POTENTIAL UTILITY AND SUSCEPTIBILITY OF TRANSGENIC B.t. COTTON AGAINST BOLLWORMS, EUROPEAN CORN BORERS AND STINK BUGS IN NC

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Abstract

Replicated field studies to evaluate the efficacy of transgenic cotton express-ing the delta endotoxin protein of Bacillus thuringiensis (B.t. cotton) against the cotton bollworm (Helicoverpa zea) (Boddie), the European corn borer (Ostrinia nubilalis) (Hubner) and stink bugs, primarily Acrosternum hilare (Say) and Euschistis servus (Say), were carried out in several representative cotton-producing areas in North Carolina from 1992 to 1995 under conditions of generally late planting (early to late May) and moderate to high bollworm pressure. In keeping with normal North Carolina cotton production practices, plots were neither irrigated nor treated with a disruptive overspray to enhance larval establishment. Overall, averaged over years and locations, the B.t. (Bollgard™) cotton lines evaluated (MON-81, MON 757 [BT-02], NuCOTN 33 and NuCOT35) expressed bollworm control and yields almost identical to the pyrethroid-protected, non-transformed varieties (Coker 312, DP-5415 and DP-5690) in both small plot and in larger-scale (2.5 to 5-acre) tests. European corn borer stem and boll damage were virtually non-existent in any of the studies. Stink bug damage to bolls in the B.t. lines, similar to the untransformed, unprotected lines, was at times significantly greater than that found in the pyrethroid- protected varieties (though typically, the damage was low to moderate), confirming that the Bollgard lines offered no protection against stink bugs, and also suggesting that stink bugs could become an economic consideration in some areas of North Carolina with the adoption of this technology. In comparing the Bollgard cotton lines with the untrans-formed, pyrethroid-protected lines for total boll damage (bollworm, European corn borer, fall armyworm and stink bug), averaged over all tests, years and locations (8 replicated comparisons), overall means were essentially the same.

Introduction

Although a number of literature references can now be cited from the southeastern cotton belt on the effect of transgenic Bollgard cotton on various lepidopterous larvae, most of these component-oriented studies deal with the bollworm complex or with only single species (Mahaffey, et al., 1995; Durant, 1994; Turnipseed et al., 1995). Only one preliminary published study focused on the complex of boll-damaging pests (Mahaffey, et al., 1994). Additionally, several evaluations were conducted under either multiple irrigations, and/or were treated with a beneficial arthropod-disrupting overspray (Mahaffey, et al., 1994 and 1995), both practices which can serve to significantly increase the survival and establishment of caterpillars beyond that anticipated in a commercial cotton production setting. At present, less than 4% of North Carolina's cotton crop is irrigated (Keith Edmisten, pers. comm.), and a disruptive treatment for either thrips, cotton aphids, plant bugs, or boll weevils just prior to the major mid to late July bollworm moth flight would be rare.

This report presents the results of a series of field trials conducted from 1992 through 1995 to evaluate the efficacy of several lines of Bollgard cotton on the major boll-damaging pests of cotton in NC (Bacheler, 1995) under conditions which could be reasonably anticipated under commercial cotton production.

