2005 Beltwide Cotton Conferences, New Orleans, Louisiana - January 4 - 7, 2005

ACTIVITY OF VIPCOT AGAINST HELICOVERPA ZEA AND HELIOTHIS VIRESCENS IN ARKANSAS

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Abstract

Replicated small plot studies were conducted in Tillar, Arkansas from 2002 – 2004 to compare the efficacy of VipCot™ cotton to its parent Coker line. Reductions in heliothine numbers were as great or greater than that observed for the Coker line treated as needed with insecticide. Yield increases over untreated Coker cotton ranged from 23 to 37% depending upon the specific experimental comparison and the use of insecticide. In more detailed examinations of the cottons via within-season plant mapping and end-of-season box mapping procedures, VipCot had higher retention of first position fruit and reached physiological cutout sooner that the parent Coker lines that sustained some insect damage. Detailed study of the cumulative patterns of insect infestation and subsequent impacts of these insects on seasonal patterns of fruit retention and end-of-season survival of fruit illustrate the descriptive power of detailed plant mapping procedures. Based on comparative estimates of insect densities and damage in these VipCot studies, a cumulative insect density equal to a larva per plant per season would cause a potential loss of seven to ten grams of seedcotton per plant. These estimates obviously need more experimental verification and understanding before they are extrapolated to practical management systems, but the experimental comparison of genetically similar cottons with and without the unique insecticidal protein illustrates a powerful method of valuing insect damage and technologies that limit insect damage. Temporal relationships associated with the value of insect damage across the course of a growing season have implications to more refined management systems.

Introduction

Cotton insect control, especially that focused on the economically important heliothines, has been revolutionized with the commercial development and successful deployment of transgenic cottons expressing insecticidal proteins. Bollgard® cotton from Monsanto Company dominates contemporary approaches to control of heliothines in much of the US Cotton Belt. The success of this technology is further influencing conceptual and strategic approaches to future pest management efforts. Many of the previous variable costs of production are being strategically targeted as future fixed costs of technology and seed at planting.

The success of Bollgard cotton and the efficacy of the Cry1Ac protein are enormous and perhaps unparalleled in recent history of insect control technologies. However, entomologists have long recognized the dangers of unilateral dependence on single or similar modes of action (Luttrell et al. 2004). Bollgard II® from Monsanto Company expresses Cry1Ac but also expresses Cry2Ab (Sivasupramaniam et al. 2003). This is welcomed additional technology to our growing arsenal of plant incorporated insecticidal proteins, as is the Widestike® cotton from Dow Agrosciences that expresses CryIF along with CryIAc (Huckaba et al. 2004).

VipCot™ from Syngenta Biotechnology is an exciting new technology in that it offers even a more diverse insecticidal protein and mode of action (Shotkoski et al. 2004). The Vip3A protein is different from the Cry proteins and could be a very important addition to our overall diversity of plant incorporated insecticidal proteins. Several researchers around the US Cotton Belt (Bradley et al. 2004, Cook et al. 2004, Mascarenhas et al. 2003) previously reported good activity of VipCot cottons against a range of different lepidopteran pests of cotton. Presented in this report are detailed field studies of the activity of VipCot cottons against heliothines in Arkansas over the past three years.

Materials and Methods

Syngenta Biotechnology and Delta Pine and Land Company provided the experimental cottons and support for these studies. Our approach was influenced by two major objectives. We wanted to compare the efficacy of VipCot cottons that were engineered to express Vip3A insecticidal protein (Cook and Prince 2004) to that of their parent line under unsprayed (NT) and spray as needed environments (TAN). Given the uniqueness of the experimental comparisons and the similar genetic background of the cottons being studied, we also wanted to explore the potential value of more detailed planting mapping and box mapping efforts in better understanding the potential management value of the technology. The similar genetic background of the experimental cottons and the infrastructure of the standard efficacy comparison provided a unique opportunity to collect a detailed set of comparative insect and insect damage data.

Pheromone traps for bollworm (*Helicoverpa zea* (Boddie)) and tobacco budworm (*Heliothis virescens* F.) were located near the plots and sampled weekly throughout the course of the study (Allen et al. 2004). When plots were infested with larvae, collections were made and larvae were placed on a pinto bean diet to measure survival and determine species. Most of the insects were *H. zea* throughout the three-year period, but *H. virescens* were present in relatively low densities, especially late in the growing season.

In 2003, laboratory colonies of *H. zea* were established from larvae surviving on conventional cotton in the test area, larvae surviving on Coker and VipCot cottons in the experimental plots, and larvae surviving on Bollgard cotton in nearby experimental plots. These colonies were exposed to Cry1Ac in a diet incorporation assay (Ali et al. 2004). Resulting LC50s were similar for colonies from conventional non-Bt cotton and colonies from VipCot research plots. Those from Bollgard study areas were slightly elevated (Ali et al. 2004).

Traditional Measurements of Insecticidal Activity

Replicated small plot studies were located on Tillar and Company in Drew County, Arkansas to compare the insecticidal efficacy of VipCot cottons from Syngenta Biotechnology and Delta Pine and Land Company to that of a parent Coker line. This location was chosen as a test site because of historically high insect pressure. The bollworm and the tobacco budworm were the targeted insects and plots were routinely oversprayed for other insects. Numerous sprays were made each year for plant bug control. Occasional oversprays of malathion were made by the active Boll Weevil Eradication program in the area. All oversprays were targeted at non-lepidopteran pests and were assumed to have no direct effect on the heliothines in the test area.

