Pink Bollworm and Cabbage Looper Mortalities and NuCOTN 33B (Bt) Cry1Ac Contents in Cotton Fruiting Forms and Leaves on Increasing Numbers of Days After Planting

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Abstract

Studies were conducted to follow seasonal susceptibility of feral pink bollworm (PBW), Pectinophora gossypiella (Saunders) larvae to NuCOTN 33B (Bt) and Deltapine (DPL) 5414 in furrow and furrow plus supplementary drip-irrigated cotton field plots. Laboratory bioassays of laboratory - reared PBW larvae to flower buds and bolls and cabbage looper (CL), Trichoplusia ni (Hübner), larval mortality feeding on DPL 5415 and Bt cottons leaves were also conducted. Cry1Ac insect toxic protein contents in the different plant tissue were determined by Enzyme Linked ImmunoSorbent Assay (ELISA) throughout the season to compare in relation to PBW and CL mortality data.

Irrigation type had no effect on PBW or CL larval mortality parameters measured. DPL 5415 bolls had 0.15 feral live larvae per boll and no dead larvae per boll compared with no live and 0.12 dead feral larvae per Bt boll. Whole plant samples showed 0.5 to 8.6% live larvae boll infestations compared to no live PBW life stages and no exit holes for Bt bolls. No PBW larvae survived on day four following bioassay infestation of one-third grown Bt flower buds with PBW neonate larvae as compared to 90% larval survival on DPL 5415 flower buds. Immature bolls harvested in the field and artificially infested with PBW larvae in the laboratory showed averages of 3 to 52% live larvae per boll, all in fourth instar of development, for DPL 5415 bolls compared to no live larvae, no development beyond the first instar, and no exit holes for Bt bolls. Cry1Ac protein level in flower buds were 0.11 to 0.16 ppm and 0.14, 0.11 and 0.05 ppm, in each case, per wet weight gram of boll tissue in bolls during the season.

For CL leaf bioassays, larval mortalities after 7 days feeding on Bt leaves were variable ranging from 82 to 94% from node 8 on 61 and 82 days after planting (DAP) to 32, 38 and 7% on leaves from node 16 on 82, 117, and 159 DAP, respectively, and 28 and 6% on leaves from node 24 on 117 and 159 DAP. Cry1Ac amounts were 0.96 and 0.85 ppm (wet wgt per g of Bt leaf tissue), from leaves from node 8 (61 and 82 DAP), 0.53, 0.50 and 0.22 ppm (node 16, 82, 117, and 159 DAP) and 0.44 and 0.18 ppm (node 24, 117 and 159 DAP).

Numbers of cotton bolls, lint and seed per acre were significantly greater from plots that were furrow plus drip irrigated as compared to furrow irrigated alone. DPL 5415 and Bt cotton yields were not significantly different.

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Introduction

Bollgard® cottons (Bt) have had a dramatic impact on improvement of pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), population management. Insecticide use has been reduced benefiting the environment and grower income increased by lowering production costs and increasing cotton yields (Frisvold et al. 2000). In spite of this progress concern has been expressed for some transgenic cotton limitations such as the potential for resistance development, non-expression of toxic protein under certain environmental and temporal conditions and potential for losing the efficacy of the Bt toxins per se in other forms of application for insect control (Mellon and Rissler 1998). Other concerns include possible Bt toxin effects on insect natural enemies, potential of genetic modifications that may result in reduced yields, increased resistance to other toxins, and obtaining crop marketability with a high level of consumer acceptance (van Emden 1999).