Materials and Methods

Small Plot Tests

In 1992, a series of replicated, small plot studies was initiated in Onslow County to evaluate the efficacy of Bollgard cotton against this state's major boll-damaging pests. In 1992 through 1995 the 8-row x 50 ft x 4-replicate tests were hand-planted with a Jab Planter (Almaco, Inc., Nevada, IA) to a depth of 0.75 inches at 3-4 seeds/ft. In 1992, MON-81 served as the transformed B.t. line, while in 1993 through 1995, the transformed line was 757. Coker-312 served as the non-transformed line in each of the studies. Although not reported herein, additional treatments included seed mixes (1994) and various thresholds (1994-95). The 3 treatments primarily reported here are the unprotected transgenic and unprotected and pyrethroid-protected Coker-312 lines. Data taken included stand population, plant height, disease incidence, bollworm egg deposition in terminals and on squares, blooms and bolls, live bollworm, European corn borer (ECB), fall armyworm, beet armyworm, and stink bug on squares, blooms and bolls and their damage, and yields. Bollworms, ECB, and armyworms were classified by instar. The bollworm/tobacco budworm ratio was determined from the outer check (untreated Coker-312) rows. The above larval information was gathered by checking 25 each of terminals, squares, and bolls (including pink blooms and "bloom tags" in proportion to their occurrence in the overall boll population) weekly from the 3rd week in July through either the last week in August or the point at which larval establishment had ended (approx. 3-4 nodes above highest 1st position white flower). The final (and typically most revealing) larval and stink bug damaged boll assessment was conducted approx. 2 wk after the final weekly evaluation. Also at this time, 60 ft of row/plot (240 ft/treatment) were examined for main stem penetrations from ECB.

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Treatments for the protected plots were triggered by the NCSU Ext. Serv. recommended 10% eggs in terminals at the onset of the major bollworm moth flight. Subsequent treatments were made based on finding 3% live bollworms on fruit. Karate 1E with 8 gpa water and 50 psi was applied via CO_2-powered back pack sprayer. The protected plots required 3, 4, 5 and 4 applications of Karate in 1992, 93, 94 and 95, respectively. In 1995, the initial 2 sprays of Karate were at the high rate of 0.04 lb. ai/acre; the remaining 2 sprays and all the treatments for 1992 through 1994 were at the 0.025 lb. ai/acre rate.

Cotton plots were harvested for yield determination from the middle 2 rows (240 row ft/treatment) with a 1-row (1992-93) or a 2-row (1994-95) mechanical John Deere harvester.

All data were analyzed via Gylling's PRM software and analyzed via ANOVA with means separated by Fisher's Protected LSD test ($P = 0.05$) (Gylling and Gylling, 1992).

Producer Trials

A series of producer-planted evaluation trials was conducted in 1994-95 to evaluate the efficacy of 2 Delta and Pine Land Company varieties, DP-5415 and DP-5690, against their transformed, B.t. (Bollgard™) counterparts, NuCOTN 33 and NuCOTN 35, respectively. The studies were conducted in Halifax, Robeson and Jones counties in 1994-95, areas representative of different major cotton production regions of NC. Plots were 4 to 8 rows by the length of the field (approx. 600 to 1,500 ft.). Within an untreated zone were the 2 transformed (NuCOTN 33 and 35) and untransformed (DP-5415 and 5690) varieties (along with the other non-reported seed mixes and varieties). Both DP-5415 and 5690 were planted also just outside the untreated area on either side of the test and were protected by the producer with a pyrethroid as needed- 3 to 5 times, primarily with either Karate 1E (0.025 lb. ai/acre) or Baythroid (0.028 lb. ai/acre). Locations (3) served as replications for each set of varietal comparisons (2) for each year (2). Several other treatments were part of these studies, including a mixed seed evaluation (90:10) in 1994 and additional varieties in 1995, but are not be reported herein.

Cotton was grown under typical NC conditions, including an at-planting insecticide and PPI, pre-plant and post-directed weed control. Recommended fertility and other agronomic practices were followed.

Although the plots were evaluated throughout the growing season, the early to mid September late-season damaged boll assessment served as the primary index of damage. One hundred bolls were examined for damage from bollworms, ECB, fall armyworm, and stink bug. All bolls suspected to have been damaged by stink bugs were cut into with a knife. Both the presence of exterior spotting and either interior staining and/or hard lock had to be confirmed for the boll to be classified as stink bug-damaged.

Results

Small Plot Tests

As can be seen in Figure 1, the bollworm is North Carolina's primary boll damaging lepidopterous pest, with the European Corn borer accounting for most of the remaining damage, and the fall armyworm a distant 3rd and generally modest pest (Bacheler, 1995).

