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# ARTHROPOD MANAGEMENT

## Maturity and Yield Responses of Non-transgenic and Transgenic Bt Cotton to Simulated Bollworm Injury

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### INTERPRETIVE SUMMARY

The bollworm is a damaging pest of cotton throughout the United States. In the past, this pest has been largely controlled with commercial insecticides. Recently, transgenic cotton cultivars expressing the insecticidal protein from *Bacillus thuringiensis* Berliner var. *kurstaki* (Bt) have been commercialized and are effective at controlling the tobacco budworm and pink bollworm. However, Bt cotton fields across Texas, the Mid-South and the U.S. Southeast have required insecticide applications to suppress bollworm populations.

Currently, no precise economic injury levels or action thresholds have been established for pests infesting transgenic Bt cotton. The specific objective of this study was to quantify the effects of injury by simulating bollworm feeding during flowering of non-transgenic and transgenic Bt cotton. We hoped to learn what effects selected levels of simulated bollworm injury would have on maturity and yield of non-transgenic and transgenic Bt cotton.

Simulated bollworm injury applied during weeks 2, 3, and 4 of flowering in 1997, and weeks 3 and 4 of flowering in 1998 significantly delayed crop maturity of non-transgenic cotton. Seedcotton yields were reduced significantly when injury was applied during weeks 3 and 4 of flowering in 1997, and week 4 of flowering in 1998 on non-transgenic cotton.

Results for transgenic Bt cotton were similar to those observed for non-transgenic cotton. Simulated bollworm injury applied during weeks 3 and 4 of flowering in 1997 and 1998 resulted in a significant delay in crop maturity. Injury levels applied during weeks 3 and 4 of flowering in 1997 significantly reduced seedcotton yields of transgenic Bt cotton.

These data provide valuable information about economic injury levels for bollworm populations on non-transgenic and transgenic Bt cotton, which will allow for the development of more precise action thresholds that will improve integrated pest management strategies on non-transgenic and transgenic Bt cotton.

### ABSTRACT

**No precise economic injury levels or action thresholds have been established for pests infesting transgenic Bt cotton, *Gossypium hirsutum* L. This study was designed to quantify the effects of injury to cotton bolls by simulating feeding by the bollworm, *Helicoverpa zea* (Boddie), during flowering stages on non-transgenic (cv. Stoneville 474) and transgenic Bt (cv. NuCOTN 33B) cotton. Field tests were conducted in Northeast Louisiana during 1997 and 1998 on upland cotton to study crop maturity, seedcotton yields, and boll injury associated with insect pests. Boll injury was produced by drilling a 63.5 mm hole completely through bolls. Yield and maturity responses of non-transgenic and transgenic, *Bacillus thuringiensis* Berliner var. *kurstaki* (Bt), cotton were measured after seven levels (0, 2.5, 5, 10, 20, 40, and 80%) of mechanical boll injury were applied to the total boll population during the first 4 wk of flowering. Boll injury during week 2 of flowering on Stoneville 474 in 1997 and during weeks 3 and 4 of flowering on Stoneville 474 and NuCOTN 33B in 1998 significantly delayed crop maturity at the time of harvest aid application. Significant reductions in seedcotton yield of Stoneville 474 were observed when bolls were damaged during weeks 3 and 4 of flowering in 1997 and during week 4 of flowering in 1998. Significant (*P* = 0.05) yield reductions of NuCOTN 33B were observed during weeks 3 and 4 of flowering in 1997. These data define economic injury**

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**levels for boll-feeding insects during the first four weeks of flowering.**

Cotton is a perennial plant that has been adapted for commercial production as an annual crop (Landivar and Benedict, 1996). Plant development follows an indeterminate fruiting cycle, with excessive production of fruiting structures by individual plants. All fruiting structures produced cannot be matured, and excess structures abscise from the plant. Loss of fruiting forms results from biotic causes such as insects and pathogens, or abiotic causes such as natural abscission, nutrient deficiency, water stress, temperature, and mechanical injury (Landivar and Benedict, 1996). Kennedy et al. (1991) found that only 24 to 36% of flowers produced on a plant during a growing season mature to harvestable bolls.