After six years of commercial Bt cotton production in Arizona, there has been no decrease in field performance for PBW control (<1% boll infestations) (Flint and Parks 1999, Simmons et al. 1998, Patin et al. 1999) or in susceptibility to the Cry1Ac toxic protein (Sims et al. 2001). The efficacy of Bt cotton recently appears to have been further protected with insertion of a second gene that mediates production of second Bt toxic protein (Greenplate et al. 2000). The two-Bt-gene cotton showed 10-fold or better PBW efficacy compared to either single gene cotton (Marchosky et al. 2001). Although Cry1Ac toxic protein levels in different cotton plant tissue are affected by field site, sampling time, and variety (Greenplate et al. 2000), in 3 years of study in Arizona, no difference in efficacy against PBW occurred that was related to irrigation type (furrow vs. furrow plus drip), days after planting (DAP), or different age cotton fruiting forms (Henneberry and Jech 2000, Henneberry et al. 2000, 2001). Since significant reductions in Cry1Ac protein level occurs with increasing days after planting (Greenplate et al. 1998, Greenplate 1999), without loss in efficacy against PBW, the continued outstanding seasonal efficiency appears to be due, at least in part, to the extreme susceptibility of PBW larvae to the variable expression levels of the Cry1Ac toxic protein (Henneberry et al. 2000). In 2001, we investigated PBW larval mortality in early-, mid-, and late-season in relation to Cry1Ac toxic protein levels occurring in 21 day-old Bt cotton bolls. Cotton yields were compared in DPL 5415 and Bt cotton. We also used a cabbage looper (CL), *Trichoplusia ni* (Hübner), bioassay to compare to Cry1Ac contents and CL larval mortalities for leaves of different ages from plants on days 61, 82, 117, and 159 DAP. CL larvae in previous studies have been less susceptible to Bt than PBW or tobacco budworm, *Heliothis virescens* (F.), but more susceptible than beet armyworm, *Spodoptera exigua* (Hübner) (Henneberry et al. 2002).

Materials and Methods

Field plot design and culture. DPL 5415 and Bt cotton seeds were planted on 19 April 2001 in seven rows wide by 19 m (60 feet) long plots at the Western Cotton Research Laboratory at Phoenix, AZ. Plots were arranged in a split plot design with four replications. Whole plots were NuCOTN 33B and DPL 5415 cultivars. Split plots were irrigation treatments, (1) furrow every 14 days or (2) furrow every 14 days plus 2 h daily supplementary surface drip irrigations. Furrow irrigations alone supplied about 13 cm of water per irrigation and the 2 h of additional drip irrigation about 102 liters of water per hour per 31 m of row. Last irrigations occurred on 21 September and plots were defoliated on 1 November.

Cry1Ac toxic protein determinations. The amounts of toxic protein in squares (139 DAP), bolls (83, 118 and 152 DAP), or leaves (61, 82, 117 and 159 DAP) of Bt cotton were determined using ELISA for all the laboratory bioassay studies described below. DPL 5415 plant tissue samples in all cases were controls. The determinations were made to identify possible differences in expression of the toxic protein in different age cotton plants and to compare PBW and CL larval mortalities feeding on the different age plant tissues. Materials, sample preparations, solutions, extractions, dilutions and assays were as described in the Envirologix, Inc. Cry1Ab/Cry1Ac plate kit (Envirologix, Inc., Portland, ME). For flower buds and bolls, 0.6 cm wide slices across the center and through the entire fruiting form were cut with razor blade from each of eight fruiting forms picked at random from each plot on the same sampling dates and same plant locations as described for PBW larval bioassays. Each 0.6 cm flower bud or boll piece was weighed before placing in a 1.5-ml microcentrifuge tube and homogenized by hand in extraction buffer with a fitted pestle. For leaves and CL mortality comparisons, a 1.0 cm diameter leaf disk punch was taken

from the leaves described for the CL bioassay weighed and placed in a 1.5-ml microcentrifuge tube and homogenized as described.

Pink bollworm moths. PBW male moths were monitored with four Delta traps (Flint and Merkle 1983) placed at random in each quadrant of the 2.5 acre experimental field. Traps were baited with one gossyplure (Hummel et al. 1973) impregnated rubber stopper (1,000 µg) and placed at the top of the cotton plant canopy. The traps were collected and replaced weekly with new traps. Gossyplure-impregnated rubber stoppers were replaced every other week.