Experiments were conducted in 2002, 2003, and 2004. Plot size was usually 4 to 8 rows wide (38” rows) and 40 to 50 ft long. They were always replicated 4 to 5 times in a randomized complete block or latin square design. Charlie Guy and co-wokers (G&H Associates) planted the plots each year with a cone planter and mechanically harvested the center rows of each plot late in October or early November. Planting dates were usually late in May or early in June, a few weeks later than normal cotton planting times for the area. Weed control and fertility were based on standard practices for the area, and the plots received furrow irrigation on an as-needed basis.

Each year the treatments in the experiment varied but an untreated VipCot (NT) was always compared to untreated Coker (NT) cotton. In 2003 and 2004, VipCot and Coker cottons were also treated as needed for heliothines or when sprays were applied to the Coker parent line. In both years, the treat as needed plots (VipCot TAN and Coker TAN) received two applications of a pyrethroid insecticide for heliothine control. Studies in 2004 included comparisons of two advanced lines or events from Delta Pine and Land Company. VipCot 102 (the original line and the same as that studied in 2002 and 2003) was compared to VipCot 202 and VipCot 203.

Observations varied from year to year based on specific protocols and our academic interests, but routine estimates of insect infestation, plant damage and yield were collected all three years. Additional, more detailed, information from plant mapping and box mapping procedures are described below.

Data from individual dates and sample periods were studied by analysis of variance and means were separated by Fisher’s Protected LSD (p=0.05). Cumulative information summarized for the entire season was studied by correlation and regression analyses as defined below. In this report, we graphically illustrate major trends in cumulative numbers of heliothines and relative impact on yield.

Comparative Estimates of Insect Damage Rates

To compare relative differences in temporal patterns of insect infestation and fruit retention as indexed by date of mainstem node initiation, we had to develop descriptive relationships between time expressed as calendar date and observed date of initiation of different mainstem nodes. Observed mainstem nodes then became an index of time. Insect infestation data were typically collected on different dates using methods other than whole plant samples. Total nodes per plant in 2002 and 2003 studies were fit to polynomial regressions with calendar (or Julian) date on the x axis. Plant mapping was done on only two dates in 2004 but the temporal patterns appeared to closely match the polynomial equation for 2003. The 2002 and 2003 equations were used to temporally align insect and plant observations for subsequent regression analyses.

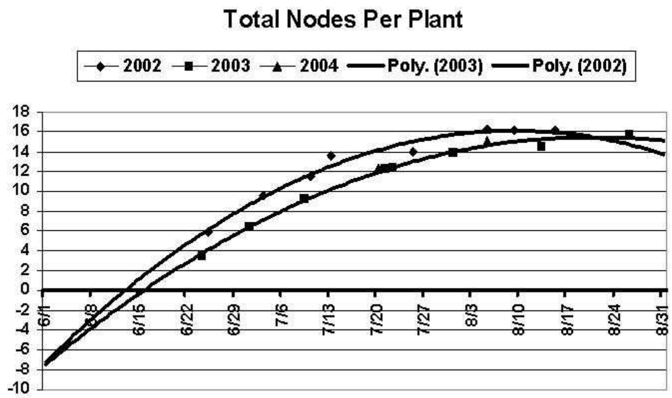


Figure 1. Temporal relationships between mainstem node initiation and calendar date in 2002 and 2003 VipCot studies in Tillar, Arkansas.

The routine plant maps and the end-of-season box maps provided sequential estimates of fruit retention, maturity and eventual yield by mainstem node location. By comparing average information for a given position with that obtained for the same position at a later date, we estimated survival of fruit for a given period of time. Figure 2 provides an example of the temporal patterns of fruit per plant observed for unsprayed Coker (NT) cotton in 2003 studies.

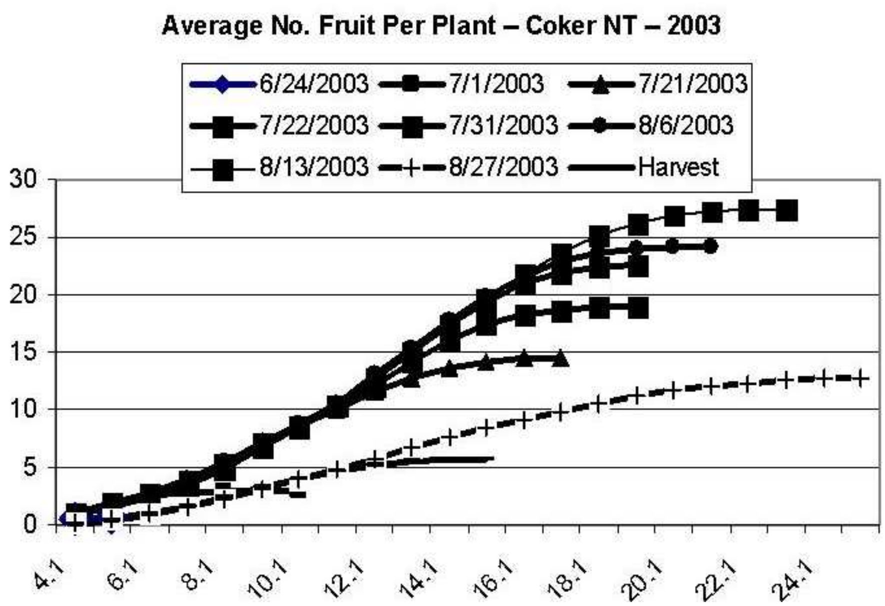


Figure 2. Average number of fruit per plant on Coker NT (not treated) cotton measured on different plant mapping dates and at harvest via box mapping procedures in 2003 VipCot studies in Tillar, Arkansas.