The indeterminate growth pattern of cotton enables it to withstand the loss of many fruiting structures without significant reductions in yield. Abscission of undamaged fruiting forms is an inherent process by which the fruit load on a plant is brought into balance with the available nutrient and water supplies (Guinn, 1982). Excess fruiting structures abscise from the plant when the available assimilate load is low and physical stresses (nutrient deficiency, water stress, and climate) are high.

Cotton plants compensate for lost fruiting forms by redirecting assimilates from the subtending leaf at that site to adjacent fruiting structures that probably would have abscised (Kerby and Buxton, 1981). Retention of bolls that would have abscised under normal conditions can adequately replace fruiting forms lost within a few days beyond anthesis (flower opening) (Jones et al., 1996b).

First position fruiting structures on sympodial branches are considered to be the most valuable for total yields (Mulrooney et al., 1992) and, under ideal environmental conditions, may produce as many as 35% more harvestable bolls than sites at or beyond the second position (Jenkins et al., 1990a,b). A higher percentage of fruiting forms at the second and subsequent fruiting positions abscise from the plant when the structure at the first position is retained (Kerby and Buxton, 1981).

Current pest management strategies incorporate economic factors such as potential crop value and insecticide application costs into insect management decisions. Stern et al. (1959) first introduced the concept of economic decision making in pest management. More recently, other scientists (Chiang, 1979; Poston et al., 1983; Pedigo et al., 1986; Onstad, 1987) have attempted to modify the philosophy introduced by Stern et al. (1959), but the general concept has remained the same.

Mi et al. (1998) developed a plant-based economic injury level for cotton. This economic injury level utilizes the same principles as the model proposed by Norton (1976) for the potato cyst eelworm (*Globodera* spp.), but includes the effects of other variables, such as the ability of the cotton plant to compensate for fruiting-form loss. Currently, the initiation of control measures in cotton production is based on experience of the pest manager and general guidelines for economic thresholds provided by individual state cooperative extension services. However, these thresholds are static and do not reflect changes in production costs, crop prices, or physiological susceptibility of cotton plants to pests (Mi et al., 1998). With new technologies (i.e. transgenic crops), more selective insecticides, and insect resistance, pest management is becoming more complex.

Transgenic cotton cultivars, containing the Bollgard gene (Monsanto Co., St. Louis, MO), produce a *B. thuringiensis* Berliner var. *kurstaki* CryIA(c) d-endotoxin (Bt) providing resistance to key insect pests such as the pink bollworm, tobacco budworm, and the bollworm, (MacIntosh et al., 1990; Perlak et al., 1990). Although the toxin suppresses bollworm populations, economic injury may occur in the presence of high bollworm populations. During the first 3 yr of commercial production of transgenic Bt cotton, bollworm populations have required foliar insecticide applications to prevent economic injury (Bacheler and Mott, 1997; Layton et al., 1997, 1998; Leonard et al., 1997, 1998; Roof and DuRant, 1997; Smith, 1997, 1998).

Although some states have adopted action thresholds for bollworms on transgenic Bt cotton that are different from those on non-transgenic cotton, no precise economic injury levels or action thresholds have been established. The objective of this study was to quantify the effects of injury designed to simulate bollworm feeding on cotton bolls and to define the level at which economic injury occurs during each of the first four weeks of flowering for a non-transgenic and transgenic Bt cotton cultivar.

## MATERIALS AND METHODS

Non-transgenic cotton (Stoneville 474, Stoneville Pedigreed Seed Co., Memphis TN ) was planted on 14 May 1997 and 7 May 1998 at the Northeast Research Station near St. Joseph, LA. Transgenic Bt cotton (Deltapine NuCOTN 33B, Delta & Pine Land Co., Scott, MS) containing the Bollgard gene (Monsanto Co., St. Louis, MO) was planted 7 May 1997 and 6 May 1998 at the Macon Ridge location of the Northeast Louisiana Research Station near Winnsboro.

Fertilization rates and general agronomic practices from Louisiana Cooperative Extension Service recommendations were used to minimize sources of variability within the test areas. Injury from insect pest infestations was suppressed with weekly applications of insecticides at the recommended rates prescribed in the Louisiana Insect Control Guide (Bagwell et al., 1997; 1998).

Plot size was three 3.1-m rows (1.02-m centers). One non-planted border row was maintained between plots to reduce mechanical injury to plants by worker movement. Plant densities on the center row of plots were thinned to two plants per 0.3 m (64 582 plants $ha^{-1}$) within 2 wk of plant emergence.