Pink bollworm larval bioassays. Flower buds. On 6 September (139 days after planting) we randomly collected 8 to 10 third grown flower buds from each plot of Bt and DPL 5415 cotton. In the laboratory, pieces of oviposition substrate with an average of 20 PBW eggs near hatch (Henneberry et al. 2000) were placed on each flower bud. All PBW larvae used for artificially infesting field grown flower buds or cotton bolls were from a laboratory colony reared on artificial diet (Bartlett and Wolf 1985) at the Western Cotton Research Laboratory. Flower buds were dissected and examined for living and dead PBW larvae on day 4 following infestations. Similarly, third grown Bt and DPL 5415 squares were picked from greenhouse plants on day 88 after planting. PBW eggs infestations and examinations for live and dead larval were as described.

Immature green bolls. We tagged 50 flowers per plot on 20 June, 21 July, and 28 August 2001. The first set of tagged flowers were on plant nodes six to eight. Approximately three weeks following each flower tagging, bolls (21 days old) were picked and taken to the laboratory. Five neonate larvae were placed on each boll that developed from a tagged flower. The bolls were placed individually in 30 cc plastic cups with covers and held in the laboratory (≈ 26-27°C) for two weeks. All larvae exited from the bolls and found as larvae, pupae, or adults in the cups were recorded. Also each boll was examined with the aid of a microscope. All larval entrance and exit holes in the carpel walls were counted. Bolls were then dissected and all living and dead larvae and pupae were recorded.

Cabbage looper bioassays. Larvae were from the Western Cotton Research Laboratory colony reared on artificial diet (Henneberry and Kishaba 1966). Leaves were picked at random from each plot on days 61, 82, 117 and 159 after planting. The leaves were trimmed to fit in 15.0 cm diameter x 1.5 cm deep plastic petri dishes lined on the bottom with moist filter paper. Prior to placing leaves in the petri dishes, one 1.0 cm diameter leaf disk was punched from each leaf for quantification of Cry1Ac protein. Leaf disks were frozen until analyses were conducted. Five, 1st instar CL larvae were placed on the identical leaves in each of four petri dishes. Living and dead larvae were counted after seven days.

Feral pink bollworm infestations. Immature green bolls. On 28 September and 24 October, 25 immature green bolls (21 to 26 days old) were randomly sampled from each plot to determine boll infestations in Bt and DPL 5415 cottons from feral PBWs. Each boll was examined under the microscope and larvae on the exterior of the bolls and in entrance and exit holes recorded. Each 25 boll sample was placed in a screen ventilated plastic incubation box held for two weeks in the laboratory. Larvae exited from bolls and found in incubation boxes as larvae, pupae, or adults were recorded. Bolls were then dissected and examined as described.

Whole plant samples. On 28 November 2001, we harvested five defoliated whole plants from each Bt and each DPL 5415 plot. All mature-open cotton bolls and all immature green cotton bolls were counted and recorded on each fruiting branch. Fruiting branches were numbered consecutively from 1 to 30 beginning with the first branch on the mainstem above the cotyledon nodes. All open mature and immature green bolls were examined as described for PBW entrance and exit holes. They were also dissected and examined for living and dead PBW larvae and other life stages.

Cultivar yields. Bt and DPL 5415 cotton yields in furrow and furrow plus drip irrigations were compared by picking all open cotton bolls in 4 meters of row in each plot. Numbers of bolls were recorded. Seed cotton was weighed and ginned followed by separate weighings of lint and seed from each plot.

ANOVA were conducted and contingent upon a significant F test, means were separated using the method of least significant differences ($P \leq 0.05$). All percentages were transformed to arcsines before analysis. Means (± SE) are presented for all data.

Results

Field plot design and culture. There were no significant differences for furrow vs. furrow plus drip irrigation for PBW infestation data (F values, df = 1,9; $P > 0.05$ ranged from 0.01 to 3.45 for infestations on all sampling dates) or amounts of Cry1Ac protein detected in bolls (F = 0.09; df = 1,15; $P > 0.05$ for all sampling dates). Thus, data were combined across cultivars for furrow vs. furrow plus drip irrigation in further analyses.

Pink bollworm moths. Numbers of male moths per gossyplure-baited trap per night increased during early August, peaked in late August and decreased in mid-September (Figure 1). Trap catches reached a second peak in early-October and remained high until late November.

