LARVAL MORTALITY OF PINK BOLLWORM AND OTHER LEPIDOPTEROUS PESTS ON NUCOTN 33B AND DELTAPINE 5415 COTTONS

T. J. Henneberry, L. Forlow Jech and T. de la Torre
USDA-ARS, Western Cotton Research Laboratory
Phoenix, AZ

Abstract

Studies were conducted in Phoenix, AZ cotton plots to evaluate the efficacy of NuCOTN 33B® (Bt) (Monsanto Co., St. Louis, MO) for pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), control in the field and to define the relationship between numbers of days of PBW larval feeding on bolls and mortality. We also determined, in the laboratory, larval mortality of cabbage looper, *Trichoplusia ni* (Hübner), beet armyworm, *Spodoptera exigua* (Hübner), and tobacco budworm, *Heliothis virescens* (F.) feeding on Bt cotton.

For PBW, over 80 and 2 percent of DPL 5415 and Bt cotton bolls, respectively, were found with live PBW larvae after artificial egg infestations. Less than 0.02 of the live larvae per Bt boll developed to the last instar compared to 2.7 larvae per boll developing to the last instar for DPL 5415 bolls. In another experiment, no first instar PBW survived after feeding for more than 48 h on Bt bolls compared to an average of 1.5 larvae per boll surviving in DPL 5415 for feeding periods of 8 days.

Tobacco budworm larvae were highly susceptible to Bt cotton; none survived greater than 3-day feeding periods on Bt cotton leaves. Beet armyworm and cabbage looper were less susceptible with 84, 68 and 47% of the beet armyworm and 100, 38, and 0% of the cabbage looper larvae, respectively, surviving 3, 6, or 9 days feeding on Bt leaves.

Introduction

The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) has been an economic pest of cotton, *Gossypium hirsutum* L. in AZ and southern CA for over 35 years. Severe economic losses due to reduced yields, low lint quality, and increased costs of insecticides have occurred (Watson and Fullerton 1969, Burrows et al. 1982, Gonzales 1990). Control costs alone have been estimated to exceed 1.2 billion dollars (Roberson et al. 1998). Commercial plantings of transgenic cotton carrying the Bollgard® gene have been highly resistant to PBW infestations (Flint and Parks 1999, Simmons et al. 1998, Patin et al. 1999, Flint et al. 1995, Flint and Parks 1995, Watson 1995, Ellsworth et al. 1995a,b, 1996). Henneberry et al. 2000 found that Bt cotton bolls developing on plants 180 days after planting (DAP) were as toxic to PBW larvae as bolls developing on plants 83 DAP. Greenplate et al. (1998) found that the toxic Cry1Ac protein in different cotton fruiting structures declined with increasing DAP. Our results suggest a high degree of susceptibility for PBW larvae to immature bolls over the entire growing season irrespective of the suggested reduction in the amount of toxic protein in fruiting form tissues late in the season. High percentages (60-75%) of the total upland cotton production acreages in the growing seasons of 1997 through 1999 have resulted in dramatic decreases in the use of insecticides on cotton in AZ (Simmons et al. 1998). Carrière et al. (2000) reported Arizona pesticide use analysis by W. Sherman and K. Agnew (USDA, Arizona Agricultural Statistics) and P. B. Baker (Arizona Pesticide Information Training Office) that indicated 2.2 insecticide applications per cotton acre in 1999 as opposed to 6.3 in 1995. Fewer applications for PBW control were a major factor in the reduced insecticide use pattern.

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In addition to PBWs, Wilson et al. (1992), Watson (1995), and Flint et al. (1995) found early experimental Bt lines and the advanced Bt breeding lines highly resistant to cotton leafperforator, *Bucculatrix thurberiella* Busck, saltmarsh caterpillar, *Estigmene acrea* (Drury), beet armyworm, *Spodoptera exigua* (Hübner), and cabbage looper, *Trichoplusia ni* (Hübner), and tobacco budworm, *Heliothis virescens* (F.). Flint et al. (1996) reported an 87% reduction in leaf feeding damage by cotton leafperforator on the commercial cultivar NuCOTN 33 (Bt). Economic infestations of these species, except for PBW, in Arizona have been sporadic in recent years and extensive evaluations of Bt effectiveness in the field for their control has not been possible.