Treatments were placed in a split-plot arrangement within a randomized complete block design with four replications. The main-plot factor consisted of week of flowering and included each of the first 4 wk. The first week of flowering was defined as the time when 50% of plants within the test area had at least one flower or boll. Boll injury treatments for each week of flowering were applied during July or August in 1997 and June or July in 1998 (Table 1). The sub-plot factor consisted of boll injury at seven levels and included 0, 2.5, 5, 10, 20, 40 and 80% damage to the total boll population within the respective plots.

Total boll densities on the center row of the plots for each respective week of flowering were determined immediately prior to injury. Bolls selected to be injured were tagged with yellow “snap-on” tags (A.M. Leonard, Inc., Piqua, OH). Boll selection for mechanical injury was based on their position within the fruiting profile and proximity to the main stem. First position bolls low in the fruiting profile were selected first. Bolls at more distal fruiting positions on sympodial branches and occasionally those on monopodial branches were selected to establish higher injury level treatments (>20%). In each instance every effort was made to injure the oldest bolls on plants. Boll injury was produced by drilling a hole completely through the bolls using a Black & Decker cordless drill (model no. 2236) and metal bit (63.5 mm diameter) to simulate feeding by late (>L4) instar larvae. Injury was independently applied to plots so that plants in those plots were only injured once.

**Table 1. Dates cotton bolls were injured at two locations in Louisiana during 1997 and 1998 in a study of simulated bollworm damage.**

| | Week of Flowering | | | |
|---|---|---|---|---|
| **Location/Year†** | **1** | **2** | **3** | **4** |
| **MRRS 1997** | **11 July** | **18 July** | **25 July** | 31 July |
| **MRRS 1998** | **2 July** | **9 July** | **16 July** | **22 July** |
| **NERS 1997** | **14 July** | **21 July** | **28 July** | **5 August** |
| **NERS 1998** | **30 June** | **7 July** | **15 July** | **21 July** |

**† MRRS = Northeast Res. Stn., Macon Ridge Location, Winnsboro (NuCOTN 33B); NERS = Northeast Res. Stn., St. Joseph (Stoneville 474).**

At the end of the season, data on percent open bolls were collected as an estimate of crop maturity prior to defoliation (>70% open bolls in the undamaged plots). Plots were hand-harvested and seedcotton weight was recorded. Data for each week of flowering were analyzed independently using analysis of variance, PROC GLM (SAS Institute, 1989). Means for the various injury levels (2.5-80%) were compared with the non-damaged means using Dunnett’s procedure (Dunnett, 1955).

## RESULTS AND DISCUSSION

Insect pest management in many crops relies on integrating multiple control strategies. These strategies are based on the economic injury level/economic threshold concept introduced by Stern et al. (1959). Prior to the introduction of the integrated pest management concept, cotton researchers attempted to determine the influence of insect feeding on subsequent yield by simulating insect injury. Dunnam et al. (1943) removed from 0 to 50% of squares from cotton plants-to simulate insect feeding-with no reduction in yields. In the present study, bollworm feeding was simulated

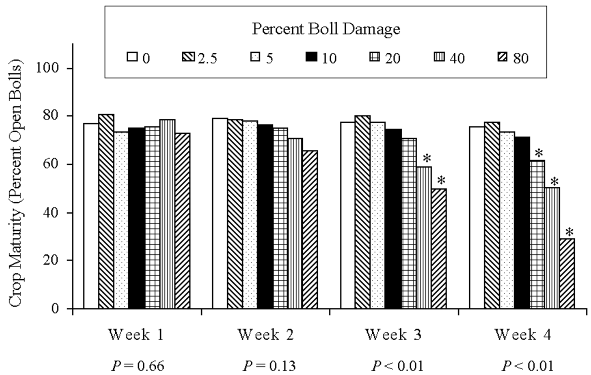


**Fig. 1. Effects of boll injury during four flowering stages on Stoneville 474 crop maturity, measured by percent open bolls, in 1997 and 1998. Asterisks indicate boll injury levels that are significantly different from the non-injured plots according to Dunnett's procedure (α = 0.05) within each week of flowering.**