Similarly, information on numbers of harvestable bolls and weight per harvestable boll that were obtained in box mapping procedures at harvest were used to develop temporal patterns of fruit surviving to harvest and yield expressed as grams of seedcotton per plant. This provides a direct estimate of value of fruit associated with each mainstem node location and each date of fruit initiation.

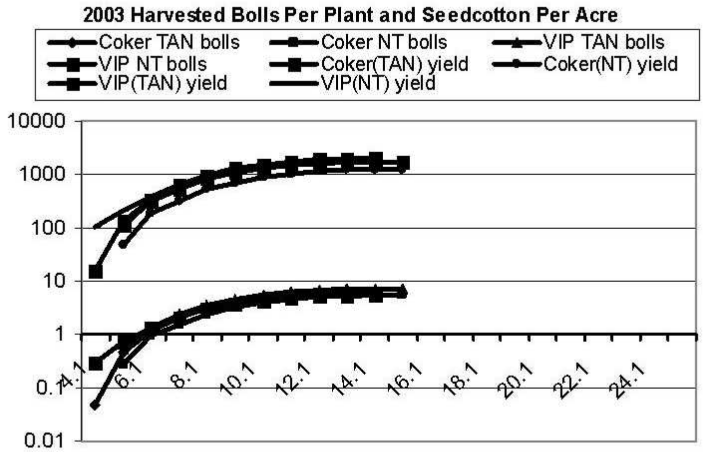


Figure 3. Cumulative grams of seedcotton and harvested bolls per plant for sprayed (TAN) and unsprayed (NT) Coker and VipCot cottons in 2003 VipCot studies in Tillar, Arkansas.

Since the VipCot cotton was always compared to its parent Coker line, experimental differences between the genetically similar treatments should be indicative of the suppressive activity on targeted insects, specifically the heliothines. By comparing cumulative differences between VipCot and Coker cottons and cumulative differences between sprayed and unsprayed cottons, we estimated the temporal influence of insect densities on fruit retention and final yield at harvest. An example of the cumulative differences in larval densities, fruit and bolls recorded on different plant mapping dates, and number of harvestable bolls and grams of seedcotton at harvest for unsprayed VipCot (NT) and Coker (NT) cottons in 2004 are shown in Figure 4. These types of collective data were obtained for comparisons of VipCot (NT) minus Coker (NT) for all three years, Coker (TAN) minus Coker (NT) for 2003 and 2004, and VipCot (TAN) minus VipCot (NT) for 2003 and 2004. In this paper we concentrated on the relationships between larval numbers and resulting retention of fruit. One could look more closely at the relative value of different fruit over time.

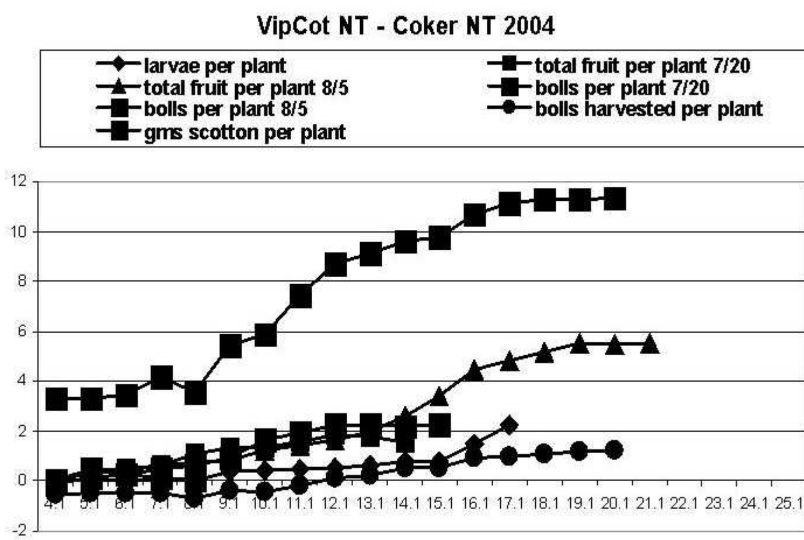


Figure 4. Differences in cumulative numbers of larvae, total fruit, bolls, and grams of seedcotton per plant between unsprayed VipCot (NT) and Coker NT cottons on different sample dates in 2004 VipCot studies in Tillar, Arkansas.

Results

Traditional Measurements of Insecticidal Activity

Cumulative numbers of heliothine larvae per acre in unsprayed VipCot (NT) cotton were dramatically reduced over those observed for the Coker (NT) parent line and the conventional buffer in 2002 studies (Figure 5). Similarly, cumulative numbers of larvae in untreated VipCot (NT) cotton in 2003 were reduced over those observed for untreated Coker (NT) cotton. However, differences were small and non-existent late in the growing season. Sprayed Coker (TAN) and VipCot (TAN) cotton had reduced numbers as compared to the unsprayed Coker (NT) and VipCot (NT) treatments. Sprayed VipCot (TAN) cotton had no recorded larvae and sprayed Coker (TAN) cotton had reduced cumulative numbers as compared to unsprayed Coker (NT) cotton.

