2002

Bollworm (Lepidoptera: noctuidae) ecology on genetically engineered Bollgard and Bollgard II cottons

Jeffrey Gore
Louisiana State University and Agricultural and Mechanical College

BOLLWORM (LEPIDOPTERA: NOCTUIDAE) ECOLOGY ON GENETICALLY ENGINEERED BOLLGARD AND BOLLGARD II COTTONS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by
Jeffrey Gore
B.S. Auburn University, 1995
M.S. Louisiana State University, 1999
May 2002

ACKNOWLEDGEMENTS

I express sincere appreciation to the Louisiana State University Agricultural Center, the faculty and staff of the Department of Entomology, and the personnel at the Northeast Research Station. I would also like to thank my major advisor Dr. Roger Leonard, my minor professor Dr. James Griffin, as well as Drs. David Boethel, Michael Stout, and Thomas Moore, other members of my graduate committee. Sincere appreciation is expressed to former department head Dr. Frank Guillot and current interim head Dr. James Fuxa. I would also like to express a special thank you to Dr. Jerry B. Graves for his guidance and support and to Ms. Gabbie Church for assistance with statistical analyses. Assistance provided by fellow graduate students Don Cook, Richard Costello, Hunter Fife, Stacy Hall, Hank Jones, and Mellissa Willrich as well as numerous student workers is greatly appreciated. Without their help, this project would not have been possible. Finally, I would like to thank my parents and family for their support and understanding while I achieved this goal. Cotton Incorporated and the Louisiana Cotton Producers provided funding for this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The ecology of bollworms, *Helicoverpa zea* (Boddie), on Bollgard and Bollgard II cottons, *Gossypium hirsutum* L., was studied in field and laboratory experiments. Neonate bollworm larvae were placed on selected components of cotton squares and flowers from non-Bollgard, Bollgard, and Bollgard II cotton varieties. Larval survival was higher on flower anthers and square anthers than on other floral parts for each cultivar. Bollworm survival was lowest on all Bollgard II floral structures. To evaluate larval behavior on Bollgard cotton, first instar bollworms were placed on terminals of non-flowering and flowering cotton plants. Larvae were recovered lower on Bollgard cotton than on non-Bollgard cotton. Larvae remained near the terminals of non-Bollgard plants feeding on terminal foliage and squares. On Bollgard cotton, more larvae were recovered from white flowers and bolls. To quantify injury from bollworms on Bollgard and Bollgard II cottons, first instar larvae were placed in white flowers of non-Bollgard, Bollgard, and Bollgard II cottons. Bollworms damaged approximately two and three times more fruiting forms on non-Bollgard cotton than on Bollgard and Bollgard II cottons, respectively. To evaluate the influence of alternate hosts on bollworm sensitivity to non-Bollgard and Bollgard cottons, host colonies were established on field corn, *Zea mays* L.; grain sorghum, *Sorghum bicolor* (Moench); soybean, *Glycine max* (Merrill); non-Bollgard cotton; and meridic diet. Field corn and grain sorghum were better hosts for bollworms than cotton. Neonates from each colony were placed on terminal foliage from non-Bollgard and Bollgard cottons in petri dishes. Mortality of larvae from the cotton colony was higher than mortality from the soybean, corn, and meridic diet colonies on non-Bollgard cotton. Mortality from the corn colony was higher than from the soybean and grain sorghum colonies on Bollgard cotton. Differences in bollworm larval behavior and development on Bollgard cotton

suggest that changes are needed in the scouting protocols and management decisions for bollworms on Bollgard cotton compared to those on non-Bollgard cotton. Insecticide applications will be needed for bollworms on Bollgard cotton when populations persist over extended periods of time or when other boll feeding pests are present. Furthermore, alternate hosts may influence bollworm management with Bollgard cotton.

CHAPTER 1

INTRODUCTION

Bollworm Biology

The bollworm, *Helicoverpa zea* (Boddie), occurs throughout North and South America as well as the West Indies (Hardwick 1965). It is a member of the Noctuidae family in the order Lepidoptera (Borror et al. 1989). This species previously was included in the genus, *Heliothis*, but was removed based on characters of the genitalia (Hardwick 1965). It currently is included in the genus, *Helicoverpa* (Hardwick1970).

The bollworm is a multivoltine species with multiple generations occurring each year (King and Coleman 1989). Development from the egg to the adult stage requires approximately 30 days. The egg is circular shaped with distinct lateral ridges and creamy to white in color (Barber 1936). Neonate larvae are creamy-white with a distinct black head (Barber 1936). The head capsule of later instars is light brown. The thorax and abdomen colors vary from greens and yellows to shades of brown. It is distinguished from tobacco budworm larvae by the absence of a well-defined molar region on the interior mandible surface and the absence of spinules on chalazas one and two of abdominal segments one, two, and eight (Oliver and Chapin 1981). The larva develops through four or five stadia for a total of five to six instars (King and Coleman 1989). The last instar larva migrates from the plant, burrows into the soil, forms an escape tunnel for the adult stage, and pupates 2.5 to 25.4 cm below the soil surface (Anonymous 1967). The last generation overwinters as a diapausing pupa in the soil. The adult is approximately 1.9 cm long with a 3.8 cm wingspan. It is light brown in color with shades of olive green, orange, or brown (Oliver and Chapin 1981). The orbicular spot on the hind wing is faint with a dark spot in the center and the reniform spot is

more distinct than the orbicular spot (Oliver and Chapin 1981). In contrast, tobacco budworm moths have olive-green bands running across the fore wings (Oliver and Chapin 1981).

Bollworm moths emerge from overwintering pupae during late-March to mid-April (Barber 1936, Anonymous 1967). Adults are crepuscular with most feeding, mating, and oviposition activity occurring at dusk (Anonymous 1967). Each female may oviposit as many as 1,000 eggs over a 10 to 20 day period (Barber 1936). Pubescent leaf surfaces are preferred over glabrous leaf surfaces for oviposition by adult females (Hillhouse and Pitre 1976, Wilson et al. 1980, Farrar and Bradley 1985).

Bollworm Larval Behavior

The bollworm is a polyphagous species that feeds on a wide variety of cultivated and wild host plants. Larvae have been reported to feed on over 100 hosts (King and Coleman 1989). Sudbrink and Grant (1995) collected bollworm larvae from 34 plant species in 11 families. Non-cultivated leguminous plants such as *Trifolium incarnatum* L., *Trifolium* spp., *Geranium* spp., *Vicia* spp., and *Lupinus* spp., are the principle hosts of the first generation in April and May until other spring and summer plant species become available (Anonymous 1967). Field corn, *Zea mays* L.; cotton, *Gossypium hirsutum* L.; tomato, *Lycopersicon esculentum* Miller; sorghum, *Sorghum* spp.; soybeans, *Glycine max* (L.); wheat, *Triticum aestivum* L.; and tobacco, *Nicotiana tabaccum* L., are some of the more common agronomic crop hosts for bollworm (King and Coleman 1989). Silking stage field corn is a preferred host of the bollworm in the southern United States, but during mid- to late- summer, cotton is a more available host.

Bollworm larvae exhibit different feeding behaviors among the different host plants and in response to various biotic and abiotic factors. On corn, bollworms typically oviposit on fresh silks within 100 mm of the ear tip (Barber 1941). Barber (1941) investigated feeding behavior

immediately after larval eclosion from the egg on corn silks. Of 41 larvae observed, 19 attempted to feed on silks immediately after emergence, but only four were successful. The majority of initial feeding was done on the empty egg shell and on setae from the corn silks (Barber 1941). Barber (1941) reported that after initial feeding, larvae exhibited a period of rest. After the period of rest, larvae moved toward the tip of the ear. The time required for larvae to reach the developing seed ranged from eight to 98 minutes with a mean of 29.6 minutes (Barber 1941). Once at the corn ear, larvae feed on immature corn kernels near the tips of ears and complete larval development on one ear (Cohen et al. 1988).

On soybeans, bollworm oviposition is generally concentrated on foliage in the top 70 cm of plants (Hillhouse and Pitre 1976). Pitre and Hillhouse (1981) reported that small larvae prefer to feed on leaf buds and flowers. Larger larvae typically feed on foliage and developing seed pods near the middle third of soybean plants (Pitre and Hillhouse 1981). Pitre and Hillhouse (1981) reported that small first and second instar larvae on older leaves and stems often fell from plants because of the heavy pubescence associated with those structures. Also, larvae that fed in blooms and unfolded trifoliates had a higher survival rate past the second instar compared to those feeding on older foliage.

Similar studies of bollworm behavior have been conducted on crimson clover (Ellsbury et al. 1989); snap beans, *Phaseolus vulgaris* L. (Naeem et al. 1992); and tomato (Burkett et al. 1983, Juvik et al. 1994). On tomato, neonates initially feed on the structure near the site of oviposition and usually remain within one node of that site for the first week of development (Burkett et al. 1983). Larvae placed in the terminals of tomato plants moved an average of 1.3 nodes down the plants within seven days under field conditions (Burkett et al. 1983). In a similar study, plant allelochemicals (acylglucoses) from wild tomato, *Lycopersicon pennelli*

(Correll), altered feeding behavior of *H. zea* when applied as a foliar spray to cultivated tomato (Juvik et al. 1994). Larvae avoided acylglucose treated tissue, indicating increased mobility compared to non-treated tissue (Juvik et al. 1994).

Behavioral adaptations to host allelochemicals have been documented with several insects (Bernays and Chapman 1994). *Locusta migratoria* (L.) avoidance of wheat-flour wafers increased as the concentration of the feeding deterrent tomatine increased (Bernays and Chapman 1978). Berenbaum and Zangerl (1992) evaluated behavioral responses to host furanocoumarins in the parsnip webworm, *Depressaria pastinacella* (Duponchel). Sixth instar parsnip webworm larvae avoided diets containing high levels of different furanocoumarins. Similar research has shown that bollworm and tobacco budworm avoid *B. thuringiensis* insecticidal proteins. Greenplate et al. (1998) observed feeding preferences for a non-treated laboratory diet by bollworm larvae. Benedict et al. (1992, 1993) and Parker (1997) observed differences in tobacco budworm behavior on conventional and Bollgard cotton. Increased movement and dispersal were observed with this insect on Bollgard cotton lines compared with conventional cotton.

Bollworm Feeding and Movement on Cotton Plants

The majority of bollworm eggs are generally deposited in the top one-third of the cotton plant canopy (Wilson et al. 1980). However, Mistric (1964) found eggs scattered over all areas of cotton plants and noted that large numbers could be found on the bracts of squares and bolls. Bollworm larvae have a preference for specific feeding sites on cotton plants. The majority of larval feeding takes place in the upper portions of the plant canopy. Young larvae feed primarily on the anthers of developing flower buds (squares) that are < 2 mm in diameter (Reese et al. 1981). Farrar and Bradley (1985) found flowers (white or red) and small bolls

with dried flower corollas to be preferred by small and large larvae with bolls being more preferred by larger larvae. Fye (1972) found that 78 to 100% of damaged fruit at any given time could be found in the upper 0.6 m of the plant. Other studies have shown that large larvae (> third instar) feed lower in the plant canopy on older bolls (Farrar and Bradley 1985). Wilson and Gutierrez (1980) found that second instar bollworms migrated down the plant feeding on older fruiting forms as larval development progressed. From mid-July through August, bollworm larvae prefer older squares compared to younger squares (Slosser et al. 1978). In addition, Slosser et al. (1978) found that bolls are susceptible to bollworm attack for at least five weeks (35 d) after anthesis. Bagwell (1994) determined that third instar bollworm larvae were not capable of penetrating the carpel wall of bolls that accumulated at least 350 heat units beyond anthesis. Gore et al. (2000) found that bollworm injury to conventional cotton bolls caused abscission until bolls accumulated 281 heat units. Weights of conventional cotton bolls infested with first instar bollworm larvae were significantly lower compared with non-infested bolls until 426.5 heat units had been accumulated (Gore et al. 2000).

Damage from the bollworm involves direct entry into the fruiting form, which violates the integrity of the exterior wall. Larvae generally migrate from one fruiting structure to another, but have been reported to feed on the same structure for more than one instar (Wilson and Gutierrez 1980). Studies in Arkansas indicated an average of 3.8 damaged squares and 2.2 damaged bolls per bollworm larva (Anonymous 1967). Similarly, Adkisson et al. (1964b) reported 3.8 and 5.7 damaged squares per larva during 1961 and 1962, respectively, in Texas.

Low population densities of bollworm larvae are capable of causing significant levels of damage in cotton. Adkisson et al. (1964a) reported that eight to ten bollworm larvae per 100 plant terminals were capable of causing significant yield losses, and that control measures are

recommended when four to five young larvae or eggs per 100 plant terminals are present. In Louisiana, insecticidal control is usually recommended when squares are at least one third grown and five live larvae per 100 plants plus eggs are present (Bagwell et al. 2000).

Bollworm Pest Status

The bollworm and the tobacco budworm comprise the heliothine pest complex of cotton in the United States. During 1998, Louisiana cotton producers averaged 3.5 insecticide applications to control these pests at a average cost of over thirty-nine dollars per acre (Williams 1999). Injury by heliothine larvae resulted in yield losses of more than fifteen thousand bales of cotton in 1998 (Williams 1999). During 1999, an average of 1.8 and 0.8 insecticide applications per acre were necessary on conventional cotton and Bollgard cotton, respectively, to control heliothines (Williams 2000).

Cotton Host Plant Resistance

Cotton plants produce numerous secondary plant chemicals that affect insect development and survival (Hedin et al. 1983). Perhaps the most widely recognized and studied of these chemicals is the sesquiterpenoid, gossypol (Hanny 1980, Hedin et al. 1983, Stipanovic 1983). The presence of gossypol in diet can reduce the weight and delay development of bollworm larvae (Lukefahr et al. 1966, Bottger and Patana 1966, Lukefahr and Houghtaling 1969, Wilson 1971, Shaver et al. 1977). Pigment glands are distributed throughout all above ground portions of cotton plants (Lukefahr et al. 1966). Gossypol is the primary constituent of these glands, and its concentration in cotton foliage and seed is directly related to gland density (Lukefahr et al. 1966). Bollworm and tobacco budworm larvae have demonstrated the ability to avoid gossypol. Parrott et al. (1983) showed that tobacco budworm neonates avoid pigment glands when feeding on terminal foliage. Similarly, bollworm and tobacco budworm larvae avoid

feeding on gossypol-rich ovaries of glanded cottons and selected to feed on anthers (Lee 1976). McMichael (1959, 1960) developed the first glandless cottons to reduce the amount of gossypol present in cotton meal and minimize discoloration in cottonseed oil. However, several investigators found that glandless cottons were more susceptible to selected insect pests such as boll weevil, *Anthonomus grandis grandis* Boheman, than glanded cottons (Lukefahr et al. 1966, Oliver et al. 1970, Parrott et al. 1978).

In addition to gossypol, several other secondary plant chemicals are present in cotton. These compounds include tannins, phenolics, flavonoids, catechin, anthocyanin, and many others (White et al. 1982). Chan et al. (1978) determined ED_{50} (effective dose that reduced the weights of 50% of the population) values for gossypol, hemigossypolone, $heliocide_1$, $heliocide_2$, methyl malvalate, methyl sterculate, condensed tannin, D-catechin, quercitrin, isoquercitrin, gossypin, rutin, and kaempferol against bollworm, tobacco budworm, and pink bollworm. ED_{50} values (as percent of diet) ranged from <0.05% to >0.40% among the different compounds and insect species. Bollworm ED_{50}'s were lowest for gossypol (0.074%) and hemigossypolone (<0.07%) (Chan et al. 1978). Guerra et al. (1990) found that the phenolic compounds trans-cinnamic acid, catechol, and catechin incorporated into a meridic diet for bollworm increased larval mortality and time required for the larvae to reach pupation and reduced larval developmental rates. Selection of cultivars with high levels of secondary plant chemicals can be an important component of cotton integrated pest management (IPM), but such cultivars are not likely to maintain insect populations below established action thresholds under field situations. Also, cultivars resistant to bollworms and tobacco budworms may suppress other pests present in cotton. Therefore, other components such as insecticides remain important in IPM when insect pest pressure is high.

Chemical Control of Heliothines

In many cropping systems, insecticides are an integral part of IPM strategies. Insecticides usually are utilized only when other management strategies (biological control, host plant resistance, etc.) fail to maintain pest populations below an economic threshold (Stern et al. 1959, Pedigo et al. 1986, Graves et al. 1999). However, in systems such as cotton, with multiple key pests, insecticides remain the primary component of IPM (Graves et al. 1999). In cotton, bollworm and tobacco budworm control relies heavily on commercial insecticides. However, widespread resistance of these insects to several classes of insecticides makes acceptable control difficult at best. Bollworm populations were first reported resistant to chlorinated hydrocarbons in Louisiana by Graves et al. (1963). Organophosphorous and carbamate insecticides ultimately replaced chlorinated hydrocarbons due to this resistance. However, resistance to these compounds was reported within a few years as a result of widespread indiscriminate use (Wolfenbarger and McGarr 1970, Harris 1972, Wolfenbarger et al. 1973). Pyrethroid insecticides were introduced for field use in the 1970's as a result of *Heliothis* control failures with other insecticides. However, resistance to pyrethroid insecticides was first reported in populations of tobacco budworm larvae from West Texas during 1985 (Plapp and Campanhola 1986, Luttrell et al. 1987), and is now widespread throughout the United States. Currently, bollworm populations are readily controlled with pyrethroids in Louisiana. Bollworm resistance to pyrethroids, however, has been reported in other areas of the cotton-belt (Brown et al. 1997, Walker et al. 1998).

There are three mechanisms associated with insecticide resistance (Georghiou 1972, Sparks et al. 1989). Insecticide resistance can occur as a result of biochemical mechanisms (enhanced detoxification, target-site insensitivity), physiological mechanisms (altered

penetration, transport, storage, or excretion) and behavioral adaptations. Mechanisms for insecticide resistance mentioned previously in the bollworm and tobacco budworm are probably the result of physiological or biochemical mechanisms. The effects of behavioral adaptations on insecticide resistance in bollworm are not well defined.

The lack of information about behavioral resistance is primarily due to the difficulty in quantifying behavioral adaptations. Standard laboratory procedures (i.e. topical bioassay) do not reflect behavioral changes in a species, and novel approaches are usually required (Pluthero and Singh 1984, Sparks et al. 1989). In addition, baseline data may not be available to measure changes in the behavior of a population over time. Behavioral resistance has been categorized as having either stimulus-dependent mechanisms or stimulus-independent mechanisms (Lockwood et al. 1984, Sparks et al. 1989). Stimulus-dependent mechanisms require contact of the insect with the toxicant and include increased repellency or irritancy. Stimulus-independent mechanisms do not require contact with the toxicant, and may include exophily (inhabiting areas other than those that are normally treated). Both of these mechanisms act to reduce exposure to the toxicant or toxic residues, thus increasing survival. Therefore, behavioral mechanisms may be an important aspect of insecticide resistance in heliothine spp. and should be more closely evaluated.

Despite the mechanism involved, the widespread occurrence of insecticide resistance in tobacco budworms and localized control failures of bollworms is renewing interest in other IPM strategies. However, before economic management of these insects can be achieved, novel strategies need to be developed that provide little disruption to the overall agro-ecosystem. One such strategy has been the advent of recombinant DNA technology and the

introduction of genetically engineered (Bollgard®) cottons that resist bollworms and tobacco budworms.

Bollgard Cotton

Recent advances in crop improvement have allowed the introduction of recombinant DNA from *B. thuringiensis* Berliner var. *kurstaki*, which codes for the production of δ-endotoxin proteins into cotton and other crop plants (Umbeck et al. 1987, Gasser and Fraley 1989, Perlak et al. 1990). The CryIA(c) protein produced by Bollgard cotton has insecticidal activity against the larvae of selected lepidopteran pests (Gowron-Burke and Baum 1991). When ingested by susceptible insects, the endotoxin disturbs the osmotic balance of the epithelial cells in the midgut causing rapid paralysis of the gut. Cessation of feeding usually occurs within minutes and death usually occurs within two to three days after exposure (Dulmage et al. 1978, Gowron-Burke and Baum 1991). The CryIA(c) protein produced by commercially available Bollgard varieties provides acceptable control of the tobacco budworm and pink bollworm (MacIntosh et al. 1990). In addition, acceptable control of bollworms can be expected from Bollgard cottons during years of light to moderate pressure. The CryIA(c) protein provides little, if any, control of other lepidopteran insects. Beet armyworms, *Spodoptera exigua* (Hübner), fall armyworms, *S. frugiperda* (J. E. Smith), and soybean loopers, *Pseuoplusia includens* (Walker), are not readily controlled by this protein. Less than adequate control of the pest spectrum, coupled with concerns about resistance management, has prompted scientists with Monsanto Co. to develop other genetically engineered cottons that contain two separate crystalline proteins (Greenplate et al. 2000a). This new technology has been termed Bollgard II®. Bollgard II cotton was developed by incorporating the CryIIA(b) protein from *B. thuringiensis* into commercially available Bollgard (CryIAc) cotton varieties (Greenplate et al.

2000a, b). The second protein increases the insecticidal activity of Bollgard cotton against target pests and broadens the spectrum of total pests controlled. Bollworms are less susceptible to CryII proteins compared to CryI proteins (Sims 1997). Bollgard II cotton contains CryIIA(b) in addition to CryIA(c); therefore, overall protein expression is much higher in Bollgard II compared to Bollgard (Voth et al. 2001, Penn et al. 2001). Greenplate et al. (2000b) observed a 4-fold increase in the total amount of insecticidal proteins in Bollgard II cotton compared to that in Bollgard cotton.

Bollgard cotton cultivars have minimal activity against beet armyworms, fall armyworms, and soybean loopers and in some situations provides less than adequate control of bollworms. The CryIIA(b) protein present in Bollgard II cotton cultivars improves control of these insects compared to Bollgard (Stewart and Knighten 2000, Stewart et al. 2001). In a laboratory study, no bollworm larvae survived to pupation on Bollgard II compared to seven percent surviving to pupation on Bollgard cotton (Stewart et al. 2001). In field and laboratory studies, Jackson et al. (2000, 2001) observed a reduction in the number of bollworm damaged terminals, squares, and bolls on Bollgard II cotton lines compared to Bollgard and non-Bollgard cotton cultivars. In a field study in South Carolina, Bollgard II cotton lines had lower densities of bollworms and soybean loopers compared to Bollgard cotton cultivars (Ridge et al. 2000). These initial data support the use of Bollgard II cotton lines in areas where multiple lepidopteran pest species reach damaging levels during most years. However, more research is necessary to determine if bollworm control in Bollgard II will be significantly increased over Bollgard cotton. Also, Bollgard II cotton may be better adapted for current high dose/refugia resistance management strategies against bollworms and other lepidopterous pests.

Insect Resistance to *Bacillus thuringiensis* and Resistance Management

Bollgard cotton provides a valuable tool for producers throughout the United States. However, because of the genetic plasticity and adaptability of insect pests to adverse conditions, they have the ability to develop resistance to the protein produced by Bollgard cotton plants. Several cotton insect pests have been artificially selected in the laboratory to tolerate higher doses of purified Cry proteins than laboratory colonies (Stone et al. 1989; Gould et al. 1992, 1995; Moar et al. 1995; Bartlett et al. 1997). Following selection in the laboratory, Burd et al. (2000) observed 46.0% survival of G_{13} larvae at a 40 μg/ml dose of purified CryIA(c) toxin in meridic diet compared to 56.2% survival of G_0 individuals at 0.1 μg/ml.

The widespread planting of *B. thuringiensis* cotton increases the risk of resistance developing in all lepidopteran pests of cotton. The protein is produced throughout the plant during the entire season. Therefore, cotton insect pests are exposed to the protein for more than one generation each year. This constant selection pressure increases the frequency of resistance alleles in the population and could lead to high levels of tolerance in a relatively short amount of time (Gould 1998). A similar situation occurred with widespread plantings of wheat cultivars resistant to the Hessian fly, *Mayetiola destructor* (Say). Insect resistant wheat cultivars are similar to *B. thuringiensis* cotton in that they use antibiosis (Painter 1951) as a resistance mechanism against Hessian fly larvae (Foster et al. 1991). Resistance in Hessian fly populations developed within a few years after widespread commercialization of the cultivars. Resistance of the greenbug, *Schizaphis graminum* (Rondani), is another example of an insect pest becoming resistant to an antibiotic host plant (Wood 1971). When a resistant sorghum cultivar was planted over large acreages in the United States, the greenbug rapidly developed resistance to the antibiotic trait in the cultivar.

Field resistance to formulated *B. thuringiensis* was first documented in the diamondback moth, *Plutella xylostella* (L.), in Hawaii, the continental United States, and Asia (Tabashnik 1994). Because of the threat of insect pests developing resistance to *B. thuringiensis* cotton, government agencies, industry, farmers, and academic researchers have adopted resistance management plans (Gould and Tabashnik 1998). These plans are based on a high dose/refuge strategy derived from population genetics theory. The purpose of the refuge is to limit the exposure of a specified proportion of the population to the selection pressure (the protein toxin) (Gould 1998). A high dose with this strategy is defined as that which will kill homozygous susceptible as well as heterozygous individuals within the population (Gould 1998). The initial resistance management (IRM) plan approved by the environmental protection agency (EPA) offered cotton producers two refuge options. Option one required that for every 100 acres of genetically transformed Bollgard cotton planted; 25 acres of conventional cotton should be planted. The 25 acres of conventional cotton in option one could be treated with foliar insecticides (other than foliar *B. thuringiensis* products) that are efficacious against lepidopteran pests. Option two required that for every 100 acres of Bollgard cotton planted, four acres of conventional cotton should be planted. In option two, the four-acre refuge could not be treated with any insecticide that has activity against lepidopteran pests. Recently, concerns have been expressed about the effectiveness of these refuge options. Also, producers are concerned about significant yield losses in the non-treated refuges. Consequently, EPA revised the refuge options prior to the 2001 growing season (Matten 2001). These new plans focus on refuge size, structure, and deployment. The new requirements include a 95:5 external unsprayed refuge, 95:5 embedded sprayed refuge, or an 80:20 external sprayed refuge. With external options, the refuge must be planted within a specified distance to the Bollgard cotton

(one half mile for 95:5 and one mile for 80:20) (Matten 2001, Mullins 2001). With the 95:5 embedded option, the refuge can be treated with lepidopteran active insecticides; however, the Bollgard cotton must be treated at the same time with the same chemicals (Matten 2001, Mullins 2001). In theory, these strategies are sound in that susceptible moths emerging from the refuge areas will mate with moths emerging from the Bollgard cotton. This, in turn, should effectively dilute the resistance alleles in the population and delay the onset of widespread resistance. However, some biological factors may interfere with this strategy. For instance, emergence of surviving adults may not be synchronized between Bollgard cotton and conventional cotton (Gould 1998). Delays in the developmental time of fall armyworm (Adamczyk et al. 1998), and bollworm (Lambert et al. 1998) larvae fed Bollgard cotton have been observed compared with those fed conventional cotton. If emergence of moths from Bollgard cotton is significantly later than that on conventional cotton, moths carrying resistance alleles may be unavailable to mate with moths carrying susceptible alleles, thereby increasing the frequency of resistance alleles in the population.

The current resistance management plan was designed specifically for tobacco budworm and pink bollworm. The plan may not be suitable for preventing resistance in the bollworm or other cotton pests. For example, if the 20% refuge is treated with a pyrethroid, sufficient numbers of tobacco budworm larvae that carry the *B. thuringiensis* susceptible allele will survive to dilute resistant individuals emerging from the Bollgard cotton. However, in most areas of the United States, bollworm larvae are more susceptible to pyrethroids than tobacco budworm larvae and nearly all individuals in the refuge area will be killed. This would decrease the number of *B. thuringiensis* susceptible bollworms emerging from refuge areas that would mate with *B. thuringiensis* resistant individuals. Also, bollworm larvae are inherently

more tolerant to the CryIA(c) protein than tobacco budworm larvae and higher survival is expected on Bollgard cotton (MacIntosh et al. 1990, Luttrell et al. 1999). Therefore, heterozygotes may not be controlled by the levels of CryIA(c) in current Bollgard cultivars.

Monitoring for Bollworm Resistance to *B. thuringiensis*

Scientists at the USDA-ARS facility in Stoneville, MS initiated bollworm resistance monitoring to *B. thuringiensis* CryIA(c) protein (Herzog et al. 1997). During 1996, field populations of bollworm and tobacco budworm from four states (Mississippi, Arkansas, Texas, and Oklahoma) were monitored for tolerance to the CryIA(c) protein in Bollgard cotton. Results from a spray chamber bioassay using MVP II (CryIA(c)) (Dow Agrosciences, Indianapolis, IN) showed no detectable changes in bollworm susceptibility to CryIA(c) (Herzog et al. 1997). Lambert et al. (1998) and Moar et al. (1998) determined that field populations of bollworm collected during the 1997 growing season did not have high frequencies of individuals adapted to CryIA(c). In contrast, USDA-ARS monitoring indicated that bollworms were more tolerant to CryIA(c) during 1998 in relation to 1996 (Summerford et al. 1999). Bollworm mortality averaged 59%, 52%, and 43% during 1996, 1997, and 1998, respectively, from colonies collected in Washington Co., MS. Also, tobacco budworm tolerance was significantly higher in 1998 compared to 1997. Monitoring for bollworm and tobacco budworm resistance to *B. thuringiensis* is an important factor that will help ensure the longevity of genetically modified cotton cultivars. Early detection of low levels of resistance in these insects will allow pest managers to alter resistance management plans before resistance levels become too high or control failures occur.

Effects of *B. thuringiensis* Cotton on Bollworm Larvae

Bollworm larvae are susceptible to the CryIA(c) endotoxin produced by Bollgard cotton plants (MacIntosh et al. 1990; Benedict et al. 1993, 1996; Halcomb et al. 1996). However, cotton lines that produce the endotoxin are not entirely resistant to bollworm injury and significant damage may occur in some instances (Ring et al. 1993, Mahaffey et al. 1994). Leonard et al. (1997) found no significant differences in mortality of third instar bollworm larvae feeding on Bollgard cotton squares compared to that for conventional cotton lines in a laboratory experiment. In a greenhouse study, Benedict et al. (1993) observed that when bollworm eggs were infested on individual Bollgard plants, 1.6 to 18.2% of squares and 6.0 to 29.5% of bolls were damaged. Mahaffey et al. (1995) observed > 30% damage to Bollgard cotton bolls by bollworm larvae and significant yield losses occurred in unsprayed Bollgard cotton when compared with sprayed Bollgard cotton. All stages of bollworm larvae have been collected from Bollgard cotton plants. This further indicates that they are capable of completing larval development on Bollgard cotton (Mahaffey et al. 1995). Since the introduction of Bollgard cotton in 1996, bollworm infestations have required supplemental applications of foliar insecticides in many fields across the Southeast and mid-South to prevent economic losses (Bacheler and Mott 1997; Layton et al. 1997, 1998; Leonard et al. 1997, 1998; Roof and DuRant 1997; Smith 1997, 1998). White flowers of Bollgard cotton appear to be the most susceptible structures to bollworms (Greenplate et al. 1998). The CryIA(c) toxin was observed to be expressed at lower levels in the pollen of white flowers. Bollworm larvae appear to be able to avoid *B. thuringiensis* toxins by showing a preference for diet without the toxin. Greenplate et al. (1998) utilized a diet bioassay with CryIA(c) and CryIA(b) endotoxins to illustrate the ability of bollworm larvae to avoid the toxins through selection of non-treated

diet. In greenhouse experiments, Benedict et al. (1992, 1993) and Parker (1997) observed increased movement of tobacco budworm larvae on Bollgard cotton plants compared with conventional plants. Larvae were observed spinning-down and crawling from the terminal of Bollgard plants more readily than on conventional plants. Avoidance of plant tissues containing *B. thuringiensis* proteins also may occur with bollworm larvae, increasing their occurrence in white flowers. In addition, lower expression of the protein in flower anthers and pollen may provide a mechanism allowing damaging numbers of bollworm larvae to survive in Bollgard cotton fields.

Currently, little information is available concerning the levels of fruiting form injury that can be expected from bollworm larvae feeding on Bollgard® reproductive structures, or effects on bollworm scouting and management. Also, data necessary for estimating treatment threshold levels is lacking. The focus of this dissertation is to present data that will contribute to our knowledge about bollworm population dynamics in relation to genetically engineered (Bollgard) cotton. The following objectives were proposed:

Objectives

I. To quantify bollworm survival on selected components of conventional and Bollgard® cotton reproductive structures.

II. To evaluate bollworm intra-plant movement and behavior in conventional and Bollgard® cotton.

III. To determine the injury potential of bollworm larvae feeding on white flowers of Bollgard® cotton.

IV. To determine host plant effects on susceptibility of subsequent generations of bollworm larvae to Bollgard® cotton.

References Cited

Adamczyk, J. J., Jr., V. J. Mascarenhas, G. E. Church, B. R. Leonard, and J. B. Graves. 1998. Susceptibility of conventional and transgenic cotton bolls expressing the *Bacillus thuringiensis* CryIA(c) δ-endotoxin to fall armyworm (Lepidoptera: Noctuidae) and beet armyworm (Lepidoptera: Noctuidae) injury. J. Agric. Entomol. 15: 163-171.

Adkisson, P. L., C. F. Bailey, and R. L. Hanna. 1964a. Effect of bollworm, *Heliothis zea*, on yield and quality of cotton. J. Econ. Entomol. 57: 448-450.

Adkisson, P. L., C. F. Bailey, and R. L. Hanna. 1964b. Estimates of the number of *Heliothis* larvae per acre in cotton and their relation to the fruiting cycle and yield of the host. J. Econ. Entomol. 57: 657-663.

Anonymous. 1967. The bollworm-tobacco budworm problem in Arkansas and Louisiana. Louisiana Agric. Expt. Sta. Bull. 720. 66 pp.

Bacheler, J. S., and D. W. Mott. 1997. Efficacy of grower-managed Bt cotton in North Carolina, pp. 858-861. *In* P. Dugger and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Bagwell, R. D. 1994. Monitoring the cotton plant for insecticide effects and late-season insecticide use termination. Ph.D. dissertation, University of Arkansas, Fayetteville.

Bagwell, R. D., J. W. Barnett, B. R. Leonard, E. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2000. Cotton insect control 2000. Louisiana Cooperative Extension Service, Pub. 1083. 7 pp.

Barber, G. W. 1936. The corn ear worm (sic) in southeastern Georgia. Georgia Expt. Sta. Tech. Bull. 192. 18 pp.

1941. Observations on the egg and newly hatched larva of the corn ear worm (sic) on corn silk. J. Econ. Entomol. 34: 451-456.

Bartlett, A. C., T. J. Dennehy, and L. Antilla. 1997. An evaluation of resistance to Bt toxins in native populations of the pink bollworm, pp. 885-888. *In* P. Dugger and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Benedict, J. H., D. W. Altman, P. F. Umbeck, and D. R. Ring. 1992. Behavior, growth, survival, and plant injury by *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) on transgenic Bt cottons. J. Econ. Entomol. 85: 589-593.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone, and S. R. Sims. 1993. Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with

Heliothis virescens and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Benedict, J. H., E. S. Sachs, D. W. Altman, W. R. Deaton, R. J. Kohel, D. R. Ring, and S. A. Berberich. 1996. Field performance of cottons expressing transgenic CryIA insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. 89: 230-238.

Berenbaum, M. R. and A. R. Zangerl. 1992. Genetics of physiological and behavioral resistance to host furanocoumarins in the parsnip webworm. Evolution 46: 1373-1384.

Bernays, E. A., and R. F. Chapman. 1978. Plant chemistry and acridoid feeding behaviour, pp. 91-141. *In* J. B. Harborne (ed.), Biochemical aspects of plant and animal coevolution. Academic Press, London.