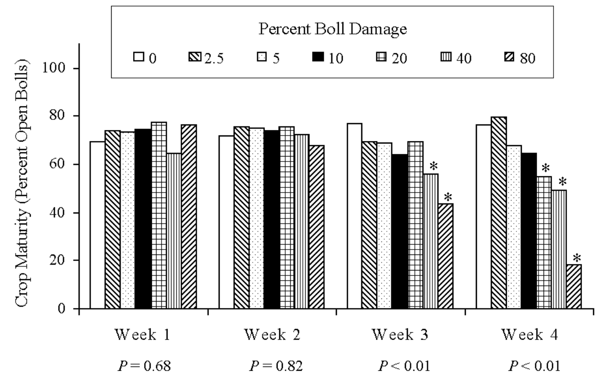


**Fig. 3. Effects of boll injury during four flowering stages on NuCOTN 33B crop maturity, measured by percent open bolls, in 1997 and 1998. Asterisks indicate boll injury levels that are significantly different from the non-injured plots according to Dunnett's procedure (α = 0.05) within each week of flowering.**

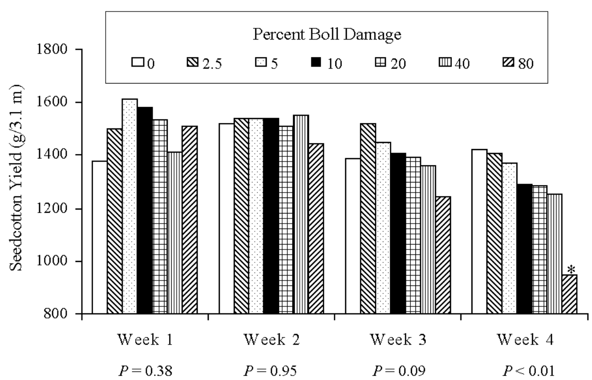


**Fig. 2. Effects of boll injury during four flowering stages on Stoneville 474 seedcotton yield in 1997 and 1998. Asterisks indicate boll injury levels that are significantly different from the non-injured plots according to Dunnett's procedure (α = 0.05) within each week of flowering.**

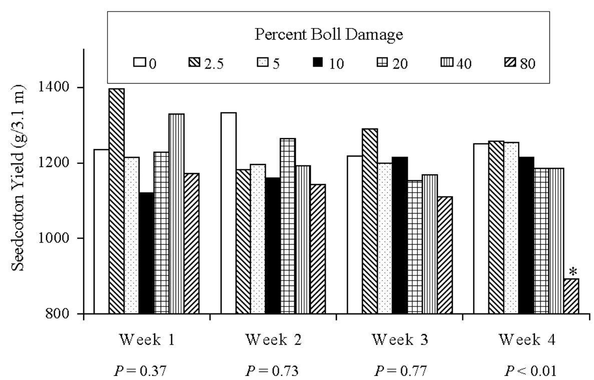


**Fig. 4. Effects of boll injury during four flowering stages on NuCOTN 33B seedcotton yield in 1997 and 1998. Asterisks indicate boll injury levels that are significantly different from the non-injured plots according to Dunnett's procedure (α = 0.05) within each week of flowering.**

during four discrete periods of cotton plant reproductive development as an initial effort for developing economic injury levels in transgenic Bt cotton and non-transgenic cotton.

There were no year-by-damage interactions for percent open bolls or seedcotton yield during any week of flowering on Stoneville 474 and NuCOTN 33B ($P > 0.13$). Therefore, data for 1997 and 1998 were combined within each week of flowering. Cotton plants compensated for boll injury during each of the first 4 wk of flowering. Jones et al. (1996b) found that fruiting forms lost as a result of abiotic or biotic factors are adequately replaced by the retention of bolls that would have abscised under normal conditions. Assimilates from the subtending leaf at lost fruiting positions are redirected to adjacent fruiting structures, thereby increasing their chance of retention (Kerby and Buxton, 1981).

## Effects of injury to Stoneville 474 bolls

During the first 2 wk of flowering, Stoneville 474 cotton plants compensated for all levels of boll injury. No delays in crop maturity were observed during week 1 ($F = 0.69$; df = 6, 28; $P = 0.66$) or week 2 ($F = 1.82$; df = 6, 28; $P = 0.13$) of flowering compared with the non-damaged plots for each of those weeks of flowering (Fig. 1).