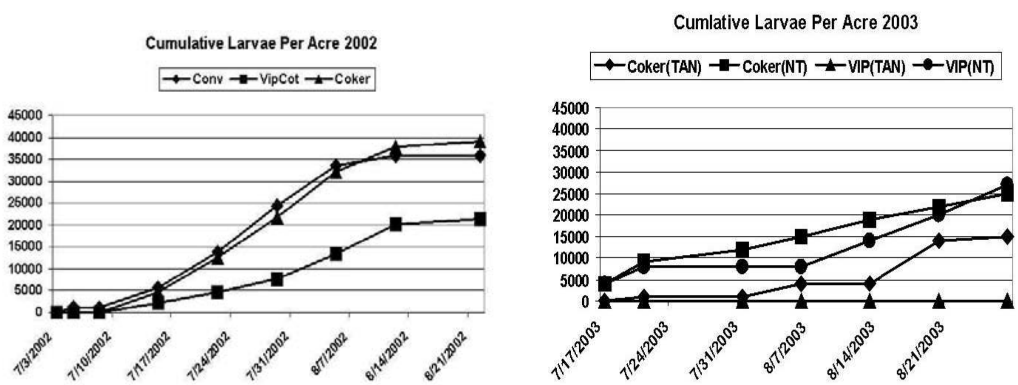


Figure 5. Cumulative heliothine larvae per acre in 2002 and 2003 VipCot studies in Tillar, Arkansas.

In 2004 studies, heliothine larvae were not observed in VipCot 202 and VipCot 203 cottons. Cumulative numbers observed on sprayed (TAN) and unsprayed (NT) VipCot cotton were reduced over those observed on sprayed and unsprayed Coker cotton. Cumulative numbers of larvae on sprayed Coker (TAN) and VipCot (TAN) cottons were reduced over those observed on unsprayed Coker (NT) and VipCot (NT) cottons.

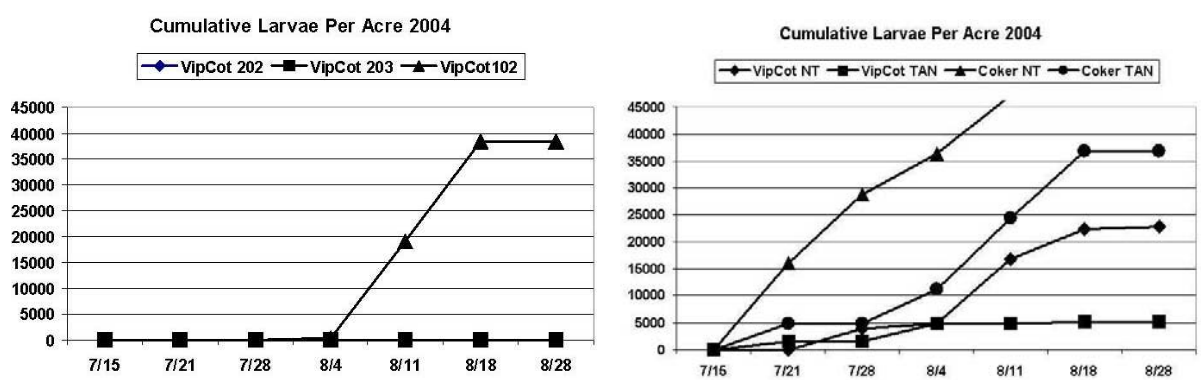


Figure 6. Cumulative heliothine larvae per acre in 2004 VipCot studies in Tillar, Arkansas.

Over the three year test period, yields from unsprayed VipCot (NT) cotton were increased ~25% over those for unsprayed Coker (NT) cotton (Figure 7). During 2003 and 2004, yields of sprayed VipCot (TAN) and Coker (TAN) cotton were increased ~37% and ~23%, respectively over those of unsprayed Coker (NT) cotton. Comparatively, yields of unsprayed VipCot (NT) were increased ~32% over those of unsprayed Coker (NT) cotton when average data for 2003 and 2004 are considered as a direct comparison to the sprayed treatments.

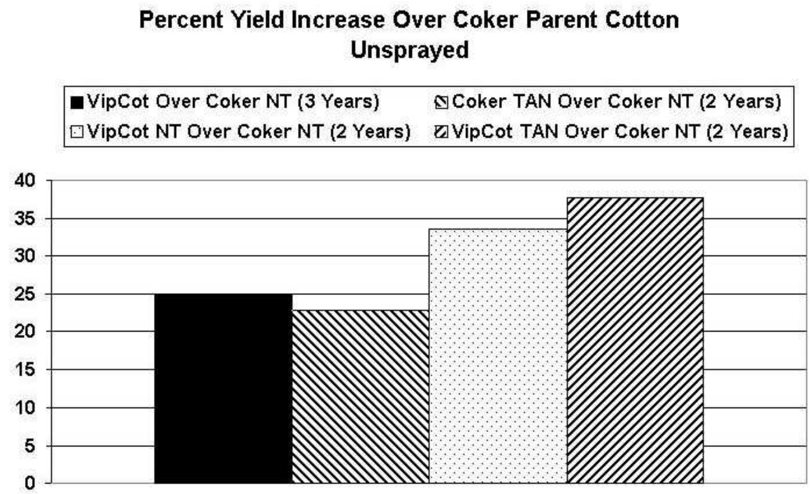


Figure 7. Percent yield increase of VipCot NT (not treated), VipCot TAN (treated as needed) and Coker TAN (treated as needed) over that for Coker NT (not treated) in 2002-2004 VipCot studies in Tillar, Arkansas.