Pink bollworm larval bioassays. Flower Buds. For flower buds collected on 6 September (139 DAP), there were no live and 2.18 ± 0.24 dead PBW larvae per flower bud in Bt bolls compared to 2.23 ± 0.24 live and 0.33 ± 0.7 dead larvae (10.2% mortality) in DPL 5415 flower buds. ELISA determined CrylAc content was 0.16 ppm (wet weight gram of flower bud tissue) (t = 38.85; df = 14; $P \leq 0.01$ for mortality differences). These results were similar to those obtained with flower buds picked from greenhouse grown cotton plants on day 88 after planting when there were 2.10 ± 0.40 dead larvae per flower bud (100% mortality) on day four following infestation of Bt cotton. ELISA analyses detected 0.11 ± 0.01 ppm of CrylAc protein. For DPL 5415 cotton on day four following infestation there were 0.5 ± 0.2 dead larvae found per flower bud (19.5 ± 6.5% mortality (t = 12.40; df = 24; $P \leq 0.01$ for mortality differences).

Immature green bolls. There were no significant differences between cultivars for numbers of PBW entrance holes in bolls (Table 1). Total numbers of larvae found in DPL 5415 and Bt bolls were variable, but no live larvae were found in Bt bolls compared to 3.2 to 52% live larvae per boll found in DPL 5415 bolls. Also, no larvae developed beyond the first instar in NuCOTN 33B bolls compared with 0.06 to 0.08, 0.01 to 0.04, and 0.01 to 0.45 live second, third and fourth instar larvae, respectively, per boll found in DPL 5415 bolls. Amounts of Cry1Ac toxic protein found in 21 to 26 day old cotton bolls on days 83, 118, and 152 days after planting were 0.14, 0.11 and 0.05 ppm (per wet weight gram of boll tissue), respectively. PBW larval mortality was 100% in bolls at all estimated Cry1Ac levels measured using the ELISA procedures.

Cabbage looper bioassays. CL larval mortalities feeding on leaves from node eight that were harvested 61 and 82 days after planting were 82 and 94%, respectively (Table 2). Larval mortalities after feeding for seven days on leaves harvested from plant nodes 16 and 24 on days 82, 117 and 159 days after planting were 32, 38, and 73%, respectively, and 28 and 5.6%, respectively for larvae feeding on leaves from node 24 harvested 117 and 159 days after planting. Cry1Ac ppm per gram of wet weight tissue from leaves harvested from node eight on days 61 and 82 after planting were not significantly different (Table 2). However, ppm of Cry1Ac protein in leaves harvested from node 16 on 82, 117 or 159 days after planting or from plant node 24 on days 117 or 159 after planting decreased with increasing days after planting. Cry1Ac protein determinations were 0.53, 0.50 and 0.22 ppm, respectively, from leaves harvested from node 16 and 0.44 and 0.18 ppm for leaves harvested from node 24. Except for leaves from node 16 harvested 159 days after planting, CL larval mortalities appeared to be related to the amount of Cry1Ac protein measured in leaf tissue. In contrast, CL larval mortalities for larvae feeding on DPL 5415 leaves ranged from 2.3 to 18.4% over all sampling dates (Table 2). The only significant differences occurred between larvae feeding on leaves from node 16 (18.4%) vs. larvae feeding on leaves from node 24 (2.3%) at 159 days after planting.

Feral pink bollworm infestations in immature green bolls. PBW infestations from feral populations were low (Table 3). Numbers of PBW entrance holes in Bt or DPL 5415 bolls were not significantly different. No live larvae were found in Bt bolls compared with 0.15 ± 0.03 live larvae per DPL 5415 boll. Also, no dead larvae were found in DPL 5415 bolls compared with 0.02 ± 0.02 dead larvae in Bt bolls.