When the highest level of boll damage caused by bollworms is considered (the damaged boll assessment date with the highest damage to the untreated C-312 check), the unprotected Bollgard cotton provided protection from bollworm damage statistically similar to the pyrethroid-protected C-312 cotton (Fig.2). The untreated C-312 check confirmed the generally high bollworm pressure in these tests. Bollworms constituted approx. 95% of the bollworm:budworm mix in 1992,93 and 95 and approx. 30% in the untreated C-312 check in 1994.

The B.t. cotton lines evaluated showed almost complete resistance to the European corn borer, with virtually no stem penetration, and complete protection from boll damage uncovered (Figs. 3 and 4). As can be seen in Figure 4, ECB was exceptionally light at this location in 1995 (as well as throughout the state), primarily due to significantly less corn acreage in the test area and exceptionally dry July and August weather.

Stink bug damage to bolls in the transformed cotton line was almost 2-fold numerically higher than the untreated C-312 in 1992, but was similar during the remaining years (Fig. 5). The initial year was likely atypical in that this MON-81 line matured much more slowly than did the C-312, providing more immature, susceptible bolls at the time of stink bug feeding- typically during the month of August. Boll damage in the 2 to 5% range for the remaining years would not likely be considered excessive in that not all of the locks of a boll are typically damaged from stink bug feeding (Barbour, 1988).

In Figure 6, a comparison of stink bug damage to bolls is made between an area in the western piedmont of NC characterized by limited insecticide use for bollworm and ECB (similar to what one might expect in an area planted to Bollgard cotton) and the remainder of NC, which receives an average of approx. 3 foliar pyrethroid applications for bollworms and ECB annually (Bacheler, 1995). On the average, stink bug damage to bolls was about 7-fold higher in the area of low pyrethroid use than the average of the reminder of the state (3.86 vs. 0.56%).

Although yields in the Bollgard cotton was approx. 100 lb lint higher than the protected C-312 in 1993 and this situation was reversed in 1995, 1994 yields were within

one lb in 1994, and the means averaged over the 3 yr. period were identical (Fig. 7).

Producer Trials

Because boll damage was nearly identical between the 2 Bollgard varieties (NuCOTN 33 and 35) and the 2 untransformed varieties (DP-5415 and 5690), the boll damage from the producer evaluations is averaged over the 2 yr period with locations (3) serving as replications (Fig. 8). The figure combines 4, 3-replication tests. Boll damage caused by bollworms, ECB and fall armyworms in the NuCOTN vs. standard-protected varieties was very similar. Bollworm damage in the NuCOTN lines was less than the protected lines, while ECB and fall armyworm damage was low in each case. Stink bug damage was about 2-fold higher in the NuCOTN lines but the level, at 2.2%, was probably too low to be of economic concern- an encouraging result. Overall boll damage for all species combined was identical between the NuCOTN and the DP-5415 and 5690 protected varieties. Average boll damage to the untreated standard lines was 27.4%, indicating high overall pressure, with most of it (21.0%) coming from bollworms.

Discussion

In both sets of tests, the Bollgard lines tested provided bollworm control essentially identical to the untransformed, protected varieties under variable, but generally high bollworm pressure. The 1994 test in Onslow County represents the highest natural, unenhanced (bollworm larvae not placed onto the plants, the test not oversprayed, or irrigated to encourage larval establishment) boll damage reported in the literature. In other tests conducted in North Carolina, substantial boll damage and yield penalties resulted in the Bollgard untreated line compared with the pyrethroid-protected line when the test was irrigated and/or oversprayed with a disruptive, broad spectrum insecticide (Mahaffey, et al., 1994 and 1995) These studies confirm the potential for substantial damage to transgenic cotton from bollworms under conditions of exceptionally high pressure. At the present time, however, little cotton is either sprayed just prior to the late-July bollworm moth flight or irrigated in North Carolina. However, if the boll weevil became reestablished as a treatable economic pest, if the plant bug were elevated to significant pest status into the bloom period or if the cotton aphid were no longer held in check by natural enemies, this situation could change.