Comparative Estimates of Insect Damage Rates

Routine plant mapping provided estimates of squaring nodes or sympodia with squares and retention of fruit on first fruiting positions. These data are similar to those obtained using the standard COTMAN monitoring procedures (Danforth and O'Leary 1998). Sampling procedures vary slightly from those suggested by the COTMAN procedures but the number of squaring nodes reported is comparable to NAWF (nodes above white flower) information in COTMAN. The rate of squaring node initiation was similar for VipCot and Coker cottons in 2002 studies (Figure 8). Both reached apogee during the second week of July. The conventional cotton buffer reached apogee about 10 days later. Cutout defined as NAWF 5 was reached during the second week of August on the VipCot and Coker cottons. Cutout for the conventional buffer was again delayed. In 2003 studies comparing sprayed and unsprayed VipCot and Coker cottons, unsprayed Coker cotton reached apogee slightly later than the sprayed Coker (TAN) and VipCot (TAN) treatments (Figure 8). This also resulted in delayed cutout. During 2004, plant mapping data were collected only on two dates. Differences in squaring nodes among VipCot 102, VipCot 202, and VipCot 203 were small and similar trends were observed on both sample dates (Figure 9). Sprayed VipCot (TAN) and unsprayed VipCot (NT) cottons had a steeper decline in squaring nodes between the two sample dates than those observed for sprayed Coker (TAN) and unsprayed Coker (NT) cottons. This indicates that the VipCot cottons were setting more fruit and reaching physiological cutout earlier than the parent Coker lines. The Coker cottons were trying to compensate for fruit lost earlier in the season. These trends in squaring node initiation among the different experimental cottons were also evident in the retention of first position fruit (Figures 10 and 11). VipCot treatments consistently had higher retention than those measured for the Coker parent line. Sprayed Coker (TAN) and VipCot (TAN) had smaller, but measurable, increases in retention of first position fruit (Figures 10 and 11).

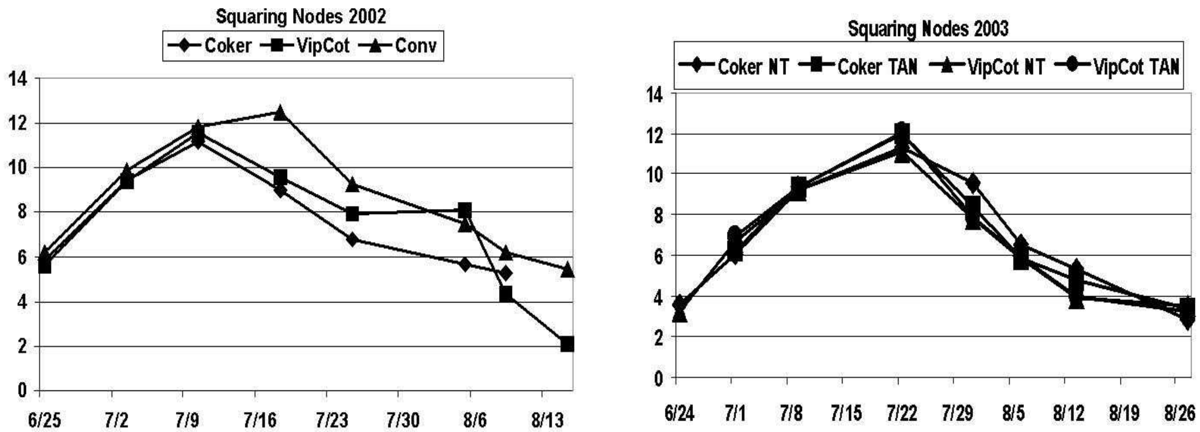


Figure 8. Number of squaring nodes (sympodia with squares) in 2002 and 2003VipCot studies in Tillar, Arkansas.

