QUANTIFYING TARNISHED PLANT BUG, LYGUS LINEOLARIS (PALISOT DE BEAUVOIS), RESISTANCE TO ACEPHATE IN LOUISIANA

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The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is one of the most yield-limiting insect pests attacking cotton in the Mid-Southern region of the U.S. This pest is almost exclusively managed with chemical control strategies. The organophosphate insecticide, acephate, has been one of the most important insecticides recommended to control tarnished plant bugs. In recent years, reports of unsatisfactory acephate performance have become common and actual field rates have been increased to improve control. The objective of this study was to survey acephate susceptibility in Louisiana populations of tarnished plant bug using laboratory bioassays and evaluate acephate efficacy in field trials. Insecticide residual on glass (vial tests) bioassays were used to estimate acephate dose mortality responses (LC$_{50}$‘s) for five, nine, and six populations during 2007, 2008, and 2009, respectively. The LC$_{50}$‘s for these collections ranged from 1.63-32.36 µg/vial. Resistance ratios (RR) were calculated relative to a susceptible standard population (LC$_{50}$ = 3.1 µg/vial) and ranged from 0.52-10.44 among populations. Field control failures with acephate are likely when RR’s >3.0 and when persistent infestations exceed the action threshold for foliar sprays. Twenty field trials were conducted during 2007-2009 to determine acephate performance against native infestations. Five treatments (0[control], and acephate at 0.54, 0.82, 1.1, 1.34 kg AI/ha) were arranged in a Latin square design and were placed in commercial production fields and on LSU AgCenter Research Stations. Acephate efficacy was collected five to seven days after treatment using a one meter black shake sheet. The lowest acephate rate (0.54 kg AI/ha) significantly reduced tarnished plant bugs compared to that in the non-treated plots at 17 locations. However, this rate only reduced numbers below the action threshold in the 2007 trials. During 2008 and 2009, acephate rates of 0.82-1.34 kg AI/ha were needed to adequately control infestations. These results indicate that
acephate susceptibility in Louisiana populations of tarnished plant bug is shifting and field performance is decreasing.
INTRODUCTION

Upland cotton, *Gossypium hirsutum* (L.), is an important agronomic crop providing fiber for clothing, and is grown in about 80 countries. In the US, the cotton belt (17 states) extends from California east to North Carolina and a northern boundary of Kansas (http://www.nass.usda.gov/QuickStats/PullData_US.jsp). In 2007 and 2008, Louisiana ranked 8th and 11th, respectively, in cotton production (bales produced). In both years, Texas was the largest producing state, whereas Kansas produced the fewest bales. In 2008, cotton played a major role in Louisiana’s economy generating > $122 million compared to rice ($423.5 million), sugarcane ($357.6 million), corn ($334.7 million), soybeans ($308.7 million) and wheat ($162.1 million) (Anonymous 2008). Cotton has consistently been one of Louisiana’s most important agriculture commodities. This crop has been produced in > 20 Louisiana parishes, and typically is planted to a large portion of row crop acreage. In recent years, cotton has suffered lower acreage as well as fewer producers. This reduction has been associated with lower cotton prices, coupled with strong prices for commodities (corn and soybean) and risk aversion to weather such as hurricanes (Anonymous 2008). In 2007, there were 330,000 acres of cotton compared to 290,000 acres in 2008 a 13% decrease (Williams 2009). Comparing 2006 planted acres (635,000) to 2008 (290,000) resulted in a decrease of 55%. Even in spite of reduced acreage cotton still remains one of Louisiana’s top commodities in some regions of Louisiana.

Cotton is an expensive crop to produce and requires intensive management of arthropod, disease, and weed pests to produce optimal yields and fiber quality. Many expenses are involved in the effective control of cotton arthropod pests including: at planting insecticides ($10.00), foliar insecticides ($44.40), boll weevil, *Anthonomous grandis grandis* Boheman, eradication ($6.00/acre), transgenic *Bacillus thuringiensis* (Bt) cotton ($26.77/acre), treatment application cost (3.45), and consulting fees ($9.34) (Williams 2008, 2009). Producers averaged $99.96/acre
on cotton arthropod pest management. For the entire state, cotton producers spent slightly over $30.2 million on arthropod management, and in spite of these expenses, arthropods decreased yield by 6.55% which translated into 38,460 bales lost, totaling approximately $12 million (Williams 2009).

Cotton in Louisiana is attacked by several important arthropod pests including the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); thrips, *Frankliniella spp.*; the heliothine complex [bollworm, *Helicoverpera zea* Boddie, and tobacco budworm, *Heliothis virescens* (F.)]; the two-spotted spider mite, *Tetranychus urticae* (Koch); several stink bugs [Pentatomidae]; and the cotton aphid, *Aphis gossypii* (Glover) (Williams 2009). In the Mid-South, several species of Heteropterans or “true bugs” attack cotton including the clouded plant bug, *Neurocolpus nubilus* (Say), and the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), but the tarnished plant bug is the predominate species (Layton 2000). The tarnished plant bug has been found in most agricultural regions of the United States, Canada, and Mexico (Snodgrass 2003). This insect historically has been considered an early season cotton pest in Mississippi Delta Regions of the Mid-South (Tugwell et al. 1976). With the widespread adoption of transgenic Bt cotton cultivars and the success of the boll weevil eradication program, the tarnished plant bug has emerged as a primary cotton pest. These once primary pests of Mid-South cotton have been nearly eliminated as a problem (Layton 2000, Steede et al. 2003). These successes have contributed to fewer insecticide sprays that would have provided collateral tarnished plant bug control (Roberts 1999b, Layton 2000, Steede et al. 2003). Furthermore, the use of more target-specific insecticides applied for Lepidopteran pests has helped the tarnished plant bug evolve into a primary pest of cotton production in the Mid-South (Layton 2000, Leonard 2006).
The cost of control strategies and cotton yield reductions caused by the tarnished plant bug has increased considerable since 1996. During the same time period Louisiana’s other major pests, the heliothine complex, exhibited a reduction in these values when compared to averages from 1990-1995. More recently, since 2004 the tarnished plant bug has significantly exceeded costs of control and yield reductions when compared with the heliothine complex (Table 1). The changes in control cost and yield loss associated with the tarnished plant bug has propelled this cotton pest into the forefront of research efforts in developing an effective integrated pest management system.

Table 1*. Comparison of average control cost and percent yield reduction of the tarnished plant bug with the heliothine complex at different time periods.

<table>
<thead>
<tr>
<th></th>
<th>Tarnished Plant Bug</th>
<th>Heliothine Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Control Cost</td>
<td>% Yield Reduction</td>
</tr>
<tr>
<td>1990-1995</td>
<td>$2.92/Acre</td>
<td>0.41/Acre</td>
</tr>
<tr>
<td>1996-2008</td>
<td>$19.51/Acre</td>
<td>1.88/Acre</td>
</tr>
<tr>
<td>2004-2008</td>
<td>$30.57/Acre</td>
<td>2.72/Acre</td>
</tr>
</tbody>
</table>

* table adapted from Head (1990-1992) and Williams (1993-2008)

Various integrated pest management (IPM) strategies are used for tarnished plant bug control. These include area-wide control of alternate hosts (Able et al. 2007), host plant resistance traits (Temple et. al 2009), and entomopathogenic fungi, *Beuvaria bassiana* (Steinkraus et. al 2006). However, chemical control has been relied upon as the most effective means for controlling the tarnished plant bug in cotton. Several insecticides are recommended by the LSU AgCenter for tarnished plant bug control (Bagwell et al. 2008). However, the tarnished plant bug has developed some degree of resistance to several classes of insecticides. This insect has exhibited some degree of resistance to organophosphates, pyrethroids, carbamates, cyclodienes (Cleveland and Furr 1979, Cleveland 1985, Snodgrass and Scott 1988, Snodgrass 1994, Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2002,
Snodgrass 2006, Snodgrass et al. 2009). Snodgrass has been monitoring tarnished plant bug susceptibility to acephate in the Mississippi River Delta of Arkansas, Louisiana and Mississippi since 1998. The first year that several populations were detected with acephate resistance was 2005 (Snodgrass 2006). Since 2005, the number of resistant populations has increased, and populations were able to overwinter with resistance (Snodgrass and Gore 2007a, Snodgrass et al. 2009).

Acephate is the most frequently used insecticide for tarnished plant bug control in cotton (Snodgrass 2006). Fortunately, acephate has remained a viable option for tarnished plant bug control even after 2006 when several populations were reported with resistance levels high enough to potentially cause control failures in isolated areas of the Mississippi River Delta region during 2005 (Snodgrass 2006). Recommended use rates of acephate have been increasing during the previous decade indicating a general degredation of field efficacy (Bagwell personal communication). The LSU AgCenter recommended acephate rates 0.22 to 0.27 kg AI/ha in 1992 (Table 2). By 1996, recommended acephate rates increased to these rates from 0.36 to 0.54 kg AI/ha (Bagent et al. 1992, Bagwell et al. 1996). The LSU AgCenter currently recommends rates ranging from 0.54 to 87 kg AI/ha (Bagwell et al. 2009).

Table 2. Evolution of acephate rates in Louisiana.  

<table>
<thead>
<tr>
<th>Year</th>
<th>kg AI/ha</th>
<th>% Formulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>0.125-0.25</td>
<td>75 SP</td>
<td>Tynes et al. 1984</td>
</tr>
<tr>
<td>1988</td>
<td>0.22-0.27</td>
<td>75 SP</td>
<td>Bagent et al. 1988</td>
</tr>
<tr>
<td>1994</td>
<td>0.27</td>
<td>90 SP</td>
<td>Bagent et al. 1994</td>
</tr>
<tr>
<td>1995</td>
<td>0.27-0.54</td>
<td>90 SP</td>
<td>Barbour et al. 1995</td>
</tr>
<tr>
<td>1996</td>
<td>0.36-0.54</td>
<td>90 SP</td>
<td>Bagwell et al. 1996</td>
</tr>
<tr>
<td>2000</td>
<td>0.54-0.87</td>
<td>90 or 97 SP</td>
<td>Bagwell et al. 2000</td>
</tr>
<tr>
<td>2006</td>
<td>0.54-0.87</td>
<td>90 or 97 SP</td>
<td>Bagwell et al. 2006</td>
</tr>
<tr>
<td>2009</td>
<td>0.54-0.87</td>
<td>90 or 97 SP</td>
<td>Bagwell et al. 2009</td>
</tr>
</tbody>
</table>

* This table was adapted from Louisiana Insect Control Guides.