Jones et al. (1996a) observed no reductions in the number of mature bolls at any rating interval when white flowers were removed during the first 2 wk of flowering. However, when white flowers were removed for the first 3 wk of flowering they observed a reduction in the number of mature bolls, compared with all other flower removal treatments, at 133 d after planting.

In the present study, no delays in crop maturity were observed for injury levels of 2.5 to 20% during week 3 of flowering or 2.5 to 10% during week 4. However, injury levels of 40 to 80% and 20 to 80% during week 3 ($F$ = 7.56; df = 6, 28; $P$ < 0.01) and week 4 ($F$ = 41.43; df = 6, 28; $P$ < 0.01) of flowering delayed crop maturity, compared with non-damaged plants (Fig. 1).

Although injury levels of 40 and 80% caused a delay in crop maturity during week 3 of flowering, seedcotton yields were not reduced during week 1 ($F$ = 1.12; df = 6, 28; $P$ = 0.38), week 2 ($F$ = 0.25; df = 6, 28; $P$ = 0.95), or week 3 ($F$ = 2.06; df = 6, 28; $P$ = 0.09) of flowering (Fig. 2).

Fife (2000) removed 0 to 100% of flower buds (squares) from Stoneville 474 and BXN 47 cotton plants during each of the first 4 wk of flowering. Crop maturity was delayed and seedcotton yields were reduced during weeks 1 and 2 of flowering, but not during weeks 3 and 4 of flowering.

Ungar et al. (1987) removed 120 squares per $m^2$ during 2 wk, 200 small squares per $m^2$ during 4 wk, or 60 small bolls per $m^2$ during 2 wk without a reduction in cotton yields. Removal of 60 large bolls per $m^2$ during 2 wk or 30 large bolls per $m^2$ on a single date resulted in yield reductions.

During the fourth week of flowering, 2.5 to 40% boll injury did not reduce yields, compared with non-damaged plots in our study. Injury to 80% of the total boll population during week 4 of flowering caused a reduction in seedcotton yields, compared with the non-damaged plots ($F$ = 8.18; df = 6, 28; $P$ < 0.01) (Fig. 2). In a similar study, Jones et al. (1996a) removed all flowers (white and red) at different timings. Removal during the first 3 wk of flowering delayed boll development but did not reduce lint yields. Removal of flowers during week 4 of flowering and subsequent weeks reduced lint yields.

**Effects of injury to NuCOTN 33B bolls**

Transgenic Bt cotton plants compensated for low to moderate levels of boll injury during each of the first 4 wk of flowering. Crop maturity was not delayed at any level of boll injury during week 1 ($F$ = 0.66; df = 6, 28; $P$ = 0.68) or week 2 ($F$ = 0.48; df = 6, 28; $P$ = 0.82) of flowering (Fig. 3). Also, crop maturity was not delayed at injury levels of 2.5 to 20% or 2.5 to 10% during weeks 3 and 4 of flowering, respectively.

Fife (2000) removed squares (0-100%) from NuCOTN 33B cotton during the first 4 wk of flowering without delaying crop maturity. In our study, injury levels of 40 and 80% delayed crop maturity during the third week of flowering compared with the non-damaged plots ($F$ = 9.69; df = 6, 28; $P$ < 0.01) (Fig. 3). During the fourth week of flowering, 20 to 80% boll injury delayed crop maturity compared with the non-damaged plots ($F$ = 28.87; df = 6, 28; $P$ < 0.01).

Sloane et al. (1973, p. 58; 1974, p. 29) observed delays in crop maturity when all squares were removed during the first 5 wk of squaring. In similar studies that involved early-season removal of squares and flowers, crop maturity was also delayed (Mistric and Covington, 1968; Pettigrew et al., 1992; Terry, 1992; Jones et al., 1996a).

NuCOTN 33B seedcotton yields were not reduced by any level of boll injury during week 1 ($F$ = 1.14; df = 6, 28; $P$ = 0.37), week 2 ($F$ = 0.60; df = 6, 28; $P$ = 0.73), or week 3 ($F$ = 0.55; df = 6, 28; $P$ = 0.77) of flowering (Fig. 4). During the fourth week of flowering, 2.5 to 40% boll injury did not reduce seedcotton yields.