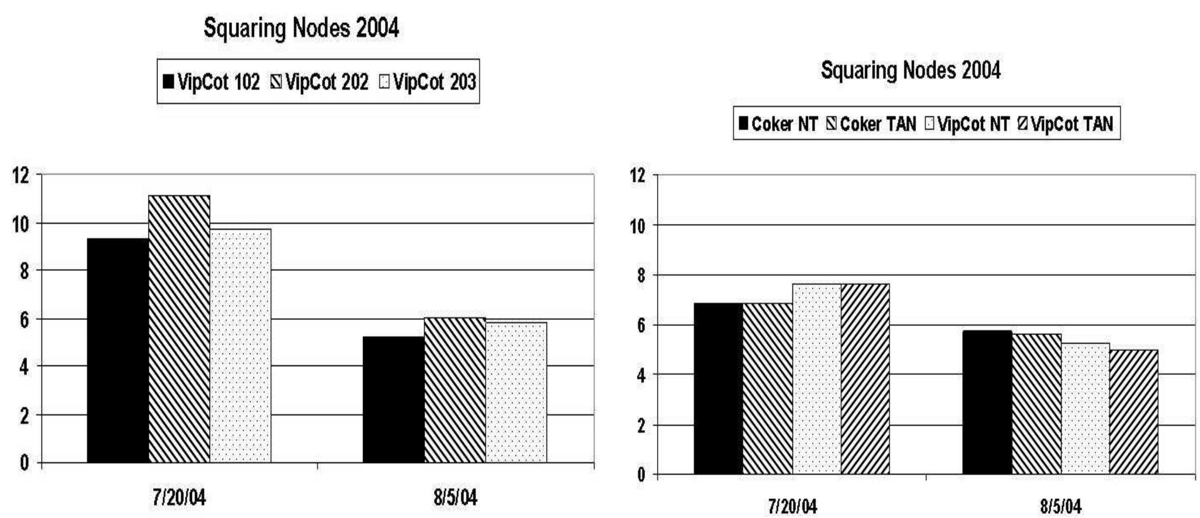


Figure 9. Number of squaring nodes (sympodia with squares) on two sample dates in 2004 VipCot studies in Tillar, Arkansas.

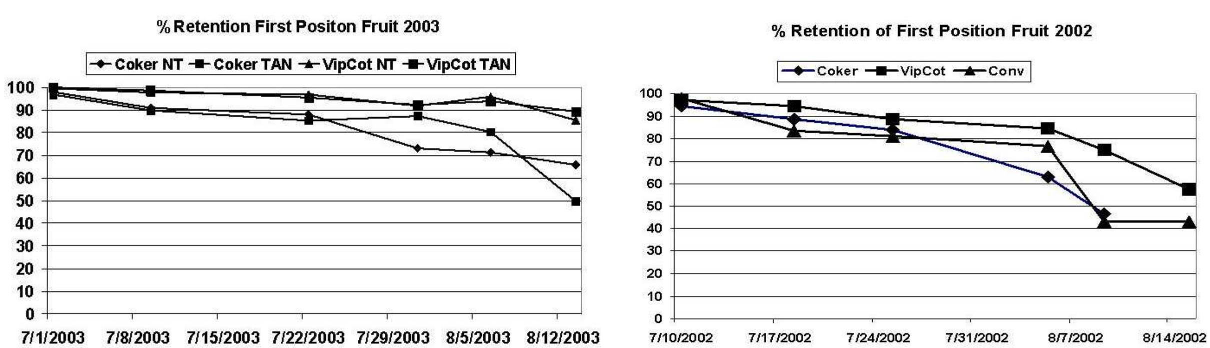
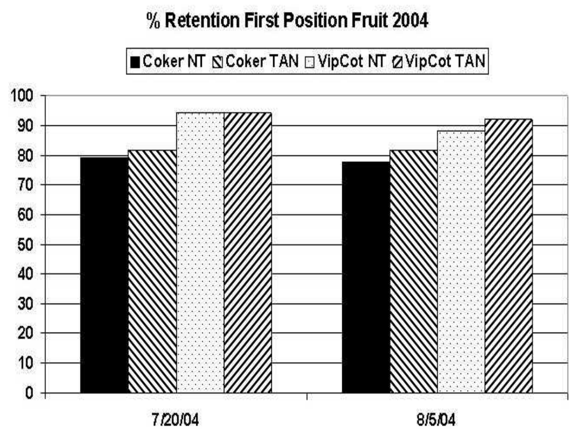


Figure 10. Percent retention of first position fruit in 2002 and 2003 VipCot studies in Tillar, Arkansas.



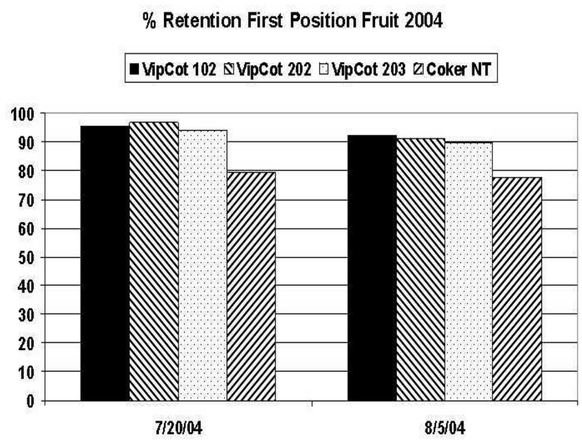


Figure 11. Percent retention of first position fruit on two sample dates in 2004 VipCot studies in Tillar, Arkansas.

Differences in cumulative larvae per acre (inverse of difference) across the three year test period (Figure 12) illustrate the season long protection provided by the VipCot insecticidal protein. Average differences in cumulative larvae between sprayed Coker (TAN) and unsprayed VipCot (NT) as compared to unsprayed Coker (NT) (Figure 12) reveal measurable effects of controlling larvae at the time of initiation of mainstem nodes 8.1 through 15.1. Peak differences between sprayed Coker (TAN) and unsprayed Coker (NT) were observed about the time of initiation of mainstem node 14.1. Peak differences between unsprayed VipCot (NT) and unsprayed Coker (NT) were a little later at time of initiation of mainstem node 17.1. Differences between VipCot TAN and VipCot NT were not noted until time of initiation of mainstem nodes 15.1 or higher.