1994. Host-plant selection by phytophagous insects. Chapman and Hall, New York.

Bottger, G. T., and R. Patana. 1966. Growth, development, and survival of certain Lepidoptera fed gossypol in the diet. J. Econ. Entomol. 59: 1166-1168.

Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1989. An introduction to the study of insects, sixth edition. Harcourt Brace College Publishers, New York.

Brown, T. M., P. K. Bryson, D. S. Brickle, J. T. Walker, and M. J. Sullivan. 1997. Pyrethroid-resistant *Helicoverpa zea* in cotton in South Carolina. Resistant Pest Manag. News Letter 9: 26-27.

Burd, A. D., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2000. Resistance of bollworm, *Helicoverpa zea*, to CryIA(c) toxin, pp. 923-926. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Burkett, G. R., J. C. Schneider, And F. M. Davis. 1983. Behavior of the tomato fruitworm, *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae), on tomato. Environ. Entomol. 12: 905-910.

Chan, B. G., A. C. Waiss, Jr., R. G. Binder, and C. A. Elliger. 1978. Inhibition of lepidopterous larval growth by cotton constituents. Entomol. Exp. Appl. 24: 94-100.

Cohen, R. W., G. P. Waldbauer, and S. Friedman. 1988. Natural diets and self-selection: *Heliothis zea* larvae and maize. Entomol. Exp. Appl. 46: 161-171.

Dulmage, H. T., H. M. Graham, and E. Martinez. 1978. Interactions between the tobacco budworm, *Heliothis virescens*, and the δ-endotoxin produced by the HD-1 isolate of *Bacillus thuringiensis* var. *kurstaki*: relationship between length of exposure to the toxin and survival. J. Invertebrate Pathol. 32: 40-50.

Ellsbury, M. M., G. A. Burkett, and F. M. Davis. 1989. Development and feeding behavior of *Heliothis zea* (Lepidoptera: Noctuidae) on leaves and flowers of crimson clover. Environ. Entomol. 18: 323-327.

Farrar, R. R., and J. R. Bradley, Jr. 1985. Within-plant distribution of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs and larvae on cotton in North Carolina. Environ. Entomol. 14: 205-209.

Foster, J. E., H. W. Ohm, F. L. Patterson, and P. L. Taylor. 1991. Effectiveness of deploying single gene resistances in wheat for controlling damage by the Hessian fly (Diptera: Cecidomyiidae). Environ. Entomol. 20: 964-969.

Fye, R. E. 1972. Preliminary investigation of vertical distributions of fruiting forms and insects on cotton plants. J. Econ. Entomol. 65: 1410-1414.

Gasser, C. S., and R. T. Fraley. 1989. Genetically engineering plants for crop improvement. Science 244: 1293-1299.

Georghiou, G. P. 1972. The evolution of resistance to pesticides. Ann. Rev. Ecol. Syst. 3: 133-168.

Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. J. Econ. Entomol. 93: 690-696.

Gould, F. A., A. Martinez-Ramirez, A. Anderson, J. Ferre, F. J. Silva, and W. J. Moar. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. Proc. Natl. Acad. Sci. 89: 7986-7990.

Gould, F. A., A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545-1559.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: Integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701-726.

Gould, F., and B. Tabashnik. 1998. Bt-cotton resistance management, pp. 65-105. *In* M. Mellon and J. Rissler [eds.], Now or Never: Serious New Plans to Save a Natural Pest Control. Union of Concerned Scientists, Cambridge, MA.

Gowron-Burke, C., and J. A. Baum. 1991. Genetic manipulation of *Bacillus thuringiensis* insecticidal protein genes in bacteria. Genetic Engineering 13: 237-261.

Graves, J. B., J. S. Roussel, and J. R. Phillips. 1963. Resistance to some chlorinated hydrocarbon insecticides in the bollworm, *Heliothis zea*. J. Econ. Entomol. 56: 442-444.

Graves, J. B., B. R. Leonard, and J. A. Ottea. 1999. Chemical approaches to managing Arthropod pests, pp. 449-486. *In* J. R. Ruberson [ed.], Handbook of Pest Management. Marcel Dekker, Inc. New York, NY.

Greenplate, J. T., G. P. Head, S. R. Penn, and V. T. Kabuye. 1998. Factors potentially influencing the survival of *Helicoverpa zea* on Bollgard® cotton, pp. 1030-1033. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J. T., S. R. Penn, J. W. Mullins, and M. Oppenhuizen. 2000a. Seasonal CryIAc levels in DP50B: The "Bollgard® basis" for Bollgard II, pp. 1039-1040. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J. T., S. R. Penn, Z. Shappley, M. Oppenhuizen, J. Mann, B. Reich, and J. Osborn. 2000b. Bollgard II efficacy: Quantification of lepidopteran activity in a 2-gene product, pp. 1041-1043. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Guerra, D. J., J. T. Cothren, and J. R. Phillips. 1990. Influence of selected phenolic compounds on development of bollworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 83: 2115-2118.

Halcomb, J. L., J. H. Benedict, B. Cook, and D. R. Ring. 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). Environ. Entomol. 25: 250-255.

Hanny, B. W. 1980. Gossypol, flavonoid, and condensed tannin content of cream and yellow anthers of five cotton (*Gossypium hirsutum* L.) cultivars. J. Agric. Food Chem. 28: 504-506.

Hardwick, D. F. 1965. The corn earworm complex. Memoirs Entomol. Soc. Can. 40: 1-248.

1970. A generic revision of the North American Heliothidinae (Lepidoptera: Noctuidae). Memoirs Entomol. Soc. Can. 73: 5-56.

Harris, F. A. 1972. Resistance to methyl parathion and toxaphene-DDT in the bollworm and tobacco budworm from cotton in Mississippi. J. Econ. Entomol. 65: 1193-1194.

Hedin, P. A., J. N. Jenkins, D. H. Collum, W. H. White, and W. L. Parrott. 1983. Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis*

virescens F., pp. 347-365. *In* P. A. Hedin, [ed.], Plant resistance to insects, ACS symposium series, American Chemical Society, Washington, D. C.

Herzog, D. D., D. A. Streett, L. C. Adams, and G. W. Elzen. 1997. Resistance monitoring in Bt cotton: first year observations, pp. 880-882. *In* P. Dugger and D. A. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Hillhouse, T. L., and H. N. Pitre. 1976. Oviposition by *Heliothis* on soybeans and cotton. J. Econ. Entomol. 69: 144-146.

Jackson, R. E., J. R. Bradley, Jr., A. D. Burd, and J. W. Van Duyn. 2000. Field and greenhouse performance of bollworm on Bollgard II cotton genotypes, pp. 1048-1051. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Jackson, R. E., J. R. Bradley, Jr., J. W. Van Duyn, and A. D. Burd. 2001. Efficacy of Bollgard and Bollgard II cottons against bollworm, *Helicoverpa zea* (Boddie) in field and greenhouse studies, pp. 815-819. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Juvik, J. A., J. A. Shapiro, T. E. Young, and M. A. Mutschler. 1994. Acylglucoses from wild tomatoes alter behavior and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Econ. Entomol. 87: 482-492.

King, E. G., and R. J. Coleman. 1989. Potential for biological control of *Heliothis* species. Ann. Rev. Entomol. 34: 53-76.

Lambert, A. L., J. R. Bradley, Jr., F. Gould, and J. W. Van Duyn. 1998. Bollworm (*Helicoverpa zea*): adaptation to Bt toxin?, pp. 1033-1037. *In* P. Dugger and D. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Layton, M. B., M. R. Williams, and S. Stewart. 1997. Bt cotton in Mississippi: The first year, pp. 861-863. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Layton, M. B., S. D. Stewart, and M. R. Williams. 1998. Performance of Bt cotton in Mississippi, 1997, pp. 970-973. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Lee, J. A. 1976. Reaction of *Heliothis* larvae to high glandulosity cottons of an improved type, p. 90. *In* Proc. 1976 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B. R., H. Fife, K. Torrey, J. B. Graves, and J. Holloway. 1997. *Helicoverpa/Heliothis* management in NuCOTN and conventional cotton cultivars in

Louisiana, pp. 863-867. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B. R., J. H. Fife, K. Torrey, E. Burris, and J. B. Graves. 1998. Evaluation of transgenic Bt cotton lines against Heliothines in Northeast Louisiana, pp. 967-970. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Lockwood, J. A., T. C. Sparks, and R. N. Story. 1984. Evolution of insect resistance to insecticides: A reevaluation of the roles of physiology and behavior. Bull. Entomol. Soc. Am. 30(4): 41-51.

Lukefahr, M. J., and J. E. Houghtaling. 1969. Resistance of cotton strains with high gossypol content to *Heliothis* spp. J. Econ. Entomol. 62: 588-591.

Lukefahr, M. J., L. W. Noble, and J. E. Houghtaling. 1966. Growth and infestation of bollworms and other insects on glanded and glandless strains of cotton. J. Econ. Entomol. 59: 817-820.

Luttrell, R., T. Roush, A. Ali, J. S. Mink, M. R. Reid, and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985-989.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebrate Path. 56: 258-266.

Mahaffey, J. S., J. S. Bacheler, J. R. Bradley. Jr., and J. W. Van Duyn. 1994. Performance of Monsanto's transgenic *B. t.* cotton against high populations of Lepidopterous pests in North Carolina, pp. 1061-1063. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1994 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Mahaffey, J. S., J. R. Bradley, Jr., and J. W. Van Duyn. 1995. *B. t.* cotton: field performance in North Carolina under conditions of unusually high bollworm populations, pp. 795-798. *In* Proc. 1995 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Matten, S. R. 2001. EPA update on insect resistance management for Bollgard cotton, pp. 840-844. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

McMichael, S. C. 1959. Hopi cotton: a source of cottonseed free of gossypol pigments. Agron. J. 61: 630.

1960. Combined effects of glandless genes gl_2 and gl_3 on pigment glands in the cotton plant. Agron. J. 52: 385-387.

Mistric, W. J., Jr. 1964. Early detection of *Heliothis* on cotton. J. Econ. Entomol. 57: 858-859.

Moar, W. J., M. Pustzi-Carey, H. Van Faassen, D. Bosch, R. Frutos, C. Rang, K. Luo, and M. J. Adang. 1995. Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Appl. Environ. Microbiol. 61: 2086-2092.

Moar, W. J., R. H. Smith, and R. Weeks. 1998. Bt resistance monitoring of tobacco budworm and Cotton Bollworm in Alabama cotton: let's get everyone involved! pp. 1054-1056. *In* P. Dugger and D. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Mullins, J. W. 2001. New Bollgard refuge requirements for 2001, pp. 844-845. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Naeem, M., G. P. Waldbauer, and S. Friedman. 1992. Selective feeding within snap bean pods by larvae of *Helicoverpa zea* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 85: 784-791.

Oliver, A. D., and J. B. Chapin. 1981. Biology and illustrated key for the identification of twenty species of economically important Noctuid pests. Louisiana Agric. Expt. Sta. Tech. Bull. 733. 26 pp.

Oliver, B. F., F. G. Maxwell, and J. N. Jenkins. 1970. Utilization of glanded and glandless cotton diets by the bollworm. J. Econ. Entomol. 63: 1965-1966.

Painter, R. H. 1951. Insect resistance in crop plants. University Press of Kansas, Lawrence, KS.

Parker, C. D., Jr. 1997. Effects of mixtures of delta-endotoxin-producing transgenic cotton and 'Coker 312' cotton on adult oviposition and larval behavior of *Heliothis virescens* (Lepidoptera: Noctuidae). M. S. thesis, Mississippi State University, Starkville.

Parrott, W. L., J. N. Jenkins, J. C. McCarty, Jr., and L. Lambert. 1978. A procedure to evaluate for antibiosis in cotton to the tobacco budworm. J. Econ. Entomol. 71: 310-312.

Parrott, W. L., J. N. Jenkins, and J. C. McCarty, Jr. 1983. Feeding behavior of first-stage tobacco budworm (Lepidoptera: Noctuidae) on three cotton cultivars. Ann. Entomol. Soc. Am. 76: 167-170.

Pedigo, L. P., S. H. Hutchins, and L. G. Higley. 1986. Economic injury levels in theory and practice. Ann. Rev. Entomol. 31: 341-368.

Penn, S. R., B. Reich, J. Osborn, K. Embry, and J. Greenplate. 2001. Quantification of lepidopteran activity in a 2-gene product: a 2-year summary of Bollgard II, pp. 830-832. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischoff. 1990. Insect resistant cotton plants. Biotechnology 8: 839-943.

Pitre, H. N., and T. L. Hillhouse. 1981. Establishment of infestation and behavior of *Heliothis zea* on soybeans in the greenhouse. J. Georgia Entomol. Soc. 16: 28-34.

Plapp, F. W., and C. Campanhola. 1986. Synergism of pyrethroids by chlordimeform against susceptible and resistant *Heliothis*, pp. 167-169. *In* T. C. Nelson [ed.], Proc. 1986 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Pluthero, F. G., and R. S. Singh. 1984. Insect behavioral responses to toxins: practical and evolutionary considerations. Can. Entomol. 116: 57-68.

Reese, J. C., B. G. Chan, N. R. Malm, and A. C. Waiss, Jr. 1981. Feeding sites of bollworm larvae on cotton. Environ. Entomol. 10: 81-84.

Ridge, R. L., S. G. Turnipseed, and M. J. Sullivan. 2000. Field comparison of genetically-modified cottons containing one strain (Bollgard) and two strains (Bollgard II) of *Bacillus thuringiensis kurstaki*, pp. 1057-1058. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Ring, D. R., J. H. Benedict, D. J. Lawlor, R. R. DeSpain, and T. B. Stone. 1993. Bollworm and tobacco budworm injury to transgenic Bt cotton and estimated density yield response using the integrated crop ecosystem management model, pp. 821-824. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1993 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roof, M. E., and J. A. DuRant. 1997. On-farm experiences with Bt cotton in South Carolina, p. 861. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Shaver, T. N., J. A. Garcia, and R. H. Dilday. 1977. Tobacco budworm: feeding and larval growth on component parts of cotton flowerbuds. Environ. Entomol. 6: 82-84.

Sims, S. R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein: effects on Lepidoptera, Diptera, and non-target arthropods. Southwest. Entomol. 22: 395-404.

Slosser, J. E., J. R. Phillips, and G. A. Herzog. 1978. Bollworm damage and population development in relation to phenology of the cotton plant. Environ. Entomol. 7: 144-148.

Smith, R. H. 1997. An extension entomologist's 1996 observation of Bollgard (Bt) technology, pp. 856-858. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

1998. Year two of Bollgard behind boll weevil eradication: Alabama observations, pp. 965-966. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Sparks, T. C., J. A. Lockwood, R. L. Byford, J. B. Graves, and B. R. Leonard. 1989. The role of behavior in insecticide resistance. Pestic. Sci. 26:383-399.

Stern, V. M., R. F. Smith, R. van den Bosch, and K. S. Hagan. 1959. The integrated control concept. Hilgardia 29: 81-101.

Stewart, S. D., and K. S. Knighten. 2000. Efficacy of Bt cotton expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner on selected caterpillar pests, pp. 1043-1048. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of Noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Stipanovic, R. D. 1983. Function and chemistry of plant trichomes and glands in insect resistance: protective chemicals in plant epidermal glands and appendages, pp. 82-100. *In* P. A. Hedin, [ed.], Plant resistance to insects, ACS symposium series. American Chemical Society, Washington, DC.

Stone, T. B., S. R. Sims, and P. G. Marrone. 1989. Selection of tobacco budworm for resistance to a genetically engineered *Pseudomonas fluorescens* containing the δ-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. J. Invert. Pathol. 53: 228-234.

Sudbrink, D. L., and J. F. Grant. 1995. Wild host plants of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) in eastern Tennessee. Environ. Entomol. 24: 1080-1085.

Summerford, D. V., D. D. Hardee, L. C. Adams, and W. L. Solomon. 1999. Status of monitoring for tolerance to CryIA(c) in populations of *Helicoverpa zea* and *Heliothis virescens*: three-year summary, pp. 936-939. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47-79.

Umbeck, P., G. Johnson, K. Barton, and W. Swain. 1987. Genetically transformed cotton (*Gossypium hirsutum*) plants. Biotechnology 5: 263-266.

Voth, R. D., J. T. Greenplate, J. E. Mann, and J. W. Mullins. 2001. Bollgard® II cotton technical review, p. 830. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Walker, J. T., M. J. Sullivan, S. Turnipseed, M. E. Roof, and T. M. Brown. 1998. Prospects for field management of pyrethroid-resistant corn earworm (cotton bollworm) populations in South Carolina, pp. 1145-1147. *In* P. Dugger and D. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

White, W. H., J. N. Jenkins, W. L. Parrott, J. C. McCarty, Jr., D. H. Collum, and P. A. Hedin. 1982. Generation mean analyses of various allelochemics in cottons. Crop Sci. 22: 1046-1049.

Williams, M. R. 1999. Cotton insect losses 1998, pp. 785-806. *In* P. Dugger and D. A. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M. R. 2000. Cotton insect losses 1999, pp.887-913. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Wilson, F. D. 1971. Response of tobacco budworm larvae to cotton seedlings carrying various combinations of gland-determining alleles. Crop Science 11: 268-270.

Wilson, L. T., and A. P. Gutierrez. 1980. Fruit predation submodel: *Heliothis* larvae feeding upon cotton fruiting structures. Hilgardia 48: 24-36.

Wilson, L. T., A. P. Gutierrez, and T. F. Leigh. 1980. Within-plant distribution of the immatures of *Heliothis zea* (Boddie) on cotton. Hilgardia 48: 12-23.

Wolfenbarger, D. A., and R. L. McGarr. 1970. Toxicity of methyl parathion, parathion, and monocrotophos applied topically to populations of lepidopteran pests of cotton. J. Econ. Entomol. 63: 1762-1764.

Wolfenbarger, D. A., M. J. Lukefahr, and H. M. Graham. 1973. LD_{50} values of methyl parathion and endrin to tobacco budworms and bollworms collected in the Americas and

hypothesis on the spread of resistance in these lepidopteran pests to these insecticides. J. Econ. Entomol. 66: 211-216.

Wood, E. A. 1971. Designation and reaction of three biotypes of the greenbug cultured on resistant and susceptible species of sorghum. J. Econ. Entomol. 64: 183-185.

CHAPTER 2

BOLLWORM (LEPIDOPTERA: NOCTUIDAE) SURVIVAL ON BOLLGARD AND BOLLGARD II COTTON FLOWER BUD AND FLOWER COMPONENTS*

Introduction

Insect pest management in cotton has traditionally relied upon synthetic insecticides to maintain insect populations below established economic injury levels (Graves et al. 1999). However, insect resistance to insecticides and increasing insecticide costs have made effective and economical insect control difficult. During the last two decades, prior to widespread resistance, organophosphate and pyrethroid insecticides provided good control of most insect pests of cotton. Currently, these compounds do not provide the same level of protection as they previously did (Graves et al. 1999).

The bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), are primary insect pests of cotton throughout much of the United States. Bollworms and tobacco budworms were highly susceptible to pyrethroid insecticides through the mid-1980's. However, widespread indiscriminate use of these insecticides has resulted in a decline in pyrethroid efficacy against tobacco budworms throughout the United States (Graves et al. 1999) and against bollworms in South Carolina (Brown et al. 1997, Walker et al. 1998). In Louisiana, pyrethroids were recently removed from the list of insecticides recommended by the Louisiana Cooperative Extension Service for tobacco budworm control (Bagwell et al. 2000). Consequently, novel approaches for controlling these insects are being developed (Greenplate et al. 2000a).

*Reprinted by permission of Journal of Economic Entomology.

Genetically modified cotton cultivars (Bollgard) that produce *Bacillus thuringiensis* Berliner insecticidal proteins have replaced or supplemented the insecticide component of integrated pest management programs throughout the cotton production regions of the United States. Since the introduction of Bollgard cotton in 1996, acreages planted to these cultivars have increased annually. In Louisiana, the percentage of acres planted to Bollgard cotton has increased from approximately 15% in 1996 (Williams 1997) to over 60% in 1999 (Williams 2000). Similar trends have been observed in other states, while the acreage has decreased in few states (Williams 2000).

Bollgard cotton consistently provides satisfactory control of tobacco budworms. However, bollworms are inherently more tolerant to the protein produced by these cultivars than are tobacco budworms (MacIntosh et al. 1990, Luttrell et al. 1999). Consequently, insecticides are often applied to Bollgard cotton to suppress bollworm populations during peak ovipositional periods (Bacheler and Mott 1997; Layton et al. 1997, 1998; Leonard et al. 1997, 1998; Roof and Durant 1997; Smith 1997, 1998). Burd et al. (1999) found that yields of several commercial Bollgard cotton cultivars were significantly increased when pyrethroids were applied. Since bollworms are readily controlled with pyrethroids, the improvement in yields observed by Burd et al. (1999) may have been the result of bollworm control with the pyrethroids.

Bollworms are more often found in white flowers than other plant parts (Smith 1998, Pietrantonio and Heinz 1999). During the first year of commercial Bollgard production, large numbers of bollworm larvae were observed feeding on white flowers in many Bollgard fields across the United States. White flowers of Bollgard cotton appear to be the plant structures most susceptible to bollworm feeding. Gore et al. (2000) infested white flowers and various

aged bolls with first instar bollworm larvae. Abscission rates of Bollgard bolls that were infested as white flowers were higher compared to bolls that were infested during later stages of development.

Unacceptable control of bollworms and other lepidopteran pests such as beet armyworms [*Spodoptera exigua* (Hübner)], fall armyworms [*S. frugiperda* (J. E. Smith)], and soybean loopers [*Pseudoplusia includens* (Walker)] prompted scientists with Monsanto Co. to develop a new genetically modified cotton (Bollgard II) that contains two separate crystalline proteins (Greenplate et al. 2000b). Bollgard II cotton was devoloped by incorporating the CryIIA(b) protein from *B. thuringiensis* into a commercially available Bollgard cotton cultivar, Deltapine 50B which contains the CryIA(c) protein (Greenplate et al. 2000a, b). The CryIIA(b) protein was added to provide greater insecticidal activity against target pests and broaden the spectrum of total pests controlled. A 3- to 6-fold increase was observed in bioactivity of Bollgard II compared to Bollgard against tobacco budworm (Greenplate et al. 2000b).

The addition of CryIIA(b) protein expressed in Bollgard II cotton provides satisfactory control of beet armyworms, fall armyworms, and soybean loopers (Stewart and Knighten 2000). Also, efficacy of Bollgard II was improved over Bollgard against bollworms (Stewart and Knighten 2000). Other investigators observed improved bollworm control in Bollgard II cotton compared to Bollgard cotton during 1999 (Jackson et al. 2000, Ridge et al. 2000). These initial data indicate that Bollgard II will be beneficial in areas where multiple lepidopteran pest species reach economically damaging levels during most years. However, more research is needed to determine if satisfactory bollworm control will consistently occur in Bollgard II cotton.

Currently, little information is available on why bollworms are more commonly observed on white flowers compared with other plant parts. Possible explanations for differences in bollworm survival may include lower expression of the protein and/or lower levels of secondary plant chemicals in white flowers. Also, the nutritional value of white flowers may be such that bollworm larvae are capable of overcoming the adverse effects of CryIA(c) toxicity. The study reported here utilized two separate experiments to investigate these possibilities. The first experiment was initiated to determine the levels of bollworm survival that can be expected on white flowers of Bollgard cotton and to determine protein expression levels of white flowers. In the second experiment, white flowers from Bollgard II cotton were evaluated to determine if bollworm control would be significantly improved over that for Bollgard cotton.

Materials and Methods

Bollworm Survival on Floral Components of Conventional and Bollgard Cotton.

Plots of a genetically modified cotton cultivar (NuCOTN 33B, Delta and Pine Land Co., Scott, MS) producing an insecticidal protein from *Bacillus thuringiensis* Berliner var. *kurstaki* (Bollgard, Monsanto Co., St Louis, MO.) and a parental cultivar (Deltapine 5415) were planted from 2 through 21 May at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA, during 1998 and 1999. Fertilization rates and general agronomic practices followed current Louisiana Cooperative Extension Service recommendations.

Bollworm colonies were established with larvae collected from clover, *Trifolium* spp., during late April and from sweet corn, *Zea mays* L., during early June of each year. Bollworms were reared in the laboratory for a minimum of one generation to eliminate parasitoids, minimize pathogens, and to obtain sufficient numbers of larvae for bioassays. Larvae were fed

an artificial soy protein, wheat germ-based diet (*Heliothis* premix, Stonefly Industries Inc., Bryan, TX) in individual 29.5-ml. plastic cups (Solo Co., Urbana, IL) with matching lids. Larvae were maintained at 27 ± 2^{0}C and 85 ± 2% relative humidity with a 14:10 h light:dark photoperiod until pupation. Pupae were placed into 3.79-liter cylindrical cardboard containers at 27 ± 2^{0}C and 85 ± 2% relative humidity. Upon eclosion, moths were fed a 10% sucrose:water solution. A single layer of cheesecloth was placed on top of each container to provide an adequate surface for oviposition. Oviposition sheets were harvested daily and placed into 118x59x354-cm plastic bags until larval eclosion.

Flower buds (squares) and white flowers were harvested from conventional and Bollgard cottons and transported to the laboratory during three stages of cotton plant reproductive development. Cotton plant stages were determined by counting the number of main stem nodes between the upper-most first position white flower and the last unfolded leaf in the plant terminal. Plant stages included main stem nodes above white flower 8 to 9, 6 to 7, and 4 to 5. Floral components included whole squares with the bracts removed, immature reproductive organs (square anthers), white flower bracts, white flower petals, and mature reproductive organs (flower anthers). Flower anthers and square anthers also included the female style and stigma. These structures were placed into 9.0-cm petri dishes along with moistened filter paper. Five neonate bollworm larvae were transferred to each dish and allowed to feed for 72 h. Five dishes were infested per treatment per block (n=100 larvae per treatment). Treatments were arranged in a randomized complete block design (blocks were infested on four successive days). Larval mortality was rated at 24, 48, and 72 h after initial exposure. Percentage survival data within each cultivar was subjected to repeated measures analysis of variance (SAS Institute 1989), and means were separated according to Fisher's

protected least significant difference. Individual comparisons were made between structures of NuCOTN 33B and Deltapine 5415 using paired t-tests from bioassays conducted in 1998 and 1999 (SAS Institute 1989).

Enzyme linked immunosorbent assays (ELISA) were conducted at the United States Department of Agriculture – Agricultural Research Service, Southern Insect Management Research Unit (USDA-ARS SIMRU) at Stoneville, MS, to quantify CryIA(c) expression in floral structures used for insect bioassays. ELISA techniques were similar to those described by Adamczyk et al. (2000). Squares and white flowers were removed from plots of Deltapine 5415 and NuCOTN 33B. Structures were dissected into individual components as described for insect bioassays. Fifty to 100 mg of each structure were placed into 1.5-ml Eppendorf® tubes and homogenized in extraction buffer. A commercial quantification plate kit (EnviroLogix, Inc., Portland, ME) was utilized for assays. This ELISA method utilizes color changes that are proportional to CryIA(c) concentration. Quantification of CryIA(c) was determined spectrophotometrically (Benchmark®, Bio-Rad, Hercules, CA) by comparison to a standard curve. Samples were arranged in a randomized complete block design and replicated four times. Data were converted to parts per million and subjected to analysis of variance (SAS Institute 1989). Means were separated according to Fisher's protected least significant difference. Also, correlation analyses were conducted on CryIA(c) expression and bollworm survival at each rating interval (PROC REG, SAS Institute 1989).

Bollworm Survival on Floral Components of Bollgard and Bollgard II Cotton. Plots of Bollgard II (Deltapine 50BII), Bollgard (Deltapine 50B), and conventional (Deltapine 50) cotton cultivars were planted on 11 June 2000. Bioassays conducted during 2000 with Deltapine 50, Deltapine 50B, and Deltapine 50BII utilized the methods described for the first

experiment except they were conducted only at one growth stage (nodes above white flower 6 to 8). Bollworm survival within each cultivar and comparisons of bollworm survival among structures of the cultivars were subjected to repeated measures analysis of variance (SAS Institute 1989), and means were separated according to Fisher's protected least significant difference.

Results

Bollworm Survival on Floral Components of Conventional and Bollgard Cotton.

Bollworm survival varied among floral structures on Deltapine 5415 (conventional). No cotton stage by floral structure (F<2.08; df =8, 45; P>0.06) or year by floral structure (F<0.59; df =4, 70; P>0.67) interactions were significant at any rating interval for bollworm survival on Deltapine 5415 cotton; therefore, data were combined across cotton stages and years. Survival averaged 93 to 100%, 81 to 98%, and 71 to 97% at 24, 48, and 72 h after infestation, respectively (Table 2.1). At 24 h, bollworm survival was different among floral structures (F=4.37; df =4, 75; P<0.01). Bollworm survival was lowest on flower bracts. Bollworm survival at 48 h (F=7.20; df =4, 75; P<0.01) and 72 h (F=15.8; df =4, 75; P<0.01) was higher on flower anthers and square anthers than on flower bracts and petals. Bollworm survival on anthers (flower and square) also was higher than on squares at 72 h.

Bollworm survival on NuCOTN 33B (Bollgard) cotton varied among floral structures. No cotton stage by floral structure (F<1.43; df =8, 45; P>0.21) or year by floral structure (F<2.25; df =4, 70; P>0.07) interactions were significant for bollworm survival at any rating interval; therefore, data were combined across cotton stages and years. Bollworm survival ranged from 85 to 97%, 57 to 96%, and 19 to 91% at 24, 48, and 72 h, respectively (Table 2.1). At 24 h, bollworm survival was lower on flower bracts than all other structures (F=3.94; df =4, 75;

Table 2.1. Comparisons of bollworm survival at 24, 48, and 72 h after infestation with neonates on Deltapine 5415 and NuCOTN 33B floral components.

		Mean (±SD) % Survival				
24 h	Floral Structure	DP 5415	NuCOTN 33B	*df*	*t*	*P>t*
	Bracts	93 ± 8A	85 ± 15B	30	-1.82	0.08
	Petals	96 ± 4ABC	94 ± 7A	30	-1.23	0.23
	Flower Anthers	98 ± 5AB	97 ± 7A	30	-0.40	0.69
	Square Anthers	100 ± 0A	97 ± 10A	30	-1.34	0.19
	Squares	95 ± 6BC	93 ± 8A	30	-1.01	0.32
	F	4.37	3.94			
	df	4, 75	4, 75			
	P>F	<0.01	0.01			
48 h						
	Bracts	81 ± 16C	57 ± 21D	30	-3.74	<0.01
	Petals	89 ± 12BC	82 ± 13B	30	-1.53	0.14
	Flower Anthers	98 ± 5A	96 ± 4A	30	-0.90	0.38
	Square Anthers	98 ± 6A	94 ± 10A	30	-1.32	0.20
	Squares	91 ± 8AB	70 ± 21C	30	-3.71	<0.01
	F	7.20	18.9			
	df	4, 75	4, 75			
	P>F	<0.01	<0.01			
72 h						
	Bracts	71 ± 18C	19 ± 15D	30	-8.76	<0.01
	Petals	76 ± 12BC	58 ± 15B	30	-3.67	<0.01
	Flower Anthers	97 ± 5A	91 ± 6A	30	-2.59	0.01
	Square Anthers	96 ± 6A	88 ± 9A	30	-2.87	0.01
	Squares	83 ± 12B	37 ± 23C	30	-7.12	<0.01
	F	15.8	71.3			
	df	4, 75	4, 75			
	P>F	<0.01	<0.01			

Means within columns followed by a common letter are not significantly (α=0.05) different according to Fisher's protected least significant difference. Means within rows are compared using paired t-tests (α=0.05).

P=0.01). At 48 h (*F*=18.9; *df* =4, 75; *P*<0.01) and 72 h (*F*=71.3; *df* =4, 75; *P*<0.01), bollworm survival was higher on flower anthers and square anthers than on other floral structures.

There were no differences between bollworm survival on Deltapine 5415 and NuCOTN 33B for any structure at 24 h (Table 2.1). At 48 h, bollworm survival was lower on NuCOTN 33B flower bracts and squares compared with the corresponding structures on Deltapine 5415. Bollworm survival was lower on all NuCOTN 33B structures compared with the corresponding structures on Deltapine 5415 at 72 h.

ELISA tests of floral structures used in these bioassays indicate that *B. thuringiensis* protein expression varies among plant parts (*F*=32.6; *df* =4, 10; *P*<0.01). Protein expression was highest in flower bracts and petals compared with other structures. In addition, protein expression was lowest on squares and square anthers. CryIA(c) expression averaged (± standard deviation) 0.59±0.03, 0.56±0.12, 0.34±0.03, 0.17±0.03, and 0.19±0.01 ppm on flower bracts, flower petals, flower anthers, square anthers, and squares, respectively. CryIA(c) levels did not correlate (24 h: R=-0.21; *F*=0.63; *df*=1, 13; *P*=0.44; 48 h: R=-0.30; *F*=1.33; *df*=1, 13; *P*=0.27; 72 h: R=-0.29; *F*=1.18; *df*=1, 13; *P*=0.30) with variation in bollworm survival.

Bollworm Survival on Floral Components of Bollgard and Bollgard II Cotton. Bollworm survival on flower anthers and square anthers was generally highest and lowest, respectively, on flower bracts on Deltapine 50, Deltapine 50B, and Deltapine 50BII (Table 2.2).

Bollworm survival on Bollgard II appeared to follow a trend similar to that observed on Bollgard. However, bollworm survival, in general, was much lower on Bollgard II than on Bollgard. At 24 h, there were no differences in bollworm survival among the three cotton cultivars on any structure (Table 2.2). At 48 h, bollworm survival on squares was lower on

Table 2.2. Mean (± standard deviation) bollworm survival on Deltapine 50, Deltapine 50B (Bollgard), and Deltapine 50BII (Bollgard II) floral structures at 24, 48, and 72 h after infestation.

		Mean (±SD) % Survival					
24 h	Floral Structure	DP 50	DP 50B	DP 50 BII	*F*	*df*	*P>F*
	Bracts	83 ± 13Aa	80 ± 13Ba	89 ± 3Ba	0.66	2, 8	0.54
	Petals	98 ± 3Aa	100 ± 0Aa	99 ± 3Aa	0.62	2, 8	0.56
	Flower Anthers	98 ± 3Aa	100 ± 0Aa	99 ± 3Aa	0.68	2, 8	0.53
	Square Anthers	98 ± 3Aa	100 ± 0Aa	100 ± 0Aa	1.45	2, 8	0.29
	Squares	85 ± 6Aa	96 ± 4Aa	97 ± 4Aa	2.09	2, 8	0.19
	F	2.39	7.84	10.49			
	df	4, 10	4, 15	4, 15			
	P>F	0.12	<0.01	<0.01			
48 h							
	Bracts	67 ± 7Ca	57 ± 23Cb	29 ± 19Cb	4.16	2, 8	0.06
	Petals	95 ± 6Aa	90 ± 10ABa	81 ± 15Aa	1.28	2, 8	0.33
	Flower Anthers	98 ± 3Aa	98 ± 3Aa	88 ± 17Aa	0.43	2, 8	0.30
	Square Anthers	98 ± 3Aa	97 ± 3Aa	72 ± 19Ab	6.18	2, 8	0.02
	Squares	80 ± 13Ba	77 ± 12Ba	38 ± 28Bb	5.20	2, 8	0.04
	F	11.1	7.39	6.89			
	df	4, 10	4, 15	4, 15			
	P>F	<0.01	<0.01	<0.01			
72 h							
	Bracts	48 ± 9Ca	18 ± 6Db	6 ± 2Cc	42.7	2, 8	<0.01
	Petals	81 ± 9Aba	67 ± 13Ba	36 ± 21Bb	7.58	2, 8	0.01
	Flower Anthers	95 ± 5Aa	93 ± 2Aa	63 ± 9Ab	33.3	2, 8	<0.01
	Square Anthers	97 ± 5Aa	92 ± 3Aa	50 ± 10ABb	49.9	2, 8	<0.01
	Squares	75 ± 17Ba	49 ± 14Cb	8 ± 4Cc	25.9	2, 8	<0.01
	F	11.2	45.8	19.9			
	df	4, 10	4, 15	4, 15			
	P>F	<0.01	<0.01	<0.01			

Means within a column followed by the same uppercase letter and within a row followed by the same lowercase letter are not significantly (α=0.05) different.