These tests further confirmed that Bollgard cotton can be expected to provide exceptional control of European corn borer, a pest which has accounted for approx. 27% of NC's overall boll damage averaged over the past 11 years (Bacheler, 1995). However, with the anticipated introduction of B.t. corn slated for the spring of 1996, the potential risk of resistance development in populations of both bollworms and ECB must be addressed where significant acreage of both transformed corn and cotton are produced in close proximity. Corn presently serves as the major untreated host for the first 2 bollworm generations and 2 generations of ECB., and has been a major part of the diversified agriculture which has helped to forestall the development of pyrethroid resistance in this area (Bradley, 1993).

Bollgard cotton showed no activity against stink bug species. However, the degree to which stink bugs may become an economic problem is presently largely a matter of speculation, and will likely vary significantly from area to area and from year to year.

Our data suggest that stink bugs will at least merit increased diligence, and will probably require scouting in a number of situations. Data from our small plot studies revealed average overall stink bug damage of 3.75% from 1993-95 in the Bollgard treatment, while in the Producer Trials, the mean was 2.2% and in the relatively untreated area in the western piedmont, the mean was 3.86%. Given that a stink bug-damaged boll often has between 1 and 3 undamaged locks, the actual total boll damage can be regarded as somewhat less (Barbour, 1988).

Fall armyworm damage to bolls throughout the study period was minimal, in keeping with long term observations (Bacheler, 1995). It was interesting to note that in the Producer Trials, fall armyworm (FAW) damage to bolls in the transgenic cotton was about half of that observed in the untreated, NuCOTN varieties, suggesting some resistance to FAW feeding. With our relatively light pressure from FAW in a typical year, if the Bollgard cotton provides some resistance to this pest, the fall armyworm may remain an occasional, relatively light pest of the cotton in NC.

At this point in time it would appear that Bollgard cotton under most conditions encountered in NC can be expected to provide protection from boll damage on a par with pyrethroid-protected, non-transformed lines. Under extreme conditions of high bollworm pressure coupled with a broad-spectrum, disruptive spray prior to the major bollworm moth flight and/or under irrigation, the pyrethroid-protected cotton may well show lower boll damage and higher yields (Mahaffey, et al., 1995). On the other side of the coin, under conditions of rainy weather which preclude the producer of being able to treat in a timely fashion, transgenic Bollgard cotton will have a decided advantage.

Presently, the 2 main impediments to significant adoption of this new technology by NC producers are 1) the full season, late maturity of the NuCOTN varieties under this area's shorter production window (Bowman, 1995) particularly NuCOTN 33, and 2) the lack of a variably-priced, realistic licensing fee more in keeping with the value of Bollgard in different regions.

References

Bacheler, J.S. and D.W. Mott. 1995. Cotton insect management in North Carolina: IPM adoption following boll weevil eradication, pp. 1067-1069. In, D.J. Herber (ed.) Proc., 1995 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Ibid. Annual fluctuations in late season boll damage from major lepidopterous pests in North Carolina: a ten-year perspective, pp. 783-786. In, D.J. Herber (ed.) Proc., 1995 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Barbour, K.S., J.R. Bradley, Jr. and J.S. Bacheler. 1988. Phytophagous stink bugs in North Carolina cotton: an evaluation of damage potential, pp. 280-283. In, J.M. Brown and D.A. Richter (eds.), Proc. 1988 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Bowman, D. . 1995. Variety..... pp. 33-51. In. Edmisten (ed.), Cotton Information, North Carolina Cooperative Extension Service. North Carolina State University, Raleigh, NC.

Bradley, J.R., Jr. 1993. Influence of habitat on the pest status and management of Heliothis species on cotton in the southern United States, Chapter 19, In, K.C. Kim and B.A. McPheron (eds.) Evolution of Insect Pests, pp. 375-391.

DuRant, J.A., 1994. Evaluation of treatment thresholds for control of bollworms and tobacco budworms in transgenic B.t. cotton in South Carolina, pp. 1073-1075. In, D.J. Herber (ed.), Proc., 1994 Beltwide Cotton Conferences. National Cotton Conferences, Memphis, TN.