Whole plant samples. PBW larvae per boll and percentage of bolls infested were higher in furrow plus drip irrigated compared with furrow alone irrigated DPL 5415 but not Bt cotton plots (Table 4). No live larvae were found in Bt bolls compared with DPL 5415 0.04 to 0.46 live larvae per boll and percentages of infested bolls were higher in DPL 5415 compared with Bt bolls. Numbers of PBW larvae (plus exit holes) peaked in bolls at plant nodes 21 to 25

and decreased in bolls from plant nodes 26 to 35. Percentages of infested bolls increased at nodes 21 to 25, decreased at nodes 26 to 30 and were highest in bolls 31 to 35. No live larvae or exits holes were found in Bt bolls from plant nodes at any level (data not tabulated).

Cultivar yields. Numbers of mature cotton bolls, and lint and seed weight per four m of row were higher in furrow plus drip irrigated Bt and DPL 5415 cottons compared with furrow alone irrigated cotton (Table 5). Variation was high and the difference between cultivars was not statistically significant. Numbers of mature cotton bolls, lint and seed weights for the higher overall averages of furrow plus drip plots compared with the averages for furrow alone were significantly different in each case.

Discussion

The results of our present study agree with our earlier reports suggesting the high susceptibility of PBW larvae to the Bt Cry1Ac toxic protein with no measurable differences in susceptibility of different age cotton fruiting forms or with increasing days after planting in laboratory bioassays or in field studies (Henneberry et al. 2000, 2001). Our earlier conclusions were based on high mortalities following minimal PBW larval feeding times, and over 99% mortality occurring in the first larval instar. In this study, ELISA determined Cry1Ac content in 21 to 26 day old cotton bolls were 0.14, 0.11, and 0.05 ppm per wet weight g of boll tissue on 1 July, 15 August and 18 September, respectively. Larval mortalities were 100% on all sampling dates corroborating the susceptibility of PBW larvae to low levels of the Cry1Ac protein. All PBW larvae died in the first instar feeding on bolls containing 0.05 ppm of Cry1Ac protein.

A previous report (Henneberry et al. 2002, in press) also indicated that CL larvae were less susceptible to Cry1Ac toxic protein than tobacco budworm, *Heliothis virescens* (F), or PBW but more susceptible to the protein than beet armyworm, *Spodoptera exigua* (Hübner). In the present studies, high CL mortalities (82 to 94%) occurred when larvae fed for seven days on leaves containing 0.96 to 0.85 ppm of the Cry1Ac protein compared to lower mortalities (5.6 to 38.4%) when feeding on cotton leaves containing 0.18 to 0.53 ppm of Cry1Ac toxic protein. For CL, one exception occurred with 73.0 percent mortality occurring with larvae feeding on leaves 159 dap that contained 0.22 ppm of Cry1Ac protein. This result remains unexplained but may reflect a leaf age effect, independent of Cry1Ac protein content, that has been observed in other studies (Henneberry et al. 2002). Higher cabbage looper larval mortality occurred on senescing cotton leaves compared with young cotton leaves.

The precise quantification of Cry1Ac and other Bt crystal proteins in relation to insect mortality may be subject to further definition with improved detection methods. Greenplate (1999) called attention to the potential influences of protein extraction efficiency on ELISA assays as reported by Miksic (1992) and Sachs et al. (1998). Greenplate (1999) also suggested that ELISA activity based on antigen-antibody interactions may not always reflect insect activity. Even so, the results of these studies with CL and those of Adamczyk et al. (2001) with fall armyworm, *Spodoptera fungiperda* (J. E. Smith), suggest different larval dose mortality responses to different levels of the toxic protein extracted from cotton tissues using a commercially available ELISA detection system.

Lint cotton yields for Bt and DPL 5415 cotton were not significantly different. PBW feral populations were low partially explaining the lack of Bt yield benefits (about 6% to 9% PBW boll infestations for DPL 5415). Yield differences for both cultivars were associated with furrow plus supplemental drip irrigation as opposed to furrow irrigation alone. These results probably masked any positive Bt yield advantage due to PBW suppression.

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TABLE 1. Mean (± SE) numbers of pink bollworm larval entrance holes, total larvae, percentages of dead pink bollworm and parts per million of Cry1Ac protein in NuCOTN 33B (Bt) bolls on increasing days after planting.