Bollgard II than on squares from the other cotton cultivars. Bollworm survival at 72 h was lower on all flower structures from Bollgard II than on the corresponding structures on the other two cotton cultivars.

Discussion

Bollworm larvae prefer specific feeding sites on cotton plants. Farrar and Bradley (1985) found that *Heliothis* larvae showed a preference for white and red flowers of conventional cotton. In that study, bollworm larvae showed a greater preference than tobacco budworm larvae for white flowers. Non-photosynthesizing (non-green) structures of cotton may be more common feeding sites for bollworm larvae. These structures, which are mostly reproductive, may be more nutritionally suitable for bollworm larvae than other plant parts. Another possible explanation for bollworm preferences for flowers could be that there are lower levels of secondary plant chemicals in non-photosynthesizing tissues. Hedin et al. (1983) reported varying levels of secondary plant chemicals (tannins, gossypol, and chrysanthemin) among different plant parts. Gossypol concentrations ranged from 0.04% in bolls to 0.50% in squares. Tannins ranged from 6.02% in terminals to 17.1% in bolls, while chrysanthemin ranged from 0.05% in bolls to 0.18% in leaves. Stipanovic (1983) reported that cotton foliage produces numerous terpenoids and other compounds in addition to gossypol. Many of the compounds found in cotton have antibiotic activity and are toxic to several insect pests. Little information is available concerning levels of secondary plant chemicals in square anthers. However, Hanny (1980) reported variation in levels of selected chemicals in flower anthers among cotton cultivars. Also, yellow flower anthers contained more gossypol than cream-colored flower anthers. Studies comparing the concentrations of secondary chemicals in flower anthers to those in other plant parts have not been conducted. It is likely that bollworm

mortality on flower structures is associated with more than one allelochemical within an individual structure and differences in chemical complexes among cotton plant parts may explain the variation in bollworm survival on those plant parts.

Differences in *B. thuringiensis* CryIA(c) protein expression among different plant parts may partially explain differences in bollworm survival on those structures (Adamczyk 2000). However, similar differences in bollworm survival among floral structures were observed on conventional cotton, which indicates that factors other than protein expression alone are involved. For example, interactions between plant secondary compounds and the CryIA(c) protein may have occurred. If there is an interaction between CryIA(c) and plant allelochemicals, then there would be an expected minimum critical level of protein that fluctuates based on allelochemical concentrations. For instance, structures with low allelochemical concentrations would require a higher level of CryIA(c) expression to provide the same level of bollworm mortality as structures with high allelochemical concentrations. Therefore, the interactions of these factors would be dynamic, where a decrease in one factor may require an increase in the other factor to provide the same level of protection.

Although statistical differences were observed between conventional and Bollgard cotton, bollworm survival averaged ≥88% on Bollgard flower anthers and square anthers. With this level of pest pressure, insecticide applications may be needed to prevent economic losses. Differences in bollworm survival on conventional and Bollgard cotton support the presence of CryIA(c) protein in those structures of Bollgard cotton with high levels of bollworm survival. However, expression in those structures may be low.

Bollgard II contains an additional gene that codes for the production of the CryIIA(b) protein from *B. thuringiensis* in addition to CryIA(c). The addition of the CryIIA(b) protein

with CryIA(c) increased the insecticidal activity against bollworm larvae. Sims (1997) found that bollworm larvae appear to be less sensitive to CryIIA than CryIA(c). The addition of the CryIIA(b) protein into Bollgard cotton, however, would most likely increase the total amount of protein present in the plant. Greenplate et al. (2000b) measured levels of Cry proteins present in Bollgard II. They found approximately a 10X higher level of CryIIA(b) over CryIA(c); however, there was only a 3-6X increase in bioactivity against tobacco budworms. In the present study, increases in bioactivity against bollworms of 3.2X, 1.6X, 1.4X, 1.8X, and 4.6X for flower bracts, flower petals, flower anthers, square anthers, and squares, respectively, were observed.

Bollgard cotton cultivars are valuable integrated pest management tools for cotton systems in the United States. Good control can be expected for the tobacco budworm and pink bollworm, *Pectinophora gossypiella* (Saunders). This new technology has not always provided sufficient levels of bollworm control, however. Data reported here support field observations made by agricultural consultants and researchers throughout the southeastern United States concerning high numbers of bollworm larvae feeding on white flowers. It was originally assumed that white flowers express lower levels of CryIA(c) protein than other plant parts. However, other factors may be involved based on the ELISA data and bollworm survival trends on conventional cotton floral structures. Similar trends in bollworm survival were observed on conventional and Bollgard floral structures. Significantly fewer larvae survived on flower bracts of conventional cotton compared with survival on other conventional cotton floral structures. This finding suggests that biochemical factors associated with bracts have adverse effects on bollworm development.

The addition of a second protein into Bollgard cotton to create Bollgard II appeared to significantly increase protection against bollworms. Despite these improvements, however, bollworm survival averaged over 50% on flower anthers and square anthers of Bollgard II at 72 h. These survival rates suggest that economic injury may occur on Bollgard II during bollworm outbreaks; however, these experiments were terminated after 72 h. Our data suggest that the possibility for injury exists, but this has not been observed for Bollgard II cotton grown under field conditions. Field studies indicate that Bollgard II cottons will consistently provide satisfactory bollworm control (Jackson et al. 2000, Stewart and Knighten 2000, Ridge et al. 2000). However, these were small plot studies conducted in relatively isolated locations and no definitive predictions can be made as to the level of bollworm protection that can expected from Bollgard II when it is planted over large acreages.

In conclusion, these data provide a baseline of information describing the levels of bollworm survival that can be expected on white flowers of Bollgard and Bollgard II cotton. This information indicates that current scouting protocols for conventional cotton may not be appropriate for Bollgard cotton. Because high levels of bollworm survival can be expected on white flowers of Bollgard cotton, those structures need to be closely examined for small larvae. Also, these data provide valuable information for improving management decisions for bollworms on Bollgard cotton. Further research is needed to determine if larvae feeding on white flowers are capable of moving to other structures, causing additional injury. Also, future research in this area should focus on quantifying secondary plant chemicals and assessing nutritional quality among selected components of white flowers and squares to determine their influence on CryIA(c) efficacy. Finally, bollworm management in genetically modified cottons (Bollgard and Bollgard II) is a complex situation that involves multiple factors. Plant

biochemistry and nutrition appear to be important for bollworm mortality, in addition to *B. thuringiensis* protein expression in genetically modified cottons. Levels of secondary plant chemicals and *B. thuringiensis* protein expression need to be determined for different genetically modified cultivars and among different plant parts so that bollworm survival can be predicted during periods of high population densities.

References Cited

Adamczyk, J. J., Jr., L. C. Adams, and D. D. Hardee. 2000. Quantification of CryIA(c) δ-endotoxin in transgenic Bt cotton: correlating insect survival to different protein levels among plant parts and varieties, pp. 929-932. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Bacheler, J. S., and D. W. Mott. 1997. Efficacy of grower-managed Bt cotton in North Carolina, pp. 858-861. *In* P. Dugger and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Bagwell, R. D., J. W. Barnett, B. R. Leonard, E. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2000. Cotton insect control 2000. Louisiana Cooperative Extension Service Pub. 1083.

Brown, T. M., P. K. Bryson, D. S. Brickle, J. T. Walker, and M. J. Sullivan. 1997. Pyrethroid-resistant *Helicoverpa zea* in cotton in South Carolina. Resistant Pest Manag. Newsletter 9: 26-27.

Burd, T., J. R. Bradley, Jr., and J. W. Van Duyn. 1999. Performance of selected *Bt* cotton genotypes against bollworm in North Carolina, pp. 931-934. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Farrar, R. R., Jr., and J. R. Bradley, Jr. 1985. Within-plant distribution of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs and larvae on cotton in North Carolina. Environ. Entomol. 14: 205-209.

Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. J. Econ. Entomol. 93: 690-696.

Graves, J. B., B. R. Leonard, and J. A. Ottea. 1999. Chemical approaches to managing arthropod pests, pp. 449-486. *In* J. R. Ruberson [ed.], Handbook of pest management. Marcel Dekker, Inc., New York.

Greenplate, J. T., S. R. Penn, J. W. Mullins, and M. Oppenhuizen. 2000a. Seasonal CryIAc levels in DP50B: The "Bollgard® basis" for Bollgard II, pp. 1039-1040. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J. T., S. R. Penn, Z. Shappley, M. Oppenhuizen, J. Mann, B. Reich, and J. Osborn. 2000b. Bollgard II efficacy: Quantification of lepidopteran activity in a 2-gene product, pp. 1041-1043. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Hanny, B. W. 1980. Gossypol, flavonoid, and condensed tannin content of cream and yellow anthers of five cotton (*Gossypium hirsutum* L.) cultivars. J. Agric. Food Chem. 28: 504-506.

Hedin, P. A., J. N. Jenkins, D. H. Collum, W. H. White, and W. L. Parrott. 1983. Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis virescens* (F.), pp. 347-365. *In* P. A. Hedin, [ed.], Plant resistance to insects, ACS symposium series. American Chemical Society, Washington, DC.

Jackson, R. E., J. R. Bradley, Jr., A. D. Burd, and J. W. Van Duyn. 2000. Field and greenhouse performance of bollworm on Bollgard II cotton genotypes, pp. 1048-1051. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Layton, M. B., M. R. Williams, and S. Stewart. 1997. Bt cotton in Mississippi: The first year, pp. 861-863. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Layton, M. B., S. D. Stewart, and M. R. Williams. 1998. Performance of Bt cotton in Mississippi, 1997, pp. 970-973. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B. R., H. Fife, K. Torrey, J. B. Graves, and J. Holloway. 1997. *Helicoverpa/Heliothis* management in NuCOTN and conventional cotton cultivars in Louisiana, pp. 863-867. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B. R., J. H. Fife, K. Torrey, E. Burris, and J. B. Graves. 1998. Evaluation of transgenic Bt cotton lines against Heliothines in Northeast Louisiana, pp. 967-970. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebrate Path. 56: 258-266.

Pietrantonio, P. V., and K. Heinz. 1999. Distribution of Heliothine larvae in *B. t.* and non-*B. t.* cotton in Texas, pp. 945-948. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Ridge, R. L., S. G. Turnipseed, and M. J. Sullivan. 2000. Field comparison of genetically-modified cottons containing one strain (Bollgard) and two strains (Bollgard II) of *Bacillus thuringiensis kurstaki*, pp. 1057-1058. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roof, M. E., and J. A. Durant. 1997. On-farm experiences with Bt cotton in South Carolina, p. 861. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

SAS Intitute. 1989. SAS/STAT user's guide, version 6, fourth ed., vol. 1. SAS Institute, Cary, NC.

Sims, S. R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein: effects on Lepidoptera, Diptera, and non-target arthropods. Southwestern Entomol. 22: 395-404.

Smith, R. H. 1997. An extension entomologist's 1996 observation of Bollgard (Bt) technology, pp. 856-858. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Smith, R. H. 1998. Year two of Bollgard behind boll weevil eradication: Alabama observations, pp. 965-966. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Stewart, S. D., and K. S. Knighten. 2000. Efficacy of Bt cotton expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner on selected catterpillar pests, pp. 1043-1048. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Stipanovic, R. D. 1983. Function and chemistry of plant trichomes and glands in insect resistance: protective chemicals in plant epidermal glands and appendages, pp. 82-100. *In* P. A. Hedin, [ed.], Plant resistance to insects, ACS symposium series. American Chemical Society, Washington, DC.

Walker, J. T., M. J. Sullivan, S. Turnipseed, M. E. Roof, and T. M. Brown. 1998. Prospects for field management of pyrethroid-resistant corn earworm (cotton bollworm) populations in South Carolina, pp. 1145-1147. *In* P. Dugger and D. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M. L. 1997. Cotton insect losses, 1996, pp. 834-853. *In* P. Dugger and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M. L. 2000. Cotton insect losses, 1999, pp. 887-913. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

CHAPTER 3

BEHAVIOR OF BOLLWORM (LEPIDOPTERA: NOCTUIDAE) LARVAE ON GENETICALLY ENGINEERED COTTON

Introduction

Genetically engineered plants are rapidly becoming important components of integrated pest management (IPM) programs in many cropping systems. Cotton cultivars (Bollgard®, Monsanto Co., St. Louis, MO) that have been genetically engineered to express the Cry1A(c) protein from the soil bacterium, *Bacillus thuringiensis kurstaki* Berliner (Perlak et al. 1990) are environmentally friendly tools for selective pest management and provide a significant economic return in many areas. Bollgard cotton was introduced for commercial production in 1996, and its adoption rate in the United States has increased from 749,431 hectares during that year (Williams 1997) to over 2.1 million hectares during 2000 (Williams 2001). Numerous lepidopteran pests including tobacco budworms, *Heliothis virescens* (F.); bollworms, *Helicoverpa zea* (Boddie); and pink bollworms, *Pectinophora gossypiella* (Saunders), are susceptible to the CryIA(c) protein in Bollgard cottons (MacIntosh et al. 1990, Luttrell et al. 1999). Bollgard cottons have continued to provide satisfactory control of tobacco budworm and pink bollworm populations and suppress other lepidopteran pests when densities are low to moderate. However, when high population densities of bollworms persist for several days, supplemental control with insecticides is often needed to prevent economic injury (Stewart et al. 2001).

Bollworm larvae are commonly observed in white flowers of Bollgard plants (Smith 1998, Pietrantonio and Heinz 1999). In a laboratory bioassay, Stewart et al. (2001) observed 10 to 48% survival of bollworm larvae on Bollgard flowers and bolls at 4 d. In field tests,

bollworm feeding caused 55% abscission of bolls infested at anthesis (Gore et al. 2000). During 1996, bollworm populations were extremely high in most cotton producing areas of the United States (Williams 1997). Consequently, crop advisors in those regions observed the presence of large numbers of bollworm larvae in Bollgard cotton fields. The majority of these populations consisted of small larvae (≤ second instar) feeding within white flowers and under dried flower corollas on small bolls.

Currently, there is no clear explanation of why bollworms are more commonly found in white flowers of Bollgard cotton than non-Bollgard cotton. Ovipositional preferences of bollworm between Bollgard and non-Bollgard have been evaluated as a possibility. Differences in sites of oviposition would not be expected between Bollgard cotton and non-Bollgard cotton because the Cry1Ac protein in Bollgard cotton does not affect bollworm adults (MacIntosh et al. 1990). Furthermore, the morphology of Bollgard cottons should be similar to the parental non-Bollgard breeding lines. Parker and Luttrell (1998) found no differences in tobacco budworm egg density or vertical distribution of eggs on plants on Bollgard cottons compared with the non-Bollgard parental cottons. Similarly, no differences in sites of bollworm oviposition were detected on Bollgard cotton compared to conventional cotton (Roof et al. 2001). In Louisiana, egg densities of the soybean looper, *Pseudoplusia includens* (Walker), were not different on a Bollgard cultivar and a non-Bollgard cotton cultivar (Hall 2000).

Dispersal of early instar bollworm larvae may be different on Bollgard cotton plants compared to non-Bollgard cotton plants. In laboratory bioassays, bollworm larvae moved from cotton leaves treated with foliar *B. thuringiensis* formulations and were found at other locations in the test arena (Jyoti et al. 1996). Also, bollworm larvae avoided feeding on meridic diets

containing purified *B. thuringiensis* proteins or lyophilized Bollgard plant tissues (Greenplate et al. 1998, Akin et al. 2001). Tobacco budworm larval movement has been observed to be different on Bollgard cotton plants compared to non-Bollgard plants in field and greenhouse studies (Benedict et al. 1993, Parker and Luttrell 1999). In both of these studies, tobacco budworm larvae moved from Bollgard plant terminals more rapidly than from non-Bollgard plant terminals. However, the fate of larvae after leaving the terminals was not reported. Larvae are the developmental stage controlled by the Cry1A(c) protein in Bollgard cotton, and differences in larval behavior could result in feeding preferences on specific plant parts. Terminal foliage expresses higher levels of CryIA(c) than other plant parts (Greenplate 1999, Greenplate et al. 2000). Levels of CryIA(c) expression in terminal foliage and fruiting forms on node nine averaged 68.1 and 26.5 μg/g dry weight, respectively (Greenplate 1999). In a similar study, CryIA(c) expression was higher in white flowers compared with squares and bolls (Adamczyk et al. 2001). Although protein expression was not measured in foliage, CryIA(c) expression was higher in bracts compared to flowers, squares, and bolls (Adamczyk et al. 2001). Variation in protein expression among different plant parts combined with bollworm detection and avoidance of the protein could result in bollworm populations becoming established on those structures with low protein expression. Field studies were conducted to evaluate bollworm larval behavior on Bollgard cotton plants compared to non-Bollgard plants and to determine their preferred feeding sites. Data from three studies conducted during pre-flowering and flowering stages are presented.

Materials and Methods

Blocks (16 rows x 100 ft.) of a Bollgard cotton cultivar (NuCOTN 33B, Delta and Pine Land Co. Scott, MS) and a non-Bollgard parental cultivar (Deltapine 5415, Delta and Pine

Land Co. Scott, MS) were planted at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA from 7 May to 23 May during 1999, 2000, and 2001. Fertilization rates and general agronomic practices for cotton production followed current Louisiana Cooperative Extension Service recommendations.

Bollworms were collected from clover, *Trifolium* spp., during April and sweet corn, *Zea mays* L., (cv. SG 90) during June. Colonies were maintained in the laboratory for at least one generation to eliminate parasitoids, minimize pathogens, and obtain sufficient numbers of larvae at the proper stage for infestations on cotton plants. Larvae were fed a wheat germ/soy protein diet (*Heliothis* premix, Stonefly Industries, Bryan, TX) until pupation. Adults were held in 3.79-L cardboard containers and fed a 10% sugar-water solution. A single layer of cheesecloth was placed over the containers to provide an adequate surface for moth oviposition. Egg sheets were harvested daily and placed into plastic bags until larval eclosion. Upon eclosion, larvae were fed meridic diet in 236-ml cups (ca. 50 larvae/cup) for ca. 48 h. After 48±3 h, bollworm larvae were placed in the terminals of cotton plants during vegetative or reproductive developmental stages.

Infestation of Pre-flowering Cotton Plants. Individual Bollgard and non-Bollgard cotton plants were isolated by removing all adjacent plants before infestation so that no interplant movement of larvae could occur. A single bollworm larva (first instar, 48±3 h old) was placed in the terminal of a cotton plant with a small paintbrush. A 40.6-cm x 40.6-cm sticky trap was placed on the soil surface at the base of each infested plant. Sticky traps were used to recover larvae that apparently left plants by "spinning-down" on a silken thread. This experiment consisted of twelve replications over two years (1999 and 2000) in a completely randomized design. Replications were represented by day of infestation and 25 plants of

Bollgard and non-Bollgard cotton were infested each day. Numbers of larvae recovered from sticky traps and remaining in plant terminals were recorded at 1, 3, 6, and 24 h after infestation. Data were converted to percentages based on the number of plants infested on a given day and analyzed using repeated measures analysis of variance (SAS, PROC MIXED, Littell et al. 1996).

Infestation of Flowering Cotton Plants. First instar bollworm larvae (48±3 h old) were infested on individual Bollgard and non-Bollgard cotton plants (one larva/plant) during flowering growth stages in 2000 and 2001. Individual plants were isolated by removing all adjacent plants before infestation so that no interplant movement could occur. Procedures and experimental design for larval infestations were similar to those described for pre-flowering cotton plants except sticky traps were not used. Bollworm infested plants were examined at 3, 6, and 24 h after infestation. The number of main stem nodes that a larva moved from the plant terminal and plant structure (terminals, squares, flowers, bolls) infested with a larva was recorded. Data were analyzed using repeated measures analysis of variance (SAS, PROC MIXED, Littell et al. 1996).

In addition to single plant infestations, micro-plots (1 row x 1-m) were established within blocks of Bollgard and non-Bollgard cotton cultivars during 2000 and 2001. Plants in micro-plots were infested with 20 first instar bollworm larvae. Larvae were placed in the terminals of plants using a small paintbrush and were evenly distributed across all plants within the micro-plots. A total of 20 and 25 micro-plots were infested during 2000 and 2001, respectively, for non-Bollgard and Bollgard cotton. The experimental design was a randomized complete block and dates of infestation represented blocks. Whole plants within each micro-plot were inspected at 24 and 48 h after infestation. Plant, square, flower, and boll

densities were recorded from each micro-plot. Numbers of plant terminals, squares, flowers, and bolls infested with larvae were recorded. Data were converted to percentages of infested structures and analyzed using repeated measures analysis of variance (SAS, PROC MIXED, Littell et al. 1996).

Results

Bollworm Movement on Pre-flowering Cotton Plants. Bollworm movement was different on Bollgard plants compared to non-Bollgard plants at all rating intervals. Cotton type (F=25.47; df=1,10; P<0.01) and time of evaluation (F=54.15; df=3,30; P<0.01) effects were significant for the percentage of larvae remaining in cotton plant terminals (Figure 3.1). More larvae remained in the terminals of non-Bollgard cotton plants compared to Bollgard cotton plants at all rating intervals. At 1, 3, and 6 h, 47.8, 39.4, and 20.9% of larvae, respectively, remained in the terminals of non-Bollgard cotton plants. In contrast, only 28.7, 11.4, and 6.3% of the larvae remained in Bollgard cotton terminals at 1, 3, and 6 h, respectively. Within 24 h, nearly all (98.7%) larvae had left the terminals of Bollgard cotton plants while 87.0% of the larvae left the terminals of non-Bollgard cotton plants. Cotton type (F=41.70; df=1,10; P<0.01), time of evaluation (F=6.79; df=3,30; P<0.01), and the cotton type by time of evaluation interaction (F=3.63; df=3,30; P=0.02) was significant for percentages of larvae recovered from sticky traps (Figure 3.1). Higher percentages of bollworm larvae were observed on sticky traps beneath Bollgard plants compared to traps beneath non-Bollgard plants at all rating intervals. At 1 h after infestation, 17.8% of the total number of larvae placed on Bollgard cotton were recovered on sticky traps beneath plants compared to 6.1% beneath non-Bollgard plants. At 3 h after infestation, 36.6% of the total number of bollworm larvae placed on Bollgard plants were found on sticky traps compared to

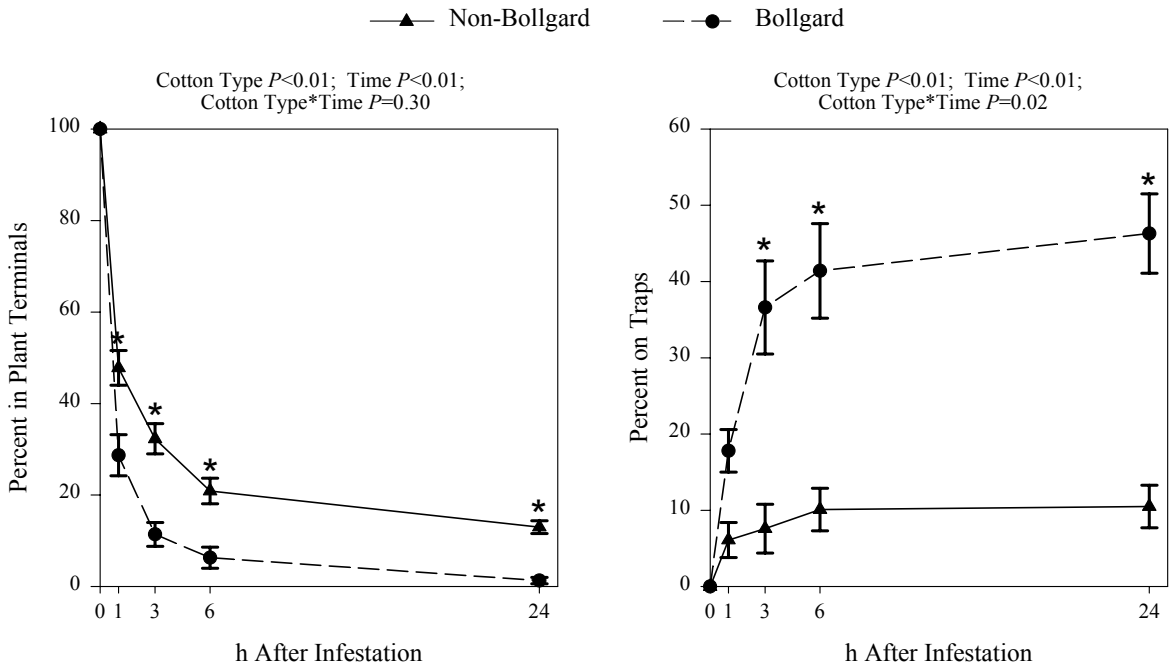

Figure 3.1. Percentage (± standard error) of bollworm larvae remaining in plant terminals and recovered from sticky traps for infestations on non-flowering Bollgard and non-Bollgard cottons (asterisks indicate rating intervals at which significant differences (α=0.05) occurred in the percentage of larvae recovered on Bollgard cotton and non-Bollgard cotton).

7.6% on non-Bollgard plants. At 6 h after infestation, 41.4% and 10.1% of the total number of larvae were recovered from sticky traps beneath Bollgard and non-Bollgard cotton plants, respectively. At 24 h after infestation, 46.3% of the larvae were recovered from sticky traps beneath Bollgard cotton plants compared to 10.5% beneath non-Bollgard cotton plants.

Bollworm Movement on Flowering Cotton Plants. Similar to the results for individual pre-flowering cotton plants, bollworm larvae moved significantly more on individual flowering Bollgard plants compared to non-Bollgard plants. Cotton type (F=59.67; df=1,8; P<0.01), time of evaluation (F=29.76; df=2,16; P<0.01), and the cotton type by time of evaluation interaction (F=5.16; df=2,16; P=0.02) was significant for numbers of main stem nodes larvae were found below terminals (Figure 3.2). Within 3 h, larvae moved 2.8 nodes below the terminal on Bollgard cotton whereas those larvae on non-Bollgard cotton moved 1.2 nodes below the terminal. Bollworm larvae were found 4.1 main stem nodes below plant terminals on Bollgard cotton compared to 1.8 main stem nodes below plant terminals on non-Bollgard cotton at 6 h. At 24 h, larvae were found an average of 5.7 main stem nodes below the terminals on Bollgard plants compared to 2.4 main stem nodes below the terminals on non-Bollgard cotton.

Cotton type (F=24.20; df=1,8; P<0.01) and time of evaluation (F=9.14; df=2,16; P<0.01) effects were significant for numbers of bollworm infested terminals (Figure 3.3). For numbers of bollworm infested squares (F=5.59; df=2,16; P=0.01) and bolls (F=5.34; df=2,16; P=0.02) there were cotton type by time of evaluation interactions. Also, there was a cotton type effect for numbers of bollworm infested white flowers (F=36.42; df=1,8; P<0.01). On Bollgard cotton, fewer larvae remained in plant terminals compared to non-Bollgard cotton at all rating intervals. Fewer larvae were observed on Bollgard squares than on non-Bollgard

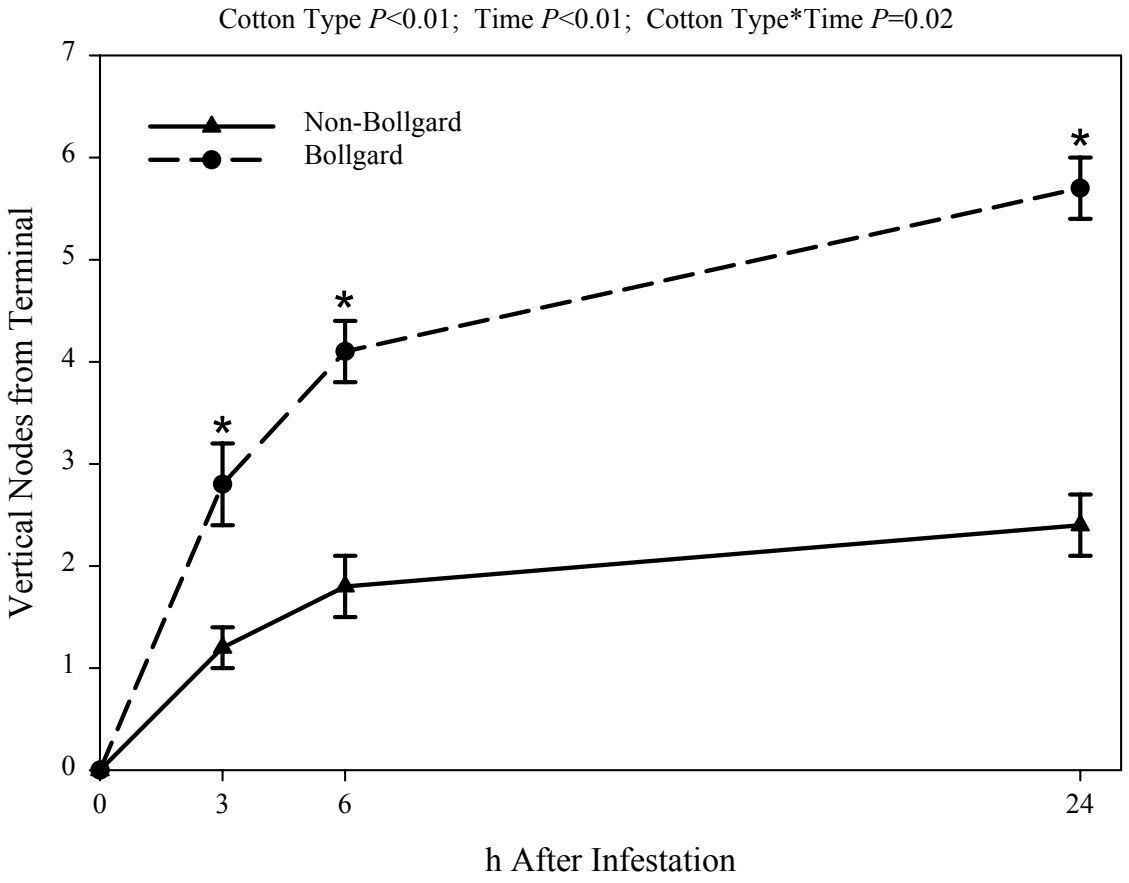

Figure 3.2. Vertical movement of bollworm larvae from Bollgard and non-Bollgard cotton terminals (asterisks indicate rating intervals at which significant differences (α=0.05) occurred in the percentage (± standard error) of larvae recovered on Bollgard cotton and non-Bollgard cotton).

squares at 24 h. Consequently, more larvae were recovered lower in the plant canopy in white flowers (1.0 vs 0.1) and bolls (4.7 vs 0.8) on Bollgard plants than on non-Bollgard plants at 24 h. No larvae were recovered from non-Bollgard white flowers at 3 and 6 h.

In the micro-plots, numbers of plants, squares, flowers, and bolls ranged from 5 to 10, 56 to 153, 0 to 9, and 24 to 87, respectively, within Bollgard and non-Bollgard micro-plots during the infestation period. There was a cotton type by time of evaluation interaction for the percentage of bollworm infested terminals (F=14.78; df=1,88; P<0.01) (Figure 3.4). Also, percentages of infested squares (F=12.09; df=1,88; P<0.01), white flowers (F=14.15; df=1,88; P<0.01), and bolls (F=28.20; df=1,88; P<0.01) were different between cotton types (Figure 3.4). Fewer bollworm larvae remained in plant terminals of Bollgard cotton (1.8%) compared to that of non-Bollgard cotton plants (20.3%) at 24 h. Within 48 h, the percentage of bollworm infested terminals decreased to 8.6% for non-Bollgard cotton; however, this remained higher than for Bollgard cotton (1.5%). Also, the percentage of bollworm infested squares was lower on Bollgard cotton (1.1 to 1.5%) than on non-Bollgard cotton (2.2 to 3.1%). Similar to the previous experiment, the percentages of infested white flowers and bolls were higher on Bollgard cotton than on non-Bollgard cotton. On Bollgard cotton, the percentages of bollworm infested white flowers was 8.0% at 24 h and 6.8% at 48 h; whereas, the percentage of bollworm infested white flowers on non-Bollgard cotton was less than 1.5%. Similarly, the percentage of infested bolls exceeded 7.5% at 24 and 48 h on Bollgard cotton and remained less than 2.0% on non-Bollgard cotton.

Discussion

Large numbers of bollworm larvae have been observed in white flowers of Bollgard cotton every year since its introduction in 1996. Bollworm eggs are generally concentrated in

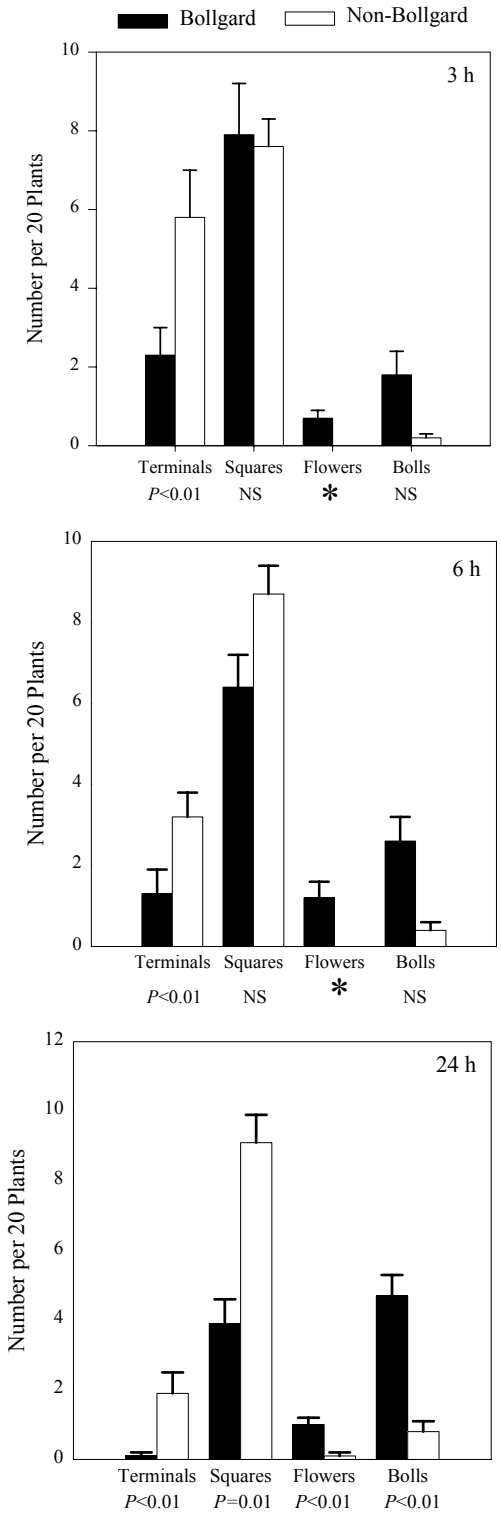

Figure 3.3. Percentage (± standard error) of bollworm infested plant structures within the canopies of individual flowering Bollgard and non-Bollgard cotton plants at 3, 6, and 24 h after infestation (* no larvae were recovered from non-Bollgard white flowers).