The resistance of the tarnished plant bug to many classes of insecticides has become a great

last tarnished plant bug resistance survey in Louisiana was conducted by Pankey et al. (1996). Mississippi has conducted extensive research involving acephate resistance in tarnished plant bug populations (Snodgrass 2006, Snodgrass and Gore 2007a, Snodgrass et al. 2009). Research in Mississippi indicates that acephate resistance is a problem producers are facing. Insecticide applications targeting tarnished plant bug have increased recently in Louisiana (Williams 2001-2009). Insecticide applications averaged 1.5 and 3.2 from 1997 to 2002 and 2003 to 2008, respectively. The increasing application frequency indicates that insecticides are losing efficacy against the tarnished plant bug, and growers have few effective insecticide alternatives for tarnished plant bug control. Changing farm landscape (Conservation Reserve Program/Wetland Reserve Program acreage) provides untreated reservoirs where large tarnished plant bug populations can build and move into cultivated fields. These factors demonstrate the need for examining acephate susceptibility in Louisiana populations of tarnished plant bug.
REVIEW OF LITERATURE

Plant Bugs Infesting Cotton

The group of insects known as “plant bugs” is classified in the order Hemiptera, suborder Heteroptera, and family Miridae (Wheeler 2001a). Miridae is the largest heteropteran family and contains nearly 10,000 species in 1,400 genera (Wheeler 2001b). There are eight subfamilies and 25 tribes in the Miridae family. The term “mirid” is also used interchangeably with “plant bug” (Wheeler 2001a).

Numerous species of mirids are phytophagous (plant feeding), but some are also predatory (facultative) and six species feed on fungi (Wheeler 2001c,d,e,f,g,h). Mirid diets range from polyphagous to monophagous. Hosts can range from trees and shrubs to floricultural and agricultural crops (Wheeler 2001c,d,f). Many field crops are attacked by at least one mirid species (Wheeler 2001f).

Mirid injury to plants is classified into broad categories ranging from: tissue discoloration (chlorosis: bleaching, spotting and stippling) and necrosis (blasting: small necrotic abscised bud or fruit), wilting of new growth, leaf crinkling and crumpling, leaf tattering and “shot holing”, and secondary symptoms (lesions and cankers) (Wheeler 2001c).

Cotton is damaged by several species of plant bugs (Wheeler 2001d, Layton 2000). Among the most prevalent and devastating of these mirids in the Mid-South (Arkansas, Louisiana, Mississippi, and Missouri) are the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter); the clouded plant bug, *Neurocolpus nubilus* (Say); and the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Layton 2000). The tarnished plant bug is the most widely distributed *Lygus* species in North America ranging from central Alaska and Newfoundland to Southern Mexico (Schwartz and Foottit 1992). This species bug is found on cotton throughout the Mid-Southern and Southeastern U.S., as well as parts of Texas (Layton 2000). Cotton fields
in the Delta regions (counties adjacent to the Mississippi river) of the Mid-South (Arkansas, Louisiana, Mississippi, and Missouri) typically support the greatest numbers of tarnished plant bug (Layton 2000).

**Tarnished Plant Bug Description and Biology**

Adult tarnished plant bugs are soft bodied, elongate (4.5 to 5.5 mm long) and reddish brown in color with a conspicuous yellow-brown triangle in the center of the back (Leigh et al. 1996). The antennae and proboscis (mouthpart) both consist of four segments (Borror et al. 1984, Leigh et al. 1996). Eggs are white, elongate (approximately one mm long), and slightly curved (Crosby and Leonard 1914). They are deposited individually in flowers, buds, bracts, and stems of plants (Crosby and Leonard 1914, Bariola 1969, Fleischer and Gaylor 1988). Usually eggs are partially inserted into the plants tissue. Eggs hatch in approximately eight days (Bariola 1969, Fleischer and Gaylor 1988, Leigh et al. 1996). Nymphs are oval and yellowish-green in color with relatively long legs compared to their body size. Nymphs develop through five instars (Crosby and Leonard 1914, Layton 2000) during a period of approximately 17 days before reaching the adult stage (Bariola 1969). Third, fourth, and fifth instars have four distinct black dots on the thoracic dorsum and one in the middle of the abdomen (Metcalf and Flint 1962, Leigh et al. 1996, Stewart 2004). Upon eclosion (adult emergence from cuticle of last instar) to the adult stage the tarnished plant bug feeds and mates before oviposition (Layton 2000). Approximately eight days are required between adult eclosion and oviposition (Bariola 1969). The reproductive organs of non-diapausing adults are fully developed by the time they are approximately seven days in age (Snodgrass 2003). Bariola (1969) determined that 33 days at 80°F are required for the completion of one generation of tarnished plant bugs on cotton plants.

The reproductive activity of tarnished plant bugs is sensitive to photoperiod. Bariola (1969) demonstrated that nymphs are the life-stage sensitive to the day length induction of
diapause, and photoperiod has a greater influence on the induction of diapause than temperature (Bariola 1969). Furthermore, nymphs exposed to 12.5:11.5 (hours light: dark) or shorter photoperiod matured into diapausung adults. Nymphs subjected to long days (≥ 13.5 hours of light) and temperatures of 21-27°C matured into reproductive adults (Bariola 1969). Snodgrass (2003) demonstrated similar results to that of Bariola (1969), determining that a photoperiod of 12.5 hours light and 11.5 hours dark or approximately September 12 for Washington County, Mississippi (Delta) induces diapause in field populations of tarnished plant bugs.

Reproduction is typically initiated in March with output increasing in April and May (Snodgrass et al. 1984). Snodgrass (2003) observed that in normal to mild winters, tarnished plant bug adults overwintering on henbit would break diapause (become reproductive) in December (photoperiod of 10:14 hours light: dark) producing adults by early March. Tarnished plant bug populations peak in September and October (Snodgrass et al. 1984). Populations decline in response to fewer host plants and adults entering reproductive diapause. With the onset of diapause, tarnished plant bug migrates to overwintering sites such as ground trash or winter hosts (Crosby and Leonard 1914, Cleveland 1982, Snodgrass et al. 1984).

The tarnished plant bug has a host range of over 380 plant species. Most hosts are broad leaf plants, and 21 of 30 most important agricultural crops have been documented as hosts for the tarnished plant bug (Young 1986). In the Northern Blackland prairies of Texas, 33 of 56 plant species surveyed were found to be hosts of tarnished plant bug (Womack and Schuster 1987). Snodgrass et al. (1984) found 169 host plant species of tarnished plant bug in the Mississippi River Delta region of Arkansas, Louisiana, and Mississippi. Populations of tarnished plant bug tend to be greater on weed hosts rather than crops. The main crop attacked by the tarnished plant bug in this region is cotton (Snodgrass et al. 1984). Two or more generations can be produced on alternate hosts before tarnished plant bugs migrate into cotton fields (Luttrell et al. 1998). As
weed hosts senesce, however, tarnished plant bug will migrate to crops or other hosts (Tugwell et al. 1976, Cleveland 1982, Snodgrass et al. 1984, Fleisher and Gaylor 1987). The movement of tarnished plant bug into cotton may correspond with host plant senescence or when herbicides terminate weed growth during the spring (Coy et al. 2001, Snodgrass et al. 1984). Snodgrass et al. (1984) observed that tarnished plant bug populations are highest from May to July in cotton. Cotton becomes a primary host of the tarnished plant bug in the Mississippi River Delta Region during June and July mainly due to a lack of available weed hosts (due to weed senescence) (Snodgrass et al. 1984). Two or more generations of tarnished plant bug often develop on cotton making this crop an important mid-summer host for population development (Luttrell et al. 1998).

Spring and early summer weed hosts are probably the most important factor in tarnished plant bug population development (Luttrell et al. 1998). Snodgrass et al. (1984) studied the dynamics of tarnished plant bug and weed hosts from September 1981 through October 1982 in the Mississippi River Delta regions of Arkansas, Louisiana, and Mississippi. Primary winter and spring hosts are classified in the families of Brassicaceae, Fabaceae, and Onagraceae. Summer and fall host families primarily include Amaranthaceae and Polygonaceae (Snodgrass et al. 1984). Winter and spring (January, February, and March) reproductive hosts included curly dock, *Rumex crispus* L.; narrowleaf vetch, *Vicia angustifolia* Reichard; and crimson clover, *Trifolium incarnatum* L. Other reproductive host species during the spring (March, April and May) were: burclover, *Medicago arabica* L.; shepherds purse, *Capsella bursa-pastoris* (L.) Medicus; daisy fleabane, *Erigeron philadelphicus* L.; cutleaf geranium, *Geranium dissectum* L.; cutleaf evening-primrose, *Oenothera laciniata* Hill; and showy evening-primrose, *O. speciosa* Nuttall (Snodgrass et al. 1984).
Some common early summer (June) non-crop hosts include annual fleabane, *E. annuus* (L.) Persoon; hedge-parsley, *Torilis arvensis* (Hudson) Link; tickseed, *Coreopsis tinctoria* Nuttall; and curly dock. Tarnished plant bugs can also be collected from crop-hosts including cotton, soybean, corn, grain sorghum, and rice (Snodgrass et al. 1984, Able and Snodgrass 2003, Bagwell and Sharp 2006). Corn tissue (milk stage corn kernels and silks but not efficiently) can be utilized for development and egg production (Able and Snodgrass 2003). Available hosts in mid to late summer (July, August, and September) are horseweed, *E. canadensis* L.; verbena, *Verbena brasiliensis* Vellozo; Pennsylvania smartweed, *Polygonum pensylvanicum* L.; and ragweed, *Ambrosia spp.* (Snodgrass et al. 1984).

Common fall (September, October, and November) hosts of the tarnished plant bug are Pennsylvania smartweed, horseweed; and two species of ragweed (giant and common); goldenrod, *Solidago altissima* L.; white heath aster, *Aster piolus* Willdenow; slender aster, *A. subulatus* Michaux var. *ligulatus* Shinners; and common lambsquarter, *Chenopodium album* L. Overwintering generations are produced on late summer and fall hosts (Snodgrass et al. 1984). Curly dock was the only host species that the tarnished plant bug could be collected off of every month out of the year (Luttrell et al. 1998).

Host plant can influence the duration of tarnished plant bug instars. Cotton has been found to negatively impact tarnished plant bug development compared to specific weed hosts. Lower survivorship, longer generation time, and higher nymphal mortality on cotton compared to annual fleabane. Total fecundity and adult survivorship was greater on cotton, but net fecundity was higher on annual fleabane (Fleischer and Gaylor 1988).