Cultivar	Entrance Holes/Boll[1/] (No.)	Larvae/boll		CrylAc (ppm)
		Total %	Dead	
		July 11, 83 days after planting[2/]		
DPL 5415	2.6 ± 0.7 a	1.3 ± 0.4 b	61.2 ± 10.9 b	0.00 ± 0.00
Bt	3.0 ± 0.2 a	2.5 ± 0.2 a	100.0 ± 0.0 a[4/]	0.14 ± 0.01
F(P)[3/]	0.55 (>0.05)	7.35 (≤ 0.05)	35.39 (≤ 0.01)	- -
		August 15, 118 days after planting		
DPL 5415	1.4 ± 0.2 a	2.1 ± 0.1 b	96.8 ± 1.1 b	0.00 ± 0.00
Bt	1.0 ± 0.2 a	2.7 ± 0.2 a	100.0 ± 0.0 a	0.11 ± 0.01
F(P)	2.4 (>0.05)	6.2 (≤ 0.05)	9.3 (≤ 0.01)	- -
		September 18, 152 days after planting		
DPL 5415	6.3 ± 0.15 a	2.9 ± 0.3 a	48.1 ± 3.5 b	0.00 ± 0.00
Bt	4.8 ± 1.40 a	1.2 ± 0.5 b	100.0 ± 0.0 a	0.05 ± 0.01
F(P)	1.1 (> 0.05)	10.2 (≤0.01)	517.9 (≤0.01)	- -

[1/] All bolls 21 to 26 days old from tagged flowers.
[2/] Planted April 19, 2001. Means of 4 replication in a column in the same date not followed by the same letter are significantly different.
[3/] df = 1,9 for all F values.
[4/] 1 fourth instar larva was found in 147 Bt bolls, ELISA was negative for Cry1Ac protein suggesting plant was from a non-Bt seed contaminant.

TABLE 2. Mean (± SE) Cry1Ac parts per million (ppm) per wet weight gram of NuCOTN 33B (Bt) cotton leaf tissue in relation to cabbage looper larval mortality.

Sampled Plant Node (No.)	Days after planting[1/]			
	61	82	117	159
	Percent Cabbage Looper Mortality Feeding on Bt Leaves[2/]			
8	81.5 ± 4.7 b	94.0 ± 3.4 a	- -	- -
16	- -	32.1 ± 2.7 c	38.4 ± 5.4 c	73.0 ± 8.8 b
24	- -	- -	28.0 ± 8.2 c	5.6 ± 2.0 d
	Percent Cabbage Looper Mortality Feeding on DPL 5415 Leaves[3/]			
8	2.5 ± 1.3 a	15.1 ± 3.0 a	- -	- -
16	- -	12.0 ± 3.5 a	2.9 ± 1.4 a	18.4 ± 4.7 a
24	- -	- -	3.0 ± 2.2 a	2.3 ± 1.2 b
	Cry1Ac PPM Per Wet Weight Gram of Bt Leaf Tissue[4/]			
8	0.96 ± 0.09 a	0.85 ± 0.08 a	- -	- -
16	- -	0.53 ± 0.05 b	0.50 ± 0.05 b	0.22 ± 0.02 c
24	- -	- -	0.44 ± 0.08 b	0.18 ± 0.04 c

[1/] Means of 8 replications not followed by the same letter are significant different. Method of least significant difference ($P \leq 0.05$).
[2/] F = 33.58; df 6, 42; $P > 0.05$.
[3/] F = 1.68; df 6, 42; $P > 0.05$.
[4/] F = 20.55; df 6, 42; $P > 0.05$.

TABLE 3. Means (± SE) numbers of pink bollworm entrance holes and live and dead larvae per boll found in immature green cotton boll of DPL 5415 and NuCOTN 33B cotton bolls.