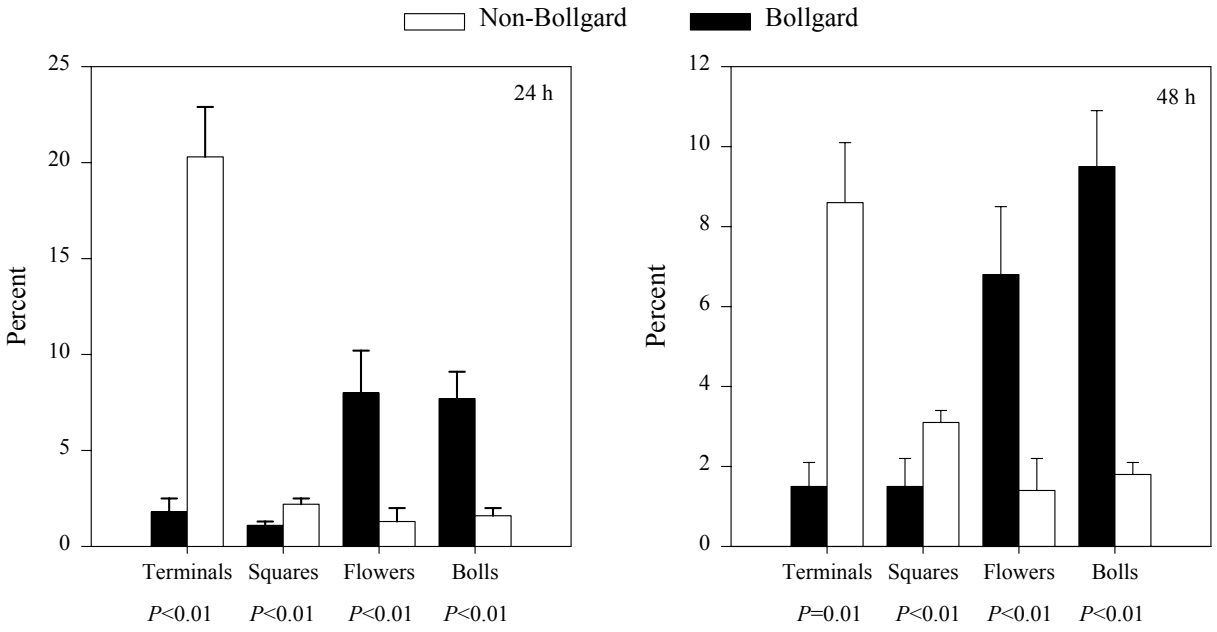

Figure 3.4. Percentage (± standard error) of infested Bollgard and non-Bollgard plant structures within micro-plots at 24 and 48 h after infestation.

the top one third of cotton plants and the majority of eggs are usually near plant terminals (Wilson et al. 1980, Farrar and Bradley 1985). Small bollworm larvae remain near the terminals of non-Bollgard cotton plants feeding on small squares (Reese et al. 1981). Fye (1972) found that 78 to 100% of damaged fruiting forms could be found in the top 0.6-m of plants at any given time. As larvae develop, they typically move down the plants feeding on larger squares and bolls (Wilson and Gutierrez 1980). Data in the present study indicate that bollworm larvae disperse more rapidly on Bollgard cotton compared to non-Bollgard cotton. Bollworm larvae moved 2.9 main stem nodes below Bollgard plant terminals within 3 h, but only moved 2.5 main stem nodes within 6 h on non-Bollgard plants. Also, those larvae ultimately moved a greater vertical distance on Bollgard cotton (5.7 nodes at 24 h) than on non-Bollgard cotton (2.4 nodes at 24 h). Larvae remained near the top of non-Bollgard cotton plants feeding on terminal foliage and small squares. In contrast, larvae were observed lower in the plant canopy on Bollgard cotton feeding on white flowers and bolls.

Results similar to those found in the present study have been observed previously. Benedict et al. (1992, 1993) and Parker and Luttrell (1999) found that tobacco budworm larvae exhibit different dispersal patterns on Bollgard cotton than on non-Bollgard cotton. In those studies, higher numbers of tobacco budworm larvae left the terminals of Bollgard cotton than non-Bollgard cotton. Bollworm larvae may exhibit this same behavior because they have demonstrated the ability to detect and avoid *B. thuringiensis* proteins in foliar sprays (Jyoti et al. 1996, Greenplate et al. 1998). In the present study, bollworm larvae began migrating away from Bollgard cotton terminals within 1 h. Within 6 h, less than 10% of larvae remained in Bollgard terminals. In a laboratory bioassay, Gould et al. (1991) found that tobacco budworm larvae were able to avoid *B. thuringiensis* proteins. Also, previous studies have shown that *B.*

thuringiensis proteins elicit avoidance behavior in other insects including the light brown apple moth larvae, *Epiphyas postvittana* (Walker), (Harris et al. 1997); gypsy moth larvae, *Lymantria dispar* (L.), (Yendol et al. 1975); and several insect pests of corn (Mohd-Salleh and Lewis 1982). In addition, bollworm larval behavior is affected by natural allelochemicals in cotton (Schmidt et al. 1988) and tomato (Cosenza and Green 1979, Binder and Bowers 1991, Juvik et al. 1994).

Cotton pest management consultants have experienced difficulties in making decisions about when to apply foliar insecticides to manage bollworms in Bollgard cotton. Currently, action thresholds to initiate heliothine (bollworm/tobacco budworm) control with foliar sprays are based on numbers of eggs and/or larvae in terminals, and numbers of larval infested/damaged squares on non-Bollgard cotton. In Louisiana, insecticide applications are recommended when at least 5 live larvae per 100 plants plus eggs are present on non-Bollgard cotton (Bagwell et al. 2000). If current thresholds for bollworm and tobacco budworm in non-Bollgard cotton are used, the assumption that bollworm damage potential is the same on Bollgard and non-Bollgard cotton would have to be met. Gore et al. (in press) found that an individual bollworm larva damaged as many as 3.5 fruiting forms on Bollgard cotton compared to 6.6 on non-Bollgard cotton. Therefore, current thresholds for non-Bollgard cotton are not appropriate for Bollgard cotton because damage potentials are not the same.

Currently, non-Bollgard cotton fields are scouted by examining plant terminals and squares. Current scouting methods are not appropriate for Bollgard cotton because larvae feeding on white flowers and bolls may be overlooked. For the 1-m row infestations, the percentage of infested terminals averaged 12.2% on non-Bollgard cotton at 48 h. This level is above the current action threshold and the non-Bollgard plots would be treated with foliar

insecticide applications. Also, 3.2% of non-Bollgard squares were infested with larvae. In contrast, 1.2% and 0.8% of Bollgard terminals and squares were infested with larvae, respectively, within 48 h. Based on current action thresholds, Bollgard cotton would not require treatment. However, if the percentages of infested flowers (9.7%) and bolls (4.2%) are also considered, Bollgard cotton may require insecticide applications to prevent economic yield loss.

In addition, bollworm larvae began moving out of plant terminals within 1 h on Bollgard cotton. Therefore, when eggs hatch, there is a narrow period of time when larvae can still be observed in or near plant terminals. Over 90% of larvae that were originally infested on pre-flowering Bollgard plants migrated away from plant terminals within 6 h. Field scouts searching for bollworm infestations in Bollgard cotton are likely not to find larvae in the terminals when sampling more than 6 h after larval eclosion.

These data suggest that current scouting protocols and action levels to initiate insecticide treatments for bollworms on non-Bollgard cotton are not appropriate for Bollgard cotton. Scouts should look at white flowers and small bolls in addition to terminals and squares when scouting Bollgard cotton because bollworm larvae migrate to those structures in a relatively short time. This information is necessary to further refine action thresholds for bollworms in Bollgard cotton.

References Cited

Adamczyk, J. J., Jr., D. D. Hardee, L. C. Adams, and D. V. Summerford. 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ-endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 94: 284-290.

Akin, D. S., S. D. Stewart, and K. S. Knighten. 2001. Behavioral response of lepidopteran pests on cotton expressing insecticidal proteins of *Bacillus thuringiensis*, (in press). *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Bagwell, R. D., J. W. Barnett, B. R. Leonard, E. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2000. Cotton insect control 2000. Louisiana Cooperative Extension Service, Baton Rouge, Pub. 1083. 7 pp.

Benedict, J. H., D. W. Altman, P. F. Umbeck, and D. R. Ring. 1992. Behavior, growth, survival, and plant injury by *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) on transgenic Bt cottons. J. Econ. Entomol. 85: 589-593.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone, and S. R. Sims. 1993. Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Binder, B. F., and W. S. Bowers. 1991. Behavioral changes and growth inhibition in last instar larvae of *Heliothis zea* induced by oral and topical application of precocene II. Entomol. Exp. Appl. 59: 207-217.

Cosenza, G. W., and H. B. Green. 1979. Behavior of the tomato fruitworm, *Heliothis zea* (Boddie), on susceptible and resistant lines of processing tomatoes. Hortscience 14: 171-173.

Farrar, R. R., and J. R. Bradley, Jr. 1985. Within-plant distribution of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs and larvae on cotton in North Carolina. Environ. Entomol. 14: 205-209.

Fye, R. E. 1972. Preliminary investigation of vertical distributions of fruiting forms and insects on cotton plants. J. Econ. Entomol. 65: 1410-1414.

Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. J. Econ. Entomol. 93: 690-696.

Gore, J., B. R. Leonard, and R. H. Gable. 2002. Damage potential of bollworm, *Helicoverpa zea* (Boddie), larvae feeding on white flowers of genetically engineered *Bacillus thuringiensis* var. *kurstaki* Berliner cotton. J. Econ. Entomol. (in press).

Gould, F., A. Anderson, D. Landis, and H. V. Mellaert. 1991. Feeding behavior and growth of *Heliothis virescens* larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. Entomol. Exp. Appl. 58: 199-210.

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92: 1377-1383.

Greenplate, J. T., G. P. Head, S. R. Penn, and V. T. Kabuye. 1998. Factors potentially influencing the survival of *Helicoverpa zea* on Bollgard® cotton, pp. 1030-1033. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J. T., S. R. Penn, J. W. Mullins, and M. Oppenhuizen. 2000. Seasonal CryIAc levels in DP50B: the "Bollgard® basis" for Bollgard II, pp. 1039-1040. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Hall, T. S. 2000. Performance of selected insecticides against soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) when applied to different host plants. M.S. Thesis, Louisiana State University Agricultural Center, Baton Rouge.

Harris, M. O., F. Mafile'o, and S. Dhana. 1997. Behavioral responses of lightbrown apple moth neonate larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. Entomol. Exp. Appl. 84: 207-219.

Juvik, J. A., J. A. Shapiro, T. E. Young, and M. A. Mutschler. 1994. Acylglucoses from wild tomatoes alter behavior and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Econ. Entomol. 87: 482-492.

Jyoti, J. L., S. Y. Young, D. T. Johnson, and R. W. McNew. 1996. *Helicoverpa zea* (Lepidoptera: Noctuidae): larval location, mortality, and leaf area consumption on *Bacillus thuringiensis*-treated cotton. Environ. Entomol. 25: 1438-1443.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® system for mixed models. SAS Institute, Cary, NC.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebrate Path. 56: 258-266.

Mohd-Salleh, M. B., and L. C. Lewis. 1982. Feeding deterrent response of corn insects to β-exotoxin of *Bacillus thuringiensis*. J. Invertebrate Path. 39: 323-328.

Parker, C. D., Jr., and R. G. Luttrell. 1998. Oviposition of tobacco budworm (Lepidoptera: Noctuidae) in mixed plantings of non-transgenic and transgenic cottons expressing δ-endotoxin protein of *Bacillus thuringiensis* (Berliner). Southwestern Entomol. 23: 247-257.

Parker, C. D., Jr. and R. G. Luttrell. 1999. Interplant movement of *Heliothis virescens* (Lepidoptera: Noctuidae) larvae in pure and mixed plantings of cotton with and without expression of the Cry1Ac δ-endotoxin protein of *Bacillus thuringiensis* Berliner. J. Econ. Entomol. 92: 837-845.

Pietrantonio, P. V., and K. Heinz. 1999. Distribution of Heliothine larvae in *B. t.* and non-*B. t.* cotton in Texas, pp. 945-948. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischoff. 1990. Insect resistant cotton plants. Biotechnology 8: 839-943.

Reese, J. C., B. G. Chan, N. R. Malm, and A. C. Waiss, Jr. 1981. Feeding sites of bollworm larvae on cotton. Environ. Entomol. 10: 81-84.

Roof, M. E., and J. A. Durant. 1997. On-farm experiences with Bt cotton in South Carolina, p. 861. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roof, M. E., J. A. DuRant, and G. McCutcheon. 2001. Ovipositional-site preferences of bollworm/budworm on Bt and non-Bt cotton plants, p. 969. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Schmidt, K. M., J. H. Benedict, and M. H. Walmsley. 1988. Behavioral responses (time budgets) of bollworm (Lepidoptera: Noctuidae) larvae for three cotton cultivars. Environ. Entomol. 17: 350-353.

Smith, R. H. 1998. Year two of Bollgard behind boll weevil eradication: Alabama observations, pp. 965-966. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival on Noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Wilson, L. T., and A. P. Gutierrez. 1980. Fruit predation submodel: *Heliothis* larvae feeding upon cotton fruiting structures. Hilgardia 48: 24-36.

Wilson, L. T., A. P. Gutierrez, and T. F. Leigh. 1980. Within-plant distribution of the immatures of *Heliothis zea* (Boddie) on cotton. Hilgardia 48: 12-23.

Williams, M. R. 1997. Cotton insect losses 1996, pp. 834-853. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

2001. Cotton insect loss estimates – 2000, pp. 774-777. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Yendol, W. G., R. A. Hamlen, and S. B. Rosario. 1975. Feeding behavior of gypsy moth larvae on *Bacillus thuringiensis*-treated foliage. J. Econ. Entomol. 68: 25-27.

CHAPTER 4

DISTRIBUTION OF BOLLWORM (LEPIDOPTERA: NOCTUIDAE) DAMAGED REPRODUCTIVE STRUCTURES ON GENETICALLY ENGINEERED, *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* BERLINER, COTTON

Introduction

Cotton insect pest management is becoming more complex with the introduction of genetically engineered (Bollgard) varieties. Bollgard cottons produce the insecticidal Cry1Ac protein from *Bacillus thuringiensis kurstaki* Berliner (Perlak et al. 1990) that is toxic only to larvae from the insect order Lepidoptera (MacIntosh et al. 1990). Tobacco budworms, *Heliothis virescens* (F.); pink bollworms, *Pectinophora gossypiella* (Saunders); and bollworms, *Helicoverpa zea* (Boddie) all are susceptible to Cry1Ac and have been the primary targets of Bollgard cotton. However, under certain situations, the CryIAc protein produced by Bollgard cotton has provided less than adequate control of bollworms (Stewart et al. 2001).

Several factors contribute to bollworm infestations becoming established in Bollgard cultivars. Bollworms are inherently less toxic to the CryIAc protein in Bollgard (Luttrell et al. 1999). Also, CryIAc levels vary both temporally (Greenplate 1999) and spatially (Greenplate 1999, Adamczyk et al. 2001). In general, protein expression in bolls is lower than in squares (Greenplate et al. 1998, Adamczyk et al. 2001). Bollworm populations feeding on older reproductive structures low in the plant canopy during the late season may not be controlled as effectively by Bollgard cotton as populations during the early fruiting period or on younger structures in the upper portions of the plant canopy. Bollworm survival is higher in white flowers than in other reproductive structures (Gore et al. 2001). Consequently, injury to white flowers is greater than for other plant structures (Gore et al. 2000a). The temporal and spatial variability in protein expression in Bollgard plants coupled with differences in larval behavior

(Gore et al. in press), result in bollworm populations becoming established low in the plant canopy. Approximately 25% of the Bollgard acreage in the United States receives at least one insecticide application annually targeting bollworm populations (Williams 2001). However, no information is currently available characterizing the levels of injury those populations cause.

Bollgard has not provided acceptable levels of bollworm control in all situations. Therefore, a second generation of genetically engineered insect resistant cotton is being developed. These experimental cotton lines (Bollgard II, Monsanto Co., St. Louis, Mo.) express two separate *B. thuringiensis* proteins (Cry1Ac and Cry2Ab) to improve efficacy against bollworms and other lepidopteran pests (Greenplate et al. 2000a). Cry1Ac protein expression in Bollgard II is similar to the level of Cry1Ac expressed in Bollgard. The addition of Cry2Ab in Bollgard II increases the amount of total insecticidal protein produced above that produced by Bollgard (Greenplate et al. 2000b). Bollgard II cotton lines have demonstrated significantly better control of bollworms and other lepidopteran pests than that observed with Bollgard cotton (Gore et al. 2001, Stewart et al. 2001).

Before Bollgard and Bollgard II cottons can be fully integrated into pest management systems, research needs to be conducted to determine if and when insecticide applications should be initiated for bollworm control. Currently, the levels of fruiting form injury caused by bollworms after they leave white flowers have not been quantified on Bollgard cotton. Therefore, this study was conducted to determine the level of fruiting form injury from bollworm larvae feeding in white flowers of Bollgard and Bollgard II cottons.

Materials and Methods

Two studies were conducted at the Macon Ridge location of the Northeast Research Station near Winnsboro, Louisiana in field plots during 2000 and 2001. The first experiment

evaluated a commercial Bollgard variety (cv. NuCOTN 33B, Delta and Pine Land Co., Scott, MS) that produces a single *B. thuringiensis* protein (Cry1Ac) and its closest conventional (non-transgenic) parental variety (cv. Deltapine 5415, Delta and Pine Land Co., Scott, MS). In the second experiment, an experimental genetically engineered cotton that produces two *B. thuringiensis* proteins (Bollgard II, Cry1Ac + Cry2Ab, Monsanto Co., St. Louis, MO) was evaluated along with a similar commercial Bollgard variety (cv. Deltapine 50B, CryIAc) and conventional parental variety (cv. Deltapine 50). Multiple seeding dates were used in the first study so that plants at the proper stage for infestations would be available over an extended period of time, thereby increasing the overall sample size. Plots (16 rows by 15 m) of Deltapine 5415 and NuCOTN 33B were planted on 2 and 21 May during 2000 and 26 April and 15 May 2001. Plots (1 row by 20 m) of Deltapine 50, Deltapine 50B, and experimental Bollgard II were planted on 12 June during 2001. Bollworm larvae were collected from clover, *Trifolium* spp., during late April and from sweet corn, *Zea mays* L., during early June each year. Colonies were maintained in the laboratory for one generation to obtain sufficient numbers of larvae at the proper developmental stage for infestations. Bollworm larvae were fed meridic diet (*Heliothis* premix, Stonefly Industries, Bryan, TX) in individual 29.5 ml plastic cups (Solo Co., Urbana, IL). Adults were maintained in 2.79 L cardboard buckets and fed a 10% sucrose solution. The tops of the buckets were covered with a single layer of Veratec graphic arts cheesecloth (BBA Nonwovens, Walpole, MA) to provide a surface for oviposition. Egg sheets were harvested daily and placed into plastic bags. Upon eclosion, neonates (ca. 50/cup) were fed meridic diet in 236 ml waxed paper cups (Chinet Co., East Providence, RI) for 24 ± 3 h. Larvae were fed diet for 24 h before being placed onto plants to minimize mortality from handling neonates in the field.

Infestation Procedures. Cotton plants used in these experiments were at the six to nine main stem nodes above white flower growth stages. Plant growth stages were determined by counting the number of main stem nodes from the uppermost first position white flower to the last unfolded leaf in plant terminals (Bourland et al. 1992). Individual plants were randomly selected within field plots and isolated by removing surrounding plants so that no interplant movement could occur. White flowers at the site of infestation were tagged with a yellow snap on tag (A. M. Leonard, Inc., Piqua, OH).

In the first experiment, two first instar bollworm larvae (24 ± 8 h old) were placed in first position white flowers on single plants from each variety using an artists paint brush. Fifty Deltapine 5415 (conventional) and NuCOTN 33B (Bollgard) plants were infested on each d. Infestations were arranged in a randomized complete block design with d of infestation representing blocks (replicates). Larvae were placed on separate cohorts of plants on three and five dates during 2000 and 2001, respectively.

In the second study, plant availability was limited; therefore, only 10 plants were infested on each d for Deltapine 50, Deltapine 50B, and the Bollgard II line. Infestation procedures followed those described for the first experiment. Plants were infested on six different dates for these varieties in 2001. Plants in both experiments were visually inspected at 3 d for damage to the fruiting structure at the infested site and for the presence of larvae. Thereafter, entire plants were inspected every 2 d for cumulative damage to fruiting structures (squares, white flowers, bolls) until larvae were no longer present. In addition to the infested plants, non-infested plants were monitored for natural abscission of fruiting structures. Data for cumulative numbers of damaged fruiting forms were analyzed using repeated measures analysis of variance where damage was recorded from the same experimental units over

different rating intervals (PROC MIXED, Littell et al. 1996). Data for total number of damaged fruiting forms by an individual larva was analyzed using analysis of variance (PROC MIXED, Littell et al. 1996).

Results

Bollworm Damaged Fruiting Forms on Bollgard (cv. NuCOTN 33B) Cotton.

Bollworms damaged more fruiting forms on non-Bollgard cotton than on Bollgard cotton. Effects for cotton type (F=7.17; df=1, 8; P=0.03) and time of evaluation (F=7.54; df=4, 32; P<0.01) were significant for bollworm damage to fruiting forms (Figure 4.1). The interaction between cotton type and time of evaluation (F=3.22; df=4, 32; P=0.02) also was significant. Initial damage (3 d) at the site of infestation was 18.4 (36.8%) and 12.6 (25.2%) fruiting forms per 50 plants on non-Bollgard and Bollgard cotton, respectively. At 5 d on non-Bollgard cotton, bollworms damaged 30.9 fruiting forms per 50 plants consisting of 8.2 squares and 22.5 bolls. Cumulative damage increased to 40.3 fruiting forms per 50 plants for non-Bollgard cotton at 7 d (Figure 4.1). Damaged fruiting forms consisted of 11.9 squares and 28.3 bolls. Beyond 7 d, damage began to decrease on non- Bollgard cotton because bollworms were beginning to complete larval development. At 9 d on non-Bollgard cotton, cumulative bollworm damage averaged 46.6 fruiting forms per 50 plants and consisted of 13.3 squares and 32.9 bolls (Figure 4.1). No additional damage was observed at 11 d. Damage to white flowers was minimal in this study.

Additional damage beyond that at the site of infestation was observed on Bollgard cotton; however, damage did not increase as rapidly as it did on non-Bollgard cotton. Bollworms damaged a mean of 16.8 fruiting forms per 50 plants at 5 d (Figure 4.1). Numbers of damaged squares and bolls ranged from 0.0 to 7.0 (μ=2.0) and 6.0 to 24.0 (μ=14.5), respectively. At 7 d,

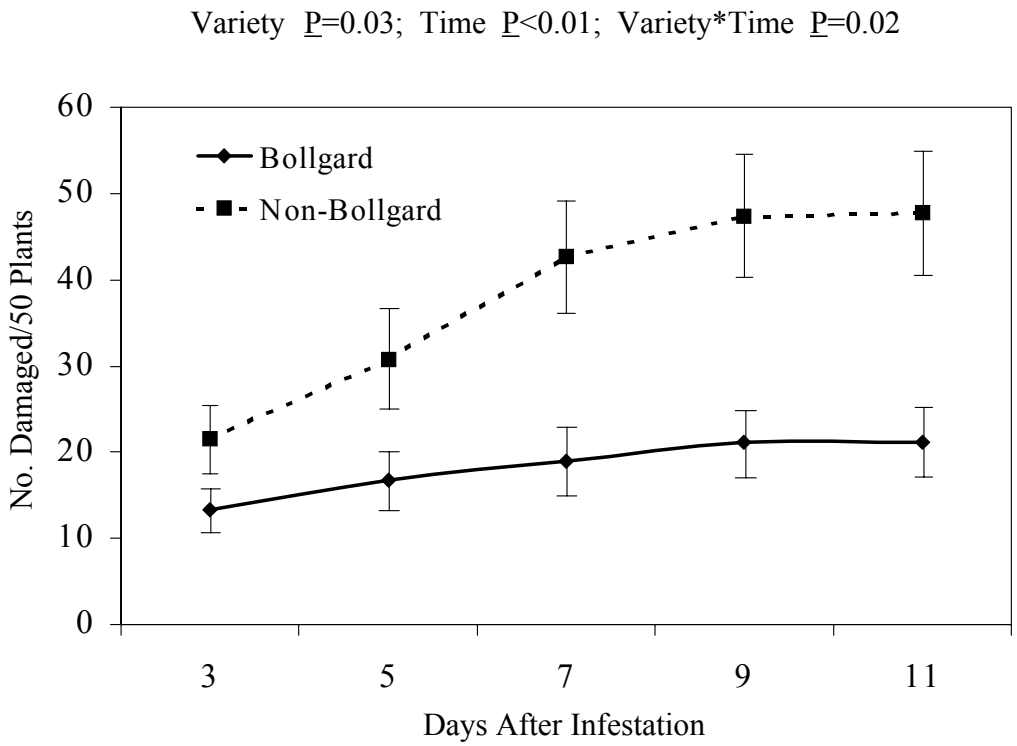

Figure 4.1. Numbers ± standard errors of damaged fruiting forms (squares, flowers, and bolls) by all larval instars of bollworms on Deltapine NuCOTN 33B (Bollgard) and Deltapine 5415 (non-Bollgard) over time.

bollworms damaged a mean of 17.9 fruiting forms per 50 plants on Bollgard cotton. Numbers of damaged squares and bolls per 50 plants ranged from 0.0 to 7.0 (μ=2.5) and 3.0 to 26.0 (μ=15.0), respectively. At 9 d, bollworms damaged a mean of 18.9 fruiting forms per 50 plants. Numbers of damaged squares and bolls ranged from 0.0 to 7.0 (μ=2.9) and 3.0 to 27.0 (μ=16.0), respectively.

At 5 d, a lower percentage of bollworms were recovered from Bollgard (72.3%) cotton than non-Bollgard (97.5%) cotton (Figure 4.2). Beyond 5 d, the percentage of bollworms recovered from non-Bollgard cotton declined more rapidly than on Bollgard cotton. Consequently, percentage recovery of larvae was similar between Bollgard (59.1%) cotton and non-Bollgard (56.7%) cotton at 7 d. At 9 d, a higher percentage of larvae remained on Bollgard (31.6%) plants than on non-Bollgard (2.8%) plants. No larvae were recovered beyond 9 d on either cotton variety.

An individual bollworm damaged more squares (F=45.18; df=1, 7; P<0.01), bolls (F=20.17; df=1, 7; P<0.01), and total fruiting forms (F=46.05; df=1, 7; P<0.01) through all larval stadia on non-Bollgard cotton than on Bollgard cotton (Figure 4.3). On non- Bollgard cotton, 4.3 fruiting forms (2.9 bolls, 0.1 white flowers, and 1.3 squares) were damaged per larva. On Bollgard cotton, 2.7 fruiting forms (2.1 bolls, 0.1 white flowers, and 0.5 squares) were damaged per larva.

Bollworm Damaged Fruiting Forms on Bollgard (cv. Deltapine 50B) and Bollgard II (experimental) Cotton. Similar to the previous study, bollworms damaged more fruiting forms on non-Bollgard cotton than on Bollgard cotton. Also, bollworms damaged more fruiting forms on Bollgard cotton than on Bollgard II cotton. Effects for cotton type (F=18.98;

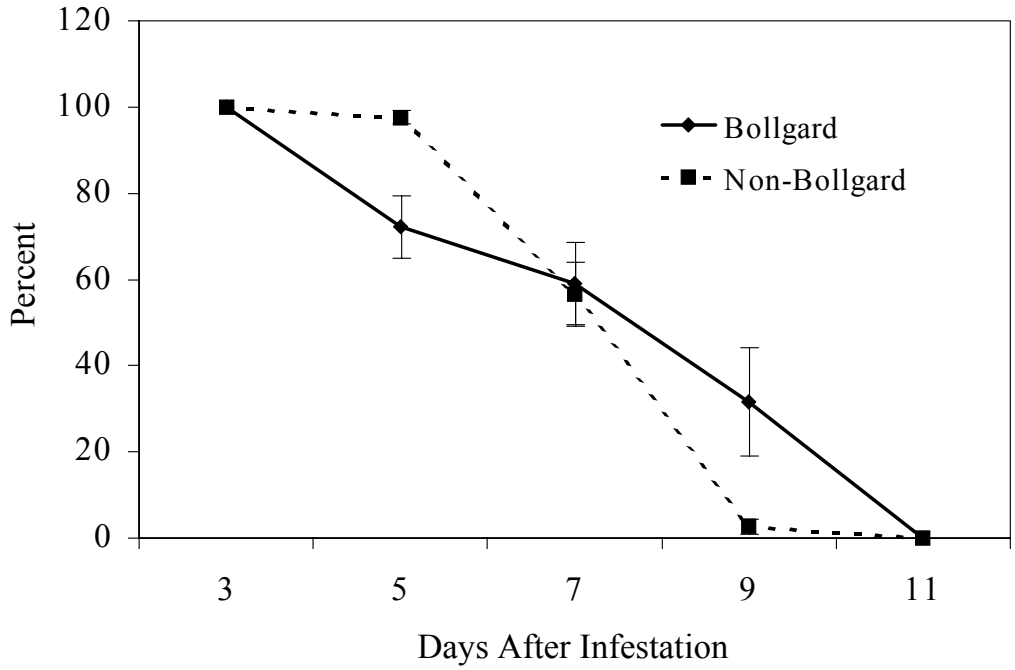

Figure 4.2. Bollworm larvae remaining on Deltapine 5415 (non-Bollgard) and NuCOTN 33B (Bollgard) cotton plants after feeding on white flowers (points represent the percentage of larvae ± standard error recovered during the initial 72 h rating period).

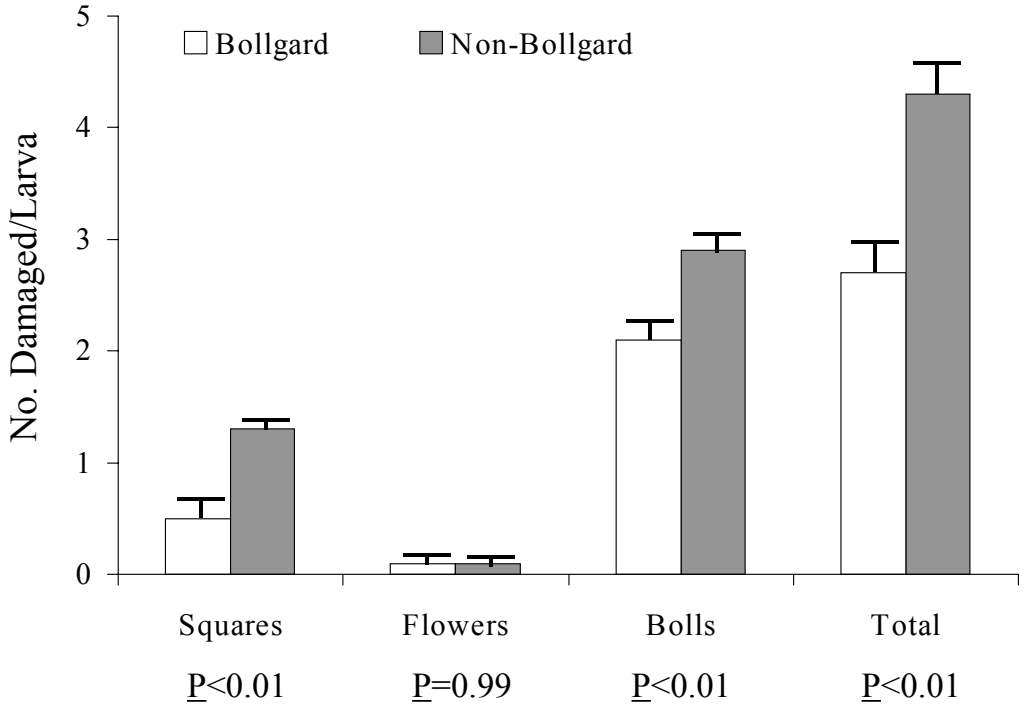

Figure 4.3. Bollworm injury to Deltapine NuCOTN 33B (Bollgard) and Deltapine 5415 (non-Bollgard) cotton after feeding on white flowers (bars represent means ± standard errors numbers of damaged structures by a bollworm larva during the completion of all larval stadia).

df=2, 15; *P*<0.01) and time of evaluation (*F*=26.14; *df*=4, 60; *P*<0.01) for bollworm damage to fruiting forms were observed. A cotton type by time of evaluation (*F*=9.89; *df*=8, 60; *P*<0.01) interaction also was observed. At 3 d, bollworms damaged 8.5 (85%), 6.3 (63%), and 5.7 (57%) bolls per 10 plants on non-Bollgard, Bollgard, and Bollgard II, respectively, at the site of infestation (Figure 4.4). After the initial 3 d period, bollworm damaged fruiting forms increased rapidly on non-Bollgard cotton. At 5 d, bollworms damaged 18.2 fruiting forms per 10 plants on non-Bollgard cotton. Damaged fruiting forms included 5.8 squares and 12.0 bolls per 10 plants. At 7 d on non-Bollgard cotton, 23.6 fruiting forms (8.2 squares and 14.7 bolls) per 10 plants were damaged by bollworms. Bollworm damage decreased after 7d; however, 25 fruiting forms were damaged per 10 plants by bollworms at 9 d on non-Bollgard cotton. Numbers of bollworm damaged squares and bolls averaged 9.0 and 15.3, respectively, on non-Bollgard cotton. No additional injury was observed beyond 9 d on non-Bollgard cotton. Cumulative bollworm damage increased slightly after the initial 72 h period on Bollgard cotton (Figure 4.4). At 5 d, bollworm larvae damaged nine fruiting forms per 10 plants consisting of 0.7 squares and 8.0 bolls. At 7 d, bollworms damaged a mean of 1.0 squares and 8.7 bolls for a total of 10.9 fruiting forms per 10 plants on Bollgard cotton. At 9 d, 11.5 fruiting forms per 10 plants were damaged by bollworms on Bollgard cotton. Bollworm damaged fruiting forms consisted of 1.3 squares and 9.0 bolls per 10 plants. No additional damage was observed beyond 9 d on Bollgard cotton.

On Bollgard II cotton, little damage was observed beyond the initial 72 h period (Figure 4.4). At 5 d, cumulative bollworm damage included 0.2 squares, 0.2 white flowers, and 6.0 bolls per 10 plants. Only one larva survived beyond 5 d on Bollgard II. The larva damaged one additional white flower and one additional boll, but did not complete development.