**Tarnished Plant Bug Injury to Cotton Plants**

Tarnished plant bug damage to cotton can occur from plant emergence through early boll development. Mirids in the *Lygus* genus typically feed upon flower buds, inflorescences (petals
and pollen), and fruit (Wheeler 2001d). Tarnished plant bug adults cause more damage to cotton than nymphal stages (Pack and Tugwell 1976). The tarnished plant bug can cause damage to 0.6 to 2.1 squares per insect per day (Wilson 1984). Plant injury from third and fifth instar nymphs has been found to be significantly greater than injury produced from second instar nymphs (Coy et al. 2001). On cotton seedlings, the presence of wilted leaves or a “flag” (small dead terminal leaf that has turned black) can indicate the migration of tarnished plant bug adults into fields (Scales and Furr 1968). Abortion of the cotton terminal associated with tarnished plant bug feeding will release apical dominance, causing secondary terminals to develop creating a phenomenon known as “crazy cotton” (Scales and Furr 1968; Hanney et al. 1977). Developing floral buds (squares) and meristematic parts of cotton are preferred feeding sites of the adult and nymph tarnished plant bug (Pack and Tugwell 1976). Tarnished plant bugs prefer to feed on small squares (three mm or < in diameter) rather than larger squares or bolls (Pack and Tugwell 1976). Tarnished plant bug feeding on squares causes “blasting” (small necrotic square) or abscission from the plant (Crosby and Leonard 1914, Wene and Sheets 1964). Pollen and anthers are the principal feeding sites for tarnished plant bug (Pack and Tugwell 1976). Anther damage is the most common and definitive damage symptom for injured squares. Damaged anthers will appear dark or necrotic (Pack and Tugwell 1976). The saliva of the tarnished plant bug contains digestive enzymes that are responsible for the necrosis of the pollen sac and anther (Reid 1965). Small squares tend to abscise in one to four days after exposure to and feeding tarnished plant bugs. Larger squares (> three mm diameter) are usually retained after feeding but when the flower opens visual feeding injury can be observed on anthers. Pack and Tugwell (1976) showed that flowers with 60 percent anther damage increased the occurrence of malformed bolls and abscised bolls (capsule). This is probably the result of poor pollination, but lower levels of damage had little or no effect on normal boll development (Pack and Tugwell
Damage to bolls may not be visible on the outer boll surface (exocarp). Tarnished plant bug feeding on bolls is described as dull, dark and slightly sunken lesions on the exocarp (Pack and Tugwell 1976). This entry site on the boll is usually a glossy, pinpoint-sized, black spot. The extent of damage incurred by a boll is correlated to boll age. In small to medium sized bolls, the tissue inside can become a jelly-like substance indicating complete loss of all locules. Damage caused to larger bolls rarely destroys them, but it can result in reduced seed quality, stained lint and a reduction in lint weight (Pack and Tugwell 1976). Bolls that sustained internal damage were often smaller in size and malformed. Mature bolls rarely show any internal damage to seed or lent when fed upon by tarnished plant bug. A boll is considered safe from tarnished plant bug damage after it has acquired approximately 250-300 degree days (DD 60s) after anthesis (Horn et al. 1999, Russell et al. 1999).

The severity of damage to cotton can be influenced by time (early to mid-season) of tarnished plant bug infestation (Tugwell et al. 1976). Most cotton yield loss and delays in fruiting occur during the period of peak squaring through early flowering (bloom) (Tugwell et al. 1976, Layton 2000). Excessive levels of tarnished plant bug infestations can cause unnecessary square loss, delay crop maturity, and alter normal crop fruiting patterns of the crop (Layton 2000, Coy et al. 2001). Cotton plants seem to be less susceptible to tarnished plant bug damage during early season (pre-squaring to the second week of squaring) mainly because plants can compensate for square loss and produce normal yields (Tugwell et al. 1976, Layton 2000). Tarnished plant bug control appears to be most important during mid-season (peak fruit set and early boll development). Cotton is not susceptible to economic injury from tarnished plant bug feeding once the plant has accumulated >150 heat units after physiological cutout (five nodes above the upper most white flower on the first position of a main-stem sympodial branch) (Teague et al. 2001).
Sample Protocols for the Tarnished Plant Bug in Cotton

Knowledge of tarnished plant bug infestation levels in cotton fields is important for successful control strategies (Tugwell et al. 1976). Sampling protocols and action thresholds serve as the basis for initiating control measures. Two types of samples (absolute and relative) are used to estimate plant bug densities (Snodgrass 1993). However, absolute samples are not used by producers or consultants because of the time and effort required for this sampling method (Snodgrass 1993). Therefore relative samples with direct and indirect methods have been recommended for estimating tarnished plant bug levels. The sweep-net, black shake sheet, visual observation and square retention are all effective methods of sampling this insect in cotton (Snodgrass 1993, Layton 1995, Bagwell et al. 2009). Musser et al. (2007) determined that the sweep-net and black shake sheet were the most efficient (based on sampling time) direct methods compared to whole-plant, square, and flower inspections. It was also concluded that the dirty bloom (evidence of feeding; necrotic anthers) was the most efficient indirect sampling method compared to sampling dirty square, external, and internal bolls. However, there is the concern that damage observed open flowers is greater than one week old, and this method would not be effective after an insecticide application. The sweep-net is most effective for sampling adults; whereas the black shake sheet is most effective for estimating nymphs (Snodgrass 1993, Musser et al. 2007). Fontenot et al. (2008) observed that an action threshold of 10-20% damaged squares (internal and external) could be used effectively to reduce insecticide applications without incurring yield losses. Visual sampling can be an easier method to use when cotton is blooming and the sweep-net and shake sheet are more difficult to use (Layton 1995). Square retention rates can also be an effective means for making control decisions during the period of square initiation to early flowering, with treatment thresholds being adjusted based on tarnished plant bug numbers and square retention (Layton 1995, Bagwell et al. 2008). Current action
thresholds are based upon square retention, sweep-net, and black shake sheet with each method being used during different cotton growth stages.

**Tarnished Plant Bug Management in Cotton**

An integrated approach to tarnished plant bug management has not been adopted but is being promoted (Gore et al. 2007). Insecticides are the primary means of managing tarnished plant bugs in cotton; however other management options have been suggested. Isolates of entomopathogenic fungi, *Beauveria bassiana*, have demonstrated potential as possible biological control agents under laboratory conditions and in caged tests (Liu et al. 2002, Steinkraus et al. 2006). However, Snodgrass and Elzen (1994) observed unsatisfactory levels of control of the tarnished plant bug using Naturalis-L (*B. bassiana* conida formulation) in field conditions. Cotton cultivars have been screened for resistance against the tarnished plant bug. Varieties expressing frego-bract and glabrous traits are more attractive and sensitive to injury from tarnished plant bugs than other varieties (Laster and Meredith 1974, Bailey 1982, Studebaker and Bourland 2009, Teague and Bourland 2009). However, the nectariless trait has been shown to reduce tarnished plant bug numbers in cotton (Bailey 1982, Bailey et al. 1984, Temple et al. 2009). This reduction in numbers is due to reduced egg laying ability (fecundity) and host non-preference (Schuster et al. 1976, Bailey 1982, Bailey et al. 1984). An area-wide program controlling weed hosts of the tarnished plant bug has proven effective in reducing tarnished plant bug numbers. Applying a single broad-spectrum herbicide in late February to marginal areas and ditches around cotton fields controls broad leaf hosts of the tarnished plant bug. This approach has been found economically effective in reducing tarnished plant bug numbers and insecticide applications (Snodgrass et al. 2006, Gore et al. 2007). Other cultural control practices effective in reducing insecticide applications or improving insecticidal efficacy involve planting shorter season varieties and varieties with an open canopy (okra leaf trait) (Gore et al. 2007).
Insecticide use strategies have been the most effective means of controlling high tarnished plant bug populations in cotton. The LSU AgCenter recommends the following chemicals for tarnished plant bug control: organophosphates (acephate, dicrotophos); neonicotinoids (acetamiprid, imidacloprid and thiamethoxam); carbamate (oxamyl); pyridinecarboxamide (flonicamid); and novaluron (insect growth regulator) (Bagwell et al. 2008). Of these, acephate and dicrotophos are the most common insecticides used to control tarnished plant bugs in cotton (Snodgrass 2006). Nozzle selection for delivery of the insecticide can have an effect on control as well. Hollow cone nozzles used to deliver recommended insecticides provide greater efficacy compared to air induction nozzles (Leonard et al. 2006, Gore et al. 2007). Shortening spray intervals (≤ 5) in combination with rotation of chemistries is effective in achieving adequate control of tarnished plant bugs (Gore et al. 2007). Tarnished plant bug populations exhibiting resistance to many of the recommended class of insecticides (pyrethroids, organophosphates, carbamates) is proving difficult to control in cotton. To obtain consistent control multiple insecticide applications are needed to reduce tarnished plant bug numbers below economic levels (Figure 1).

Figure 1. Frequency of tarnished plant bug insecticide applications. Table adapted from Head 1990-1992 and Williams 1993-2008.
Tarnished Plant Bug and Insecticide Resistance

Surveying insect populations for susceptibility/resistance to chemicals is critical to insecticide resistance management (Dennehy and Garnett 1984, Staetz 1985). The precision and accuracy of a monitoring program is influenced by its purpose (Roush and Miller 1986). Some programs are designed to determine if control failures with a pesticide are due to resistance. Other protocols are used to detect a change in susceptibility before control failures occur (Roush and Miller 1986).

Insecticide susceptibility monitoring programs usually involve comparisons of lethal dose (LD<sub>values</sub>) or lethal concentration (LC<sub>values</sub>) and slopes of dose/mortality lines from field-collected individuals and laboratory susceptible strains, or between independent field populations (Twine and Reynolds 1980, Staetz 1985). This method can be sufficient when high levels of resistance are suspected, but not for detecting minor shifts in susceptibility (Roush and Miller 1986). For consistent results, the susceptible strain should remain constant throughout the duration of the survey. The susceptibility of populations from areas of low or no insecticide use can vary between generations. These natural variations in susceptibility make comparison and interpretation of dose mortality lines difficult (Roush and Miller 1986).

Discriminating dose or diagnostic tests reveal what proportion of the population is resistant to an insecticide (Plapp et al. 1992). One difficulty with discriminating dose bioassays is that sample sizes must be large in order to detect resistance when resistance frequencies are low (< 10%) (Roush and Miller 1986). Several hundred specimens are needed to detect resistance with 95% probability at a 0.1% frequency (Roush and Miller 1986).