Sampling date /Treatment [2/]	Entrance holes/boll	Larvae / boll[1/]		% Mortality
		live	dead	
NuCOTN 33B (Bt)	0.21 ± 0.05 a	0.0 ± - - b	0.12 ± 0.02 a	100.00 a
DPL 5415	0.41 ± 0.10 a	0.15 ± 0.03 a	0.00 ± - - b	0.00 b
t[3/], (*P*)	1.85 (> 0.05)	4.48 (≤ 0.05)	5.35 (≤ 0.05)	65.6 (≤ 0.05)

[1/] Mean of 10 observations in a column and date not followed by the same letter are significantly different. Twenty-five bolls of each cultivar picked per replicate on each sampling date.
[2/] Furrow alone compared with furrow plus supplementary drip irrigation.
[3/] df = 20 for all t tests.

TABLE 4. Mean (± SE) numbers of open cotton bolls per plant per node after whole plant samples of Deltapine 5415 and NuCOTN 33B cottons.

Cultivar / Treatment	Number of[1/] Open bolls	PBW larvae plus exit holes per boll	Bolls % infested[1/]
NuCOTN 33B (Bt)[2/]			
Furrow	3.53 ± 0.34 a	0.01 ± 0.01 b	0.17 ± 0.17 b
Furrow plus drip	3.86 ± 0.33 a	0.03 ± 0.07 b	0.46 ± 0.34 b
DPL 5415 (DPL)			
Furrow	4.23 ± 0.42 a	0.64 ± 0.02 b	1.36 ± 0.79 b
Furrow plus drip	3.17 ± 0.41 a	0.46 ± 0.12 a	11.61 ± 3.49 a
F[3/], (*P*)	2.46 (> 0.05)	14.79 (≤ 0.05)	11.15 (≤ 0.05)
Main Effects			
Cultivar			
Bt[2/]	3.70 ± 0.73 a	0.02 ± 0.01 b	0.32 ± 0.19 b
DPL 5415	3.70 ± 0.30 a	0.25 ± 0.07 a	6.60 ± 1.94 a
F[3/], (*P*)	0.00 (> 0.05)	20.88 (≤ 0.05)	19.63 (*P* ≤ 0.05)
Irrigation			
Furrow	3.35 ± 0.27 b	0.03 ± 0.01 b	0.75 ± 0.40 b
Furrow plus drip	4.40 ± 0.27 a	0.24 ± 0.07 a	5.92 ± 1.90 a
F[3/], (*P*)	8.78 (≤ 0.05)	17.70 (≤ 0.05)	13.94 (≤ 0.05)
Nodes			
6-10	4.03 ± 0.20 b	0.03 ± 0.02 b	0.48 ± 0.33 e
11-15	4.93 ± 0.26 a	0.05 ± 0.03 b	0.83 ± 0.57 e
16-20	3.95 ± 0.37 b	0.13 ± 0.07 b	2.34 ± 1.29 d
21-25	5.29 ± 0.42 a	0.33 ± 0.15 a	5.77 ± 2.47 b
26-30	3.13 ± 0.29 c	0.15 ± 0.09 b	3.61 ± 2.18 c
31-35	0.86 ± 0.20 d	0.13 ± 0.19 b	8.61 ± 5.39 a
F[4/], (*P*), df = 5,95	30.77 (≤ 0.05)	2.69 (≤ 0.05)	104.55 (≤ 0.05)

[1/] Means of 4 replications in the same column and category not followed by the same letter are significantly different $P \leq 0.05$.

[2/] No exit holes, all dead larvae.

[3/] df = 1,95

[4/] df = 5,95

TABLE 5. Mean (± SE) numbers of open mature cotton bolls and cotton seed and lint weights per four meters of DPL 5415 and NuCOTN 33B cotton cultivars in furrow and furrow plus drip irrigated plots.