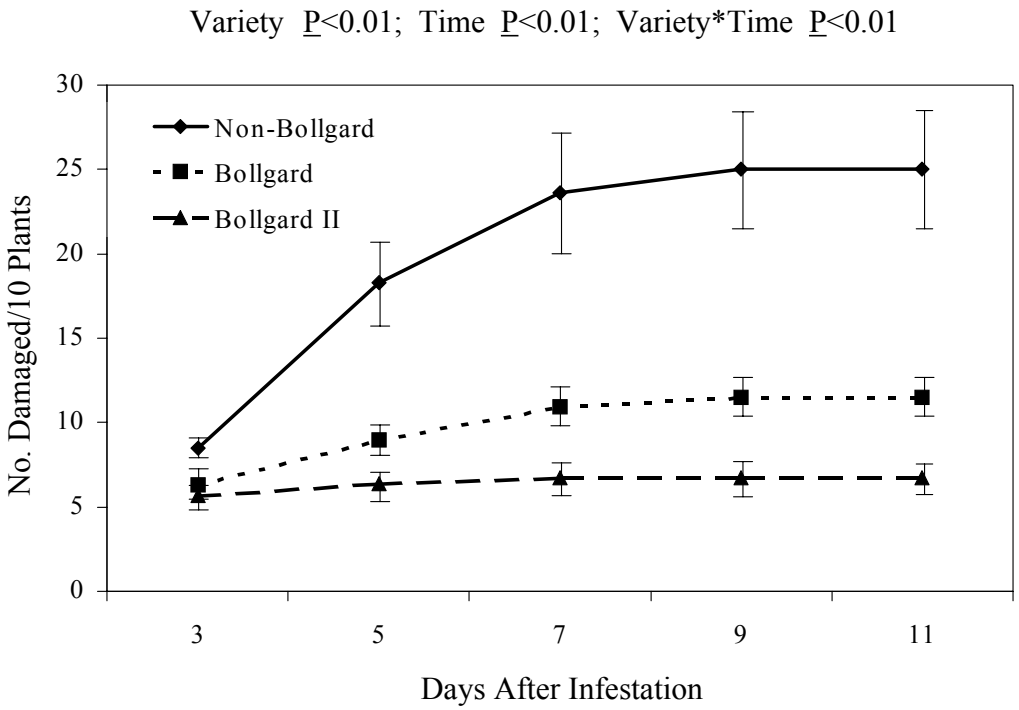

Figure 4.4. Damaged fruiting forms ± standard errors (squares, flowers, and bolls) by bollworms on Deltapine 50 (non-Bollgard), Deltapine 50B (Bollgard), and an experimental (Bollgard II) cotton variety.

The percentage of bollworm larvae recovered from non-Bollgard and Bollgard cotton followed a similar trend to that in the previous study (Figure 4.5). At 5 d, a lower percentage of bollworms were recovered on Bollgard (73.6%) cotton than on non-Bollgard (96.1%) cotton. At 7 d, the percentages of larvae recovered were similar on Bollgard (59.7%) cotton and non-Bollgard (67.8%) cotton. At 9 d, a higher percentage of larvae remained on Bollgard (40.3%) plants compared to non-Bollgard (8.1%) plants. No larvae were recovered beyond 9 d on either variety. The percentage of bollworms recovered on Bollgard II declined very rapidly. At 5 d, 25.0% of bollworm larvae were recovered. No larvae were recovered beyond 7 d on Bollgard II cotton.

An individual bollworm larva damaged more fruiting forms on non-Bollgard cotton (6.6) than on Bollgard (3.5) or Bollgard II (0.8) cotton during all larval stadia (F=20.76; df=2, 12; P<0.01) (Figure 4.6). Also, more fruiting forms were damaged by an individual bollworm on Bollgard cotton than on Bollgard II cotton. An individual bollworm larva damaged more squares on non-Bollgard cotton (2.6) than on Bollgard (0.4) or Bollgard II (<0.1) (F=38.76; df=2, 12; P<0.01). More white flowers were damaged by an individual bollworm on Bollgard cotton (0.5) than on non-Bollgard (0.3) or Bollgard II (0.1) cotton (F=8.63; df=2, 12; P<0.01). Also, more white flowers were damaged on non-Bollgard than on Bollgard II cotton. However, white flower damage was minimal during this study. An individual bollworm larva damaged fewer bolls on Bollgard II (0.8) cotton than on non-Bollgard (3.4) or Bollgard (2.7) cotton (F=13.23; df=2, 12; P<0.01).

Discussion

Bollworms damaged more fruiting forms on non-Bollgard cotton than on Bollgard or Bollgard II cotton. However, additional structures beyond those at the site of infestation were damaged

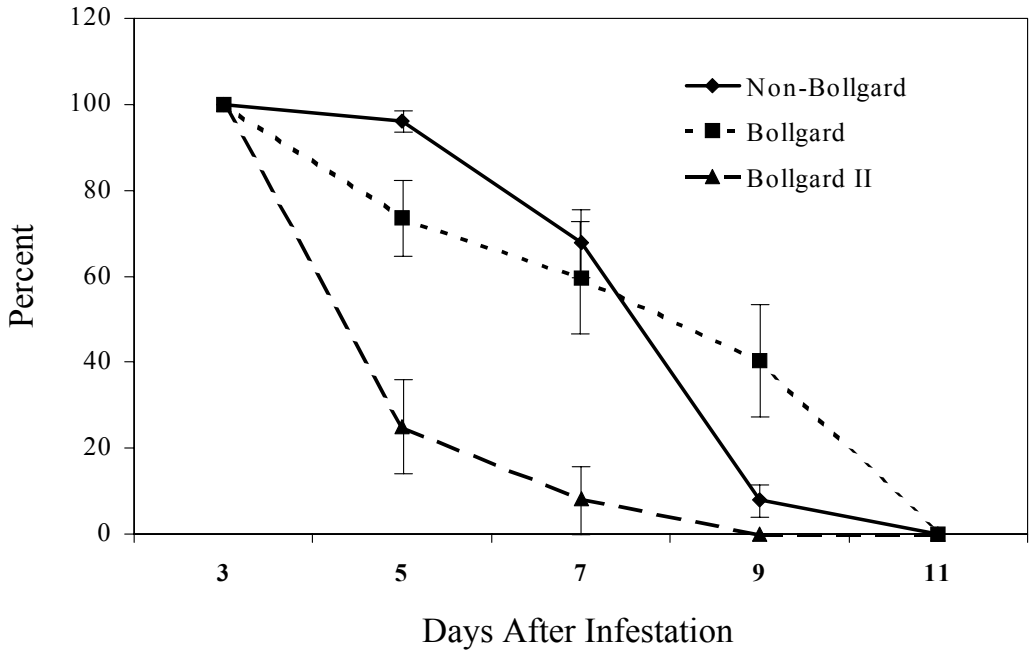

Figure 4.5. Bollworm larvae remaining on Deltapine 50 (non-Bollgard), Deltapine 50B (Bollgard), and experimental (Bollgard II) cotton plants after feeding on white flowers (points represent the percentage of larvae ± standard errors recovered during the initial 72 h rating period).

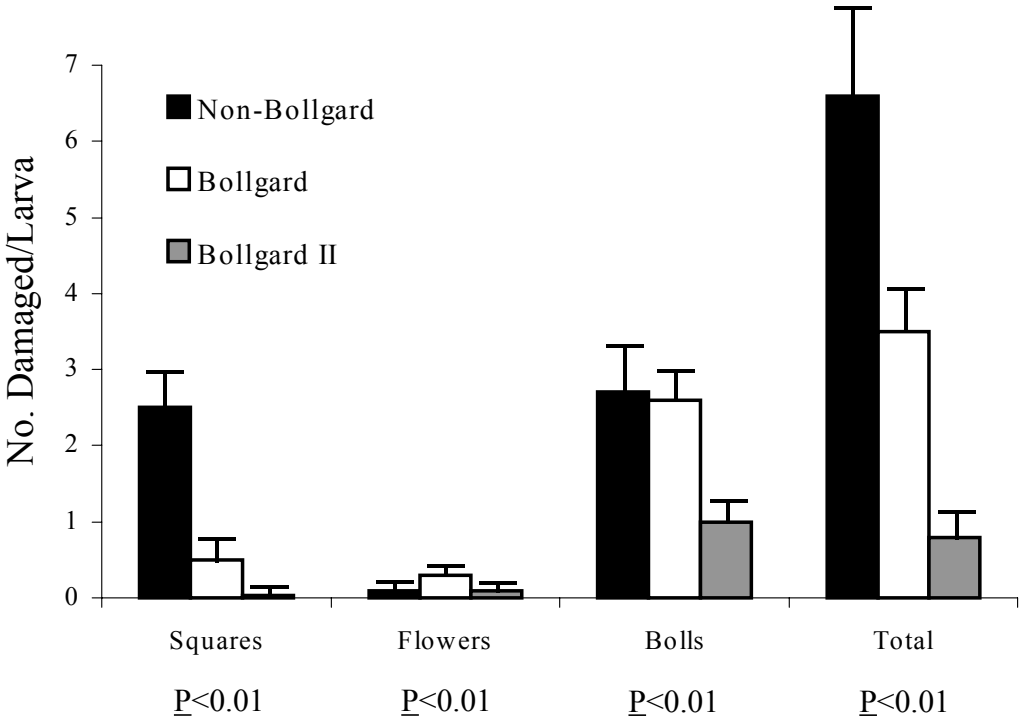

Figure 4.6. Bollworm injury to Deltapine 50 (non-Bollgard), Deltapine 50B (Bollgard), and an experimental (Bollgard II) cotton variety after feeding on white flowers (bars represent means ± standard errors numbers of damaged structures by a bollworm larva during the completion of all larval stadia).

by bollworms on Bollgard cotton. Bollworm damage at 3 d on Deltapine 5415 (non-Bollgard, 36.8%) and NuCOTN 33B (Bollgard, 25.2%) was lower than those observed by Gore et al. (2000a). Gore et al. (2000a) observed 69.7% and 48.5% boll abscission 72 h after infestation of Deltapine 5415 and NuCOTN 33B white flowers, respectively. In contrast, initial damage on Deltapine 50 (non-Bollgard, 85.0%) and Deltapine 50B (Bollgard, 63.0%) was higher than that observed by Gore et al. (2000a). Differences in damage between the two current studies may be attributed to differences in cotton variety. Also, environmental conditions may have been more conducive for bollworm feeding and survival during the Bollgard II study because of the later planting date.

In general, bollworm damaged fruiting forms increased more rapidly on non-Bollgard cotton than on Bollgard or Bollgard II cottons. Bollworm damage on non-Bollgard cotton peaked at 7 d. Although damage never reached the same levels on Bollgard cotton as those observed on non-Bollgard cotton, some additional damage did occur. Therefore, insecticide applications may be required to control bollworms feeding in white flowers. To further support this, Burd et al. (1999) found that yields of Bollgard cotton were significantly improved following applications of a pyrethroid for bollworm control. However, pyrethroids have a broad spectrum of activity and some of the yield increase may be attributed to control of other pests such as tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois) or stink bugs (Hemiptera: Pentatomidae).

Gore et al. (2000b) determined that cotton plants compensate for relatively high levels of boll damage during the early flowering period and low to moderate levels of boll damage during later flowering periods. However, in that study, boll damage levels were applied at a specific point in time and did not consider additional damage over time or damage to other

fruiting structures (squares and flowers). Therefore, based on the available data, insecticide applications may be warranted for bollworm control in Bollgard cotton, especially when other pests are present or when insect pressure persists over time. In contrast, little damage was observed on Bollgard II cotton beyond the initial damage observed at the site of infestation. Therefore, insecticide applications targeting bollworms may not always provide significant yield increases and thus may not be economical for Bollgard II cotton.

Numbers of damaged fruiting forms for non-Bollgard cotton were similar to those observed in previous studies (Adkisson et al. 1964, Anonymous 1967). Studies in Arkansas revealed that an individual bollworm larva damaged an average of 3.8 squares and 2.2 bolls (Anonymous 1967). Similarly, in Texas, bollworms damaged 3.8 and 5.7 squares per larva during 1961 and 1962, respectively (Adkisson et al. 1964). Bollworms damaged fewer fruiting forms on Bollgard cotton compared to non-Bollgard cotton. However, larvae on Bollgard cotton fed for a longer period of time. Consequently, more larvae remained on Bollgard cotton plants at 9 d, while on non-Bollgard cotton most of the larvae had left the plants to pupate. On Bollgard II cotton, bollworms damaged very few squares and white flowers. The majority of damage to the Bollgard II cotton line consisted of small bolls at the site of infestation.

In conclusion, these data support the application of insecticides to control bollworms in Bollgard cotton. Protein expression in Bollgard plants varies both temporally and spatially and causes bollworm injury to vary accordingly. Action thresholds for bollworms on Bollgard cotton are currently listed in the insect control guides for Mississippi (Mississippi State University Extension Service 2001), Georgia (Guillebeau 2001), and South Carolina (Roof 2001). Insecticides are recommended for Bollworm control when seven to eight live larvae greater than one fourth of an inch long are found per 100 plants in Mississippi (Mississippi

State University Extension Service 2001) and Georgia (Guillebeau 2001). In Mississippi, treatment is also recommended if damaged fruit counts exceed five percent. In South Carolina, insecticide treatments are recommended when 30 or more live bollworms smaller than one fourth of an inch are found, three larvae greater than one fourth of an inch are found, or when damaged boll counts exceed five percent (Roof 2001). These estimates appear to be conservative given the ability of cotton plants to compensate for relatively high levels of boll damage. Currently, the Louisiana Cooperative Extension Service recommends growers plant a significant portion of their farms with a Bollgard cultivar; however, there are no recommendations listed in the Louisiana Insect Control Guide for bollworm control on Bollgard cotton (Bagwell et al. 2001). Based on these data, action thresholds should be higher for Bollgard cotton than for non-Bollgard cotton because bollworms damaged approximately two times as many fruiting forms on non-Bollgard cotton. Also, action thresholds should be dynamic and change throughout the season based on changes in plant susceptibility to bollworms and levels of previous fruiting form injury. These data will be important for establishing accurate thresholds for bollworms on Bollgard cotton in Louisiana and refining current thresholds throughout the mid-South and Southeast.

References Cited

Adamczyk, J. J., Jr., D. D. Hardee, L. C. Adams, and D. V. Summerford. 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ-endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 94: 284-290.

Adkisson, P. L., C. F. Bailey, and R. L. Hanna. 1964. Estimates of the number of *Heliothis* larvae per acre in cotton and their relation to the fruiting cycle and yield of the host. J. Econ. Entomol. 57: 657-663.

Anonymous. 1967. The bollworm-tobacco budworm problem in Arkansas and Louisiana. Louisiana Agric. Expt. Sta. Bull. 720. 66 pp.

Bagwell, R. D., J. W. Barnett, B. R. Leonard, E. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2001. Cotton insect control 2001. Louisiana Cooperative Extension Service, Pub. 1083, 7 pp.

Bourland, F. M., D. M. Oosterhuis, and N. P. Tugwell. 1992. Concept for monitoring the growth and development of cotton plants using main-stem node counts. J. Prod. Agric. 5: 532-538.

Burd, T., J. R. Bradley, Jr., and J. W. Van Duyn. 1999. Performance of selected *Bt* cotton genotypes against bollworm in North Carolina, pp. 931-934. *In* P. Dugger and D. Richter [eds.], Proc. 1999 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000a. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. J. Econ. Entomol. 93: 690-696.

Gore, J., B. R. Leonard, E. Burris, D. R. Cook, and J. H. Fife. 2000b. Maturity and yield responses of non-transgenic and transgenic Bt cotton to simulated bollworm injury. J. Cotton Sci. 4: 152-160, http://www.jcotsci.org.

Gore, J., B. R. Leonard, and J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) Survival on Bollgard® and Bollgard II® Cotton Flower Bud and Flower Components. J. Econ. Entomol. 94: 1445-1451.

Gore, J., B. R. Leonard, G. E. Church, and D. R. Cook. Behavior of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. J. Econ. Entomol. (in press).

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92: 1377-1383.

Greenplate, J. T., G. P. Head, S. R. Penn, and V. T. Kabuye. 1998. Factors potentially influencing the survival of *Helicoverpa zea* on Bollgard® cotton, pp. 1030-1033. *In* P. Dugger and D. A. Richter [eds.], Proc. 1998 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J. T., S. R. Penn, J. W. Mullins, and M. Oppenhuizen. 2000a. Seasonal CryIAc levels in DP50B: The "Bollgard® basis" for Bollgard II, pp. 1039-1040. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J. T., S. R. Penn, Z. Shappley, M. Oppenhuizen, J. Mann, B. Reich, and J. Osborn. 2000b. Bollgard II efficacy: Quantification of lepidopteran activity in a 2-gene product, pp. 1041-1043. *In* P. Dugger and D. Richter [eds.], Proc. 2000 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Guillebeau, P. 2001. 2001 Georgia pest control handbook. Georgia Cooperative Extension Service, Special Bulletin 28, Athens, GA.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® system for mixed models. SAS Institute, Cary, NC.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebrate Path. 56: 258-266.

Mississippi State University Extension Service. 2001. Cotton insect control guide 2001, Publication 343. Mississippi State University Extension Service, Starkville, MS.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischoff. 1990. Insect resistant cotton plants. Biotechnology 8: 839-943.

Roof, M. E. 2001. Cotton insect management. Clemson University Cooperative Extension Service, IC 97, Clemson, SC.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival on Noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Williams, M. R. 2001. Cotton insect loss estimates – 2000, pp. 774-777. *In* P. Dugger and D. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

CHAPTER 5

INFLUENCE OF AGRONOMIC HOSTS ON THE SUSCEPTIBILITY OF *HELICOVERPA ZEA* (BODDIE) (LEPIDOPTERA: NOCTIDAE) TO GENETICALLY ENGINEERED AND NON-ENGINEERED COTTONS

Introduction

For any pest management system to be effective, knowledge of the population dynamics of the target pests in relation to their various host plants is necessary (Fitt 1989, Dent 1991). Polyphagy is considered a key component of *Heliothis* (=*Helicoverpa*) population dynamics and pest status (Fitt 1989). *Helicoverpa zea* (Boddie) exploits multiple hosts concurrently or in succession. *H. zea* is unique in that it has three common names accepted by the Entomological Society of America, depending on the host plant. This insect is known as the corn earworm on corn, *Zea mays* L.; the tomato fruitworm on tomato, *Lycopersicon esculentum* Miller; and the bollworm on cotton, *Gossypium hirsutum* L. Larvae have been reported on >100 wild and cultivated plant hosts (King and Coleman 1989).

In the mid-south region of the United States, the initial *H. zea* generation emerges from overwintering pupae in April and May (Anonymous 1967). This generation infests non-cultivated hosts including *Trifolium* spp., *Geranium* spp., *Vicia* spp., and *Lupinus* spp. (Stadelbacher et al. 1986). In the southern United States, subsequent generations migrate to field corn during June. Field corn is a preferred host of *H. zea* in the southern United States during the R1 and R2 (silking) growth stages; however, during mid- to late-summer (after silking, >R2), cotton is a more attractive host (Stadelbacher et al. 1986). In addition to corn and cotton, *H. zea* also feeds on soybean, *Glycine max* L., and grain sorghum, *Sorghum bicolor* (L.), during mid- to late-summer. *H. zea* population densities on these hosts are usually not as numerous as those found in cotton (Anonymous 1967). However, *H. zea* can be an annual pest

of field corn, cotton, soybean, and grain sorghum in the southern United States. Multiple integrated pest management tactics are used to prevent *H. zea* from reaching damaging levels; however, insecticides have been the primary tool used in most production systems.

H. zea development on selected host plants has been studied extensively. Gross and Young (1977) determined the period from larval eclosion to pupation of *H. zea* larvae on field corn, various non-cultivated hosts, and a meridic diet. Larvae required a longer period to develop on corn foliage (30.6 d) compared to the meridic diet (21.8 d) at day:night temperatures of 26:15^{o}C. Also, pupal weights were lower on corn foliage (268 mg/insect) compared to meridic diet (447 mg/insect) (Gross and Young 1977). Based on the ability of *H. zea* larvae to complete development under field conditions, field corn was a better host than cotton or grain sorghum (Harding 1976). Hayes (1988) released adults into field cages that contained various host plants and determined that *H. zea* larvae developed faster on grain sorghum compared to larvae that developed on cotton and corn. Although some variation occurred in the results of these studies, the investigators rated host suitability based on a single factor rather than all of the factors that affect insect performance. Also, no information was presented about plant developmental stages. Hartstack et al. (1973) and Roach and Ray (1976) determined that the density of *H. zea* adults produced on field corn was higher than on other agronomic crops. In a similar study, *H. zea* larvae were introduced into field cages over corn; tobacco, *Nicotiana tabacum* L.; cotton; sesame, *Sesamum indicum* L.; and soybean (Sparks et al. 1971). Field corn produced more pupae than cotton or the other hosts (Sparks et al. 1971).

Little information is available about *H. zea* populations from various host plants and their subsequent development on cotton. This information will be important to effectively integrate genetically engineered Bollgard cotton into current pest management systems.

Bollgard cotton was developed by incorporating a foreign gene from the soil bacterium, *Bacillus thuringiensis* var. *kurstaki* Berliner, into cotton plants (Perlak et al. 1990). These plants produce the cryIA(c) protein from *B. thuringiensis* which is selectively toxic to the larval stages of several lepidopteran insects (MacIntosh et al. 1990, Luttrell et al. 1999). Although the primary targets were tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders), bollworms also are susceptible to the cryIA(c) protein. However, Bollgard cotton has not provided satisfactory control of *H. zea* under certain situations.

Federal and state agencies, industry, producers, and academic researchers are concerned with the development of resistance to Bollgard cottons and have adopted resistance management plans for target pests (Gould and Tabashnik 1998). These plans rely on the use of refuges (Gould 1998) to produce susceptible populations. However, concerns have been expressed about the effectiveness of this strategy (Environmental Protection Agency 2001). Initial plans were developed for pests with narrow host ranges such as *H. virescens* and *P. gossypiella* and the contribution of alternate hosts for the production of susceptible populations of these species has not been extensively considered. This is primarily due to the fact that *P. gossypiella* feeds only on cotton and *H. virescens* has a relatively limited host range in most areas of the United States. Alternate hosts may effectively serve as refugia for the production of susceptible populations of polyphagous insects such as *H. zea* (Fitt 1989). However, before the role of alternate hosts can be evaluated as refugia, information on *H. zea* development on agronomic crops such as field corn, soybean, and grain sorghum in areas adjacent to cotton should be determined. Also, the survival of subsequent *H. zea* generations on non-Bollgard and Bollgard cotton from populations surviving on those hosts needs to be evaluated. These

studies examine *H. zea* performance on selected agronomic hosts and the influence of those hosts on subsequent *H. zea* survival on non-Bollgard and Bollgard cotton.

Materials and Methods

***H. zea* Colony.** A *H. zea* colony was established from sweet corn (cv. SG 90) and maintained on meridic diet for one generation in the laboratory. Approximately 200 to 300 larvae (≥ third instar) were collected daily from sweet corn ears during 15 to 23 June 2000. Larvae were placed in 29.5 ml plastic cups (Solo Co., Urbana, IL) with a soy protein/wheat germ based meridic diet (*Heliothis* premix, Stonefly Industries, Bryan, TX) and transported to the laboratory. Moths were placed in 3.8 L cardboard containers and fed a 10% sucrose solution. A single layer of cheesecloth was placed on the top of buckets for moth oviposition. Oviposition sheets were harvested daily and placed into 118 x 59 x 354 cm plastic bags. Larvae eclosing from these eggs were separated into five host specific colonies and utilized for bioassays.

Development of *H. zea* Host Colonies. Plots of conventional cotton (cv. Deltapine 5415), field corn (cv. Pioneer 3223), grain sorghum (cv. Pioneer 8282), and soybean (cv. Deltapine 3478) were planted at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA. Plots consisted of four 9.1 m rows and included one row each of cotton, field corn, grain sorghum, and soybean. Crop hosts were planted on multiple dates (17 and 26 May, and 12 June 2000) to ensure that the plant stages preferred by *H. zea* were available at the proper timing. Neonate *H. zea* (F_1) from the field-collected colony were offered tissues from cotton, soybean, field corn, or grain sorghum or a meridic diet in individual 29.5 ml plastic cups (Solo Co.) until pupation.

H. zea reared on cotton were presented with flower buds (squares, 10 to 15 mm diameter) removed from plants at the nodes above white flower 7 to 9 (Bourland et al. 1992) growth stage. For the colony maintained on field corn, larvae were offered sections of ears (including cob and seed) harvested during the R2 (blister) (Ritchie et al. 1993) growth stage until larvae reached the second instar. Larvae then were offered sections of R3 (milk) stage ears (Ritchie et al. 1993) until pupation. Larvae of the soybean colony were initially offered foliage harvested from plants at the R5 growth stage (Fehr et al. 1971) until the second instar. Subsequent instars were offered soybean pods harvested from plants during the R6 (Fehr et al. 1971) growth stage. Larvae reared on grain sorghum were offered pieces of seed heads in the soft-dough stage (Vanderlip 1993) throughout larval development. A separate control colony also was maintained on the meridic diet used for the original collection from sweet corn. Two separate cohorts of insects were maintained on each host and meridic diet. Cohorts served as blocks in a randomized complete block design and were initiated with 1000 neonates for each host on 5 and 6 August 2000, respectively. Plant tissue was changed every 48 h until pupation. Meridic diet (ca. 8g) was not changed throughout the duration of larval development. Larval survival, time to pupation, and pupal weights for each host and meridic diet were recorded. Data were analyzed with analysis of variance using the MIXED procedure in SAS (Littell et al. 1996) and means were separated according to Tukey's Studentized Range Test (Tukey 1977).

Mortality of *H. zea* Host Colonies on Bollgard and Non-Bollgard Cotton. Plots (four rows by 9.1 m.) of Bollgard (cv. Deltapine 50B) and non-Bollgard (cv. Deltapine 50) cotton were planted on 11 June 2000 for bioassays conducted on F_2 *H. zea* neonates. Pupae surviving from each host specific colony were maintained as previously described. Egg sheets were harvested daily and placed into 5.1 x 10.2 x 30.5 cm plastic bags. Upon eclosion, 200 F_2

neonates (ca. 50 per d for four d) were offered leaves (<5 cm diameter) harvested from Bollgard or non-Bollgard cotton plant terminals and held in 5.5 cm petri dishes. Treatments (host specific colonies) were arranged in a randomized complete block design where d of larval eclosion constituted blocks. All *H. zea* developmental stages were maintained at 27±2°C and 85±5% relative humidity. *H. zea* larval mortality on Bollgard and non-Bollgard cotton was compared among the different host specific colonies 96 h after exposure to cotton tissue. Data were subjected to analysis of variance using the MIXED procedure in SAS (Littell et al. 1996) and means were separated according to Tukey's Studentized Range Test (Tukey 1977).

Results and Discussion

Development of *H. zea* Host Colonies. *H. zea* survival varied among diets (F=37.02; df=4, 4; P<0.01) (Figure 5.1). Survival declined to less than 60% within 2d on soybean and cotton. Initial *H. zea* survival remained relatively high (>90%) on field corn, grain sorghum, and meridic diet. However, survival declined to less than 70% within 8d on field corn. At 16d, *H. zea* survival declined to less than 80% on grain sorghum. Survival remained greater than 80% on meridic diet. Total *H. zea* survival was higher on meridic diet (83%) and grain sorghum (73%) than survival on soybean (26%), and cotton (13%). Also, *H.* zea survival on field corn (55%) was higher than survival on cotton. Differences in survival of F_1 larvae among the host specific colonies may have been due to variations in levels of nutrients and/or plant secondary compounds. Meridic diets are developed to provide optimum nutrition with minimal amounts of toxic substances. In contrast, many plant species produce specific allelochemicals such as tannins, phenolics, and terpenoids, that may adversely affect insect development (Schoonhoven et al. 1998). Cotton, field corn, soybean, and grain sorghum plants

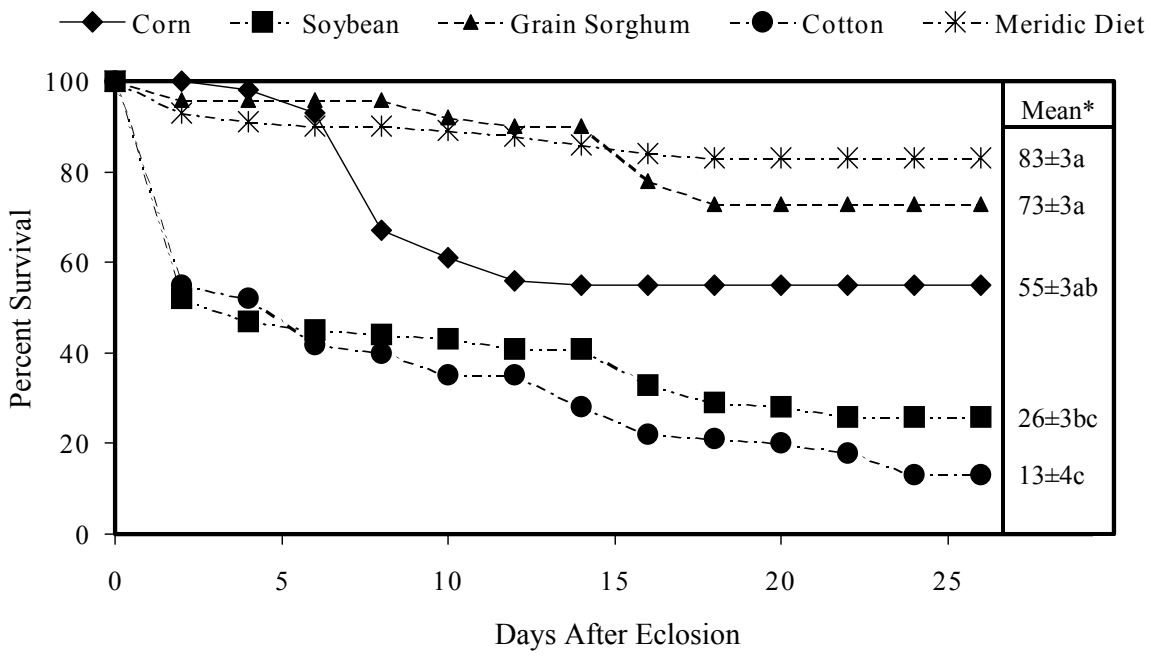

Figure 5.1. *H. zea* survival on various larval diets (*total survival means for host colonies followed by a common letter are not significantly different according to Tukey's Studentized Range Test, α=0.05).

produce numerous allelochemicals that adversely affect insect development and survival (Schoonhoven et al. 1998).

H. zea from the various host plant and meridic diet colonies varied in their times to completion of all larval stadia. Intervals to pupation were different among host plants and meridic diet (F=350.78; df=4, 4; P<0.01) (Figure 5.2). *H. zea* completed larval stadia more rapidly on field corn (12.4 d) than all other host plants or meridic diet. Completion of larval stadia for *H. zea* offered soybean (18.4 d) was shorter than for *H. zea* offered grain sorghum (15.6 d), cotton (25.0 d), or meridic diet (15.2 d). *H. zea* took longer to complete larval stadia on cotton than all other plant hosts. In addition, all larvae achieved the pupal stage over a range of 5 d, 6 d, 8 d, 7 d, and 8 d on field corn, meridic diet, grain sorghum, soybean, and cotton, respectively.

In a similar study, *H. zea* development from larval eclosion to pupation required 30.6 d on corn foliage (Gross and Young 1977). This is considerably longer than observations in the present study (12.4 d) but their experiment was conducted at lower night temperatures. Also, *H. zea* larvae prefer to feed on structures that contain high levels of nitrogen (i.e. reproductive structures) (Fitt 1989). Corn seed may have provided a higher level of nutrition for *H. zea* larvae than foliage; therefore, larvae would be expected to develop faster on seed than foliage. In our study, *H. zea* larvae fed cotton required 25.0 d to pupate. The sesquiterpene gossypol, an allelochemical found in cotton, delays development and reduces larval weight of *Heliothis* spp. and *Helicoverpa* spp. (Hedin et al. 1983). Consequently, survival and pupal weights were lowest on cotton compared to the other hosts. Also, larval developmental time was longer on cotton than the other hosts.

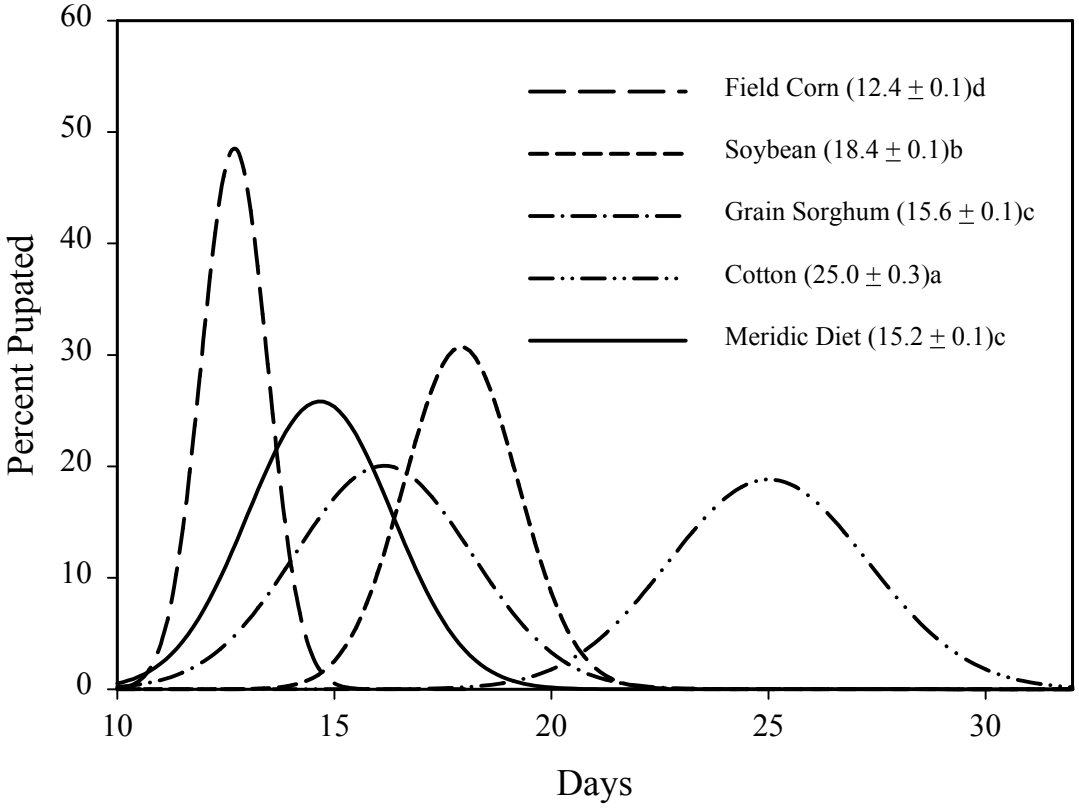

Figure 5.2. Distributions of *H. zea* developmental times to pupation on various larval diets (numbers next to crop hosts in the legend represent mean [± standard error] days to pupation; means for host colonies followed by a common letter are not significantly different according to Tukey's Studentized Range Test, α=0.05).

Pupal weights were higher for larvae reared on meridic diet (381.8 mg) compared to larvae fed cultivated host plants (F=63.07; df=4, 4; P<0.01) (Figure 5.3). Pupal weights on cotton, corn, grain sorghum, and soybean averaged 231.7, 293.5, 306.5, and 337.3 mg, respectively. Weights of pupae from larvae fed cotton were lower than pupal weights on all other larval diets.

Mortality of *H. zea* Host Colonies on Non-Bollgard and Bollgard Cotton. In addition to direct affects observed on *H. zea* development in our study, larval diet influenced the mortality of subsequent generations on cotton. Mortality on non-Bollgard cotton was different among F_2 *H. zea* larvae from the host colonies (F=4.65; df=4, 12; P=0.02) (Figure 5.4). *H. zea* mortality averaged 54.8, 35.8, 13.9, 11.0, and 8.0% for the cotton, grain sorghum, field corn, soybean, and meridic diet colonies, respectively. *H. zea* mortality on non-Bollgard cotton was higher for the cotton colony than the soybean, field corn, and meridic diet colonies.

Bollgard cotton produced variable levels of mortality among F_2 *H. zea* larvae from the host colonies (F=4.58; df=4, 12; P=0.02) (Figure 5.5). Mortality of *H. zea* from the cotton, grain sorghum, field corn, soybean, and meridic diet colonies averaged 76.8, 63.0, 89.7, 64.5, and 75.6%, respectively, on Bollgard cotton. *H. zea* mortality on Bollgard cotton was higher for the field corn colony than the soybean and grain sorghum colonies.