Insecticide resistance in the tarnished plant bug has become a great concern to cotton growers in the Mid-Southern U.S. (Luttrell et al. 1998, Snodgrass et al. 2009). Numerous surveys of tarnished plant bug susceptibility have been conducted to follow changes in
susceptibility to current and new insecticides. Several methods have been used to monitor changes in insecticide susceptibility among tarnished plant bug populations. These methods ranged from insecticide residual on glass (glass-vial bioassay) to topical applications of products.

Snodgrass (1996b) modified the glass-vial bioassay procedures to provide optimum results with tarnished plant bug. Snodgrass found that tarnished plant bug reared in the laboratory should be tested at an age of 10 days as an adult. Nymphs are not tested due to the large difference in body sizes among the five instars (Snodgrass 1996b). Sex does not influence bioassay results so male or female specimens may be used. Two to three adult tarnished plant bugs per 20 ml glass scintillation should be used and mortality assessed 24 h after exposure (Snodgrass 1996b). Mortality was found to be significantly affected by the length of time tarnished plant bug was exposed to the insecticide and whether food was present or absent (Snodgrass 1996b). Therefore, a piece of green bean, Phaseolus vulgaris L., (≈ three mm) in length should be used as a food and moisture source.

Luttrell et al. (1998) studied tarnished plant bug resistance using computer modeling. These estimates suggested that it would take 101 generations or 12.6 years for tarnished plant bug to become resistant when two generations per year were exposed to an insecticide. The model also revealed the effects of gene dominance and inheritance on the development of resistance. When the model was changed to make effective dominance gene more recessive, resistance was delayed for 244 generations. If the resistance genes become more dominant in the population time to resistance development was decreased with resistance occurring in 78 generations. Development of resistance would increase rapidly, if all generations (eight) of the tarnished plant bug within a year were exposed to selection pressure. To increase the life of an insecticide, management practices must be established that reduce insecticidal selection pressure.
within and among tarnished plant bug populations. A non-treated refuge in which no selection pressure is exerted can also help delay resistance in an insect population (Luttrell et al. 1998).

During the late 1970’s and mid 1980’s, Mississippi reported populations of tarnished plant bug with significant tolerances to 6 organophosphates (monocrotophos, methyl parathion, dimethoate, chlorpyrifos, malathion, acephate) and a carbamate (carbaryl) (Cleveland and Furr 1979, Cleveland 1985). McCaa and Schuster (1986) observed lower slope values for dicrotophos, monocrotophos, and carbaryl as compared to methomyl which indicates more variability in tolerance to these compounds in the populations. Dimethoate resistance was documented in the Mississippi Delta in 1988 (Snodgrass and Scott 1988). The first documented pyrethroid (permethrin and bifenthrin) resistance in the Mississippi Delta region was reported in 1994 (Snodgrass 1994). A population from Schlater, Mississippi exhibited multiple resistance to organophosphate and cyclodienes (Snodgrass 1996a). Another population of tarnished plant bugs was found resistant to dicrotophos, permethrin, and methyl parathion (Snodgrass and Elzen 1995). In Arkansas, Hollingsworth et al. (1997) reported field populations of tarnished plant bugs with significantly high LC$_{50}$s for endosulfan and oxamyl compared to a reference susceptible population, and also reported significant seasonal variation in susceptibility to l-cyhalothrin, endosulfan, and oxamyl. Pankey et al. (1996) reported tolerance levels to cypermethrin (37x), oxamyl (5x), and acephate (7x) in Louisiana. Tests in 1999 found several populations collected from Arkansas, Louisiana, and Mississippi demonstrated pyrethroid resistance. Seasonal changes in susceptibility of these populations were recorded with most spring collections being more susceptible than fall collections (Snodgrass and Scott 2000). Wide-spread malathion resistance was documented in Mississippi during 1999 to 2001 with resistance ratios usually increasing from spring to fall when compared to a reference-susceptible population (Snodgrass and Scott 2002).
A formal survey of tarnished plant bug susceptibility to acephate has been ongoing since 1998 in selected Arkansas, Louisiana, and Mississippi locations. Prior to 2005, most of these populations had resistance ratios (RR) 2-fold (Snodgrass and Scott 2002). Most of these sample sites are concentrated in Mississippi with two sites being located in East Carroll parish, Louisiana. Snodgrass (2006) found 10 populations from this region that expressed resistance to acephate (≥ 3-fold RR). In 2006, Snodgrass and Gore (2007a) documented 18 populations from the Mississippi River Delta and five from the Mississippi hills area to have elevated resistance to acephate (RR ≥ 3). Field tests indicate that tarnished plant bugs with RR ≥ 3 would prove difficult to control in fields with acephate (Snodgrass 2006, Snodgrass and Gore 2007a, Snodgrass et al. 2009). Tests conducted in May 2006 revealed 11 populations had successfully overwintered with resistance (RR > 3), and further tests in May 2007 exposed 18 populations were successful in overwintering with resistance. In the fall of 2007, 19 populations from this region were observed to be resistant to acephate (RR ≥ 3) (Snodgrass et al. 2009). Acephate resistance in these tarnished plant bug populations were found to be semi-dominant and not sex-linked (Snodgrass et al. 2009). Tarnished plant bugs from the tested locations have successfully overwintered with acephate resistance since the fall of 2005 indicating the persistent nature of acephate resistance (Snodgrass et al. 2009). Acephate resistance appears to be easy to select for, persistent in the populations, and probable to spread to other agricultural areas.

Objectives

I. Determine range of acephate susceptibility in Louisiana populations of tarnished plant bugs.

II. Evaluate a dose response to acephate efficacy against the tarnished plant bug in cotton.
MATERIALS AND METHODS

Two experiments were designed to address the objectives proposed in this research problem. The first experiment involved acephate susceptibility surveys of tarnished plant bug populations across Louisiana. Those areas included the cotton production regions. The second experiment was a series of field trials designed to establish acephate for native populations in cotton fields.

Insects

Tarnished plant bug adults were collected in cotton and non-cotton producing parishes during March to September during 2007 to 2009 (Table 3 and Figure 3). A standard sweep net (38 cm diameter) was used for collecting insects from native hosts or cotton. Adults were aspirated from the sweep net and held in 30 X 30 X 30 polypropylene cage with 24 mesh sides (BugDorm-1, Megaview Science Education Services Co., Ltd, Taichung, Taiwan). They were provided washed green beans for a food and moisture source, and were held at ambient room temperature until transported to Dr. Snodgrass.

Residual Insecticide on Glass Bioassays

All bioassays were done at the USDA-ARS according to protocols previously established by Snodgrass (1996b). A brief description of the methods is listed below. The glass vial bioassay developed by Snodgrass (1996b) was specifically designed to determine acephate toxicity to tarnished plant bug. Adults were held for at least 24 h to allow for any natural mortality. Technical grade acephate was diluted in acetone to achieve the desired dosage. These doses were pipetted into 20 ml scintillations and allowed to dry. Control vials were only coated with acetone. Four doses of acephate (5, 10, 15, and 20µg/vial) were diluted from the stock solution. Two tarnished plant bug adults were placed into the glass vials coated with acephate. Each vial contained a piece of washed green bean (three mm) as a food and moisture source.
Each dose was replicated three times. Sample size ranged from 109-168, 150-270, and 180 for 2007, 2008, and 2009, respectively. The test subjects were held at ambient room temperature. Assessment of mortality occurred 24 h after exposure. Insects were considered dead if they were unable to right their self after being placed on their back for 10 seconds.

During 2007, pre-treated vials were shipped from Dr. Snodgrass for the tarnished plant bug susceptibility surveys. Vials where covered with black plastic and held in an ice chest to avoid photodegradation of insecticide. They were stored in a freezer and removed only when needed during the 2007 season. For the 2008-2009 surveys, were delivered to Dr. Snodgrass and tests were conducted in the USDA-ARS lab at Stoneville, Mississippi. The reason change occurred to allow for more experienced individuals to conduct bioassays under controlled conditions and therefore reduce variability in the testing procedure.

These data were subjected to the probit regression model for analysis (SAS Institute 2003) and data was corrected for control mortality using Abbott’s (1925) formula. The results were used to establish LC$_{50}$'s and confidence intervals for each population. Locations were considered significantly different from one another if their confidence intervals did not overlap. Resistance ratios were also calculated for each population based upon the LC$_{50}$ of a susceptible population from Crosset, Arkansas (LC$_{50}$ 3.1 µg) (Snodgrass and Gore 2007a). This is the standard used to estimate if field control failures with acephate could occur with these populations.

**Field Efficacy of Acephate**

Field trials were conducted in several Louisiana cotton producers’ fields in 2007 and 2008; tests were done on the Macon Ridge Research Station (MRRS) in Winnsboro and the Northeast Research Station (NERS) in St. Joseph in 2009 (Figure 4). Recommended cotton varieties (Delta and Pine Land, Stoneville, or Phytogen) were planted at common planting dates.
(early April or May) for all tests. Native tarnished plant bug populations were utilized for these experiments. Each off-station test had a 50 ft border of cotton surrounding it (Figure 2). The border was marked using red bicycle flags (Parker Flags, Inc. Hollandale, Florida). This was to ensure over-sprays across the test area did not occur. These tests sites were located adjacent to alternate hosts of tarnished plant bugs and increased the potential for infestation levels sufficiently high to challenge acephate at selected doses (Figure 2). Tests were not initiated unless fruiting structures (squares and blooms) were present on plants and tarnished plant bug numbers average ≥ two per shake sheet sample within the test area. Tarnished plant bug populations were estimated prior to treatment by averaging the number of plant bugs obtained from four to six separate shake sheet samples across the test site. The shake sheet (0.76 m long X 0.91 m wide) covers 1.52 m of row space (0.76 m of row on each side of the sheet). During 2009, trials were performed on the research stations and allowed for more control of variability in the experiments. Mustard greens Brassica rapis, were planted at NERS as a host to generate tarnished plant bugs. Test plots were planted between the mustard and allowed for potential heavy infestations to develop. Four rows of mustard greens alternated with eight rows of cotton across the test site. The test area MRRS was located around other hosts (Amaranth spp. and horseweed) utilized by the tarnished plant bug as an alternate host. The test site was non-treated for an extended period allowing for a heavy population (average 19.4/sheet sample) to develop.