Gylling, S.R. and F.O. Gylling. 1992. Pesticide Research Manager. Gylling Data Management, Inc.,Brookings, SD.

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 eastern North Carolina, pp. 1061-1064. In, D.J. Herber and D.A. Richter (eds.), Proc., 1994 Beltwide Cotton Conferences. 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, D.J. Herber and (ed.), Proc., 1995 Beltwide Cotton Conferences, Memphis TN.

Turnipseed, S.G., M.J. Sullivan, J.E. Mann and M.E. Roof. 1995. Secondary pests in transgenic B.t. cotton in South Carolina, pp. 768-769. In, D.J. Herber (ed.), Proc. Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

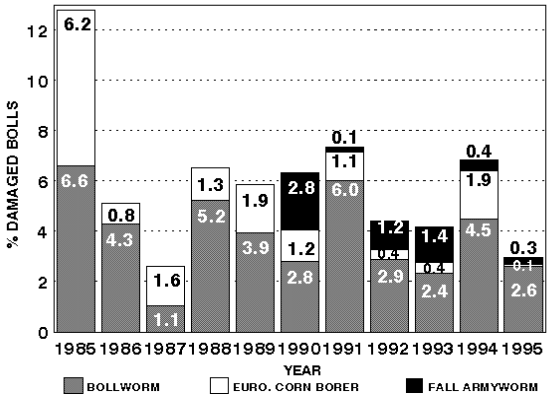

Figure 1: Statewide damaged boll survey, NC, 1985-1995, (n=132 to 312 fields/year)

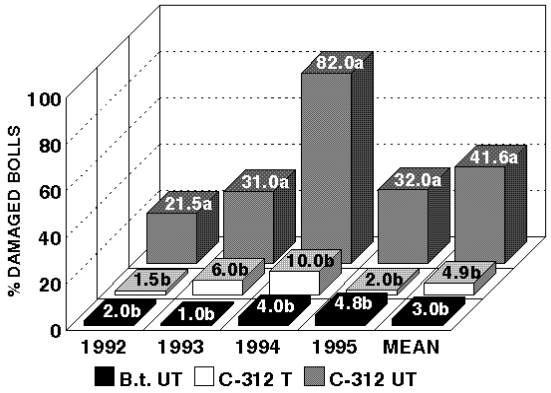

Figure 2: Bollworm damage to *B.t.* and conventional cotton in Onslow County, NC; 1992-1995