The objectives of our current study were to determine PBW larval survival following high density artificially induced egg infestations on DPL 5415 and Bt cotton bolls and to determine the relationship between number of days of feeding on Bt cotton and larval mortality. We also determined the effect of Bt cotton on cabbage looper, beet armyworm and tobacco budworm larval mortality after feeding on Deltapine 5415 and Bt cotton leaves in the laboratory.

Methods and Materials

DPL 5415 and Bt cotton seeds were planted in 16 rows wide by 19 m (60 feet) long plots at the Western Cotton Research Laboratory at Phoenix, AZ. Plots were arranged in completely random design with four observations per treatment. No insecticides were applied. Cotton bolls, 21 days old, were infested with PBW eggs by placing 2.54 cm^2 pieces of paper towels from oviposition cages under the bracts of bolls. PBWs used for infesting cotton bolls in the field and laboratory were from a colony reared on artificial diet at the Western Cotton Research Laboratory (Bartlett and Wolf 1985). Bolls were picked one week after egg infestation and placed in screen-ventilated plastic incubation boxes (Fye 1976). After three weeks in the laboratory at 26 to 27° C all larvae that had exited from the bolls and found as larvae, pupae or adults in the boxes were recorded. Also, with the aid of a microscope each boll was examined and 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. Percentages of infested bolls were calculated using living larvae, pupae and exit holes in the bolls.

In the laboratory, an experiment was conducted to compare successful larval entry into DPL 5415 compared with Bt cotton bolls and numbers of surviving larvae following feeding for 2, 4, 6, or 8 days after egg infestation. Bt or DPL 5415 seeds were placed in Jiffy Pots (Jiffy Products of America, Inc., Batavia, IL) or plastic flower pots filled with potting soil at about one week intervals to assure a continuous supply. Seedlings, after thinning to one per pot, were transplanted to 0.31 m diameter x 0.47 m tall flower pots when plants were in the four to six leaf stage of plant development. Flowers were tagged as they occurred on the plants and three weeks following each flower tagging (21 day old bolls) bolls were harvested and placed individually in 5 cm diameter x 7.5 cm tall polyethylene containers. Bolls were infested with PBW eggs as previously described. Each boll was examined with the aid of a microscope and all PBW larval entrance holes in the carpel walls were counted. The bolls were dissected on days 2, 4, 6, 8 or 10 days after infestation to determine days of feeding before death.

Cabbage looper, beet armyworm, tobacco budworm larvae of each species were from the Western Cotton Research Laboratory colonies reared on artificial diet (Henneberry and Kishaba 1966). Leaves from field grown DPL 5415 and Bt cotton plants were picked and trimmed to fit in 15.0 cm diameter x 1.5 cm deep petri dishes bottom-lined with a moist filter paper. Five first instar larvae, in each case, of the cabbage looper, beet armyworm, or tobacco budworm were placed in each of ten petri dishes with leaves of DPL 5415 or Bt cotton. Dishes were checked daily, dead larvae were recorded and new leaves added as needed to sustain larvae.

Results

Infesting 21 day bolls of field grown cotton with PBW eggs from our laboratory colony resulted in seasonal averages of 14.4 and 19.6 entrance holes per DPL 5415 and Bt boll, respectively (Table 1). Eighty-one and two percent of the DPL 5415 and Bt bolls, respectively, were infested with live PBW larvae. Of the live larvae, 2.7 and <0.02 for DPL 5415 and Bt, respectively, developed to the last instar and pupated.

In the laboratory, numbers of PBW larvae surviving after feeding for 2, 4, 6, or 8 days on DPL 5415 bolls were 2.1, 0.4, 1.0, and 1.5 and 0.6, 0.0, 0.0, and 0.0 after feeding for the same periods of time on Bt bolls (Table 3). The high mortality of larvae within 2 to 4 days of egg hatch precludes the opportunity for significant amounts of injury to bolls and subsequent lint loss. (Fry et al. 1978).