Treatments were arranged in Latin square design with five replicates (Figure 2). Orthene 97 WP (AMVAC Chemical Corporation Los Angeles, California) was evaluated in field tests at rates of (0.54, 0.82, 1.1, and 1.34 kg AI/ha). Test plots were four rows wide by 15.24 m (50 ft) in length. All off-station test plots were treated with a CO₂ powered back-pack sprayer (Bellspray, Inc. d.b.a. R&D Sprayer Opelousas, Louisiana). Teejet 11002 flat fan nozzles were calibrated to apply 93.5 liters of total spray per hectare (ha) at 2.11 kg/cm². Acephate treatments
were delivered to test plots using a John Deere 6000 high clearance sprayer located at the NERS and MRRS. The high cycles were equipped with Teejet TX-8 hollow cone nozzles (two/row) that were calibrated to apply 56.17 (Macon Ridge Research Station) and 112.34 (Northeast Research Station) liters of water per hectare at 3.52 and 3.94 kg/cm² respectively.

Acephate efficacy data was collected using black shake sheets (0.76 m x 0.76 drop cloth). Efficacy was evaluated 5-7 d after treatment by taking two samples (3.04 m of plants) within each plot using the previously mentioned protocol. Each sample was taken by vigorously shaking the cotton plants over the shake sheet and recording for tarnished plant bug nymphs. All data were subjected to Mixed Model procedures (SAS Institute 2003) to determine significant treatment effects. Treatment was the only fixed effect; whereas year and location were included as random effects in the model. Significant treatment (non-treated control and acephate rates) effects were evaluated for locations within a year and across locations for each year. Treatments were considered significant at α = 0.1. The LSMEANS and CONTRASTS procedures were used to compare results between individual treatments.
Figure 2. Experimental design for field trials evaluating acephate efficacy at selected doses against tarnished plant bugs in cotton. The perimeter was marked with red bicycle flags.
Table 3. Sample sites for Louisiana acephate susceptibility survey locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>Parish</th>
<th>Date</th>
<th>Latitude/Longitude</th>
<th>Collection Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola</td>
<td>West Feliciana</td>
<td>July 26</td>
<td>N30°58'27.48&quot; W91°37'26.77&quot;</td>
<td>Cotton</td>
</tr>
<tr>
<td>Wisner</td>
<td>Franklin</td>
<td>July 31</td>
<td>N31°58'33.69&quot; W91°34'12.71&quot;</td>
<td>Horseweed</td>
</tr>
<tr>
<td>Tallulah</td>
<td>Madison</td>
<td>August 1</td>
<td>N32°19'33.76&quot; W91°01'31.60&quot;</td>
<td>Cotton</td>
</tr>
<tr>
<td>Monroe</td>
<td>Ouachita</td>
<td>August 3</td>
<td>N32°31'24.07&quot; W91°59'40.54&quot;</td>
<td><em>Amaranth</em> spp.</td>
</tr>
<tr>
<td>Monroe</td>
<td>Ouachita</td>
<td>August 9</td>
<td>N32°31'24.07&quot; W91°59'40.54&quot;</td>
<td><em>Amaranth</em> spp.</td>
</tr>
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</table>

2007

<table>
<thead>
<tr>
<th>Location</th>
<th>Parish</th>
<th>Date</th>
<th>Latitude/Longitude</th>
<th>Collection Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vidalia</td>
<td>Concordia</td>
<td>April 28</td>
<td>N30° W91°</td>
<td>Vetch</td>
</tr>
<tr>
<td>Newellton</td>
<td>Tensas</td>
<td>April 28</td>
<td>N32°07'19.82&quot; W91°10'32.70&quot;</td>
<td>Vetch</td>
</tr>
<tr>
<td>Crowville</td>
<td>Franklin</td>
<td>April 28</td>
<td>N30° W91°</td>
<td>Crimson Clover</td>
</tr>
<tr>
<td>Vidalia</td>
<td>Concordia</td>
<td>May 16</td>
<td>N30° W91°</td>
<td>Vetch</td>
</tr>
<tr>
<td>Newellton</td>
<td>Tensas</td>
<td>May 16</td>
<td>N32°07'19.82&quot; W91°10'32.70&quot;</td>
<td>Vetch</td>
</tr>
<tr>
<td>Start</td>
<td>Richland</td>
<td>May 16</td>
<td>N32°30'02.65&quot; W91°51'32.23&quot;</td>
<td>Daisy fleabane</td>
</tr>
<tr>
<td>Wisner</td>
<td>Franklin</td>
<td>July 31</td>
<td>N31°59'12.23&quot; W91°40'37.69&quot;</td>
<td>Verbena/Horseweed</td>
</tr>
<tr>
<td>Newlight</td>
<td>Tensas</td>
<td>August 8</td>
<td>N32°02'58.66&quot; W91°24'24.16&quot;</td>
<td>Horseweed</td>
</tr>
<tr>
<td>Monroe</td>
<td>Ouachita</td>
<td>August 8</td>
<td>N32°31'24.07&quot; W91°59'40.54&quot;</td>
<td><em>Amaranth</em> spp.</td>
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2008

<table>
<thead>
<tr>
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<th>Parish</th>
<th>Date</th>
<th>Latitude/Longitude</th>
<th>Collection Host</th>
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<tr>
<td>Baton Rouge</td>
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<tr>
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<td>Franklin</td>
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<td>N31°08'30.02&quot; W91°41'00.45&quot;</td>
<td>Crimson Clover</td>
</tr>
<tr>
<td>Catahoula</td>
<td>Catahoula</td>
<td>May 18</td>
<td>N31°44'50.05&quot; W91°32'46.48&quot;</td>
<td>Red Clover/Black-eyed Susan</td>
</tr>
<tr>
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<td>Madison</td>
<td>May 22</td>
<td>N32°23'00.27&quot; W91°07'51.84&quot;</td>
<td>Curly Dock/Cutleaf Primrose</td>
</tr>
<tr>
<td>Alexandria</td>
<td>Rapides</td>
<td>June 15</td>
<td>N31°10'44.23&quot; W92°23'58.49&quot;</td>
<td><em>Amaranth</em> spp.</td>
</tr>
<tr>
<td>Gilliam</td>
<td>Caddo</td>
<td>June 16</td>
<td>N32°49'18.22&quot; W93°51'01.18&quot;</td>
<td><em>Amaranth</em> spp.</td>
</tr>
</tbody>
</table>
Figure 3. Collection sites in Louisiana production parishes (green) for tarnished plant bug susceptibility surveys for acephate during 2007, 2008, and 2009.
Figure 4. Field trial locations for acephate efficacy against tarnished plant bugs in Louisiana production parishes (green) during 2007, 2008, and 2009.
RESULTS AND DISCUSSION

Residual Insecticide on Glass Bioassays

The acephate susceptibility surveys indicate that tarnished plant bug resistance in Louisiana is widespread and apparently well-established (Tables 4-6). In 2007, the treated vials were shipped from Dr. Gordon Snodgrass at USDA-ARS in Stoneville, Mississippi to Louisiana. Surveys were accomplished during July and August of 2007 when insecticide use frequency against tarnished plant bug is highest. Dose mortality responses expressed as lethal concentrations estimated to produce 50% mortality (LC\(_{50}\)'s) ranged from 1.63-15.64 µg/vial (Table 4). The Angola and Tallulah populations demonstrated the highest LC\(_{50}\)'s (15.64 µg/vial and 13.96 µg/vial, respectively) and were significantly different from other populations tested during 2007. The Wisner collection was significantly more susceptible (1.63 µg/vial) than other populations. This unexpected and low LC\(_{50}\) for this population may have been due to inconsistent toxicity from acephate in the insecticide-treated vials. Furthermore, this value was the lowest obtained during all three years of testing. No significant difference was found between the Monroe-A (9.73 µg/vial) and Monroe-B (9.69 µg/vial) collections.

Table 4. Response of Louisiana tarnished plant bugs to acephate in laboratory tests, 2007.

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>N</th>
<th>LC(_{50})</th>
<th>95% CL(^1)</th>
<th>Slope</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola</td>
<td>Jul 26</td>
<td>109</td>
<td>15.64</td>
<td>(12.74-22.97)</td>
<td>0.85</td>
<td>0.89</td>
</tr>
<tr>
<td>Wisner</td>
<td>Jul 31</td>
<td>168</td>
<td>1.63</td>
<td>(0.10-3.13)</td>
<td>0.72</td>
<td>0.04</td>
</tr>
<tr>
<td>Tallulah</td>
<td>Aug 1</td>
<td>120</td>
<td>13.96</td>
<td>(12.27-15.61)</td>
<td>1.50</td>
<td>0.21</td>
</tr>
<tr>
<td>Monroe A</td>
<td>Aug 3</td>
<td>168</td>
<td>9.73</td>
<td>(8.75-10.59)</td>
<td>2.11</td>
<td>1.00</td>
</tr>
<tr>
<td>Monroe B</td>
<td>Aug 9</td>
<td>168</td>
<td>9.69</td>
<td>(7.93-11.26)</td>
<td>1.11</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^1\)Confidence Limits; LC\(_{50}\)'s are considered significantly different if CL do not overlap.

Four of the five populations tested during 2007 demonstrated resistance ratios (RR) greater than the critical 3.0-fold level determined by Snodgrass (2006) (Figure 5). That work showed that tarnished plant bug populations exhibiting a RR ≥ 3 would likely be difficult to
control with acephate at recommended field rates. Although the protocol designed by Snodgrass et al. (1996b) was followed in all 2007 bioassays, the results were highly variable and did not clearly relate to field observations of acephate performance against tarnished plant bugs at the sample sites. Low survival in the non-treated (control) vials (100 to 79%) was observed with inconsistent mortality across the dosage range (10, 15, 20 µg/vial) in the bioassays. These variable data resulted in the inability to replicate the results observed by Snodgrass (1996b). The doses were increased to 10, 25, and 50 µg acephate/vial and additional care was taken to only use healthy insects. Potential reasons for the inconsistent results could be related to the fact that acephate is unstable on glass. As previously stated vials used during 2007 were shipped from Mississippi to Louisiana and in many instances were stored for several days before being used. Regardless of the reasons, the test results were not inconsistent and this poor success during 2007 prompted us to ship samples of Louisiana tarnished plant bugs to the USDA-ARS laboratories in Stoneville, Mississippi for testing in 2008 and 2009.