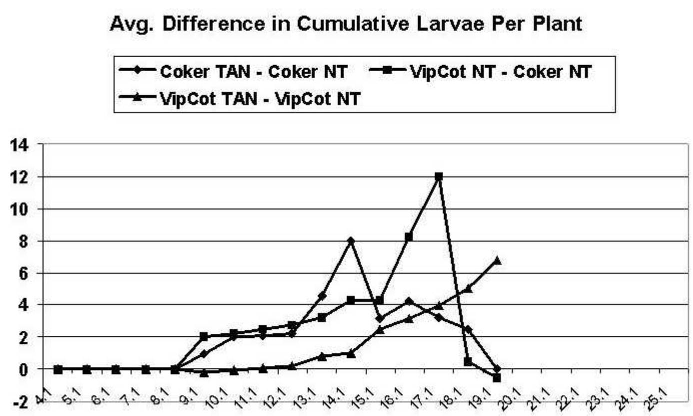


Figure 12. Average cumulative difference (inverse of difference) in densities of heliothine larvae between VipCot NT and Coker NT, VipCot TAN and VipCot NT, and Coker TAN and Coker NT treatments in 2002-2004 VipCot studies in Tillar, Arkansas.

Figure 13 graphically illustrates cumulative trends in the differences in larvae per plant and resulting grams of seedcotton per plant for unsprayed VipCot (NT) and Coker (NT) cottons, sprayed Coker (TAN) and unsprayed Coker (NT) cottons, and sprayed VipCot (TAN) and unsprayed VipCot (NT) cottons. Regression statistics associated with these relationships are reported in Table 1. Statistically significant regressions were found for the relationships between differences in cumulative larvae per plant (inverse) and cumulative grams of seedcotton per plant for Coker (TAN) minus Coker (NT) and VipCot (NT) minus Coker (NT) cottons. The relationship between cumulative differences in larvae per plant and resulting grams of seedcotton lost per plant for VipCot (TAN) minus VipCot (NT) was not statistically significant. The regression equations for Coker (TAN) minus Coker (NT) and VipCot (TAN) minus VipCot (NT) had similar slopes suggesting the loss of seven to eight grams of seedcotton for each unit of difference in cumulative larva per plant.

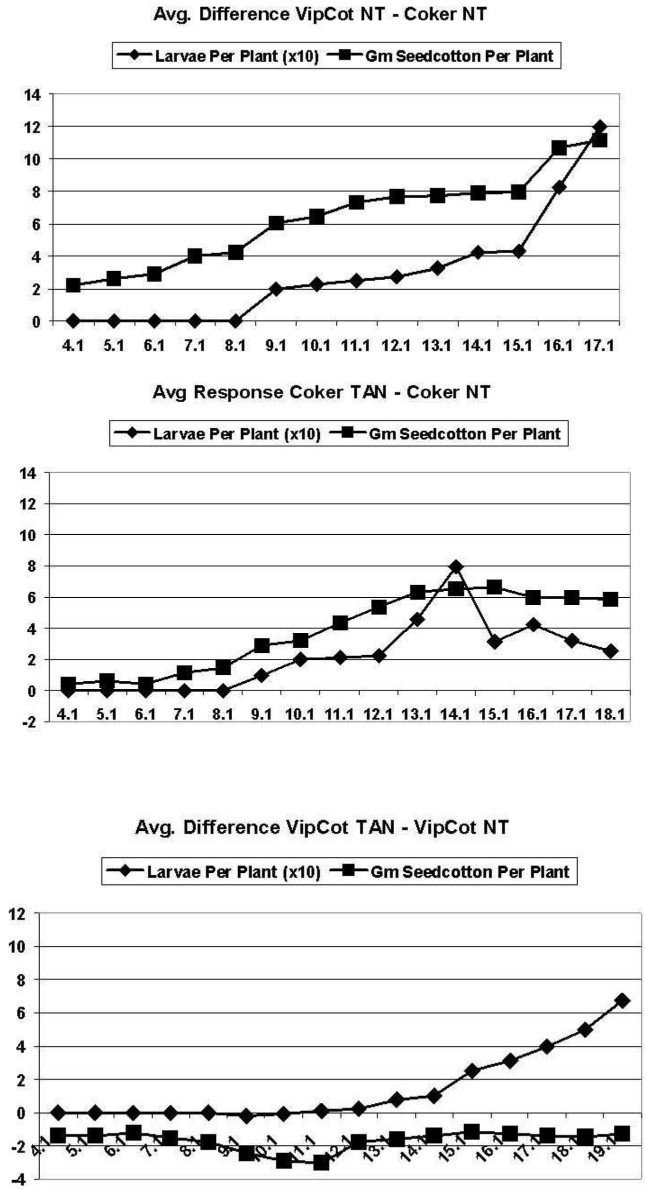


Figure 13. Cumulative differences in larvae per plant (inverse of differences) and grams of seedcotton per plant for VipCot NT minus Coker NT, Coker TAN minus Coker NT, and VipCot TAN minus Coker NT cottons in 2002-2004 VipCot studies in Tillar, Arkansas.