Fife (2000) observed no yield reductions at square removal levels of 0 to 75% during each of the first 4 wk of flowering. Reductions in seedcotton yields were observed during each of the first 4 wk of flowering when 100% of squares were removed compared with plots with no square removal.

Seedcotton yields in our study were reduced compared with the non-damaged plots only when 80% boll injury was applied ($F$ = 4.49; df = 6, 28; $P$ < 0.01) (Fig. 4). Similar studies looking at the effects of fruiting form removal on cotton yields are widespread and varied. Kletter and Wallach (1982) removed squares less than 1 cm (four per plant), squares greater than 1 cm (four per plant) and bolls less than 10 g (two per plant) at three timing intervals without reductions in yields. Removal of 0 and 45% of squares in three patterns to simulate injury by boll weevil, *Anthonomus grandis grandis*

Boheman, demonstrated no yield reductions (Kletter and Wallach, 1982). Brook et al. (1992a,b) simulated *Heliothis* spp. injury to terminal buds and fruiting forms. The only yield reductions occurred when 100% of fruiting forms were removed cumulatively during the first 3 wk of flowering.

The indeterminate fruiting cycle of cotton allows plant compensation for high levels of insect injury. A large percentage of fruiting forms on plants abscise before maturity, even in the absence of insect injury. Retention of fruit on positions that would have abscised under normal conditions allows cotton plants to mature a similar number of bolls in the presence of insect injury.

Mann et al. (1997) observed no effect on lint yields at square removal levels of 0, 50, and 100% during the first 4 wk of squaring. However, during some years there was a square-removal-by-planting-date interaction.

Late-planted cotton may not have the same ability to compensate for injury as early-planted cotton does, perhaps due to the absence of time required for bolls to mature at alternative fruiting sites. In our study, cotton was planted at the optimum time for plant development.

Sympodial branches on main stem nodes typically have the highest boll retention and are responsible for 60% of the seedcotton yield produced on a plant (Jenkins, 1990a,b). However, if bolls at those positions are lost, assimilates from the subtending leaf at that site are redirected to adjacent structures (Kerby and Buxton, 1981). The survivability of adjacent structures is increased (Stewart and Sterling, 1988) and those structures may become larger than they would in the absence of injury to other structures.

Cotton plants in our studies compensated for boll injury during each of the first 4 wk of flowering. A level of boll injury applied to a plot only rarely resulted in an equivalent amount of yield loss. During the first 2 wk of flowering, a relatively low number of bolls was injured in relation to the total boll population set on plants (Figs. 5, 6). During each of those weeks, cotton plants had sufficient time to mature bolls at alternative fruiting sites. However, plants required more time to completely mature bolls that were retained high in the plant canopy or at distal positions on fruiting branches. In these instances, crop maturity was delayed.

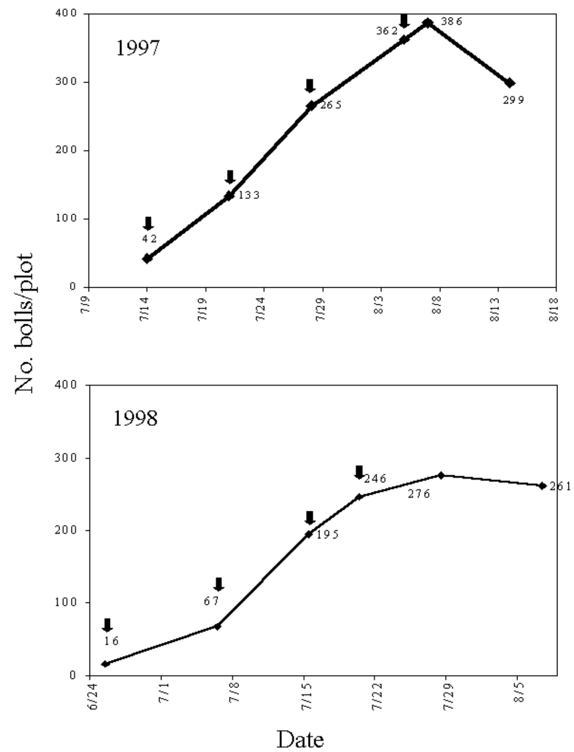


**Fig. 5. Boll density of Stoneville 474 in the undamaged plots during flowering (arrows indicate dates boll injury was applied).**