Tarnished plant bugs were collected from nine locations during 2008. These collections were made from April through August and expressed LC$_{50}$'s that varied from 9.54 to 32.36 µg/vial (Table 5). Tarnished plant bugs expressing the highest LC$_{50}$'s were collected during May at Start (32.36 µg/vial) and Vidalia (28.26 µg/vial). These values were significantly higher than that for other 2008 populations. Tarnished plant bugs collected in July and August from Newlight and Wisner expressed the lowest LC$_{50}$'s, 10.54 µg/vial and 9.54 µg/vial respectively, and were significantly lower than LC$_{50}$'s for the other 2008 populations. There was no significant difference in LC$_{50}$'s among the Newellton (both), Crowville, or Monroe collections.
Figure 5. Acephate resistance ratios (RR’s) for Louisiana tarnished plant bugs collected during 2007. RR’s were derived by comparing LC$_{50}$’s for Louisiana populations to the LC$_{50}$’s (3.1 µg/vial) of an acephate-susceptible population from Arkansas (Snodgrass and Gore 2007).

Table 5. Response of Louisiana tarnished plant bugs to acephate in laboratory tests, 2008.

<table>
<thead>
<tr>
<th>Location/Date</th>
<th>Site</th>
<th>Date</th>
<th>N</th>
<th>LC$_{50}$</th>
<th>95% CL$^1$</th>
<th>Slope</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola Jul/26</td>
<td>Vidalia</td>
<td>Apr 28</td>
<td>210</td>
<td>14.22</td>
<td>(11.56-16.96)</td>
<td>1.07</td>
<td>0.40</td>
</tr>
<tr>
<td>Wisner Jul/31</td>
<td>Newellton</td>
<td>Apr 28</td>
<td>210</td>
<td>18.97</td>
<td>(16.03-22.43)</td>
<td>1.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Tallulah Aug/01</td>
<td>Crowville</td>
<td>Apr 28</td>
<td>210</td>
<td>16.58</td>
<td>(14.24-19.11)</td>
<td>1.41</td>
<td>0.44</td>
</tr>
<tr>
<td>Monroe A Aug/03</td>
<td>Vidalia</td>
<td>May 16</td>
<td>270</td>
<td>28.25</td>
<td>(24.04-33.81)</td>
<td>1.03</td>
<td>0.17</td>
</tr>
<tr>
<td>Monroe B Aug/09</td>
<td>Newellton</td>
<td>May 16</td>
<td>210</td>
<td>19.96</td>
<td>(16.74-23.84)</td>
<td>1.11</td>
<td>0.99</td>
</tr>
<tr>
<td>Start May 16</td>
<td>Start</td>
<td>May 16</td>
<td>210</td>
<td>32.36</td>
<td>(26.85-41.95)</td>
<td>1.09</td>
<td>0.67</td>
</tr>
<tr>
<td>Wisner Jul 31</td>
<td>Wisner</td>
<td>Jul 31</td>
<td>180</td>
<td>10.54</td>
<td>(8.74-12.26)</td>
<td>1.48</td>
<td>0.57</td>
</tr>
<tr>
<td>Newlight Aug 8</td>
<td>Newlight</td>
<td>Aug 8</td>
<td>150</td>
<td>9.54</td>
<td>(7.77-11.20)</td>
<td>1.45</td>
<td>0.54</td>
</tr>
<tr>
<td>Monroe Aug 8</td>
<td>Monroe</td>
<td>Aug 8</td>
<td>180</td>
<td>16.13</td>
<td>(13.23-19.67)</td>
<td>1.05</td>
<td>0.15</td>
</tr>
</tbody>
</table>

$^1$ Confidence Limits; LC$_{50}$’s are considered significantly different if CL do not overlap.
In 2008, RR’s for all populations ranged from 3.08-10.44 (Figure 6). The only sites with RR’s that did not greatly exceed the critical level of 3.0 were for collections from Wisner (3.4 RR) and Newlight (3.08 RR). The highest RR’s were for the collections from Vidalia (9.1 RR), Newellton (6.44 RR), and Start (10.4 RR). These sites were sampled in May before any insecticide applications would have targeted tarnished plant bug control in cotton. Two of the three late-season (Jul and Aug) collections (Wisner and Newlight) demonstrated the lowest RR’s in spite of this period of the season being that when the highest frequency of insecticide applications for tarnished plant bug is occurring.

During 2009, six sites were surveyed for acephate susceptibility in tarnished plant bugs. These LC$_{50}$’s ranged from 9.59 to 19.11 µg/vial. The Tallulah, Alexandria, Gilliam, and Catahoula populations demonstrated LC$_{50}$’s that were not significantly different from each other. The lowest LC$_{50}$’s were observed for the Baton Rouge (11.10 µg/vial) and Winnsboro (9.59 µg/vial) populations and were not significantly different from one another. In addition, no significant difference was observed between the LC$_{50}$’s of the Catahoula and Baton Rouge populations.

Resistance ratios for the 2009 populations ranged from 6.16 to 3.09 (Figure 7). The Gilliam population with a RR of 5.79 is from the Red River Valley, an area in Louisiana that usually is associated with low insecticide use for tarnished plant bugs. Also, the Baton Rouge collection is well beyond the normal cotton production region in Louisiana and is another area of low insecticide use. In fact, there is almost no commercial cotton production in this or the surrounding parishes. This population demonstrated a LC$_{50}$ of 11.10 µg/vial and a RR of 3.6.

These observations suggest that selection for acephate resistance in tarnished plant bugs could be occurring in other crops, or that acephate resistance is established throughout Louisiana tarnished plant bug populations.
Figure 6. Acephate resistance ratios (RR’s) for Louisiana tarnished plant bugs collected during 2008. RR’s derived from a comparison of LC$_{50}$’s for Louisiana populations to the LC$_{50}$’s (3.1 µg/vial) of a susceptible population from Arkansas (Snodgrass and Gore 2007).

Table 6. Response of Louisiana tarnished plant bugs to acephate in laboratory tests, 2009.

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>N</th>
<th>LC$_{50}$</th>
<th>95% CL$^1$</th>
<th>Slope</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baton Rouge</td>
<td>Apr 28</td>
<td>210</td>
<td>11.10</td>
<td>(7.99-13.96)</td>
<td>0.85</td>
<td>0.89</td>
</tr>
<tr>
<td>Winnsboro</td>
<td>Apr 28</td>
<td>180</td>
<td>9.59</td>
<td>(7.51-11.49)</td>
<td>1.22</td>
<td>0.81</td>
</tr>
<tr>
<td>Catahoula</td>
<td>May 18</td>
<td>180</td>
<td>14.30</td>
<td>(12.15-16.55)</td>
<td>1.44</td>
<td>0.60</td>
</tr>
<tr>
<td>Tallulah</td>
<td>May 22</td>
<td>180</td>
<td>19.11</td>
<td>(15.16-25.48)</td>
<td>0.84</td>
<td>0.93</td>
</tr>
<tr>
<td>Alexandria</td>
<td>Jun 15</td>
<td>180</td>
<td>18.12</td>
<td>(14.29-23.91)</td>
<td>0.83</td>
<td>0.91</td>
</tr>
<tr>
<td>Gilliam</td>
<td>Jun 16</td>
<td>180</td>
<td>17.96</td>
<td>(15.04-21.76)</td>
<td>1.15</td>
<td>0.70</td>
</tr>
</tbody>
</table>

$^1$ Confidence Limits; LC$_{50}$’s are considered significantly different if CL do not overlap.
Further evidence to support this observation is that each collection made in spring 2008 and all collections in 2009 were made prior to insecticide use in cotton. All of those populations demonstrated RR’s > 3.0.

Figure 7. Acephate resistance ratios (RR’s) for Louisiana tarnished plant bugs collected during 2008. RR’s derived from a comparison of LC$_{50}$’s for Louisiana populations to the LC$_{50}$’s (3.1 µg/vial) of a susceptible population from Arkansas (Snodgrass and Gore 2007).

Selection pressure exerted by insecticides on most cotton insects including tarnished plant bugs is typically low during the fall (post-harvest) and spring (pre-plant) allowing time for insecticide-susceptible insects to mate with resistant individuals diluting the resistant genes in the population (Snodgrass and Scott 2000). This pattern has been obvious with pyrethroid resistant tarnished plant bugs (Snodgrass and Scott 2000) and other insects such as tobacco budworm (Temple et al. 2006), when the gene for resistance has been classified as recessive. However, Snodgrass et al. (2009) reported that acephate resistance genes in Mississippi tarnished plant bugs are semi-dominant resulting in those populations retaining a high frequency of acephate-resistant individuals in the population even during periods of low insecticide use. High RR’s in the spring probably indicate that acephate-resistant tarnished plant bugs are successfully overwintering in some areas. Tarnished plant bugs can utilize soybeans as a reproductive host during bloom (G. L. Snodgrass unpublished data). In early season blooming soybeans are
attractive to tarnished plant bugs before they migrate to cotton. Acephate is commonly applied to soybeans for stink bug control (Baldwin et al. 2008). When acephate is applied for stink bug control inadvertent selection pressure from acephate would occur against infesting tarnished plant bug populations, partially explaining high RR’s observed in the spring.

The results in the current study agree with those generated from recent acephate susceptibility surveys for Mississippi tarnished plant bugs (Snodgrass et al. 2009). During May 2006, the average acephate LC$_{50}$'s were 8.5 µg/vial and 8.4 µg/vial for tarnished plant bug populations from Mississippi Delta and Hill regions, respectively. During the fall of 2006, LC$_{50}$'s averaged 16.1 µg/vial and 9.9 µg/vial for tarnished plant bugs in the Delta and Hill regions, respectively. Similar results were obtained for the spring of 2007 and mean LC$_{50}$'s for the Delta and Hill regions were 10.7 µg/vial and 8.1 µg/vial respectively. The range of LC$_{50}$'s observed for Louisiana during 2007 to 2009 was 1.63-32.36 µg/vial.