Plant hosts and meridic diets can influence the activity of various mortality factors including insecticides (Berry et al. 1980, Wood et al. 1981, Muehleisen et al. 1989, Tan and Guo 1996), bacteria (Moldenke et al. 1994), nuclear polyhedrosis viruses (Richter et al. 1987; Keating et al. 1988, 1989; Santiago-Alvarez and Ortiz-Garcia 1992; Peng et al. 1997), fungi (Hare and Andreadis 1983, Ramoska and Todd 1985), and nematodes (Barbercheck et al. 1995). Multiple factors associated with host plants can influence insect susceptibility to toxic

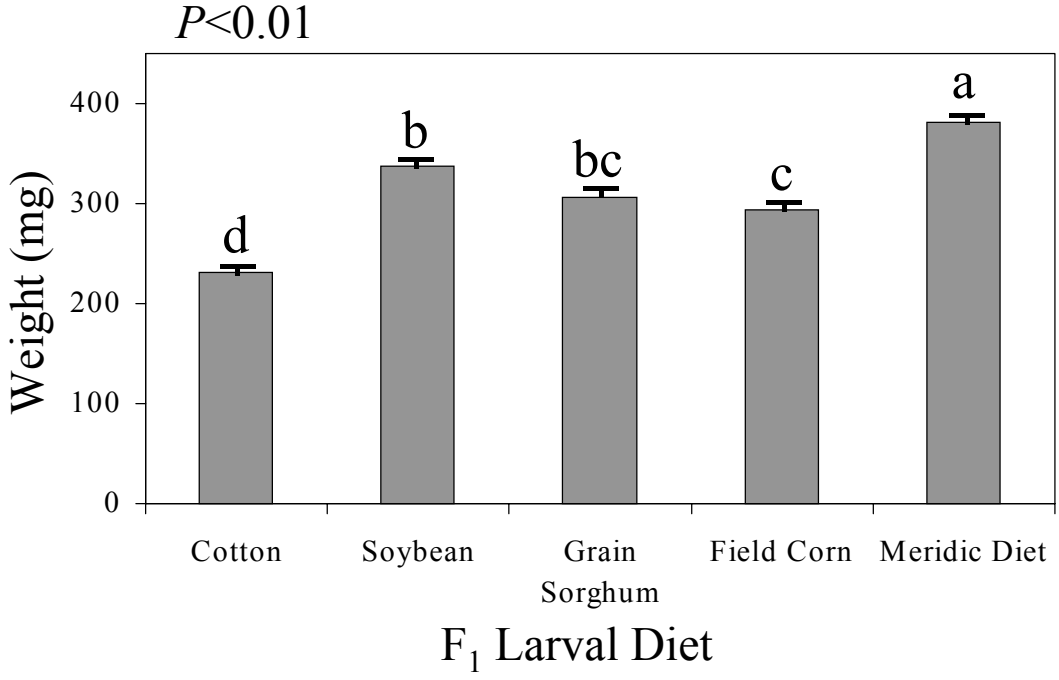

Figure 5.3. Mean (±SE) *H. zea* pupal weights on various larval diets (means for host colonies with a common letter are not significantly different according to Tukey's Studentized Range Test, α=0.05).

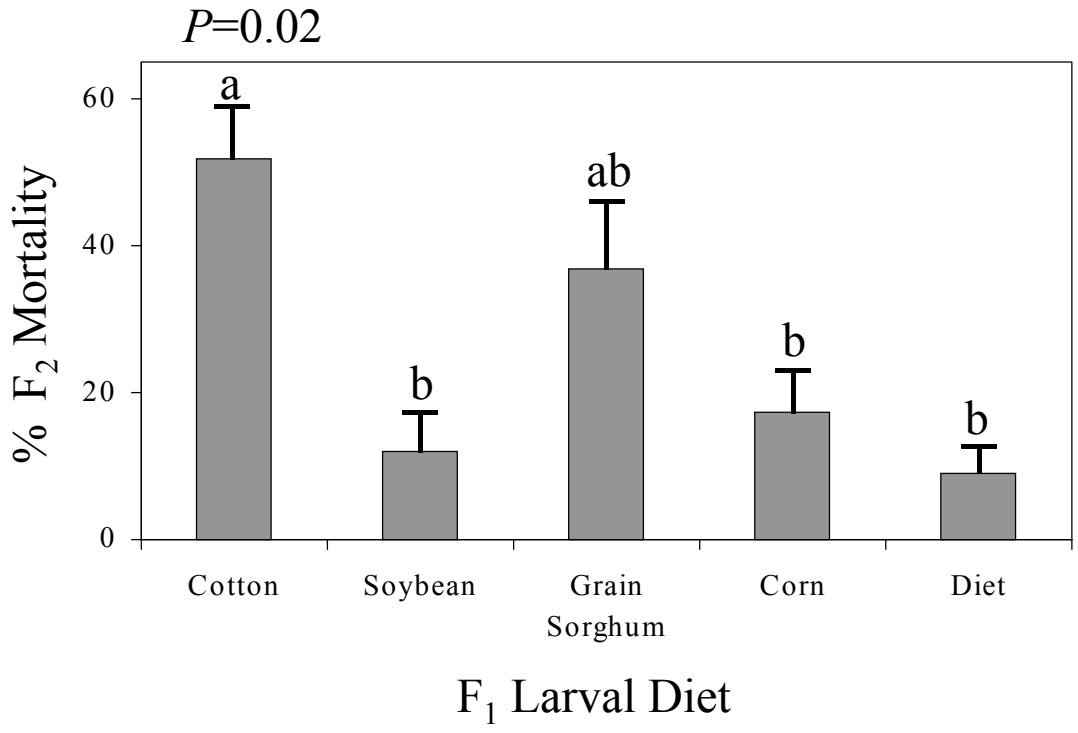

Figure 5.4. Host plant influence on subsequent *H. zea* generation susceptibility to non-Bollgard cotton. Bars represent means plus standard errors (bars with the same letter are not significantly different according to Tukey's Studentized Range Test [α=0.05]).

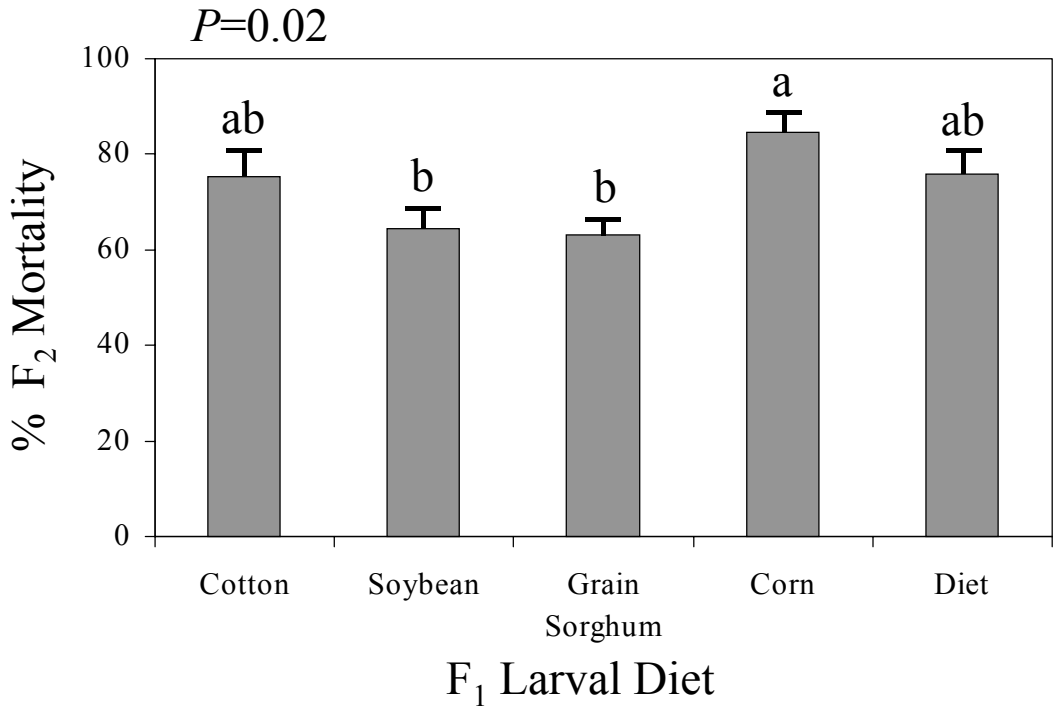

Figure 5.5. Host plant influence on subsequent *H. zea* generation susceptibility to Bollgard cotton. Bars represent means plus standard errors (bars with the same letter are not significantly different according to Tukey's Studentized Range Test [α=0.05]).

substances. Several studies have documented induction by host plants of detoxifying enzymes in insect pests (Yu et al. 1979; Berry et al. 1980; Yu 1982, 1984). This may be an important factor in reducing the effects of some toxins, especially synthetic insecticides (Berry et al. 1980, Moldenke et al. 1994). All of the previous studies were conducted during the same generation of insects that were fed the different host plants. In the current study, bioassays with non-Bollgard and Bollgard cotton were conducted on the generation (F_2) following the one (F_1) that was exposed to the different host plants. Therefore, induction of detoxifying enzymes is not a likely cause for differences observed in *H. zea* mortality on Bollgard cotton because induction is temporary and non-hereditary (Brattsten 1988). Some individuals within a population may have inherently higher enzyme levels than other individuals. In this instance, those larvae from specific host colonies may have been selected with enzymes that increase insect performance on the different hosts (Whitman 1988). The host colonies were not combined and the frequency of individuals with high enzyme levels would have increased if inheritance of that trait was recessive, thereby, resulting in differences in F_2 larval mortality among the different hosts. However, if this were the case in our study, bollworm mortality from the cotton colony would be expected to be lower than the other host colonies on both non-Bollgard and Bollgard cotton.

Nutrition is another factor that may contribute to differences in insect mortality. Moldenke et al. (1994) suggested that gypsy moth larvae fed alder, *Alnus rhombifolia* Nuttall, may have been less susceptible to *B. thuringiensis* than larvae fed Douglas fir, *Pseudotsuga menziesii* Franco, because higher levels of nitrogen were available in alder. Differences in fall armyworm (Richter et al. 1987) and velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, (Peng et al. 1997) susceptibility to nuclear polyhedrosis viruses can be attributed to host

suitability. Field corn is a preferred host for *H. zea* development (Barber 1936, Anonymous 1967). *H. zea* developed similarly on field corn, grain sorghum, and meridic diet. In contrast, F_1 larval survival, developmental time, and pupal weights were poor on cotton compared with the other hosts. Lukefahr and Martin (1964) determined that adult fecundity was influenced by larval diet. Moths that were fed cotton during the larval stage did not produce viable eggs when fed water alone during the adult stage. In contrast, 73.6 to 87.0% of eggs laid by moths from larvae that fed on corn or meridic diet were viable when the moths were fed only water. Corn and meridic diet were sufficient to produce viable eggs without the adults receiving additional nutrition. Cotton was not sufficient as a larval diet for subsequent adults to produce viable eggs unless they were provided a sugar water solution (Lukefahr and Martin 1964). Previous host plants in the current study may have influenced survival of *H. zea* on Bollgard cotton based on their relative nutritional value for F_1 larvae.

Agronomic crops other than cotton provide a source of *H. zea* during much of the season in the southeastern and mid-southern United States. Based on the combination of all developmental factors from our study as well as data from other studies, field corn appears to be the most suitable host plant for *H. zea*. During the period when corn is most susceptible to *H. zea* feeding, few larvae are present in cotton (Anonymous 1967). Therefore, field corn may not provide a source of *H. zea* adults at the proper time of year to mate with *H. zea* adults emerging from Bollgard cotton. Consequently, when *H. zea* populations peak in cotton, field corn is no longer attractive. However, large numbers of *H. zea* develop on corn and this may effectively dilute resistance alleles from the previous season before *H. zea* moves into cotton. *H. zea* moths will oviposit on soybean foliage (Hillhouse and Pitre 1976, Pitre and Hillhouse 1981) and grain sorghum seed heads (Cronholm et al. 1998) during the flowering stages of

each of these hosts. The flowering stages of these hosts correspond with the preferred ovipositional stages of cotton in the southern United States (Johnson et al. 1975). Population densities on soybean and grain sorghum are generally lower than that observed on cotton (Anonymous 1967), but a higher percentage of larvae develop to pupation on these hosts compared to cotton. Because *H. zea* survival on soybean and grain sorghum is higher than on cotton and they are present at the same time on cotton, these hosts may provide a source of *H. zea* adults to mate with moths emerging from Bollgard cotton. In a similar study, Losey et al. (2001) determined that alternative hosts would not would not support European corn borer, *Ostrinia nubilalis* (Hübner), densities at a sufficient level to contribute to a resistance management plan for Bt-corn. However, in that study the alternative hosts were not as attractive as corn for oviposition and larval survival was lower on the other hosts than on corn (Losey et al. 2001). In contrast, previous studies indicate that *H. zea* oviposition is similar among various hosts depending on the hosts growth stage (Johnson et al. 1975, Stadelbacher 1980). Also, based on the present study, more *H. zea* larvae survived on the alternate hosts evaluated than on cotton. Similarly, Craig (1998) determined that velvetleaf, *Abutilon theophrasti* (L.), could support sufficient populations of *H. zea* and *H. virescens* for consideration as a refuge for Bollgard cotton.

The role of the major cultivated host plants in the mid-southern United States on *H. zea* population densities and their relationship to cotton should not be underestimated in the design/implementation of integrated pest management and resistance management strategies. Field corn produces large numbers of *H. zea* that subsequently serve as a source of initial populations that migrate into cotton during late June and early July (Anonymous 1967). A study conducted during 1964 over a 27 square mile area in Arkansas determined that *H. zea*

populations achieve high densities on field corn during mid- to late-June and early July (Anonymous 1967). Subsequent *H. zea* populations were observed at varying densities on grain sorghum, soybean, and cotton during July and August. Grain sorghum and soybean may serve as a source of *H. zea* re-infestations during July and August after applications of foliar insecticides have reduced populations in cotton. However, before these crops can be considered for refuges in a resistance management strategy, studies need to be conducted to determine specific numbers of *H. zea* adults contributed by each of these hosts under field conditions.

References Cited

Anonymous. 1967. The bollworm-tobacco budworm problem in Arkansas and Louisiana. Louisiana Agric. Expt. Sta. Bull. 720. 66 pp.

Barber, G. W. 1936. The corn ear worm (sic) in southeastern Georgia. Georgia Expt. Sta. Tech. Bull. 192. 18 pp.

Barbercheck, M. E., J. Wang, and I. S. Hirsh. 1995. Host plant effects on entomopathogenic nematodes. J. Invert. Pathol. 66:169-177.

Berry, R. E., S. J. Yu, and L. C. Terriere. 1980. Influence of host plants on insecticide metabolism and management of variegated cutworm. J. Econ. Entomol. 73: 771-774.

Bourland, F. M., D. M. Oosterhuis, and N. P. Tugwell. 1992. Concept for monitoring the growth and development of cotton plants using main-stem node counts. J. Prod. Agric. 5: 532-538.

Brattsten, L. B. 1988. Potential role of plant allelochemicals in the development of insecticide resistance, pp. 313-348. *In* P. Barbosa and D. K. Letourneau [eds.], Novel aspects of insect-plant interactions. Wiley and Sons, New York.

Craig, Jr., C. C. 1998. Development of a trap crop for *Lygus lineolaris* (Heteroptera: Miridae) and a refuge for production of *Heliothis virescens* (Lepidoptera: Noctuidae) susceptible to cotton expressing insecticidal proteins. M.S. Thesis, Mississippi State University, Mississippi State, MS.

Cronholm, G., A. Knutson, R. Parker, G. Teetes, and B. Pendleton. 1998. Managing insect and mite pests of Texas sorghum. Texas Agric. Extension Service Bull. 1220. 26 pp.

Dent, D. 1991. Insect pest management. CAB International, Wallingford, UK.

Environmental Protection Agency. 2001. *Bt* cotton refuge requirements for the 2001 growing season. http://www.epa.gov/pesticides/biopesticides/otherdocs/bt_cotton _refuge_2001.htm.

Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11: 929-931.

Fitt, G. P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. Ann. Rev. Entomol. 34: 17-52.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann. Rev. Entomol. 43: 701-726.

Gould, F., and B. Tabashnik. 1998. Bt-cotton resistance management, pp. 65-105. *In* M. Mellon and J. Rissler [eds.], Now or Never: Serious New Plans to Save a Natural Pest Control. Union of Concerned Scientists, Cambridge, MA.

Gross, H. R., Jr., and J. R. Young. 1977. Comparative development and fecundity of corn earworm reared on selected wild and cultivated early-season hosts common to the southwestern U. S. Annals Entomol. Soc. Am. 70: 63-65.

Harding, J. A. 1976. *Heliothis* spp.: seasonal occurrence, hosts and host importance in the lower Rio Grande Valley. Environ. Entomol. 5: 666-668.

Hare, J. D., and T. G. Andreadis. 1983. Variation in the susceptibility of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) when reared on different host plants to the fungal pathogen, *Beauveria bassiana* in the field and laboratory. Environ. Entomol. 12: 1892-1897.

Hartstack, A. W., Jr., J. P. Hollingsworth, R. L. Ridgway, and J. R. Coppedge. 1973. A population dynamics study of the bollworm and the tobacco budworm with light traps. Environ. Entomol. 2: 244-252.

Hayes, J. L. 1988. A comparative study of adult emergence phenologies of *Heliothis virescens* (F.) and *H. zea* (Boddie) (Lepidoptera: Noctuidae) on various hosts in field cages. Environ. Entomol. 17: 344-349.

Hedin, P. A., J. N. Jenkins, D. H. Collum, W. H. White, and W. L. Parrott. 1983. Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis*

virescens F., pp. 347-365. *In* P. A. Hedin, [ed.], Plant resistance to insects, ACS symposium series, American Chemical Society, Washington, D. C.

Hillhouse, T. L., and H. N. Pitre. 1976. Oviposition by *Heliothis* on soybeans and cotton. J. Econ. Entomol. 69: 144-146.

Johnson, M. W., R. E. Stinner, and R. L. Rabb. 1975. Ovipositional response of *Heliothis zea* (Boddie) to its major hosts in North Carolina. Environ. Entomol. 4: 291-297.

Keating, S. T., W. G. Yendol,, and J. C. Schultz. 1988. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a baculovirus and host plant foliage constituents. Environ. Entomol. 17: 952-958.

Keating, S. T., W. J. McCarthy, and W. G. Yendol. 1989. Gypsy moth (*Lymantria dispar*) larval susceptibility to a baculovirus affected by selected nutrients, hydrogen ions (pH), and plant allelochemicals in artificial diets. J. Invert. Pathol. 54: 165-174.

King, E. G., and R. J. Coleman. 1989. Potential for biological control of *Heliothis* species. Ann. Rev. Entomol. 34: 53-76.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® system for mixed models. SAS Institute, Cary, NC.

Losey, J. E., D. D. Calvin, M. E. Carter, and C. E. Mason. 2001. Evaluation of noncorn host plants as a refuge in a resistance management program for European corn borer (Lepidoptera: Crambidae) on Bt-corn. Environ. Entomol. 30: 728-735.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

Lukefahr, M. J., and D. F. Martin. 1964. The effects of various larval and adult diets on the fecundity and longevity of the bollworm, tobacco budworm, and cotton leafworm. J. Econ. Entomol. 57: 233-235.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebrate Path. 56: 258-266.

Moldenke, A. F., R. E. Berry, J. C. Miller, J. G. Wernz, and X. H. Li. 1994. Toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to gypsy moth, *Lymantria dispar*, fed with alder or Douglas-fir. J. Invert. Pathol. 64: 143-145.

Muehleisen, D. P., J. H. Benedict, F. W. Plapp, Jr., and F. A. Carino. 1989. Effects of cotton allelochemicals on toxicity of insecticides and induction of detoxifying enzymes in bollworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1554-1558.

Peng, F., J. R. Fuxa, S. J. Johnson, and A. R. Richter. 1997. Susceptibility of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), reared on four host plants, to a nuclear polyhedrosis virus. Environ. Entomol. 26: 973-977.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischoff. 1990. Insect resistant cotton plants. Biotechnology 8: 839-943.

Pitre, H. N., and T. L. Hillhouse. 1981. Establishment of infestation and behavior of *Heliothis zea* on soybeans in the greenhouse. J. Georgia Entomol. Soc. 16: 28-34.

Ramoska, W. A., and T. Todd. 1985. Variation in efficacy and viability of *Beauveria bassiana* in the chinch bug (Hemiptera: Lygaeidae) as a result of feeding activity on selected host plants. Environ. Entomol. 14: 146-148.

Richter, A. R., J. R. Fuxa, and M. Abdel-Fattah. 1987. Effect of host plant on the susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus. Environ. Entomol. 16: 1004-1006.

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1993. How a corn plant grows, special report no. 48, 22 pp. Iowa State University of Science and Technology Cooperative Extension Service, Ames.

Roach, S. H., and L. Ray. 1976. Pattern of emergence of adult *Heliothis* from fields planted to cotton, corn, tobacco, and soybeans. Environ. Entomol. 5: 628-630.

Santiago-Alvarez, C., and R. Ortiz-Garcia. 1992. The influence of host plant on the susceptibility of *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) larvae to *Spodoptera littoralis* NPV (Baculoviridae, *Baculovirus*). J. Appl. Entomol. 114: 124-130.

Schoonhoven, L. M., T. Jermy, and J. J. A. van Loon. 1998. Insect-plant biology: from physiology to evolution, 409 pp. Chapman and Hall, New York.

Sparks, A. N., B. R. Wiseman, and W. W. McMillian. 1971. Production of corn earworms on several hosts in field cages. J. Econ. Entomol. 64: 540-541.

Stadelbacher, E. A. 1980. Oviposition preference of the bollworm for species of early-season host plants in the delta of Mississippi. Environ. Entomol. 9: 542-545.

Stadelbacher, E. A., H. M. Graham, V. E. Harris, J. D. Lopez, J. R. Phillips, and S. H. Roach. 1986. *Heliothis* populations and wild host plants in southern U. S., pp. 54-74. *In* S. J. Johnson, E. G. King, and J. R. Bradley, Jr. [eds.], Theory and tactics of *Heliothis*

population management: I – cultural and biological control. Southern Cooperative Series Bulletin 316, Tifton, GA.

Tan, W. J., and Y. Y. Guo. 1996. Effects of host plant on susceptibility to deltamethrin and detoxication enzymes of *Heliothis armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 89: 11-14.

Tukey, J. W. 1977. Exploratory data analysis. Addison-Wesley, Reading, MA.

Vanderlip, R. L. 1993. How a sorghum plant develops, 20 pp. Kansas State University Cooperative Extension Service, Manhattan.

Whitman, D. W. 1988. Allelochemical interactions among plants, herbivores and their predators, pp. 11-64. *In* P. Barbosa and D. K. Letourneau [eds.], Novel aspects of insect-plant interactions. Wiley and Sons, New York.

Wood, K. A., B. H. Wilson, and J. B. Graves. 1981. Influence of host plant on the susceptibility of the fall armyworm to insecticides. J. Econ. Entomol. 74: 96-98.

Yu, S. J. 1982. Induction of microsomal oxidases by host plants in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Pesticide Biochem. Physiol. 17: 59-67.

Yu, S. J. 1984. Interactions of allelochemicals with detoxication enzymes of insecticide-susceptible and resistant fall armyworms. Pesticide Biochem. Physiol. 22: 60-68.

Yu, S. J., R. E. Berry, and L. C. Terriere. 1979. Host plant stimulation of detoxifying enzymes in a phytophagous insect. Pesticide Biochem. Physiol. 12: 280-284.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The bollworm, *Helicoverpa zea* (Boddie), is an important pest of cotton in the United States. Traditionally, insecticides have been the primary component of cotton integrated pest management for bollworms as well as other insect pests such as the tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders). An alternative control method has recently been developed for these insects using genetic engineering in combination with recombinant deoxyribonucleic acid (rDNA) methods of crop breeding. Genetically engineered cotton was developed using rDNA techniques to incorporate a gene from *Bacillus thuringiensis* Berliner into cotton plants. This technology has created Bollgard cotton plants that produce the CryIAc protein from *B. thuringiensis* var. *kurstaki* that is selectively toxic to larvae from the order Lepidoptera. Although Bollgard cotton has consistently provided satisfactory control of tobacco budworms and pink bollworms, supplemental insecticide applications are often necessary to prevent economic yield losses from bollworms. More recently, experimental cotton varieties (Bollgard II) have been developed that produce two proteins (CryIAc + CryIIAb) from *B. thuringiensis*. These studies investigated various factors that contribute to Bollgard cottons susceptibility to bollworms and the efficacy of Bollgard II against bollworms.

White flowers and small bolls are the developmental structures on cotton plants that are most susceptible to bollworm feeding. Field studies were conducted to quantify bollworm survival on selected floral components, larval behavior, subsequent fruiting form injury after feeding in white flowers, and the influence of previous host plants on bollworm sensitivity to Bollgard cotton. Bollworm larval survival was higher on male and female reproductive organs

(stigma, style, and anther) in white flowers (flower anthers) and squares (square anthers) than that on flower bracts, flower petals, or whole squares. Bollworm survival was >90% on flower anthers and square anthers compared to ≤67% on flower bracts, flower petals, and whole squares from Bollgard cotton at 72 hours. Bollgard II was more effective against bollworms than Bollgard. On Bollgard II flower anthers and square anthers, bollworm survival was ≤63% at 72 hours. Bollworm survival was 36%, 6%, and 8% on Bollgard II flower petals, flower bracts, and squares, respectively, at 72 hours.

Bollworm larval behavior has been proposed to be different on Bollgard cotton compared to non-Bollgard cotton. On Bollgard cotton, larvae placed in the terminals of individual flowering plants were found 5.7 main stem nodes below the terminals at 24 hours compared to 2.4 main stem nodes below the terminals on non-Bollgard cotton. Significantly fewer larvae remained in the terminals of Bollgard cotton than non-Bollgard cotton at all rating intervals. At 24 hours, more larvae were found in Bollgard flowers and bolls than in non-Bollgard flowers and bolls. Where multiple plants were infested in a 1-m section of row, lower percentages of plant terminals and squares were infested on Bollgard cotton than on non-Bollgard cotton. Also, higher percentages of infested flowers and bolls were observed on Bollgard cotton than on non-Bollgard cotton. Differences in bollworm behavior result in larval infestations becoming established lower in the plant canopy on Bollgard cotton than on non-Bollgard cotton. These changes in behavior coupled with differential survival among reproductive structures results in less than adequate control of bollworms by Bollgard cotton. Currently, insecticide applications target bollworms in white flowers on Bollgard cotton. However, there is no data currently available that demonstrates the potential of bollworms to cause significant levels of fruiting form injury.

To determine the level of bollworm injury to fruiting forms on Bollgard cotton, first instar larvae were placed into white flowers on individual cotton plants. Plants were inspected after 72 hours and every 48 hours thereafter, and the numbers of damaged fruiting forms were recorded. Bollworms damaged 46.6 fruiting forms per 50 plants on non-Bollgard cotton compared to 18.9 fruiting forms per 50 plants on Bollgard cotton. An individual bollworm larva damaged 4.3 fruiting forms (2.9 bolls, 0.1 white flowers, and 1.3 squares) on non-Bollgard cotton and 2.7 fruiting forms (2.1 bolls, 0.1 white flowers, and 0.5 squares) on Bollgard cotton. Based on these data, bollworms are capable of damaging additional fruiting forms on Bollgard cotton after leaving white flowers; therefore, control measures should be directed at small larvae in plant terminals and white flowers before they become established on bolls low in the plant canopy.

The polyphagous nature of bollworms is an important factor contributing to their pest status. To determine the influence of alternate hosts on bollworm sensitivity to Bollgard cotton, bollworm colonies were allowed to complete larval development on field corn, grain sorghum, soybean, cotton, or meridic diet. Bollworm survival, larval development, and pupal weights were recorded. Neonates from the subsequent generation were offered terminal foliage from non-Bollgard or Bollgard cotton. Mortality was rated at 24, 48, 72, and 96 hours. Based on these data, field corn and grain sorghum appear to be adequate hosts for bollworms, whereas, cotton is a poor host. Bollworms required a longer period of time to complete all larval stadia on cotton than the other hosts. Also, pupal weights and survival to pupation were lower on cotton than on the other hosts. On non-Bollgard cotton, bollworm mortality was higher for F_2 larvae from the cotton colony than from the soybean, field corn, and meridic diet colonies. On Bollgard cotton, mortality was higher for F_2 larvae from the field corn colony

than from the soybean or grain sorghum colonies. Therefore, adjacent crops may influence bollworm susceptibility to Bollgard.

In conclusion, these data provide a basis for refining current scouting protocols for bollworms on Bollgard cotton. The highest levels of bollworm survival were observed on white flowers and because larvae migrate down plants faster on Bollgard cotton, scouts should sample white flowers and small bolls low in the plant canopy. Bollworms caused additional injury to fruiting forms after leaving white flowers indicating the need for insecticide applications. Finally, other crops grown adjacent to Bollgard cotton can influence bollworm management with Bollgard cotton based on these data. Large population densities can occur on field corn prior to cotton being attractive for oviposition and may serve as an initial source of bollworm infestations in cotton. Subsequent infestations also can migrate into cotton from soybean and grain sorghum later during the season. The results of these studies provide a valuable reference for the utilization of Bollgard cotton in current integrated pest management systems.

APPENDIX A

LETTER OF PERMISSION AND DATA FOR CHAPTER 2

Letter of permission from the Journal of Economic Entomology to reprint Chapter 2 and actual data for bollworm survival on non-Bollgard (Deltapine 5415 and Deltapine 50), Bollgard (Deltapine NuCOTN 33B and Deltapine 50B), and Bollgard II (experimental) cottons.

From: "Maggie Meitzler" <maggie@entsoc.org>
To: <jgore@unix1.sncc.lsu.edu>
Subject: Permission
Date: Fri, 25 Jan 2002 14:10:58 -0500
X-MSMail-Priority: Normal
X-MimeOLE: Produced By Microsoft MimeOLE V4.72.3110.3

Dear Dr. Gore,

The Entomological Society of America (ESA) grants you one-time permission to include your paper "Bollworm (Lepidoptera: Noctuidae) Survival on 'Bollgard' and 'Bollgard II' Cotton Flower Bud and Flower Components" (J. Gore, B. R. Leonard, and J. J. Adamczyk. Journal of Enonomic Entomology 94: 1445-1451) as a chapter in your dissertation.

We wish you the best of luck in your academic endeavors and hope you will publish with the ESA often.

Maggie Meitzler
Managing Editor/Manuscript Editor

Table A.1. Bollworm survival on selected floral structures of Bollgard (Deltapine NuCOTN 33B) and Non-Bollgard cotton (Deltapine 5415).

Replicate	Variety	Floral Structure	Percent Survival		
			24 hours	48 hours	72 hours
1	Bollgard	Flower Bracts	91	57	11
1	Bollgard	Flower Anthers	100	95	95
1	Bollgard	Flower Petals	100	80	50
1	Bollgard	Square Anthers	100	94	76
1	Bollgard	Squares	96	35	0
1	Non-Bollgard	Flower Bracts	100	91	91
1	Non-Bollgard	Flower Anthers	100	100	100
1	Non-Bollgard	Flower Petals	96	91	79
1	Non-Bollgard	Square Anthers	100	95	95
1	Non-Bollgard	Squares	100	94	84
2	Bollgard	Flower Bracts	100	50	11
2	Bollgard	Flower Anthers	100	95	95
2	Bollgard	Flower Petals	100	89	67
2	Bollgard	Square Anthers	100	95	89
2	Bollgard	Squares	96	90	30
2	Non-Bollgard	Flower Bracts	100	95	95
2	Non-Bollgard	Flower Anthers	95	100	100
2	Non-Bollgard	Flower Petals	100	95	95
2	Non-Bollgard	Square Anthers	100	100	100
2	Non-Bollgard	Squares	100	100	91
3	Bollgard	Flower Bracts	82	76	11
3	Bollgard	Flower Anthers	96	95	95
3	Bollgard	Flower Petals	94	63	63
3	Bollgard	Square Anthers	94	89	85
3	Bollgard	Squares	89	56	30
3	Non-Bollgard	Flower Bracts	100	91	86
3	Non-Bollgard	Flower Anthers	100	96	96
3	Non-Bollgard	Flower Petals	100	100	95
3	Non-Bollgard	Square Anthers	100	100	95
3	Non-Bollgard	Squares	94	90	85

(table A.1 continued)

(table A.1 continued)

4	Bollgard	Flower Bracts	96	90	15
4	Bollgard	Flower Anthers	100	89	89
4	Bollgard	Flower Petals	96	82	70
4	Bollgard	Square Anthers	100	94	90
4	Bollgard	Squares	89	86	27
4	Non-Bollgard	Flower Bracts	95	95	91
4	Non-Bollgard	Flower Anthers	100	100	96
4	Non-Bollgard	Flower Petals	94	91	81
4	Non-Bollgard	Square Anthers	100	100	95
4	Non-Bollgard	Squares	94	89	89
5	Bollgard	Flower Bracts	86	38	5
5	Bollgard	Flower Anthers	95	95	89
5	Bollgard	Flower Petals	89	74	27
5	Bollgard	Square Anthers	100	100	86
5	Bollgard	Squares	95	85	42
5	Non-Bollgard	Flower Bracts	91	70	53
5	Non-Bollgard	Flower Anthers	96	88	100
5	Non-Bollgard	Flower Petals	96	91	68
5	Non-Bollgard	Square Anthers	100	100	100
5	Non-Bollgard	Squares	100	92	90
6	Bollgard	Flower Bracts	88	48	14
6	Bollgard	Flower Anthers	100	100	100
6	Bollgard	Flower Petals	89	84	58
6	Bollgard	Square Anthers	100	94	84
6	Bollgard	Squares	94	50	50
6	Non-Bollgard	Flower Bracts	95	76	68
6	Non-Bollgard	Flower Anthers	100	100	100
6	Non-Bollgard	Flower Petals	100	94	81
6	Non-Bollgard	Square Anthers	100	100	100
6	Non-Bollgard	Squares	96	90	85
7	Bollgard	Flower Bracts	100	89	29
7	Bollgard	Flower Anthers	100	100	89
7	Bollgard	Flower Petals	100	95	64

(table A.1 continued)

(table A.1 continued)

7	Bollgard	Square Anthers	92	90	74
7	Bollgard	Squares	96	62	37
7	Non-Bollgard	Flower Bracts	100	100	75
7	Non-Bollgard	Flower Anthers	100	100	100
7	Non-Bollgard	Flower Petals	94	94	79
7	Non-Bollgard	Square Anthers	100	100	95
7	Non-Bollgard	Squares	100	100	87
8	Bollgard	Flower Bracts	89	53	36
8	Bollgard	Flower Anthers	100	94	90
8	Bollgard	Flower Petals	89	88	68
8	Bollgard	Square Anthers	100	100	100
8	Bollgard	Squares	100	57	48
8	Non-Bollgard	Flower Bracts	100	95	46
8	Non-Bollgard	Flower Anthers	100	95	95
8	Non-Bollgard	Flower Petals	100	89	73
8	Non-Bollgard	Square Anthers	100	100	100
8	Non-Bollgard	Squares	95	91	68
9	Bollgard	Flower Bracts	48	22	22
9	Bollgard	Flower Anthers	71	91	91
9	Bollgard	Flower Petals	71	50	33
9	Bollgard	Square Anthers	100	89	89
9	Bollgard	Squares	67	62	52
9	Non-Bollgard	Flower Bracts	74	43	43
9	Non-Bollgard	Flower Anthers	81	81	81
9	Non-Bollgard	Flower Petals	94	50	50
9	Non-Bollgard	Square Anthers	100	100	90
9	Non-Bollgard	Squares	79	75	75
10	Bollgard	Flower Bracts	60	18	18
10	Bollgard	Flower Anthers	100	87	87
10	Bollgard	Flower Petals	95	75	53
10	Bollgard	Square Anthers	100	100	95
10	Bollgard	Squares	91	87	39
10	Non-Bollgard	Flower Bracts	75	75	62

(table A.1 continued)

(table A.1 continued)

10	Non-Bollgard	Flower Anthers	100	100	100
10	Non-Bollgard	Flower Petals	100	100	89
10	Non-Bollgard	Square Anthers	100	100	100
10	Non-Bollgard	Squares	100	100	85
11	Bollgard	Flower Bracts	89	57	14
11	Bollgard	Flower Anthers	100	100	82
11	Bollgard	Flower Petals	94	90	35
11	Bollgard	Square Anthers	100	100	88
11	Bollgard	Squares	96	96	62
11	Non-Bollgard	Flower Bracts	92	82	61
11	Non-Bollgard	Flower Anthers	100	100	94
11	Non-Bollgard	Flower Petals	100	100	76
11	Non-Bollgard	Square Anthers	100	100	96
11	Non-Bollgard	Squares	95	95	90
12	Bollgard	Flower Bracts	91	74	16
12	Bollgard	Flower Anthers	96	94	94
12	Bollgard	Flower Petals	92	88	73
12	Bollgard	Square Anthers	100	100	95
12	Bollgard	Squares	95	92	92
12	Non-Bollgard	Flower Bracts	88	88	88
12	Non-Bollgard	Flower Anthers	100	100	96
12	Non-Bollgard	Flower Petals	89	81	81
12	Non-Bollgard	Square Anthers	100	100	100
12	Non-Bollgard	Squares	95	95	92
13	Bollgard	Flower Bracts	100	75	67
13	Bollgard	Flower Anthers	100	100	75
13	Bollgard	Flower Petals	100	100	57
13	Bollgard	Square Anthers	100	100	100
13	Bollgard	Squares	100	100	50
13	Non-Bollgard	Flower Bracts	91	75	68
13	Non-Bollgard	Flower Anthers	100	100	97
13	Non-Bollgard	Flower Petals	95	85	68
13	Non-Bollgard	Square Anthers	100	93	93

(table A.1 continued)

(table A.1 continued)

13	Non-Bollgard	Squares	93	87	77
14	Bollgard	Flower Bracts	87	40	7
14	Bollgard	Flower Anthers	100	100	100
14	Bollgard	Flower Petals	100	86	81
14	Bollgard	Square Anthers	100	100	100
14	Bollgard	Squares	100	71	19
14	Non-Bollgard	Flower Bracts	100	93	85
14	Non-Bollgard	Flower Anthers	100	100	100
14	Non-Bollgard	Flower Petals	93	93	53
14	Non-Bollgard	Square Anthers	100	100	100
14	Non-Bollgard	Squares	87	80	80
15	Bollgard	Flower Bracts	87	62	10
15	Bollgard	Flower Anthers	100	100	90
15	Bollgard	Flower Petals	100	73	73
15	Bollgard	Square Anthers	60	60	69
15	Bollgard	Squares	92	38	8
15	Non-Bollgard	Flower Bracts	93	53	40
15	Non-Bollgard	Flower Anthers	100	100	92
15	Non-Bollgard	Flower Petals	100	77	77
15	Non-Bollgard	Square Anthers	100	78	78
15	Non-Bollgard	Squares	100	80	50
16	Bollgard	Flower Bracts	67	58	10
16	Bollgard	Flower Anthers	100	100	100
16	Bollgard	Flower Petals	92	92	57
16	Bollgard	Square Anthers	100	100	92
16	Bollgard	Squares	93	64	8
16	Non-Bollgard	Flower Bracts	90	80	80
16	Non-Bollgard	Flower Anthers	100	100	100
16	Non-Bollgard	Flower Petals	91	85	75
16	Non-Bollgard	Square Anthers	100	100	100
16	Non-Bollgard	Squares	100	100	100

Table A.2. Bollworm survival on selected floral structures of Bollgard (Deltapine 50B), Bollgard II (experimental), and Non-Bollgard (Deltapine 50) cottons.