Cultivar Treatment	No. bolls[1/] / 4 m row	Weight grams (per 4 m of row)[1/] seed	 lint
NuCOTN 33B (Bt)			
Furrow	624 ± 68 a	1190 ± 105 a	711 ± 61 a
Furrow plus drip	760 ± 34 a	1571 ± 87 a	975 ± 54 a
DPL 5415			
Furrow	454 ± 72 a	817 ± 133 a	547 ± 90 a
Furrow plus drip	720 ± 46 a	1441 ± 89 a	991 ± 62 a
F[2/] (*P*)	107 (> 0.05)	1.05 (> 0.05)	1.38 (> 0.05)
Main Effects			
Cultivar			
Bt	692 ± 44 a	1380 ± 96 a	843 ± 63 a
DPL 5415	586 ± 64 a	1128 ± 139 a	769 ± 98 a
F[3/] (*P*)	2.81 (> 0.05)	4.43 (> 0.05)	0.93 (> 0.05)
Irrigation			
Furrow	539 ± 56 b	1003 ± 105 b	629 ± 59 b
Furrow plus drip	740 ± 28 a	1506 ± 63 a	983 ± 38 a
F[3/] (*P*)	10.31 (≤ 0.05)	17.68 (≤ 0.05)	21.3 (≤ 0.05)

[1/] x 1000 approximates per acre equivalents, means of 4 replication within a column in the same category not followed by the same letter are significantly different ($P \leq 0.05$).

[2/] df = 3, 9.

[3/] df = 1, 9.

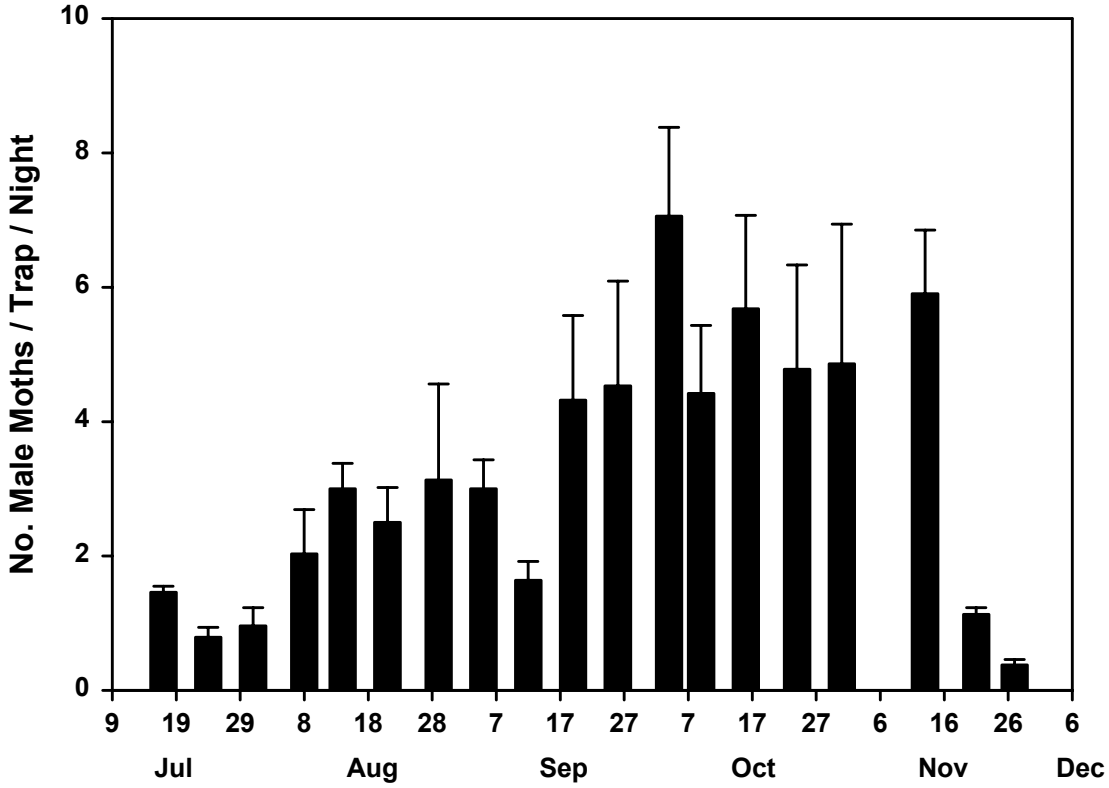

Figure 1. Mean numbers of pink bollworm male moths caught per gossyplure-baited trap per night in a 2.5 acre field of NuCOTN 33B and Deltapine 5415 cottons.