During 2006, a tarnished plant bug collection with a RR of 3.6 was caged on cotton plants treated with recommended rates of acephate (0.54 and 1.1 kg AI/ha). These rates only produced mortality levels of 39% (0.54 kg AI/ha) and 48% (1.1 kg AI/ha). These mortality levels are sufficiently low to cause in field control failures, especially under high and persistent infestations of tarnished plant bugs (Snodgrass 2006). Additional field tests in 2007 revealed that a tarnished plant bug population with a RR of 7.1 was not effectively controlled using acephate at rates up to 1.0 lb AI/acre. However, these rates as low as 0.5 lb AI/acre did provide adequate control of a tarnished plant bug collection expressing a RR of 2.3 (Snodgrass and Gore 2007a). During the three years of the present study in Louisiana, 20 populations were tested for acephate susceptibility and all but 1 demonstrated RR’s greater than 3.0. Therefore, it is likely that acephate resistance is influencing successful management of tarnished plant bug in Louisiana cotton fields.
Many of these same tarnished plant bug populations expressing acephate resistance also demonstrated elevated LC$_{50}$'s to other insecticides. In Louisiana during 2008 to 2009, 14 populations in the current study were tested for pyrethroid (permethrin) susceptibility (unpublished data, G. L. Snodgrass, USDA-ARS, Stoneville, MS). Two populations were found to be highly resistant (< 70% mortality) and two moderately resistant (70 to 90% mortality). This observation also is similar to Mississippi results that show tarnished plant bug resistance to pyrethroids is widespread in that state (Snodgrass et al. 2009). There also may be a shift in neonicotinoid (thiamethoxam and imidacloprid) susceptibility in Louisiana tarnished plant bug populations. During 2008, the susceptibilities of nine Louisiana populations to thiamethoxam were evaluated in a feeding bioassay (unpublished data, G. L. Snodgrass, USDA-ARS, and Stoneville, MS). Thiamethoxam LC$_{50}$'s ranged from 0.54 µg/ml to 3.61 µg/ml (G. L. Snodgrass unpublished data). During 2008 and 2009, nine populations were tested with imidacloprid and LC$_{50}$'s ranged from 0.77 µg/ml to 6.18 µg/ml. The LC$_{50}$'s for thiamethoxam and imidacloprid against a susceptible tarnished plant bug population are 1.40 µg/ml and 0.85 µg/ml, respectively (Snodgrass and Gore 2007b). The ranges of responses among the Louisiana collections were variable, but several populations expressed LC$_{50}$'s much higher than those observed for the neonicotinoid-susceptible tarnished plant bugs. These results are important because neonicotinoids are commonly rotated with acephate for tarnished plant bug control in cotton (Gore et al. 2007a, Bagwell et al. 2009). Gore et al. (2007a) observed that applying a neonicotinoid in rotation with acephate increased tarnished plant bug control and these plots reached threshold levels (third application) later than plots treated with just acephate.

**Acephate Rate Response Field Trials**

The results from three years of field tests (n=20) demonstrated that successful and consistent control of tarnished plant bugs with acephate is difficult unless high rates (≥ 0.75 kg
AI/ha) are used. The 2007 and 2008 trials were conducted on producer fields of commercial cotton production, whereas in 2009, all tests were performed in fields on LSU AgCenter Research Stations at St. Joseph and Winnsboro. The inclusion of field trials on the Research Stations allowed additional control of variables that could influence test results. Variability in cotton growth, field environment, production decisions, and pest distribution were all considerations in each on-farm trial capable of influencing the conduct of the trial and ultimately the interpretation of the results. In addition, the data from controlled small plot trials could be used to confirm the observations on acephate performance against tarnished plant bugs in commercial cotton fields.

In the analysis of results, years and locations (tests) were managed as a random effects variables. Differences in the initial tarnished plant bug populations prior to treatment, application timings during the season, and field environments contributed to variability of treatment performance in all field trials. The results are presented by test within each of the years (2007, 2008, and 2009), but also as a summary of treatment performance across all locations for individual years.

The 2007 field trials were conducted at nine locations across Louisiana and a significant treatment effect ($P < 0.036$) was detected in all trials (Figure 8). In the post-treatment sample (5-7 DAT), tarnished plant bug nymphs ranged from 1.6 to 32.2 insects per four row meters in the non-treated plots. Significant differences were observed between acephate rates of 0.54 to 1.34 kg AI/ha ($P \leq 0.068$). In one or more trials, each rate (0.54, 0.82, 1.1, and 1.34 kg AI/ha) significantly reduced tarnished plant bug nymphs compared to numbers in the control plots. There were no further significant reductions in numbers of nymphs with rates exceeding 0.82 kg AI/ha ($P \geq 0.267$) during 2007. At the highest rate (1.34 kg AI/ha), numbers ranged from 0.0 to 9.1 insects per four row meters all trials. This highest rate (1.34 kg AI/ha) reduced tarnished
plant bug nymphs below the current action threshold level of eight insects per four row meters (six insects per 10 row ft) in eight of nine trials.

During 2008, eight field trials were conducted in commercial cotton fields across Louisiana and treatments significantly ($P \leq 0.066$) influenced tarnished plant bug nymphs in seven trials (Figure 9). At the CH2 (Carl Haring) location, no treatment effect ($P = 0.235$) was observed. One or more rates (0.54, 0.82, 1.1, and 1.34 kg AI/ha) of acephate significantly reduced tarnished plant bug nymphs compared to numbers in the control plots in all other tests ($P \leq 0.094$). Similar to the results in 2007, there were no significant reductions in numbers of nymphs with rates exceeding 0.82 kg AI/ha ($P \geq 0.235$) in the 2008 tests. In the post-treatment samples (5-7 DAT), tarnished plant bug nymphs ranged from 6.0 to 65.6 per four row meters in the non-treated plots, and from 0.3 to 7.2 per four row meters in the plots treated with the highest rate of acephate (1.34 kg AI/ha). In the those trials with significant treatment effects, acephate at 1.34 kg AI/ha reduced tarnished plant bug nymphs below the current action threshold level of eight insects per four row meters.

During 2008, eight field trials were conducted in commercial cotton fields across Louisiana and treatments significantly ($P \leq 0.066$) influenced tarnished plant bug nymphs in seven trials (Figure 9). At the CH2 (Carl Haring) location, no treatment effect ($P = 0.235$) was observed. One or more rates (0.54, 0.82, 1.1, and 1.34 kg AI/ha) of acephate significantly reduced tarnished plant bug nymphs compared to numbers in the control plots in all other tests ($P \leq 0.094$). Similar to the results in 2007, there were no significant reductions in numbers of nymphs with rates exceeding 0.82 kg AI/ha ($P \geq 0.235$) in the 2008 tests. In the post-treatment samples (5-7 DAT), tarnished plant bug nymphs ranged from 6.0 to 65.6 per four row meters in the non-treated plots, and from 0.3 to 7.2 per four row meters in the plots treated with the highest rate of acephate (1.34 kg AI/ha).
Figure 8. Acephate efficacy against tarnished plant bug nymphs in Louisiana field trials during 2007. The * indicates significant rate effects for a specific treatment within a location.

In the those trials with significant treatment effects, acephate at 1.34 kg AI/ha reduced tarnished plant bug nymphs below the current action threshold level of eight insects per four row meters.

Acephate efficacy was evaluated at three locations during 2009 and significant treatment effects (P ≤ 0.005) were observed at two locations (Figure 10). No treatment effect was detected at the St. Joseph 1 (StJ1) location (P = 0.109). In the two tests with significant treatment effects, the low rate (0.54 kg AI/ha) of acephate significantly reduced (P < 0.001) numbers of tarnished plant bug nymphs below that in the non-treated plots at only one location, Macon Ridge (MR).
Figure 9. Acephate efficacy against tarnished plant bug nymphs in Louisiana field trials during 2008. The * indicates significant rate effects for a specific treatment within a location.

At both locations, acephate rates at 0.82, 1.1 and 1.34 kg AI/ha, significantly reduced ($P \leq 0.055$) tarnished plant bug nymphs from that in the non-treated control. During 2009, acephate at 1.34 kg AI/ha outperformed all other rates except for 1.1 kg AI/ha and reduced numbers of nymphs below that in the other acephate-treated plots ($P \leq 0.096$). In the post-treatment sample (5-7 DAT), tarnished plant bug nymphs ranged from 6.0 to 38.8 insects per four row meters in the non-treated plots. At the highest rate (1.34 kg AI/ha), numbers ranged from 2.8 to 9.8 insects per four row meters across all trials. In all three trials plots treated with 0.82 kg AI/ha had significantly fewer nymphs than that in the non-treated control plots. Acephate used at 1.34 kg AI/ha reduced tarnished plant bug nymphs below the current action threshold level in only two of the three trials.
Significant treatment effects (all rates) were detected with acephate in one or more field trials during each year. However, significant reductions in number of nymphs from the non-treated control plots with acephate did not necessarily mean that all rates provided satisfactory control and consistently reduced numbers below the action level of eight insects per four row meters (six insects per 10 row ft). A summary across years of field trials suggest that tarnished plant bug populations in Louisiana are becoming more difficult to control with recommended rates of acephate (Figure 11). Numbers in the non-treated control plots did not vary much (20.2-24.7 insects per four row meters) among the three years, but infestation levels were slightly higher during 2008 and 2009 compared to that in 2007 (Figure 11). For each rate tested, tarnished plant bug numbers were higher in 2008 than in 2007 and higher in 2009 than in 2008. Although variation in infestation levels may have contributed to acephate performance in this study, only the highest rate (1.34 kg AI/ha) reduced tarnished plant bug nymphs below the action threshold during all three years. The lowest rate (0.54 kg AI/ha) did reduce tarnished plant bug
numbers compared to that in the non-treated control plots, but not enough to delay the need for an immediate re-treatment based upon the action threshold.

Figure 11. A three year summary (2007-2009) of acephate efficacy against the tarnished plant bug in Louisiana field trials. The line indicates the action threshold of eight tarnished plant bugs / four row meters.

Acephate field rates have been increasing in Louisiana and in other areas of the Mid-Southern U.S. to maintain satisfactory tarnished plant bug control and reduce numbers below the action threshold in cotton. Tarnished plant bugs are one of the most costly cotton pests across the Southern U.S. and appear to be a pest during nearly all stages of cotton plant development. In addition, higher numbers of this pest are infesting fields compared to that in previous years. This increase in tarnished plant bug populations can be related to changing farm landscapes (large areas of natural hosts and more acreage of other host crops), insecticide resistance issues, and the reduction in broad-spectrum insecticide application frequency with the success of transgenic cultivars and boll weevil eradication (Roberts 1999a, Roberts 1999b, Layton 2000, Steede et al. 2003). Acephate rates of 0.36 to 0.54 kg AI/ha were providing sufficient control of tarnished plant bugs in the past (Gore et al. 2007). However, higher rates (0.82-1.1 kg AI/ha) are
recommended during the present time, but these rates are producing efficacy levels equal to or less than that observed for the lower rates used during previous years (Gore et al. 2007).