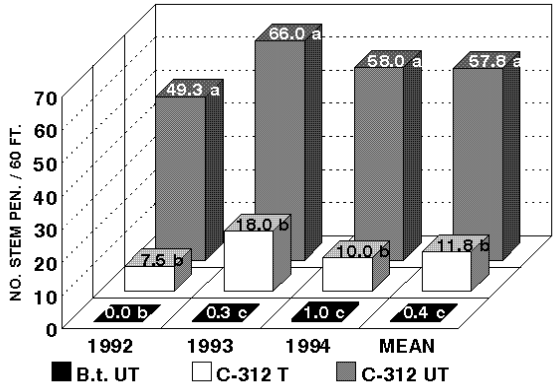

Figure 3: European corn borer stem penetrations; Onslow Co., NC, 1992-1994

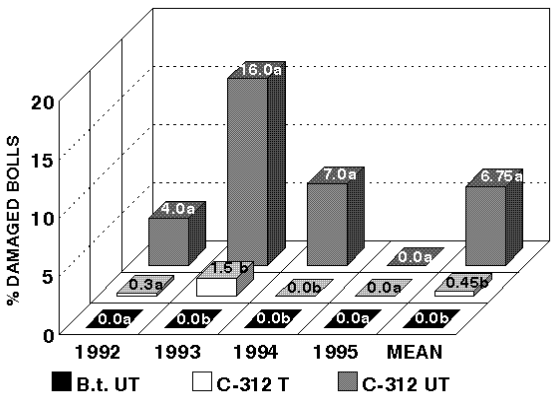

Figure 4: European corn borer damaged bolls; Onslow Co., NC, 1992-1995

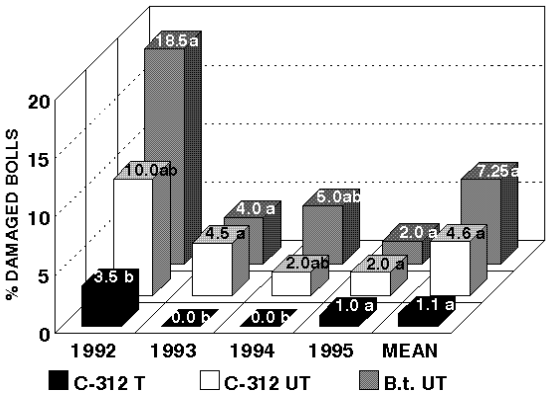

Figure 5: Stink bug damaged bolls; Onslow Co., NC, 1992-1995

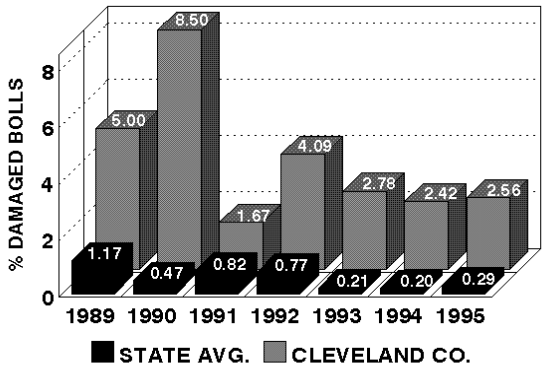

Figure 6: Stink bug damaged bolls in NC: state vs. Cleveland Co. Average, 1989 - 1995.

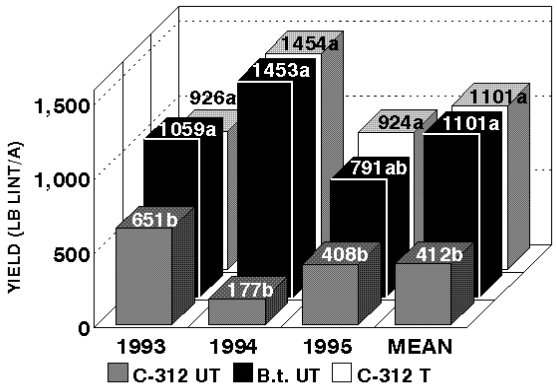

Figure 7: Yields of *B.t.* and conventional cotton under high caterpillar pressure in Onslow Co., NC, 1993-1995.

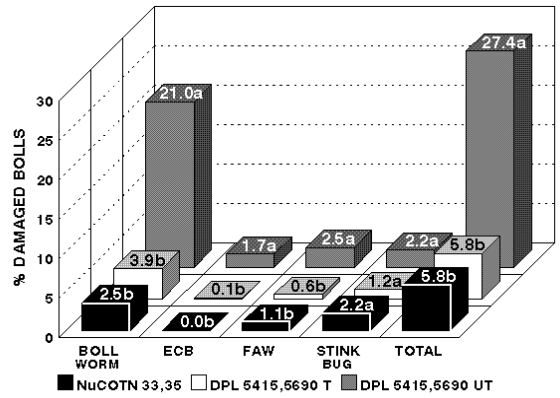

Figure 8: Caterpillar and stink bug damage to B.t. cotton in NC under grower conditions, (3 locations, 2 varieties; 1994-95).

Legend: Figures 2-5 and 7-8; C-312 T- Coker 312 pyrethroid-protected; B.t. UT- Bollgard unprotected with pyrethroid; C-312 UT- Coker 312 unprotected with pyrethroid. Means within a column followed by the same letter are not significantly different, (P = 0.05), Fisher's Protected LSD Test.