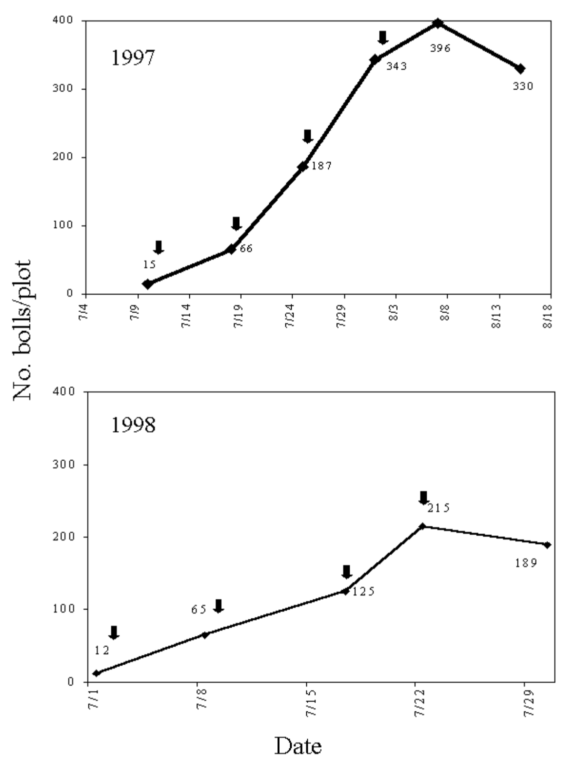


**Fig. 6. Boll density of NuCOTN 33B in the undamaged plots during flowering (arrows indicate dates boll injury was applied).**

**Table 2. Yield reduction associated with boll injury applied during four flowering stages to Stoneville 474 and NuCOTN 33B in 1997 and 1998.**

| | Yield reduction during week of flowering | | | |
|---|---|---|---|---|
| Damaged Bolls (%) | 1 | 2 | 3 | 4 |
| | -------------------%------------------- | | | |
| 0 | - | - | - | - |
| 2.5 | 1.3 | 7.0 | 0.5 | 7.0 |
| 5 | 2.3 | 6.3 | 1.8 | 1.0 |
| 10 | 4.0 | 7.0 | 2.8 | 6.0 |
| 20 | 1.5 | 6.0 | 3.8 | 6.5 |
| 40 | 1.5 | 6.3 | 5.8 | 11.0 |
| 80 | 4.8 | 6.8 | 9.5 | 27.5 |

Crop maturity is an important consideration when making management decisions. When maturity is delayed, the crop remains susceptible to late-season pests that are difficult to control and require additional insecticide applications. In addition, the crop becomes more susceptible to adverse environmental conditions later in the growing season, as compared with earlier harvest dates. The delays in crop maturity observed on Stoneville 474 and NuCOTN 33B were the result of less mature bolls at positions other than primary fruiting positions taking more time to compensate for the loss of bolls at primary fruiting positions.

In summary, significant reductions in seedcotton yield were observed for 80% boll injury applied during week 4 of flowering on Stoneville 474 and NuCOTN 33B. Overall yield reductions due to boll injury of 2.5 to 80% across both cultivars ranged from 1.3 to 4.8% when the boll injury was applied during week 1; 6.0 to 7.0% for boll injury applied during week 2; 0.5 to 9.5% for boll injury applied during week 3; and 1.0 to 27.5% for boll injury applied during week 4 of flowering during 1997 and 1998 (Table 2). Boll injury significantly delayed crop maturity during weeks 3 and 4 of flowering to Stoneville 474 and NuCOTN 33B in 1997 and 1998.

In conclusion, these data form a base of information about economic injury levels for boll loss during the peak flowering stages of cotton in northeast Louisiana. This information should allow for the development of dynamic treatment thresholds during the first 4 wk of flowering that change during the season. This information will be important for future reference with proper insect pest management on transgenic Bt cotton.

However, caution should be used in interpreting these data. Delays in crop maturity should be considered when making decisions about managing pests. Furthermore, cotton plants may not be able to fully compensate for boll loss in combination with damaged squares, damaged flowers, and/or high levels of defoliation from insect pests.

## ACKNOWLEDGMENT

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