In the late 1980’s, acephate used at rates of 0.22 to 0.27 kg AI/ha provided adequate control of tarnished plant bug (Miciński et al. 1990, Miciński et al. 1991). During the mid-1990’s, acephate was used at 0.36 to 0.54 kg AI/ha but still provided sufficient control of tarnished plant bug populations in cotton fields (Pankey et al. 1996, Russell et al. 1997). During the late 1990’s and into the early years of this century, acephate was recommended at rates of 0.54 to 0.82 kg AI/ha which was needed to provide satisfactory efficacy (Hall et al. 2000, Teague et al. 1999a and b, Snodgrass et al. 2001, Ngo et al. 2002). From 2004 to 2007 acephate rates ranging from 0.82 to 1.1 kg AI/ha was used to control of tarnished plant bug populations (Catchot et al. 2005a, b; Fontenot et al. 2007, Smith and Catchot 2007a, b, c).

Results from the current field study indicate that higher acephate rates are required for adequate tarnished plant bug control. Acephate used at 1.1 to 1.34 kg AI/ha was required to consistently reduce tarnished plant bug numbers below the action threshold. At most locations (14 of 20) tarnished plant bugs were significantly reduced below that in the non-treated control 0.54 kg AI/ha of acephate. However, during 2008 and 2009, numbers of tarnished plant bug nymphs were not reduced below the action threshold until acephate rates were increased to 0.82 kg AI/ha. In 2009, even higher rates (≥ 1.1 kg AI/ha) were needed during 2009 to reduce this pest below the action threshold. In order to achieve consistent tarnished plant bug control under high and persistent populations, higher acephate rates are required.

Acephate is the most commonly used insecticide for tarnished plant bug control in cotton (Snodgrass et al. 2009). The past three years of monitoring acephate susceptibility indicate wide-spread acephate resistance in Louisiana tarnished plant bug populations. Mississippi has reported acephate resistance problems in tarnished plant bug populations since 2005 (Snodgrass
Tarnished plant bugs with an acephate RR > 3.0 have proved difficult to control even with high rates of acephate (Snodgrass et al. 2009).

Chemical control strategies will continue to be one of the primary tools used to manage this pest in cotton, but alternative management practices are needed. Gore et al. (2007a) recommended a combination of strategies including short season varieties, nectariless cultivars, and area-wide management (controlling early spring hosts with a broad spectrum herbicide) as non-insecticidal components. Modifying insecticide use strategies including a rotation of modes of actions, co-application of insecticides (adulticides plus novaluron), reducing spray intervals (≤ five days), and nozzle selection for better foliage coverage are necessary to manage these insects in cotton fields. Results of the present study show that multiple applications will be needed in many instances when using acephate. Using high rates of acephate on a narrow spray interval in multiple applications within a season will rapidly increase selection pressure and result in more severe resistance problems with tarnished plant bugs.

The results of these laboratory bioassays and field trials show that acephate susceptibility in Louisiana populations of tarnished plant bug is decreasing. For chemical control strategies to be successful in the near future, producer reliance should shift from acephate to other chemistries with alternative modes of action such as thiamethoxam, novaluron, and flonicamid. These products should be used in conjunction with the non-insecticides strategies previously described. A tarnished plant bug susceptibility monitoring program for acephate should be expanded to include other chemistries and will provide information to better understand how populations are responding to these products. These results can then, be used to adjust recommendations to maintain or even improve insecticide efficacy against tarnished plant bug. However, it is likely that if tarnished plant bug infestations continue to increase in cotton fields then none of the
currently registered insecticides will be capable of providing consistent and satisfactory control for this important cotton pest.
SUMMARY AND CONCLUSIONS

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has become one of the most economically important insect pests attacking cotton in the Mid-South. Integrated pest management (IPM) tactics are limited for tarnished plant bug; therefore infestations are controlled almost exclusively with chemical control strategies. The tarnished plant bug is a persistent season-long pest and typically requires multiple insecticide applications to maintain adequate control and reduce economic losses. Acephate has been one of the primary products used to control the tarnished plant bug due to its relative cost-effectiveness. This product was first recommended Louisiana’s cotton IPM program during 1984. Acephate rates have increased from 0.13-0.27 kg AI/ha in 1984 to 0.54-0.87 kg AI/ha in 2009. In recent years, the actual amount of acephate applied to Mid-South cotton acreage has approached the maximum frequencies of sprays and total active ingredient allowed by the label in a season. In spite of the increased use, reports of poor field performance with acephate against tarnished plant bug have become common. Acephate resistance in Louisiana populations of tarnished plant bug could potentially reduce cotton production. Insecticide susceptibility surveys in Mississippi during 2005 have already documented the initial case of acephate resistance in a tarnished plant bug population. Therefore, the objectives of this study were to determine acephate susceptibility and field performance against Louisiana populations of tarnished plant bug.

Laboratory bioassays (insecticide residual on glass [vial tests]) were used to determine acephate LC$_{50}$'s for five, nine, and six Louisiana populations during 2007, 2008, and 2009, respectively. All samples of tarnished plant bug populations represented cotton-producing parishes except for one collected from the Baton Rouge location. The LC$_{50}$'s for these collections ranged from 1.63 to 32.36 µg/vial. The highest LC$_{50}$ was found for the Start population during spring of 2008. Resistance ratios (RR) relative to a susceptible standard
population (LC$_{50}$ = 3.1 µg/vial) of tarnished plant bug were also calculated (Snodgrass and Gore 2007). Resistance ratios (RR) for all populations ranged from 0.53 to 10.44. Tarnished plant bug populations exhibiting RR $\geq$ 3.0 have been difficult to control with recommended rates of acephate (Snodgrass 2006). All Louisiana populations surveyed during this study demonstrated RR $> 3.0$, except for the Wisner population tested in 2007. Only five other populations tested (Monroe A [3.14], Monroe B [3.13], Wisner [3.4], Newlight [3.08], and Winnsboro [3.09]) expressed RR similar to the 3.0 critical level. In addition, populations sampled during the spring on non-cotton hosts demonstrated relatively high LC$_{50}$'s and RR $> 3.0$. These results are significant observations and show that prior to acephate exposure on cotton during the spring tarnished plant bug populations are maintaining a high frequency of resistance. Furthermore, populations tested from areas (Baton Rouge and Gilliam) where few insecticides target tarnished plant bugs exhibited RR $> 3.0$. Acephate resistance levels are variable, but widespread throughout Louisiana’s tarnished plant bug populations. Additional selection pressure with acephate sprays target other insect pests (stink bugs), on a non-cotton crop (soybean) that can serve as a hosts for tarnished plant bug.

Twenty field trials were conducted during 2007-2009 to evaluate acephate efficacy against native infestations of tarnished plant bug. Five treatments including a non-treated control, and acephate (Orthene 97 SP) at four rates (0.54, 0.82, 1.1, 1.34 kg AI/ha) were arranged in a Latin square experimental design and placed in test areas on commercial production fields (17 trials) and LSU AgCenter Research Stations (three trials). All plots were rated five to seven days after treatment using a one meter black shake sheet.

Significant treatment effects ($P \leq 0.066$) were observed in 18 of the 20 trials. Seventeen tests showed acephate at 0.54 AI/ha significantly reduced tarnished plant bug nymphs below that in the non-treated control. However, based upon the action threshold of eight insects per four
row meters (six insects per 10 row feet), acephate at 0.54 kg AI/ha rate only provided acceptable control during the 2007 tests. The highest rate (1.34 kg AI/ha) of acephate was successful in reducing tarnished plant bug numbers below the action threshold in 18 trials. The results of the field efficacy trials indicate that successful control of persistent tarnished plant bug infestations that exceed action thresholds can be accomplished with acephate rates of 0.82 to 1.34 kg AI/ha.

Laboratory bioassays and field efficacy trials indicate that resistance in Louisiana populations of tarnished plant bug is partially responsible for decreasing acephate performance. Tarnished plant bug infestations in recent years have been excessively high (2-5x > action thresholds) which is also influencing overall insecticide efficacy. With few alternatives to acephate, it is likely that complete control failures of tarnished plant bug will occur without the intervention of additional control strategies. Furthermore, subtle changes in this insect’s susceptibility to other classes of insecticides have been reported (G. L. Snodgrass, USDA-ARS, Stoneville, MS, unpublished).

To maintain the value of acephate in the current cotton IPM system for control of this pests, producers and pest managers should limit the frequency of acephate sprays in a season, rotate with other recommended insecticides, and co-apply acephate with other products that demonstrate different modes of action. In addition, acephate applications that target other pest species in adjacent crops could be exposing populations of tarnished plant bugs to selection pressure prior to their migration into cotton. Therefore, chemical control strategies for pest management across the farmscape should consider the impact of acephate selection on tarnished plant bugs and include the use of alternative chemistry whenever appropriate. Additional IPM strategies such as those described by Gore et al. (2007) must be implemented for the continued satisfactory control of tarnished plant bug and mitigation of insecticide resistance in this pest.
Expanding the insecticide susceptibility surveys in combination with coordinated field efficacy trials will provide valuable information to better understand the responses of tarnished plant bugs to various insecticides, and provide valuable information for adjusting insecticide recommendations. All recommended insecticides for tarnished plant bug should be included in these laboratory trials to provide efficacy estimates and determine the ranges of susceptibility to various products. Unfortunately, the agrochemical industry has few products with novel modes of action that have demonstrated efficacy against tarnished plant in the latter phases of development and registration. For the next two-three years, consistent and effective control of tarnished plant bug with acephate even at the highest labeled rates is questionable, if the changes in susceptibility to this product continue to occur. Furthermore, complete reliance on the alternative recommended products will likely not solve this problem either.


VITA

Josh Thomas Copes is the middle child of Dan Copes and Cathy Fulton. He was born in 1983 in Vicksburg, Mississippi. He graduated from Tallulah Academy in Tallulah, Louisiana in May, 2001. He received his Bachelor of Science degree May, 2007, in agricultural business from the University of Louisiana at Monroe. Dr. Joseph Pankey arranged a meeting with Dr. Ralph Bagwell who Josh began working for the summer following obtaining his bachelor’s degree. Josh was then accepted by the Graduate School at Louisiana State University, Baton Rouge, Louisiana, to the Department of Entomology under the supervision of Dr. Ralph Bagwell. Dr. Ralph Bagwell accepted a position with Bayer Crop Science in 2008, and Josh was graciously taken on by Dr. Rogers Leonard. Presently Josh is a candidate for a Master of Science degree in the Department of Entomology at Louisiana State University under the direction of Dr. Rogers Leonard.