Replicate	Variety	Floral Structure	Percent Survival		
			24 hours	48 hours	72 hours
1	Bollgard	Flower Bracts	91	55	20
1	Bollgard	Flower Anthers	100	95	95
1	Bollgard	Flower Petals	100	85	60
1	Bollgard	Square Anthers	100	100	94
1	Bollgard	Squares	90	67	32
1	Bollgard II	Flower Bracts	89	11	6
1	Bollgard II	Flower Anthers	95	63	59
1	Bollgard II	Flower Petals	100	59	17
1	Bollgard II	Square Anthers	100	47	45
1	Bollgard II	Squares	100	12	12
1	Non-Bollgard	Flower Bracts	89	75	41
1	Non-Bollgard	Flower Anthers	94	100	91
1	Non-Bollgard	Flower Petals	100	89	74
1	Non-Bollgard	Square Anthers	94	95	91
1	Non-Bollgard	Squares	70	65	55
2	Bollgard	Flower Bracts	80	63	17
2	Bollgard	Flower Anthers	100	100	92
2	Bollgard	Flower Petals	100	95	73
2	Bollgard	Square Anthers	100	94	89
2	Bollgard	Squares	96	82	48
2	Bollgard II	Flower Bracts	90	43	9
2	Bollgard II	Flower Anthers	100	100	75
2	Bollgard II	Flower Petals	100	94	62
2	Bollgard II	Square Anthers	100	93	59
2	Bollgard II	Squares	91	74	10
2	Non-Bollgard	Flower Bracts	68	61	45
2	Non-Bollgard	Flower Anthers	100	100	100
2	Non-Bollgard	Flower Petals	95	95	77
2	Non-Bollgard	Square Anthers	100	100	100
2	Non-Bollgard	Squares	90	86	85

(table A.2 continued)

(table A.2 continued)

3	Bollgard	Flower Bracts	88	83	24
3	Bollgard	Flower Anthers	100	95	90
3	Bollgard	Flower Petals	100	100	82
3	Bollgard	Square Anthers	100	94	95
3	Bollgard	Squares	96	91	67
3	Bollgard II	Flower Bracts	84	47	5
3	Bollgard II	Flower Anthers	100	94	63
3	Bollgard II	Flower Petals	95	83	43
3	Bollgard II	Square Anthers	100	74	58
3	Bollgard II	Squares	100	47	5
3	Non-Bollgard	Flower Bracts	91	64	59
3	Non-Bollgard	Flower Anthers	100	100	95
3	Non-Bollgard	Flower Petals	100	100	91
3	Non-Bollgard	Square Anthers	100	100	100
3	Non-Bollgard	Squares	96	90	85
4	Bollgard	Flower Bracts	62	28	10
4	Bollgard	Flower Anthers	100	100	95
4	Bollgard	Flower Petals	100	78	52
4	Bollgard	Square Anthers	100	100	89
4	Bollgard	Squares	100	67	50
4	Bollgard II	Flower Bracts	91	14	4
4	Bollgard II	Flower Anthers	100	94	54
4	Bollgard II	Flower Petals	100	88	21
4	Bollgard II	Square Anthers	100	72	38
4	Bollgard II	Squares	95	20	4
4	Non-Bollgard	Flower Bracts	.	.	.
4	Non-Bollgard	Flower Anthers	.	.	.
4	Non-Bollgard	Flower Petals	.	.	.
4	Non-Bollgard	Square Anthers	.	.	.
4	Non-Bollgard	Squares	.	.	.

APPENDIX B

DATA FOR CHAPTER 3

Data for movement of bollworm larvae on non-Bollgard (Deltapine 5415) and Bollgard (Deltapine NuCOTN 33B) cottons.

Table B.1. Bollworm intraplant movement on individual non-flowering cotton plants.

Year	Replicate	Variety	HAI*	Percent		
				Terminals	Plants	Traps
1999	1	Bollgard	1	32	32	8
1999	1	Bollgard	3	8	20	20
1999	1	Bollgard	6	0	12	32
1999	1	Bollgard	24	0	4	32
1999	1	Non-Bollgard	1	58	68	5
1999	1	Non-Bollgard	3	32	58	5
1999	1	Non-Bollgard	6	26	53	5
1999	1	Non-Bollgard	24	16	26	10
1999	2	Bollgard	1	13	40	27
1999	2	Bollgard	3	0	27	47
1999	2	Bollgard	6	0	13	60
1999	2	Bollgard	24	0	0	60
1999	2	Non-Bollgard	1	20	53	7
1999	2	Non-Bollgard	3	13	27	33
1999	2	Non-Bollgard	6	7	27	33
1999	2	Non-Bollgard	24	7	27	33
1999	3	Bollgard	1	13	60	13
1999	3	Bollgard	3	7	7	67
1999	3	Bollgard	6	0	7	67
1999	3	Bollgard	24	0	0	67
1999	3	Non-Bollgard	1	40	67	13
1999	3	Non-Bollgard	3	20	60	20
1999	3	Non-Bollgard	6	13	60	20
1999	3	Non-Bollgard	24	13	53	20
1999	4	Bollgard	1	20	33	33
1999	4	Bollgard	3	20	20	80
1999	4	Bollgard	6	7	13	80
1999	4	Bollgard	24	0	0	80
1999	4	Non-Bollgard	1	40	73	0
1999	4	Non-Bollgard	3	33	73	0

(table B.1 continued)

(table B.1 continued)

1999	4	Non-Bollgard	6	13	67	7
1999	4	Non-Bollgard	24	13	60	7
1999	5	Bollgard	1	33	42	0
1999	5	Bollgard	3	0	17	33
1999	5	Bollgard	6	0	8	50
1999	5	Bollgard	24	0	0	50
1999	5	Non-Bollgard	1	58	75	8
1999	5	Non-Bollgard	3	42	67	8
1999	5	Non-Bollgard	6	20	67	8
1999	5	Non-Bollgard	24	20	67	8
1999	6	Bollgard	1	33	50	17
1999	6	Bollgard	3	17	33	17
1999	6	Bollgard	6	8	25	33
1999	6	Bollgard	24	0	8	42
1999	6	Non-Bollgard	1	67	75	0
1999	6	Non-Bollgard	3	42	75	0
1999	6	Non-Bollgard	6	27	75	8
1999	6	Non-Bollgard	24	7	58	8
2000	1	Bollgard	1	10	35	25
2000	1	Bollgard	3	0	20	50
2000	1	Bollgard	6	0	10	50
2000	1	Bollgard	24	5	5	60
2000	1	Non-Bollgard	1	55	60	15
2000	1	Non-Bollgard	3	50	60	20
2000	1	Non-Bollgard	6	25	50	20
2000	1	Non-Bollgard	24	20	45	20
2000	2	Bollgard	1	10	25	20
2000	2	Bollgard	3	5	25	40
2000	2	Bollgard	6	5	20	45
2000	2	Bollgard	24	5	20	45
2000	2	Non-Bollgard	1	55	65	0
2000	2	Non-Bollgard	3	35	60	0
2000	2	Non-Bollgard	6	30	60	0

(table B.1 continued)

(table B.1 continued)

2000	2	Non-Bollgard	24	15	60	0
2000	3	Bollgard	1	55	85	5
2000	3	Bollgard	3	25	45	15
2000	3	Bollgard	6	20	45	15
2000	3	Bollgard	24	0	25	15
2000	3	Non-Bollgard	1	55	70	0
2000	3	Non-Bollgard	3	30	65	0
2000	3	Non-Bollgard	6	30	60	0
2000	3	Non-Bollgard	24	15	60	0
2000	4	Bollgard	1	40	50	25
2000	4	Bollgard	3	15	20	30
2000	4	Bollgard	6	0	10	35
2000	4	Bollgard	24	0	10	35
2000	4	Non-Bollgard	1	40	50	25
2000	4	Non-Bollgard	3	15	65	5
2000	4	Non-Bollgard	6	5	45	10
2000	4	Non-Bollgard	24	5	40	10
2000	5	Bollgard	1	35	50	20
2000	5	Bollgard	3	20	50	20
2000	5	Bollgard	6	15	45	10
2000	5	Bollgard	24	0	30	40
2000	5	Non-Bollgard	1	35	50	0
2000	5	Non-Bollgard	3	40	50	0
2000	5	Non-Bollgard	6	20	50	10
2000	5	Non-Bollgard	24	15	50	10
2000	6	Bollgard	1	50	50	20
2000	6	Bollgard	3	20	40	20
2000	6	Bollgard	6	20	40	20
2000	6	Bollgard	24	5	35	30
2000	6	Non-Bollgard	1	50	50	0
2000	6	Non-Bollgard	3	35	70	0
2000	6	Non-Bollgard	6	35	55	0
2000	6	Non-Bollgard	24	10	55	0

* hours after infestation

Table B.2. Bollworm intraplant movement on individual flowering cotton plants.

					Number Infested per 20 Plants			
Year	Replicate	Variety	HAI*	Nodes Moved	Terminals	Squares	Flowers	Bolls
2000	1	Bollgard	3	1.8	2	13	0	1
2000	1	Non-Bollgard	3	1.4	4	11	0	0
2000	2	Bollgard	3	2.3	1	10	0	1
2000	2	Non-Bollgard	3	0.9	5	8	0	0
2000	3	Bollgard	3	4.7	0	2	2	6
2000	3	Non-Bollgard	3	2.1	2	10	0	1
2000	4	Bollgard	3	2.8	1	10	1	0
2000	4	Non-Bollgard	3	1.4	4	7	0	0
2000	1	Bollgard	6	3.1	3	8	1	0
2000	1	Non-Bollgard	6	3	1	10	0	0
2000	2	Bollgard	6	4	0	9	1	3
2000	2	Non-Bollgard	6	1.3	3	10	0	0
2000	3	Bollgard	6	5.3	0	2	1	3
2000	3	Non-Bollgard	6	3.4	1	4	0	0
2000	4	Bollgard	6	4.6	0	8	4	2
2000	4	Non-Bollgard	6	2.2	2	8	0	1
2000	1	Bollgard	24	5.2	1	6	2	4
2000	1	Non-Bollgard	24	2.8	2	10	0	0
2000	2	Bollgard	24	4.8	0	4	0	3
2000	2	Non-Bollgard	24	1.9	3	9	0	0
2000	3	Bollgard	24	6.5	0	1	1	4
2000	3	Non-Bollgard	24	4.4	0	4	1	2
2000	4	Bollgard	24	6.3	0	2	1	8
2000	4	Non-Bollgard	24	2.6	1	7	0	2
2001	1	Non-Bollgard	3	0.7	5	6	0	0
2001	1	Bollgard	3	4.3	0	11	1	2
2001	2	Non-Bollgard	3	1.3	5	7	0	0
2001	2	Bollgard	3	1.7	6	4	1	0
2001	3	Non-Bollgard	3	0.4	14	4	0	0
2001	3	Bollgard	3	2	4	10	0	2

(table B.2 continued)

(table B.2 continued)

2001	4	Non-Bollgard	3	1.4	5	6	0	1
2001	4	Bollgard	3	2.4	5	3	0	3
2001	5	Non-Bollgard	3	1	8	9	0	0
2001	5	Bollgard	3	2.8	2	8	1	1
2001	1	Non-Bollgard	6	1.2	3	8	0	0
2001	1	Bollgard	6	5.3	0	7	1	3
2001	2	Non-Bollgard	6	1.2	5	9	0	0
2001	2	Bollgard	6	2.6	3	6	1	0
2001	3	Non-Bollgard	6	1.1	4	12	0	0
2001	3	Bollgard	6	4.3	0	8	0	6
2001	4	Non-Bollgard	6	1.5	4	8	0	1
2001	4	Bollgard	6	2.8	5	3	1	4
2001	5	Non-Bollgard	6	1.1	6	9	0	0
2001	5	Bollgard	6	4.5	1	7	1	2
2001	1	Non-Bollgard	24	2.2	1	10	0	0
2001	1	Bollgard	24	6.9	0	6	1	4
2001	2	Non-Bollgard	24	1.3	5	9	0	0
2001	2	Bollgard	24	4.7	0	5	1	3
2001	3	Non-Bollgard	24	2.1	4	12	0	2
2001	3	Bollgard	24	5.7	0	6	0	6
2001	4	Non-Bollgard	24	2	1	9	0	0
2001	4	Bollgard	24	4.9	0	3	1	6
2001	5	Non-Bollgard	24	2.4	0	12	0	1
2001	5	Bollgard	24	6.6	0	2	2	4

* hours after infestation

Table B.3. Dispersal of bollworm larvae within microplots (1-m row) of Bollgard and non-Bollgard cotton.

				Percent Infested			
Year	Replicate	Variety	HAI*	Terminals	Squares	Flowers	Bolls
2000	1	Bollgard	24	0	0	0	0
2000	1	Non-Bollgard	24	0	0	0	0
2000	2	Bollgard	24	0	1.4	0	0
2000	2	Non-Bollgard	24	14.3	1.4	0	0
2000	3	Bollgard	24	0	1.8	0	0
2000	3	Non-Bollgard	24	22.2	1	0	0
2000	4	Bollgard	24	0	2	0	0
2000	4	Non-Bollgard	24	20	0	0	0
2000	5	Bollgard	24	0	0	0	0
2000	5	Non-Bollgard	24	20	2.9	0	0
2000	6	Bollgard	24	0	6.5	0	0
2000	6	Non-Bollgard	24	37.5	0	0	0
2000	7	Bollgard	24	0	3.8	0	50
2000	7	Non-Bollgard	24	22.2	0	0	0
2000	8	Bollgard	24	0	1.1	0	0
2000	8	Non-Bollgard	24	20	1.2	0	0
2000	9	Bollgard	24	0	1.1	0	0
2000	9	Non-Bollgard	24	60	0	0	0
2000	10	Bollgard	24	14.3	1.4	0	0
2000	10	Non-Bollgard	24	14.3	0	0	0
2000	11	Bollgard	24	15.4	1.3	0	3.7
2000	11	Non-Bollgard	24	83.3	1.7	0	0
2000	12	Bollgard	24	0	1.5	16.7	4.2
2000	12	Non-Bollgard	24	25	4.1	0	3.2
2000	13	Bollgard	24	0	2.4	0	3.7
2000	13	Non-Bollgard	24	40	1.5	0	4.8
2000	14	Bollgard	24	0	1.4	0	4.1
2000	14	Non-Bollgard	24	28.6	1.8	0	2.2
2000	15	Bollgard	24	0	0	8.3	4.7
2000	15	Non-Bollgard	24	66.7	5.2	0	0

(table B.3 continued)

(table B.3 continued)

2000	1	Bollgard	48	0	1.1	33.3	50
2000	1	Non-Bollgard	48	14.3	1.8	0	0
2000	2	Bollgard	48	0	0	0	0
2000	2	Non-Bollgard	48	0	4.2	0	0
2000	3	Bollgard	48	0	0	0	0
2000	3	Non-Bollgard	48	10	0	0	0
2000	4	Bollgard	48	18.1	0	16.7	0
2000	4	Non-Bollgard	48	9	5.4	0	0
2000	5	Bollgard	48	0	0	0	0
2000	5	Non-Bollgard	48	42.9	1.5	0	0
2000	6	Bollgard	48	0	3.4	50	0
2000	6	Non-Bollgard	48	0	0.9	0	0
2000	7	Bollgard	48	0	2.5	0	0
2000	7	Non-Bollgard	48	16.7	1.1	0	0
2000	8	Bollgard	48	0	1.2	0	0
2000	8	Non-Bollgard	48	8.3	2.5	0	0
2000	9	Bollgard	48	0	0	0	0
2000	9	Non-Bollgard	48	0	6.7	0	0
2000	10	Bollgard	48	0	0	0	0
2000	10	Non-Bollgard	48	14.3	4	0	0
2000	11	Bollgard	48	0	0	0	1.6
2000	11	Non-Bollgard	48	0	3.6	33.3	2.8
2000	12	Bollgard	48	0	0	0	1.9
2000	12	Non-Bollgard	48	0	2.2	0	8.3
2000	13	Bollgard	48	0	0	20	4.3
2000	13	Non-Bollgard	48	20	3.2	0	3.8
2000	14	Bollgard	48	0	3.8	25	1.7
2000	14	Non-Bollgard	48	14.3	5.8	0	7.4
2000	15	Bollgard	48	0	0	0	4.1
2000	15	Non-Bollgard	48	33.3	5.6	0	2.6
2001	16	Non-Bollgard	24	5.9	2.2	0	14
2001	16	Bollgard	24	10	0	33.3	18.2
2001	17	Non-Bollgard	24	37.5	1.1	0	0

(table B.3 continued)

(table B.3 continued)

2001	17	Bollgard	24	0	1	0	22
2001	18	Non-Bollgard	24	6.3	2.5	0	0
2001	18	Bollgard	24	0	1	0	13.3
2001	19	Non-Bollgard	24	7.7	1.4	0	0
2001	19	Bollgard	24	0	1.4	0	4.8
2001	20	Non-Bollgard	24	6.3	1	0	7.1
2001	20	Bollgard	24	0	0	0	30
2001	21	Non-Bollgard	24	16.7	2.3	0	2
2001	21	Bollgard	24	8.3	1.1	9.1	6.4
2001	22	Non-Bollgard	24	25	2.6	0	0
2001	22	Bollgard	24	0	1	0	3.8
2001	23	Non-Bollgard	24	20	3.7	10	0
2001	23	Bollgard	24	0	1.2	0	6.5
2001	24	Non-Bollgard	24	15.8	5.2	0	1.6
2001	24	Bollgard	24	14.3	0	0	5.3
2001	25	Non-Bollgard	24	26.3	1.3	0	0
2001	25	Bollgard	24	0	1.1	16.7	10.4
2001	26	Non-Bollgard	24	16.7	1.2	0	0
2001	26	Bollgard	24	0	0	0	2.7
2001	27	Non-Bollgard	24	10	0	0	1.9
2001	27	Bollgard	24	0	0	16.7	8.8
2001	28	Non-Bollgard	24	0	1.7	11.1	0
2001	28	Bollgard	24	0	0	0	3.6
2001	29	Non-Bollgard	24	10	1.9	0	0
2001	29	Bollgard	24	0	1.2	0	3.1
2001	30	Non-Bollgard	24	6.3	1.1	0	0
2001	30	Bollgard	24	0	0	0	4
2001	31	Non-Bollgard	24	5.6	3.3	0	9
2001	31	Bollgard	24	0	1	0	7.7
2001	32	Non-Bollgard	24	5.9	4.1	0	0
2001	32	Bollgard	24	0	1.3	0	7.1
2001	33	Non-Bollgard	24	14.3	2.7	0	0
2001	33	Bollgard	24	0	2.4	0	1.9

(table B.3 continued)

(table B.3 continued)

2001	34	Non-Bollgard	24	10	3.3	0	0
2001	34	Bollgard	24	0	1.6	0	1.7
2001	35	Non-Bollgard	24	25	2.7	0	3.7
2001	35	Bollgard	24	0	1.1	14.3	13.6
2001	36	Non-Bollgard	24	10	2.5	0	8.6
2001	36	Bollgard	24	0	1	75	15.6
2001	37	Non-Bollgard	24	40	3.8	0	3.1
2001	37	Bollgard	24	0	1.1	16.7	12.3
2001	38	Non-Bollgard	24	0	1.3	0	3.1
2001	38	Bollgard	24	0	1	14.3	11.1
2001	39	Non-Bollgard	24	28.6	4.3	12.5	0
2001	39	Bollgard	24	0	1.1	20	2.5
2001	40	Non-Bollgard	24	20	10	0	0
2001	40	Bollgard	24	0	0	33.3	6.9
2001	41	Non-Bollgard	24	37.5	3.5	0	0
2001	41	Bollgard	24	0	1	25	8.9
2001	42	Non-Bollgard	24	0	2.2	0	1.4
2001	42	Bollgard	24	0	0	0	17.5
2001	43	Non-Bollgard	24	22.2	4.8	0	1.6
2001	43	Bollgard	24	0	1	40	3.4
2001	44	Non-Bollgard	24	0	2.5	0	1.2
2001	44	Bollgard	24	0	0	20	11.1
2001	45	Non-Bollgard	24	10	0	25	1.3
2001	45	Bollgard	24	16.7	0	0	12.7
2001	16	Non-Bollgard	48	8.3	5.6	0	0
2001	16	Bollgard	48	10	0	0	20.8
2001	17	Non-Bollgard	48	7.6	4.4	0	1.6
2001	17	Bollgard	48	0	1	0	30.2
2001	18	Non-Bollgard	48	0	6.8	0	0
2001	18	Bollgard	48	0	0	0	13.3
2001	19	Non-Bollgard	48	0	1	0	1.3
2001	19	Bollgard	48	0	0	20	4.9
2001	20	Non-Bollgard	48	7.3	0	0	0

(table B.3 continued)

(table B.3 continued)

2001	20	Bollgard	48	0	1	0	21.6
2001	21	Non-Bollgard	48	16.7	1	0	3.9
2001	21	Bollgard	48	8.3	1.1	0	4.3
2001	22	Non-Bollgard	48	12.5	1.3	0	2.1
2001	22	Bollgard	48	0	0	0	6.4
2001	23	Non-Bollgard	48	10	5.6	0	6.7
2001	23	Bollgard	48	0	1.2	0	6.5
2001	24	Non-Bollgard	48	0	3.1	0	1.6
2001	24	Bollgard	48	0	1.2	16.7	1.8
2001	25	Non-Bollgard	48	0	3.9	0	4.5
2001	25	Bollgard	48	0	1.1	0	8.3
2001	26	Non-Bollgard	48	8.3	3.6	0	0
2001	26	Bollgard	48	0	1	0	2.7
2001	27	Non-Bollgard	48	0	4.4	0	3.8
2001	27	Bollgard	48	0	31.1	0	5.9
2001	28	Non-Bollgard	48	7.1	1.1	11.1	0
2001	28	Bollgard	48	0	1.1	0	5.3
2001	29	Non-Bollgard	48	0	3.2	0	4.4
2001	29	Bollgard	48	0	2.4	12.5	6.3
2001	30	Non-Bollgard	48	6.3	3.4	0	1.8
2001	30	Bollgard	48	0	0	0	6
2001	31	Non-Bollgard	48	5.6	2.2	0	0
2001	31	Bollgard	48	0	0	0	7.7
2001	32	Non-Bollgard	48	5.9	1	0	0
2001	32	Bollgard	48	0	0	0	21.4
2001	33	Non-Bollgard	48	0	1	0	0
2001	33	Bollgard	48	10	1.2	14.3	3.8
2001	34	Non-Bollgard	48	10	2.2	0	0
2001	34	Bollgard	48	0	0	0	8.8
2001	35	Non-Bollgard	48	0	1.8	0	6.9
2001	35	Bollgard	48	0	1.1	14.3	9.1
2001	36	Non-Bollgard	48	0	1	10	4.8
2001	36	Bollgard	48	0	0	25	3.1

(table B.3 continued)

(table B.3 continued)

2001	37	Non-Bollgard	48	20	2.4	0	4.3
2001	37	Bollgard	48	0	0	20	7
2001	38	Non-Bollgard	48	0	6.7	10	2
2001	38	Bollgard	48	11.1	1.8	0	1.6
2001	39	Non-Bollgard	48	0	1	0	2.3
2001	39	Bollgard	48	0	4.5	0	5
2001	40	Non-Bollgard	48	20	3	0	2.5
2001	40	Bollgard	48	10	0	0	1.7
2001	41	Non-Bollgard	48	12.5	4.4	0	1.1
2001	41	Bollgard	48	0	1.9	16.7	11.4
2001	42	Non-Bollgard	48	33.3	2.9	0	0
2001	42	Bollgard	48	0	1.5	0	14
2001	43	Non-Bollgard	48	11.1	3.9	0	0
2001	43	Bollgard	48	0	0	20	13.8
2001	44	Non-Bollgard	48	0	5.7	0	1.2
2001	44	Bollgard	48	0	1.1	0	13
2001	45	Non-Bollgard	48	0	5.2	0	1.4
2001	45	Bollgard	48	0	1	0	17.5

* hours after infestation.

APPENDIX C

DATA FOR CHAPTER 4

Data used for analysis of bollworm damage to fruiting forms on non-Bollgard (Deltapine 5415 and Deltapine 50), Bollgard (Deltapine NuCOTN 33B and Deltapine 50B), and Bollgard II (experimental) cottons in Chapter 4.

Table C.1. Bollworm damaged fruiting forms on Bollgard and non-Bollgard cottons.

				Number Damaged per 50 Plants			
Year	Replicate	Variety	DAI*	Squares	Flowers	Bolls	Total
2000	1	Bollgard	3	.	.	23	23
2000	2	Bollgard	3	.	.	15	15
2000	3	Bollgard	3	.	.	2	2
2001	4	Bollgard	3	.	.	21	21
2001	5	Bollgard	3	.	.	11	11
2001	6	Bollgard	3	.	.	22	22
2001	7	Bollgard	3	.	.	4	4
2001	8	Bollgard	3	.	.	9	9
2000	1	Non-Bollgard	3	.	.	36	36
2000	2	Non-Bollgard	3	.	.	28	28
2000	3	Non-Bollgard	3	.	.	1	1
2001	4	Non-Bollgard	3	.	.	26	26
2001	5	Non-Bollgard	3	.	.	28	28
2001	6	Non-Bollgard	3	.	.	13	13
2001	7	Non-Bollgard	3	.	.	10	10
2001	8	Non-Bollgard	3	.	.	14	14
2000	1	Bollgard	5	7	0	24	31
2000	2	Bollgard	5	3	1	21	25
2000	3	Bollgard	5	0	0	6	6
2001	4	Bollgard	5	1	0	23	24
2001	5	Bollgard	5	0	0	6	6
2001	6	Bollgard	5	3	1	8	12
2001	7	Bollgard	5	1	0	6	7
2001	8	Bollgard	5	1	0	22	23
2000	1	Non-Bollgard	5	17	1	26	44
2000	2	Non-Bollgard	5	18	0	19	37
2000	3	Non-Bollgard	5	3	0	2	5
2001	4	Non-Bollgard	5	2	0	32	34
2001	5	Non-Bollgard	5	16	0	40	56
2001	6	Non-Bollgard	5	4	0	30	34

(table C.1 continued)

(table C.1 continued)

2001	7	Non-Bollgard	5	4	0	12	16
2001	8	Non-Bollgard	5	2	0	19	21
2000	1	Bollgard	7	7	1	26	34
2000	2	Bollgard	7	3	1	22	26
2000	3	Bollgard	7	1	0	7	8
2001	4	Bollgard	7	1	0	24	25
2001	5	Bollgard	7	0	0	6	6
2001	6	Bollgard	7	4	1	10	15
2001	7	Bollgard	7	1	0	3	4
2001	8	Bollgard	7	3	0	22	25
2000	1	Non-Bollgard	7	27	1	32	60
2000	2	Non-Bollgard	7	23	0	21	44
2000	3	Non-Bollgard	7	3	0	4	7
2001	4	Non-Bollgard	7	3	0	34	37
2001	5	Non-Bollgard	7	18	0	50	68
2001	6	Non-Bollgard	7	5	0	35	40
2001	7	Non-Bollgard	7	14	0	22	36
2001	8	Non-Bollgard	7	2	0	28	30
2000	1	Bollgard	9	7	2	27	36
2000	2	Bollgard	9	3	1	22	26
2000	3	Bollgard	9	2	0	8	10
2001	4	Bollgard	9	3	0	27	30
2001	5	Bollgard	9	0	0	8	8
2001	6	Bollgard	9	4	1	11	16
2001	7	Bollgard	9	1	0	3	4
2001	8	Bollgard	9	3	0	22	25
2000	1	Non-Bollgard	9	27	1	37	65
2000	2	Non-Bollgard	9	23	0	29	52
2000	3	Non-Bollgard	9	3	0	4	7
2001	4	Non-Bollgard	9	3	0	35	38
2001	5	Non-Bollgard	9	22	1	54	77
2001	6	Non-Bollgard	9	6	0	39	45
2001	7	Non-Bollgard	9	16	2	34	52

(table C.1 continued)

(table C.1 continued)

2001	8	Non-Bollgard	9	6	0	31	37
2000	1	Bollgard	11	7	2	27	36
2000	2	Bollgard	11	3	1	22	26
2000	3	Bollgard	11	2	0	8	10
2001	4	Bollgard	11	3	0	27	30
2001	5	Bollgard	11	0	0	8	8
2001	6	Bollgard	11	4	1	11	16
2001	7	Bollgard	11	1	0	3	4
2001	8	Bollgard	11	3	0	22	25
2000	1	Non-Bollgard	11	27	1	37	65
2000	2	Non-Bollgard	11	24	0	31	55
2000	3	Non-Bollgard	11	3	0	4	7
2001	4	Non-Bollgard	11	3	0	35	38
2001	5	Non-Bollgard	11	22	1	54	77
2001	6	Non-Bollgard	11	6	0	39	45
2001	7	Non-Bollgard	11	16	2	34	52
2001	8	Non-Bollgard	11	6	0	31	37

* days after infestation.

Table C.2. Numbers of bollworm damaged fruiting forms per larva on non-Bollgard (Deltapine 5415) and Bollgard (Deltapine NuCOTN 33B) cotton.

			Number Damaged per Larva			
Year	Replicate	Variety	Squares	Flowers	Bolls	Total
2000	1	Bollgard	0.7	0.0	1.9	2.6
2000	2	Bollgard	1.3	0.0	2.4	3.7
2000	3	Bollgard	0.1	0.3	2.1	2.5
2000	1	Non-Bollgard	1.1	0.0	3.1	4.2
2000	2	Non-Bollgard	1.7	0.4	2.9	5.0
2000	3	Non-Bollgard	1.0	0.0	2.3	3.3
2001	1	Bollgard	0.2	0.0	2.6	2.8
2001	2	Bollgard	0.8	0.2	2.0	3.0
2001	3	Bollgard	0.0	0.0	2.0	2.0
2001	4	Bollgard	0.3	0.3	2.5	3.1
2001	5	Bollgard	0.4	0.0	1.6	2.0
2001	1	Non-Bollgard	1.0	0.0	2.8	3.8
2001	2	Non-Bollgard	1.4	0.3	3.4	5.1
2001	3	Non-Bollgard	1.4	0.0	3.3	4.7
2001	4	Non-Bollgard	1.3	0.1	2.7	4.1
2001	5	Non-Bollgard	1.2	0.0	2.8	4.0

Table C.3. Percentages of bollworm larvae recovered following infestations in white flowers on non-Bollgard (Deltapine 5415) and Bollgard (Deltapine NuCOTN 33B) cottons.

			Percentage of Larvae Recovered				
Year	Replicate	Variety	3 days	5 days	7 days	9 days	11 days
2000	1	Bollgard	100	80.0	70.0	30.0	0
2000	1	Non-Bollgard	100	94.4	50.0	5.6	0
2000	2	Bollgard	100	80.0	60.0	20.0	0
2000	2	Non-Bollgard	100	100	53.3	0	0
2000	3	Bollgard	.	.	.	.	.
2000	3	Non-Bollgard	100	100	100	0	0
2001	4	Bollgard	100	50.0	31.8	9.1	0
2001	4	Non-Bollgard	100	85.7	42.9	7.1	0
2001	5	Bollgard	100	66.7	50.0	16.7	0
2001	5	Non-Bollgard	100	100	37.5	0	0
2001	6	Bollgard	100	57.1	42.9	14.3	0
2001	6	Non-Bollgard	100	100	70.0	10.0	0
2001	7	Bollgard	100	100	100	100	0
2001	7	Non-Bollgard	100	100	50.0	0	0
2001	8	Bollgard	.	.	.	.	.
2001	8	Non-Bollgard	100	100	50.0	0	0

Table C.4. Bollworm damaged fruiting forms on non-Bollgard (Deltapine 50), Bollgard (Deltapine 50B), and Bollgard II (experimental) cottons.