Table 1. Regression statistics associated with estimates of differences in grams of seedcotton per plant as a function of differences in numbers of heliothine larvae per plant.

r^2	Intercept (SE)	Slope (SE)	Prob.
Coker TAN – Coker NT			
0.776	0.475 (.445)	8.279 (1.482)	0.000

VipCot NT – Coker NT			
0.828	0.154 (.437)	7.3891 (.974)	0.000
VipCot Tan – VipCot NT			
0.179	-1.857 (.167)	1.143 (.653)	0.102

Given similarities in the regression equations describing differences in cumulative grams of seedcotton per plant as a function of cumulative differences in larvae per plant for Coker (TAN) and VipCot (NT) cottons minus Coker (NT) cotton, we combined the data for both examples and further examined impacts of larvae on numbers of bolls in August, numbers of bolls at harvest, and averaged grams of seedcotton per plant. The noted lack of similarity in these regression lines to that of the VipCot (TAN) minus VipCot (NT) regression may be due to different damage rates and survival traits of larvae on Coker and VipCot cottons. Stunting of larvae is a common characteristic of larvae found surviving on other Bt cottons. This may have contributed to the lack of a strong statistical relationship between differences in cumulative larvae per plant and grams of seedcotton per plant between VipCot (TAN) and VipCot (NT) treatments.

The combined regression equations (Table 2) revealed strong relationships between differences in larvae per plant, bolls in August, bolls at harvest, and grams of seedcotton at harvest. Slopes on the regression lines suggest that the loss of ~4.5 bolls per plant in August could be attributed to presence of a cumulative larva per plant. At harvest each cumulative larva was related to ~1 boll per plant suggesting that many of the bolls on the plant in August do not survive to harvest. The slope on the regression of grams of seedcotton as a function of differences in cumulative larvae per plant was ~10. This is a little confusing since a single harvestable boll typically produces three to five grams of seedcotton. Using this index of weight of seedcotton, one could conclude that each cumulative larvae per plant results in the loss of two or more fruit at harvest. The actual regression of cumulative larvae versus harvestable bolls indicates a slope of 1 or less. We are uncertain about the source of this variability, but it is indicative of the need for more study and exploration of these biological relationships. One possible influence is the accumulation of fruiting forms as age cohorts. Fruit initiated by time mainstem node 9.1 would include fruit at position 9.1, fruit at position 7.2, and fruit at position 5.3. Our work included fruit on three position of each branch, and it was accumulated by comparable time interval of mainstem (3 days) and branch (6 days) node formation.

Table 2. Regression statistics associated with estimates of differences in bolls per plant in August, bolls per plant at harvest, and grams of seedcotton per plant as a function of average differences in larvae per acre for Coker TAN (treat as needed) and VipCot NT (not treated) treatments over that observed over Coker NT (not treated) cottons in 2002-2004 VipCot studies in Tillar, Arkansas.

r^2	Intercept (SE)	Slope (SE)	Prob.
Larvae per plant – bolls in August			
0.836	0.618 (.185)	4.496 (.600)	0.000
Larvae per plant – bolls at harvest			
0.824	-0.044 (.037)	0.954 (.139)	0.000
Larvae per plant – gms seedcotton per plant			
0.863	2.501 (.379)	10.211 (1.229)	0.000

Discussion

VipCot cotton has good activity against heliothines in Arkansas. It is not immune to damage but reductions in insect densities and increases in yield were greater than those observed for cotton sprayed with conventional insecticides. The bollworm was the predominant species present in these studies, although tobacco budworm may have been present at low densities later in the year. Increased efficacy was observed when VipCot was sprayed with insecticides illustrating that a few insects do escape control on the current VipCot cotton. The magnitude of the increase was much less than the magnitude of the effect of the insecticidal protein expressed in the transgenic plant. In 2004, comparisons between VipCot 102 and two newer lines VipCot 202 and VipCot 203 suggested that the newer lines have much higher insecticidal activity that the original VipCot 102 line. This has been observed and reported by others (Bradley et al. 2004, Cook et al. 2004, Mascarenhas et al. 2003).

A more detailed look at the effects of the surviving insects on plant damage and yield through routine within-season plant maps and end-of-season box maps corroborated the general observations from insect scouting and yield comparisons. Coker cotton matured later and had more vegetative growth than the VipCot cottons because of insect damage. This was supported by routine measurements of retention of first position fruit.

Exploratory comparisons of differences in the cumulative densities of larvae, fruit retention, and yield allowed us to speculate about the potential cost or loss of cotton due to season long infestations of larvae. Based on these regression equations, a single cumulative larva per season could cause a loss of seven to ten grams of seedcotton per plant. Variability in regressions that project numbers of harvestable bolls and grams of seedcotton caution against over-extension of these exploratory data. The detailed accounting of plant map information illustrates a potential approach to valuing new technologies.

Acknowledgements

This work was supported by Syngenta Biotechnology and Delta Pine and Land Company. David Black and Jay Mahaffey are acknowledged for their cooperation and support of the three-year project. Thad Freeland and Mar Miles of Tillar and Company assisted us with plot space and location of the study. Marvin and Steven Wall were very instrumental in establishing this research site and keeping the work relevant to local cotton production. Charlie Guy and associates were very helpful throughout the course of the study. We are grateful for their assistance in planting and harvesting the plots. We also acknowledge the ongoing support of the Arkansas Agricultural Experiment Station.

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