effective acephate residual begins to decay, nymphs re-infested the upper plant stratum either through eggs hatching or immigrating upwards from lower nodes. The effective residual appeared to last about 72 HAT to 96 HAT. After that time interval,

nymph survivorship in the upper two strata became similar and even higher in the upper stratum at 120 HAT. This higher survivorship in the upper stratum at 120 HAT may be due to several factors. Fewer natural enemies, less competition among nymphs, or adult preference for non-injured squares on upper nodes may support this observation. In addition, nymphs may be re-infesting the upper canopy either through migration up the plant and/or hatching of eggs that were oviposited near the time of application. It was important to document the effects of an insecticide such as acephate on the vertical distribution of tarnished plant bugs on cotton plants to better understand the differences in sampling protocols using various sampling tools.

The current requirement for multiple insecticide applications to control tarnished plant bugs throughout the season could influence the precision and efficiency of sampling techniques in cotton fields. All current research used to develop these sampling methods and action thresholds were aimed at triggering the initial insecticide application. The results from the present study show that the distribution of the nymphs is changing after this initial application and perhaps after subsequent applications. When sampling the insects directly, it is important that areas of the plant where the majority of the insects are located be sampled. The standard sweep net (15 inches diameter) sampling method is concentrating its sample on the very upper portion (probably < 10 inches) of the plant. These data have shown that nymphs are distributed throughout the upper and middle portions of the plant. Within 72 HAT for acephate-treated plants, the majority of the nymphs are located in the middle stratum (nodes 6-10) of the plant. Therefore, if a sweep net is used to estimate acephate efficacy within this time period, it may provide an inaccurate efficacy measurement. During the flowering stages of cotton development, the whole-plant sampling protocols could provide a better indication tarnished plant bugs numbers, especially nymphs. Previous research has recommended the use of a shake-sheet over a

sweep net in flowering cotton and stated that the shake-sheet is more effective in sampling the immature nymphs when compared to the sweep net (Pack and Tugwell 1976, Fleischer et al. 1985, Snodgrass 1993, Musser et al. 2007). The shake-sheet sampling method is considered a whole-plant sampling method and could reduce the chance of an inaccurate measurement of insecticide efficacy. This protocol should also be equally effective prior to an insecticide application or after the effective residual has decayed.

There is considerable ongoing research re-evaluating sampling methods and action thresholds for flowering stage cotton plants (Musser et al. 2007, Musser et al. 2009). The results in the present study support and validate whole-plant protocols used to sample tarnished plant bugs in flowering cotton, especially after insecticide applications. Acephate was the only insecticide included in this experiment and future work should examine the effects of candidate insecticides representing other classes (i.e. carbamates, IGR's, neonicotinoids, and pyrethroids) for tarnished plant bug distribution on cotton plants. These results coupled with that from ongoing studies should aid in re-defining the sampling methods and action thresholds for tarnished plant bugs in flowering cotton.

## SUMMARY AND CONCLUSIONS

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois)*,* has been the most costly and difficult to control cotton arthropod pest across the Mid-South United States during the last several years. There are limited IPM options to manage this pest and chemical control strategies remain the primary tool used in cotton. Tarnished plant bug has become a season-long pest that is requiring multiple insecticide applications to obtain satisfactory control and protect cotton yields.  To ensure effective control, efficient sampling protocols and action thresholds are critically important in triggering timely insecticide applications.  Considerable research efforts have been ongoing in recent years to re-evaluate the sampling protocols and action thresholds recommended during the flowering stages of cotton development.  Most of this work has focused on the action threshold for the initial insecticide application and used that information for any subsequent treatments.  With a need for frequent insecticide applications to control tarnished plant bugs, understanding how these applications influence the distribution of surviving tarnished plant bugs on cotton plants and may affect results obtained with selected sampling protocols is important.

Studies were performed during 2007-2008 to evaluate tarnished plant bug survivorship, age-class of nymphs, fruiting form (flower bud [square], white flower, and boll) preference, and vertical distribution along main stem branches on non-treated and acephate-treated cotton plants at 0 through 120 hours after treatment (HAT).

There were significantly ($P < 0.07$) more nymphs on the non-treated plants compared to the acephate treated plants from 48 HAT to 120 HAT.  Acephate produced mortality levels ranging from 33-61% (50% mean) across the sample periods.  The locations of 20 tarnished plant bug nymphs was surveyed within each plot on randomly selected plants at each sampling period

to provide a consistent evaluation of insect distribution. Across all sampling periods, an average of 40% more plants were sampled in the acephate-treatment compared to the non-treated to maintain a constant sample size of 20 nymphs per plot.

The percentage of small nymphs ($\leq 3^{rd}$ instars), was greater ($P < 0.1$) than large nymphs ($\geq 4^{th}$ instars) on non-treated plants at all sample periods. However, on acephate-treated plants, the ratio of small nymphs to large nymphs was equal ($P > 0.15$). These results are related to the fact that earlier instars are more sensitive to acephate than the large instars. This knowledge of differential sensitivity to acephate between nymph stages can become important.

On non-treated and acephate-treated plants during all sampling periods, the majority (>70%) of tarnished plant bug nymphs were recorded on squares. On white flowers and bolls nymphs never represented more than 8% and 18%, respectively, of the total surveyed in a sample. Tarnished plant bug preference for squares, regardless of insecticide treatment, can be used to support those sampling techniques associated with visual observations of fruiting forms on reproductive stage cotton plants. The development of cotton plants follows an indeterminate growth pattern and reproductive stage plants can have multiple fruiting form types (squares, flowers, bolls) of various ages present at the same time period. Therefore, sampling techniques that examine fruiting form infestations and injury should primarily focus on squares or sympodial branches where squares are located, regardless of acephate application timing.

The majority of tarnished plant bug nymphs were found on main stem branches in the upper 10 nodes compared that on branches in the lower five nodes for both non-treated and acephate-treated plants. On non-treated plants, at all but one sample period, the frequency of nymphs found on sympodial nodes 1-5 and nodes 6-10 were not significantly different ($P > 0.6015$). However, on acephate-treated plants from 24 to 72 HAT, there were significantly more

35

($P < 0.0356$) nymphs on sympodial nodes 6-10 compared to sympodial nodes 1-5. At 96 HAT, the distribution on acephate-treated plants was similar ($P > 0.267$) among sympodial nodes 1-10. The number of squares on sympodial braches in the upper 10 nodes is much greater than lower in the plant and likely provides a better resource for these insects. Acephate produced clear effects on the main stem vertical distribution of tarnished plant bug nymphs within 72 HAT. Based upon these results sampling protocols in flowering cotton should encompass the whole plant and not focus on just one area of the plant canopy.

With the current recommended sampling protocols and action thresholds in cotton, visual observations of squares or using the shake-sheet to sample the entire plant profile would likely provide better estimates of tarnished plant bug nymphs following an acephate application than the sweep net which samples only the upper nodes. These results should be used in conjunction with ongoing studies to further refine our recommended sampling methods and action thresholds. Future studies should examine the effects of alternative insecticide classes, including neonicotinoids and insect growth regulators, on tarnished plant bug distribution within cotton plants. In addition, the effectiveness of current sampling methods following insecticide applications should be evaluated to ensure proper monitoring of insect pest populations and damage.

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# VITA

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