			Number Damaged per 10 Plants			
Replicate	Variety	DAI*	Squares	Flowers	Bolls	Total
1	Bollgard	3	.	.	4	4
2	Bollgard	3	.	.	8	8
3	Bollgard	3	.	.	4	4
4	Bollgard	3	.	.	8	8
5	Bollgard	3	.	.	8	8
6	Bollgard	3	.	.	6	6
1	Bollgard II	3	.	.	3	3
2	Bollgard II	3	.	.	6	6
3	Bollgard II	3	.	.	6	6
4	Bollgard II	3	.	.	7	7
5	Bollgard II	3	.	.	8	8
6	Bollgard II	3	.	.	4	4
1	Non-Bollgard	3	.	.	6	6
2	Non-Bollgard	3	.	.	9	9
3	Non-Bollgard	3	.	.	8	8
4	Non-Bollgard	3	.	.	9	9
5	Non-Bollgard	3	.	.	9	9
6	Non-Bollgard	3	.	.	10	10
1	Bollgard	5	0	0	6	6
2	Bollgard	5	1	1	9	11
3	Bollgard	5	0	0	10	10
4	Bollgard	5	2	0	9	11
5	Bollgard	5	0	0	8	8
6	Bollgard	5	1	1	6	8
1	Bollgard II	5	0	0	3	3
2	Bollgard II	5	0	0	6	6
3	Bollgard II	5	0	0	7	7
4	Bollgard II	5	1	0	7	8
5	Bollgard II	5	0	1	8	9
6	Bollgard II	5	0	0	5	5

(table C.4 continued)

(table C.4 continued)

1	Non-Bollgard	5	12	0	16	28
2	Non-Bollgard	5	13	0	11	24
3	Non-Bollgard	5	2	2	14	18
4	Non-Bollgard	5	4	0	12	16
5	Non-Bollgard	5	3	0	9	12
6	Non-Bollgard	5	1	0	10	11
1	Bollgard	7	0	2	6	8
2	Bollgard	7	2	1	10	13
3	Bollgard	7	0	2	11	13
4	Bollgard	7	3	1	9	13
5	Bollgard	7	0	0	8	8
6	Bollgard	7	1	1	8	10
1	Bollgard II	7	0	0	3	3
2	Bollgard II	7	0	0	6	6
3	Bollgard II	7	0	0	7	7
4	Bollgard II	7	1	0	7	8
5	Bollgard II	7	0	1	9	10
6	Bollgard II	7	0	0	5	5
1	Non-Bollgard	7	14	1	18	33
2	Non-Bollgard	7	15	1	17	33
3	Non-Bollgard	7	8	2	19	29
4	Non-Bollgard	7	5	0	14	19
5	Non-Bollgard	7	4	0	10	14
6	Non-Bollgard	7	3	0	10	13
1	Bollgard	9	0	2	7	9
2	Bollgard	9	2	1	10	13
3	Bollgard	9	1	2	12	15
4	Bollgard	9	3	1	9	13
5	Bollgard	9	1	0	8	9
6	Bollgard	9	1	1	8	10
1	Bollgard II	9	0	0	3	3
2	Bollgard II	9	0	0	6	6
3	Bollgard II	9	0	0	7	7

(table C.4 continued)

(table C.4 continued)

4	Bollgard II	9	1	0	7	8
5	Bollgard II	9	0	1	9	10
6	Bollgard II	9	0	1	5	6
1	Non-Bollgard	9	14	1	18	33
2	Non-Bollgard	9	16	1	17	34
3	Non-Bollgard	9	8	2	19	29
4	Non-Bollgard	9	6	0	17	23
5	Non-Bollgard	9	4	0	10	14
6	Non-Bollgard	9	6	0	11	17
1	Bollgard	11	0	2	7	9
2	Bollgard	11	2	1	10	13
3	Bollgard	11	1	2	12	15
4	Bollgard	11	3	1	9	13
5	Bollgard	11	1	0	8	9
6	Bollgard	11	1	1	8	10
1	Bollgard II	11	0	0	3	3
2	Bollgard II	11	0	0	6	6
3	Bollgard II	11	0	0	7	7
4	Bollgard II	11	1	0	7	8
5	Bollgard II	11	0	1	9	10
6	Bollgard II	11	0	1	5	6
1	Non-Bollgard	11	14	1	18	33
2	Non-Bollgard	11	16	1	17	34
3	Non-Bollgard	11	8	2	19	29
4	Non-Bollgard	11	6	0	17	23
5	Non-Bollgard	11	4	0	10	14
6	Non-Bollgard	11	6	0	11	17

* days after infestation

Table C.5. Numbers of bollworm damaged fruiting forms per larva on non-Bollgard (Deltapine 50), Bollgard (Deltapine 50B), and Bollgard II (experimental) cottons.

		Number Damaged per Larva			
Replicate	Variety	Squares	Flowers	Bolls	Total
1	Bollgard	0.0	0.5	2.8	3.3
2	Bollgard	0.6	0.3	2.4	3.3
3	Bollgard	0.3	0.5	3.1	3.9
4	Bollgard	1.2	0.3	2.6	4.1
5	Bollgard	0.2	0.0	1.8	2.0
6	Bollgard	0.4	0.3	2.9	3.6
1	Bollgard II	0.0	0.0	0.8	0.8
2	Bollgard II	0.0	0.0	1.2	1.2
3	Bollgard II	0.0	0.0	0.6	0.6
4	Bollgard II	0.2	0.0	0.5	0.7
5	Bollgard II	0.0	0.3	1.4	1.7
6	Bollgard II	0.0	0.3	0.9	1.2
1	Non-Bollgard	2.3	0.3	2.9	5.5
2	Non-Bollgard	3.6	0.3	3.8	7.7
3	Non-Bollgard	2.4	0.3	3.2	5.9
4	Non-Bollgard	2.6	0.0	2.6	5.2
5	Non-Bollgard	1.9	0.0	1.8	3.7
6	Non-Bollgard	2.0	0.0	2.0	4.0

Table C.6. Percentages of bollworm larvae recovered following infestations in white flowers on non-Bollgard (Deltapine 50), Bollgard (Deltapine 50B), and Bollgard II (experimental) cottons.

			Percentage of Larvae Recovered			
Replicate	Variety	3 days	5 days	7 days	9 days	11 days
1	Bollgard	100	75.0	25.0	25.0	0.0
2	Bollgard	100	50.0	50.0	33.3	0.0
3	Bollgard	100	50.0	50.0	0.0	0.0
4	Bollgard	100	100	100	50.0	0.0
5	Bollgard	100	66.7	33.3	33.3	0.0
6	Bollgard	100	100	100	100	0.0
1	Bollgard II	100	0.0	0.0	0.0	0.0
2	Bollgard II	100	0.0	0.0	0.0	0.0
3	Bollgard II	100	50.0	0.0	0.0	0.0
4	Bollgard II	100	0.0	0.0	0.0	0.0
5	Bollgard II	100	50.0	0.0	0.0	0.0
6	Bollgard II	100	50.0	50.0	0.0	0.0
1	Non-Bollgard	100	100	75.0	0.0	0.0
2	Non-Bollgard	100	87.5	75.0	25.0	0.0
3	Non-Bollgard	100	100	100	0.0	0.0
4	Non-Bollgard	100	100	50.0	0.0	0.0
5	Non-Bollgard	100	100	62.5	12.5	0.0
6	Non-Bollgard	100	88.9	44.4	11.1	0.0

APPENDIX D

DATA FOR CHAPTER 5

Data used for analysis of *Helicoverpa zea* performance on various host plants and subsequent susceptibility to Bollgard cotton in Chapter 5.

Table D.1. *Helicoverpa zea* developmental times on field corn, cotton, grain sorghum, soybean, and meridic diet.

Host Colony	Replicate	Days
Field Corn	1	11
Field Corn	1	11
Field Corn	1	11
Field Corn	1	11
Field Corn	1	11
Field Corn	1	11
Field Corn	1	11
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	12
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	13
Field Corn	1	14
Field Corn	2	11
Field Corn	2	11
Field Corn	2	11
Field Corn	2	11
Field Corn	2	11
Field Corn	2	11
Field Corn	2	11
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	12
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	14
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	13
Field Corn	2	14
Field Corn	2	14
Cotton	1	23
Cotton	1	23

(table D.1 continued)

(table D.1 continued)

Cotton	1	23
Cotton	1	22
Cotton	1	25
Cotton	1	24
Cotton	1	24
Cotton	1	24
Cotton	1	27
Cotton	1	27
Cotton	1	27
Cotton	1	27
Cotton	1	26
Cotton	1	26
Cotton	2	23
Cotton	2	23
Cotton	2	22
Cotton	2	25
Cotton	2	25
Cotton	2	24
Cotton	2	24
Cotton	2	24
Cotton	2	27
Cotton	2	27
Cotton	2	27
Cotton	2	27
Cotton	2	26
Cotton	2	28
Meridic Diet	1	13
Meridic Diet	1	13
Meridic Diet	1	13

(table D.1 continued)

Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	15
Meridic Diet	1	17
Meridic Diet	1	17
Meridic Diet	1	17

(table D.1 continued)

Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	14
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	15
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	17
Meridic Diet	2	19
Meridic Diet	2	19
Meridic Diet	2	19
Meridic Diet	2	19
Meridic Diet	2	19
Meridic Diet	2	19
Meridic Diet	2	19
Meridic Diet	2	19
Grain Sorghum	1	13
Grain Sorghum	1	13

(table D.1 continued)

(table D.1 continued)

Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	13
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	14
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	15
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	17
Grain Sorghum	1	16
Grain Sorghum	1	16
Grain Sorghum	1	16
Grain Sorghum	1	16
Grain Sorghum	1	16
Grain Sorghum	1	16
Grain Sorghum	1	16
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19

(table D.1 continued)

(table D.1 continued)

Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	19
Grain Sorghum	1	18
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	13
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	14
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	15
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17
Grain Sorghum	2	17

(table D.1 continued)

(table D.1 continued)

Grain Sorghum	2	16
Grain Sorghum	2	16
Grain Sorghum	2	16
Grain Sorghum	2	16
Grain Sorghum	2	16
Grain Sorghum	2	16
Grain Sorghum	2	16
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	19
Grain Sorghum	2	18
Grain Sorghum	2	18
Soybean	1	15
Soybean	1	15
Soybean	1	17
Soybean	1	17
Soybean	1	17

(table D.1 continued)

Soybean	1	20
Soybean	1	21
Soybean	1	21
Soybean	1	21
Soybean	1	21
Soybean	2	15
Soybean	2	17
Soybean	2	17
Soybean	2	17

Table D.2. *Helicoverpa zea* pupal weights on field corn, cotton, grain sorghum, soybean, and meridic diet.

Host Colony	Replicate	Weight (mg)
Field Corn	1	310.2
Field Corn	1	295.1
Field Corn	1	263.5
Field Corn	1	241.9
Field Corn	1	284.7
Field Corn	1	354
Field Corn	1	245.9
Field Corn	1	363.8
Field Corn	1	393.7
Field Corn	1	331.1
Field Corn	1	298.6
Field Corn	1	288.2
Field Corn	1	305.4
Field Corn	1	325.2
Field Corn	1	270.4
Field Corn	1	371.3
Field Corn	1	236.2
Field Corn	1	321.5
Field Corn	1	222.9
Field Corn	1	319.4
Field Corn	2	276.2
Field Corn	2	376.9
Field Corn	2	248.7
Field Corn	2	331.4
Field Corn	2	215.2
Field Corn	2	283.7
Field Corn	2	246.2
Field Corn	2	254.4
Field Corn	2	248.8
Field Corn	2	318.6
Field Corn	2	331.2
Field Corn	2	293.6
Field Corn	2	271
Field Corn	2	322.8
Field Corn	2	279.8
Field Corn	2	231.7
Field Corn	2	287.2
Field Corn	2	296.4
Field Corn	2	242.1
Field Corn	2	342.5
Cotton	1	274
Cotton	1	255.4
Cotton	1	171.7
Cotton	1	288.9
Cotton	1	206.4
Cotton	1	241.2
Cotton	1	246.6
Cotton	1	218.5
Cotton	1	264.7
Cotton	1	246.5
Cotton	1	299.2
Cotton	1	214.8
Cotton	1	226.3
Cotton	1	233.2
Cotton	2	283.9
Cotton	2	267
Cotton	2	187.3
Cotton	2	304.7
Cotton	2	276.6
Cotton	2	270.7
Cotton	2	188.7
Cotton	2	139.2
Cotton	2	184.9
Cotton	2	171.6
Cotton	2	241.7
Cotton	2	199.6
Cotton	2	159.8
Cotton	2	223.6
Meridic Diet	1	373.1
Meridic Diet	1	446.2
Meridic Diet	1	427.2
Meridic Diet	1	426.9
Meridic Diet	1	369.2
Meridic Diet	1	392.6

(table D.2 continued)

(table D.2 continued)

Meridic Diet	1	377.7
Meridic Diet	1	400.2
Meridic Diet	1	355.7
Meridic Diet	1	436.7
Meridic Diet	1	341.6
Meridic Diet	1	414.1
Meridic Diet	1	336.3
Meridic Diet	1	417.8
Meridic Diet	1	365.5
Meridic Diet	1	353.2
Meridic Diet	1	381.9
Meridic Diet	1	357.3
Meridic Diet	1	400.5
Meridic Diet	1	347.6
Meridic Diet	2	335.6
Meridic Diet	2	382.1
Meridic Diet	2	416.1
Meridic Diet	2	435.1
Meridic Diet	2	391.9
Meridic Diet	2	298.2
Meridic Diet	2	359.1
Meridic Diet	2	411.5
Meridic Diet	2	358.3
Meridic Diet	2	364.4
Meridic Diet	2	443
Meridic Diet	2	371.5
Meridic Diet	2	373.6
Meridic Diet	2	336.8
Meridic Diet	2	407.1
Meridic Diet	2	357.7
Meridic Diet	2	357.9
Meridic Diet	2	397.1
Meridic Diet	2	378.3
Meridic Diet	2	377.3
Grain Sorghum	1	385.2
Grain Sorghum	1	346.8
Grain Sorghum	1	321.5
Grain Sorghum	1	280.9
Grain Sorghum	1	256.7
Grain Sorghum	1	310.8
Grain Sorghum	1	347.1
Grain Sorghum	1	231.8
Grain Sorghum	1	325.6
Grain Sorghum	1	311.4
Grain Sorghum	1	344.8
Grain Sorghum	1	324.9
Grain Sorghum	1	323.3
Grain Sorghum	1	284.6
Grain Sorghum	1	281.4
Grain Sorghum	1	255.1
Grain Sorghum	1	238.6
Grain Sorghum	1	253.6
Grain Sorghum	1	334.8
Grain Sorghum	1	343
Grain Sorghum	2	352.9
Grain Sorghum	2	282.8
Grain Sorghum	2	318.4
Grain Sorghum	2	331.2
Grain Sorghum	2	330.4
Grain Sorghum	2	295.9
Grain Sorghum	2	322.7
Grain Sorghum	2	370.8
Grain Sorghum	2	276.4
Grain Sorghum	2	289.9
Grain Sorghum	2	259.8
Grain Sorghum	2	286.4
Grain Sorghum	2	317.6
Grain Sorghum	2	344.6
Grain Sorghum	2	269.5
Grain Sorghum	2	255
Grain Sorghum	2	235.5
Grain Sorghum	2	397.7
Grain Sorghum	2	319.2
Grain Sorghum	2	303.1
Soybean	1	308.5
Soybean	1	394

(table D.2 continued)

(table D.2 continued)

Soybean	1	357.5
Soybean	1	305.7
Soybean	1	340
Soybean	1	321.3
Soybean	1	383
Soybean	1	311.9
Soybean	1	374.1
Soybean	1	290.8
Soybean	1	284.3
Soybean	1	386.1
Soybean	1	383.1
Soybean	1	263.5
Soybean	1	327.5
Soybean	1	319.5
Soybean	1	402.6
Soybean	1	325.5
Soybean	1	387.4
Soybean	1	363.7
Soybean	2	310.3
Soybean	2	337.1
Soybean	2	377.8
Soybean	2	292
Soybean	2	318.4
Soybean	2	369.7
Soybean	2	393.1
Soybean	2	402.6
Soybean	2	315.7
Soybean	2	359.2
Soybean	2	287.9
Soybean	2	349.3
Soybean	2	381.8
Soybean	2	305.8
Soybean	2	322.6
Soybean	2	324.8
Soybean	2	278
Soybean	2	325.2
Soybean	2	284
Soybean	2	326

Table D.3. *Helicoverpa zea* survival to pupation on field corn, cotton, grain sorghum, soybean, and meridic diet.

Replicate	Host Colony	Percent Survival
1	Field Corn	57
2	Field Corn	52
1	Cotton	9
2	Cotton	17
1	Meridic Diet	75
2	Meridic Diet	91
1	Grain Sorghum	70
2	Grain Sorghum	76
1	Soybean	30
2	Soybean	22

Table D.4. Mortality of *Helicoverpa zea* from host colonies on non-Bollgard (Deltapine 50) and Bollgard (Deltapine 50B) cotton.

Replicate	Host Colony	Variety	Percent Mortality			
			24 h	48 h	72 h	96 h
1	Field Corn	Bollgard	0	100	60	80
1	Field Corn	Bollgard	0	80	100	100
1	Field Corn	Bollgard	20	40	80	100
1	Field Corn	Bollgard	0	67	60	40
1	Field Corn	Bollgard	0	75	60	75
1	Field Corn	Bollgard	0	20	40	60
1	Field Corn	Bollgard	0	60	50	60
1	Field Corn	Bollgard	0	80	60	80
1	Field Corn	Bollgard	0	100	60	100
2	Field Corn	Bollgard	20	60	60	80
2	Field Corn	Bollgard	0	100	60	60
2	Field Corn	Bollgard	0	40	100	100
2	Field Corn	Bollgard	0	25	100	80
2	Field Corn	Bollgard	40	60	80	60
2	Field Corn	Bollgard	0	25	60	80
2	Field Corn	Bollgard	0	60	100	100
2	Field Corn	Bollgard	0	75	80	75
2	Field Corn	Bollgard	20	40	75	100
3	Field Corn	Bollgard	0	75	20	100
3	Field Corn	Bollgard	0	60	100	100
3	Field Corn	Bollgard	0	60	80	100
3	Field Corn	Bollgard	20	60	60	100
3	Field Corn	Bollgard	0	20	80	100
3	Field Corn	Bollgard	50	33	40	100
3	Field Corn	Bollgard	0	100	100	100
3	Field Corn	Bollgard	0	60	100	100
3	Field Corn	Bollgard	40	60	100	100
4	Field Corn	Bollgard	0	100	100	100
4	Field Corn	Bollgard	0	100	100	100
4	Field Corn	Bollgard	0	100	100	100

(table D.4 continued)

(table D.4 continued)

4	Field Corn	Bollgard	0	40	100	100
4	Field Corn	Bollgard	0	20	50	60
4	Field Corn	Bollgard	20	60	60	40
4	Field Corn	Bollgard	20	60	40	20
4	Field Corn	Bollgard	0	0	0	80
4	Field Corn	Bollgard	0	0	0	100
4	Field Corn	Bollgard	0	25	100	80
4	Field Corn	Bollgard	0	80	100	75
4	Field Corn	Bollgard	0	80	60	100
4	Field Corn	Bollgard	0	60	50	100
1	Cotton	Bollgard	60	20	20	100
1	Cotton	Bollgard	40	40	0	20
1	Cotton	Bollgard	0	40	80	60
1	Cotton	Bollgard	25	0	60	80
1	Cotton	Bollgard	20	60	20	40
1	Cotton	Bollgard	0	80	75	20
1	Cotton	Bollgard	0	20	20	60
1	Cotton	Bollgard	20	33	100	80
1	Cotton	Bollgard	60	80	0	60
1	Cotton	Bollgard	60	0	20	80
2	Cotton	Bollgard	0	25	75	100
2	Cotton	Bollgard	0	100	100	100
2	Cotton	Bollgard	25	50	100	60
2	Cotton	Bollgard	0	100	25	40
2	Cotton	Bollgard	0	40	20	60
2	Cotton	Bollgard	0	25	60	75
2	Cotton	Bollgard	100	20	100	80
2	Cotton	Bollgard	80	0	50	60
2	Cotton	Bollgard	20	33	80	60
2	Cotton	Bollgard	100	25	20	100
3	Cotton	Bollgard	0	20	60	80
3	Cotton	Bollgard	40	80	0	75
3	Cotton	Bollgard	0	80	75	100

(table D.4 continued)

(table D.4 continued)

3	Cotton	Bollgard	0	50	100	25
3	Cotton	Bollgard	0	25	20	75
3	Cotton	Bollgard	60	25	50	80
3	Cotton	Bollgard	0	0	67	100
3	Cotton	Bollgard	0	0	80	20
3	Cotton	Bollgard	0	67	80	20
3	Cotton	Bollgard	20	75	0	100
4	Cotton	Bollgard	100	0	50	100
4	Cotton	Bollgard	20	100	67	100
4	Cotton	Bollgard	20	20	20	100
4	Cotton	Bollgard	0	60	20	100
4	Cotton	Bollgard	0	0	100	100
4	Cotton	Bollgard	0	0	100	100
4	Cotton	Bollgard	0	25	100	100
4	Cotton	Bollgard	20	100	100	100
4	Cotton	Bollgard	60	100	100	100
4	Cotton	Bollgard	0	100	100	100
1	Meridic Diet	Bollgard	0	0	67	20
1	Meridic Diet	Bollgard	20	25	100	60
1	Meridic Diet	Bollgard	0	0	20	80
1	Meridic Diet	Bollgard	0	0	0	40
1	Meridic Diet	Bollgard	20	50	100	75
1	Meridic Diet	Bollgard	0	20	0	100
1	Meridic Diet	Bollgard	0	100	80	100
1	Meridic Diet	Bollgard	0	100	40	100
1	Meridic Diet	Bollgard	0	60	80	40
1	Meridic Diet	Bollgard	0	40	40	60
2	Meridic Diet	Bollgard	0	80	20	100
2	Meridic Diet	Bollgard	0	80	20	100
2	Meridic Diet	Bollgard	0	60	100	100
2	Meridic Diet	Bollgard	0	20	20	100
2	Meridic Diet	Bollgard	0	60	75	100
2	Meridic Diet	Bollgard	0	50	100	100

(table D.4 continued)

(table D.4 continued)

2	Meridic Diet	Bollgard	0	0	100	100
2	Meridic Diet	Bollgard	20	20	100	100
2	Meridic Diet	Bollgard	0	40	20	40
2	Meridic Diet	Bollgard	20	60	0	40
3	Meridic Diet	Bollgard	0	20	80	100
3	Meridic Diet	Bollgard	0	0	100	100
3	Meridic Diet	Bollgard	0	20	20	80
3	Meridic Diet	Bollgard	20	0	80	100
3	Meridic Diet	Bollgard	40	75	100	40
3	Meridic Diet	Bollgard	0	25	75	100
3	Meridic Diet	Bollgard	0	40	20	75
3	Meridic Diet	Bollgard	0	40	100	60
3	Meridic Diet	Bollgard	20	50	0	80
3	Meridic Diet	Bollgard	0	100	60	80
4	Meridic Diet	Bollgard	0	40	50	60
4	Meridic Diet	Bollgard	0	0	60	60
4	Meridic Diet	Bollgard	0	20	60	20
4	Meridic Diet	Bollgard	25	80	80	100
4	Meridic Diet	Bollgard	0	100	20	100
4	Meridic Diet	Bollgard	0	40	75	100
4	Meridic Diet	Bollgard	0	20	100	100
4	Meridic Diet	Bollgard	20	80	100	100
4	Meridic Diet	Bollgard	0	0	0	0
4	Meridic Diet	Bollgard	0	0	0	20
1	Grain Sorghum	Bollgard	0	20	100	40
1	Grain Sorghum	Bollgard	20	40	60	40
1	Grain Sorghum	Bollgard	0	20	20	60
1	Grain Sorghum	Bollgard	60	60	20	80
1	Grain Sorghum	Bollgard	0	60	20	40
1	Grain Sorghum	Bollgard	0	40	80	20
1	Grain Sorghum	Bollgard	0	40	60	100
1	Grain Sorghum	Bollgard	0	20	25	40
1	Grain Sorghum	Bollgard	0	20	40	60

(table D.4 continued)

(table D.4 continued)

1	Grain Sorghum	Bollgard	0	60	60	40
2	Grain Sorghum	Bollgard	20	40	60	60
2	Grain Sorghum	Bollgard	0	50	40	60
2	Grain Sorghum	Bollgard	80	60	60	20
2	Grain Sorghum	Bollgard	25	40	60	60
2	Grain Sorghum	Bollgard	0	40	40	60
2	Grain Sorghum	Bollgard	60	20	20	80
2	Grain Sorghum	Bollgard	40	80	0	60
2	Grain Sorghum	Bollgard	0	40	40	80
2	Grain Sorghum	Bollgard	100	80	80	80
2	Grain Sorghum	Bollgard	0	40	80	40
3	Grain Sorghum	Bollgard	75	40	100	40
3	Grain Sorghum	Bollgard	0	80	40	60
3	Grain Sorghum	Bollgard	0	40	0	60
3	Grain Sorghum	Bollgard	0	0	75	40
3	Grain Sorghum	Bollgard	0	60	20	40
3	Grain Sorghum	Bollgard	0	60	0	60
3	Grain Sorghum	Bollgard	20	40	0	60
3	Grain Sorghum	Bollgard	0	60	40	100
3	Grain Sorghum	Bollgard	20	100	20	40
3	Grain Sorghum	Bollgard	0	40	40	80
4	Grain Sorghum	Bollgard	20	20	20	80
4	Grain Sorghum	Bollgard	0	20	0	60
4	Grain Sorghum	Bollgard	0	60	20	100
4	Grain Sorghum	Bollgard	40	20	0	100
4	Grain Sorghum	Bollgard	0	0	100	100
4	Grain Sorghum	Bollgard	20	80	40	100
4	Grain Sorghum	Bollgard	0	20	100	100
4	Grain Sorghum	Bollgard	100	100	100	100
4	Grain Sorghum	Bollgard	0	100	25	20
4	Grain Sorghum	Bollgard	20	0	0	60
1	Soybean	Bollgard	0	20	75	20
1	Soybean	Bollgard	0	20	40	20

(table D.4 continued)

(table D.4 continued)

1	Soybean	Bollgard	20	0	0	100
1	Soybean	Bollgard	0	80	20	60
1	Soybean	Bollgard	0	40	60	40
1	Soybean	Bollgard	0	20	0	20
1	Soybean	Bollgard	20	0	40	20
1	Soybean	Bollgard	0	0	80	40
1	Soybean	Bollgard	20	40	40	100
1	Soybean	Bollgard	0	0	40	100
2	Soybean	Bollgard	40	40	40	40
2	Soybean	Bollgard	0	75	0	100
2	Soybean	Bollgard	0	0	0	0
2	Soybean	Bollgard	0	40	80	100
2	Soybean	Bollgard	0	40	100	60
2	Soybean	Bollgard	0	25	20	80
2	Soybean	Bollgard	0	60	80	100
2	Soybean	Bollgard	20	0	40	60
2	Soybean	Bollgard	0	40	60	80
2	Soybean	Bollgard	0	40	40	60
3	Soybean	Bollgard	0	20	40	40
3	Soybean	Bollgard	0	40	40	60
3	Soybean	Bollgard	0	20	40	40
3	Soybean	Bollgard	0	40	0	60
3	Soybean	Bollgard	0	20	0	80
3	Soybean	Bollgard	20	20	40	80
3	Soybean	Bollgard	0	0	80	60
3	Soybean	Bollgard	0	20	40	100
3	Soybean	Bollgard	0	0	40	100
3	Soybean	Bollgard	20	20	20	80
4	Soybean	Bollgard	20	80	80	80
4	Soybean	Bollgard	20	40	40	60
4	Soybean	Bollgard	0	100	60	80
4	Soybean	Bollgard	20	80	100	40
4	Soybean	Bollgard	40	100	60	100

(table D.4 continued)

(table D.4 continued)

4	Soybean	Bollgard	50	60	80	100
4	Soybean	Bollgard	0	40	100	100
4	Soybean	Bollgard	0	60	100	100
4	Soybean	Bollgard	0	0	20	20
4	Soybean	Bollgard	0	20	0	0
1	Field Corn	Non-Bollgard	0	20	0	67
1	Field Corn	Non-Bollgard	0	0	0	25
1	Field Corn	Non-Bollgard	0	0	100	0
1	Field Corn	Non-Bollgard	0	0	0	0
1	Field Corn	Non-Bollgard	0	0	0	0
2	Field Corn	Non-Bollgard	0	0	40	0
2	Field Corn	Non-Bollgard	0	0	0	0
2	Field Corn	Non-Bollgard	0	25	40	0
2	Field Corn	Non-Bollgard	0	0	0	0
2	Field Corn	Non-Bollgard	40	40	0	25
3	Field Corn	Non-Bollgard	0	0	20	0
3	Field Corn	Non-Bollgard	20	0	0	0
3	Field Corn	Non-Bollgard	0	0	20	0
3	Field Corn	Non-Bollgard	25	60	0	33
3	Field Corn	Non-Bollgard	0	40	0	20
4	Field Corn	Non-Bollgard	0	0	0	0
4	Field Corn	Non-Bollgard	0	0	0	25
4	Field Corn	Non-Bollgard	20	0	25	50
4	Field Corn	Non-Bollgard	20	20	0	0
4	Field Corn	Non-Bollgard	50	0	0	100
1	Cotton	Non-Bollgard	20	25	60	0
1	Cotton	Non-Bollgard	40	40	0	60
1	Cotton	Non-Bollgard	60	100	80	60
1	Cotton	Non-Bollgard	80	100	40	100
1	Cotton	Non-Bollgard	0	100	0	40
1	Cotton	Non-Bollgard	40	75	67	60
2	Cotton	Non-Bollgard	80	67	25	100
2	Cotton	Non-Bollgard	40	0	100	100

(table D.4 continued)

(table D.4 continued)

2	Cotton	Non-Bollgard	60	40	100	100
2	Cotton	Non-Bollgard	0	80	100	100
2	Cotton	Non-Bollgard	20	0	20	40
2	Cotton	Non-Bollgard	25	0	20	25
3	Cotton	Non-Bollgard	0	40	20	0
3	Cotton	Non-Bollgard	0	20	60	0
3	Cotton	Non-Bollgard	40	40	50	20
3	Cotton	Non-Bollgard	0	20	50	25
3	Cotton	Non-Bollgard	0	0	0	50
4	Cotton	Non-Bollgard	0	20	20	20
4	Cotton	Non-Bollgard	0	0	25	60
4	Cotton	Non-Bollgard	0	20	20	40
4	Cotton	Non-Bollgard	0	25	80	80
4	Cotton	Non-Bollgard	0	0	20	60
1	Meridic Diet	Non-Bollgard	0	0	0	0
1	Meridic Diet	Non-Bollgard	0	0	0	0
1	Meridic Diet	Non-Bollgard	0	0	0	0
1	Meridic Diet	Non-Bollgard	0	0	20	40
1	Meridic Diet	Non-Bollgard	0	0	0	0
2	Meridic Diet	Non-Bollgard	0	0	0	0
2	Meridic Diet	Non-Bollgard	0	0	0	40
2	Meridic Diet	Non-Bollgard	0	0	0	0
2	Meridic Diet	Non-Bollgard	0	50	0	0
2	Meridic Diet	Non-Bollgard	0	0	50	0
3	Meridic Diet	Non-Bollgard	0	0	0	20
3	Meridic Diet	Non-Bollgard	0	0	0	0
3	Meridic Diet	Non-Bollgard	0	0	0	20
3	Meridic Diet	Non-Bollgard	0	0	0	0
3	Meridic Diet	Non-Bollgard	0	0	0	0
4	Meridic Diet	Non-Bollgard	0	0	0	0
4	Meridic Diet	Non-Bollgard	0	0	0	0
4	Meridic Diet	Non-Bollgard	0	0	0	0
4	Meridic Diet	Non-Bollgard	40	60	40	0

(table D.4 continued)

(table D.4 continued)

4	Meridic Diet	Non-Bollgard	0	0	60	60
1	Grain Sorghum	Non-Bollgard	0	0	0	20
1	Grain Sorghum	Non-Bollgard	0	0	0	0
1	Grain Sorghum	Non-Bollgard	0	0	0	50
1	Grain Sorghum	Non-Bollgard	0	0	0	0
1	Grain Sorghum	Non-Bollgard	0	0	0	0
2	Grain Sorghum	Non-Bollgard	0	0	0	25
2	Grain Sorghum	Non-Bollgard	0	20	20	20
2	Grain Sorghum	Non-Bollgard	20	0	0	0
2	Grain Sorghum	Non-Bollgard	0	0	20	20
2	Grain Sorghum	Non-Bollgard	0	0	0	0
3	Grain Sorghum	Non-Bollgard	100	0	0	0
3	Grain Sorghum	Non-Bollgard	0	20	40	0
3	Grain Sorghum	Non-Bollgard	75	40	20	0
3	Grain Sorghum	Non-Bollgard	40	0	0	20
3	Grain Sorghum	Non-Bollgard	0	0	100	100
4	Grain Sorghum	Non-Bollgard	0	100	100	100
4	Grain Sorghum	Non-Bollgard	100	100	100	100
4	Grain Sorghum	Non-Bollgard	100	100	100	100
4	Grain Sorghum	Non-Bollgard	40	0	0	80
4	Grain Sorghum	Non-Bollgard	20	40	80	100
1	Soybean	Non-Bollgard	20	0	0	0
1	Soybean	Non-Bollgard	0	0	0	0
1	Soybean	Non-Bollgard	0	40	0	0
1	Soybean	Non-Bollgard	0	0	0	0
1	Soybean	Non-Bollgard	0	0	0	20
2	Soybean	Non-Bollgard	0	0	0	0
2	Soybean	Non-Bollgard	0	0	100	0
2	Soybean	Non-Bollgard	0	0	0	100
2	Soybean	Non-Bollgard	40	40	0	0
2	Soybean	Non-Bollgard	0	0	60	60
3	Soybean	Non-Bollgard	20	20	0	0
3	Soybean	Non-Bollgard	0	0	0	0

(table D.4 continued)

(table D.4 continued)

3	Soybean	Non-Bollgard	0	0	0	0
3	Soybean	Non-Bollgard	0	0	0	0
3	Soybean	Non-Bollgard	20	20	20	20
4	Soybean	Non-Bollgard	0	0	20	0
4	Soybean	Non-Bollgard	0	20	0	20
4	Soybean	Non-Bollgard	0	0	20	0
4	Soybean	Non-Bollgard	0	0	0	20
4	Soybean	Non-Bollgard	0	0	0	0

VITA

Jeffrey Gore, the youngest son of Charles Augustus and Shirley Mae Gore, was born in Columbus, Ohio, on March 31, 1968. He attended Southside High School in Gadsden, Alabama. He obtained his bachelor of science degree in entomology from Auburn University in 1995 and his master of science in entomology from Louisiana State University and Agricultural and Mechanical College in 1999. Jeff started working on the degree of Doctor of Philosophy in entomology in 1999 at Louisiana State University and Agricultural and Mechanical College under the supervision of Dr. B. Rogers Leonard. Currently, he is a doctoral candidate in the Department of Entomology at Louisiana State University and Agricultural and Mechanical College.