In the case of other lepidopterous larvae, on average, 100, 38 and 0% of the cabbage looper larvae and 84, 68, and 47% of the beet armyworm larvae survived on days 3, 6 and 9, respectively, feeding on Bt cotton leaves (Table 3). For tobacco budworm, 38% of the larvae survived 3 days of feeding on Bt cotton leaves and none survived, after day 6 or 9. Survival of beet armyworm and tobacco budworm larvae on DPL 5415 cotton leaves, also decreased with increasing days of feeding on DPL 5415 leaves. For cabbage looper and beet armyworm larvae, none of the surviving larvae on day 6 and 9 developed beyond the second instar, whereas larvae of each species feeding on DPL 5415 leaves were in the late third or early fourth instar of development. These studies are continuing in efforts to quantify the leaf areas of each cultivar consumed and further evaluate larval development of each species feeding on Bt and DPL 5415 cottons.

Discussion

Bt cotton technology has provided a major breakthrough for protection against PBW infestation. Overall, insecticide use for PBW in Arizona has been reduced dramatically (Simmons et al. 1998, Carrière et al., in review). Concern has been expressed about the PBW potential for resistance development to Bt (Gould and Tabashnik 1998) based on laboratory selections (Bartlett 1995, Patin et al. 1999). Under field conditions, PBW infestations in commercial Bt cotton in Arizona have been <0.1% for the growing seasons of 1996 through 1999. Our results here agree with other reports of the high toxicity of Bt cotton to PBW (Watson 1995, Flint and Parks 1999, Simmons et al. 1998, Patin et al. 1999). It appears that PBW remains highly susceptible to the Cry1Ac toxic protein and the imminent introduction of cottons with a second Cry toxic protein shows promise of delaying or preventing resistance development (Greenplate et al. 2000). Nonetheless, a continued monitoring of Bt efficacy in commercial fields and Bt resistance management development is essential to assure long-term viability of the Bt technology in cotton IPM systems for PBW control.

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Table 1. Seasonal mean numbers of pink bollworm entrance holes, percentages of bolls infested and numbers of last instar larvae in Deltapine 5415 and Bt cottons.

Cultivars	No. Sampling Dates[a]	Entrance Holes Range	Entrance Holes $\bar{x}$	% Bolls Infested[b]	No. Last Instar Larvae
DPL 5415	7	3.8-20.0	14.4	81.0	2.70
Bt	7	6.3-36.3	19.6	2.0	<0.02

[a] July 20 to October 25, total of 615 and 580 DPL 5415 and Bt cotton bolls, respectively.
[b] Presence of live larvae, pupae, or exit holes.

Table 2. Mean (± SE) numbers of pink bollworm larvae (hatching eggs) placed on DPL 5415 and Bt cotton bolls and numbers of larvae surviving per boll after feeding for 2, 4, 6, or 8 days.

Cultivars	Ave. No. Hatched Eggs/Infested Boll[a]	$\bar{x}$ Numbers[a] of Surviving Larvae Per Boll After Feeding for (days)			
		2	4	6	8
DPL 5415	12.2 ± 0.6	2.1 ± 0.2	0.4 ± < 0.1	1.0 ± 0.1	1.5 ± 0.2
Bt	12.9 ± 0.5	0.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] Means of 6-22 observations.

Table 3. Mean numbers (± SE) of cabbage looper, beet armyworm, pink bollworm or tobacco budworm larvae surviving per replication on days 0, 3, 6, and 9 following release on DPL 5415 or Bt cotton leaves in the laboratory.

Days after infestation (No.)/ Cultivars	Species		
	Cabbage loopers	Beet armyworm	Tobacco budworm
0[a]			
(Controls)			
DPL 5415	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Bt	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
3			
DPL 5415	5.0 ± 0.0	4.8 ± 0.1	4.0 ± 0.3
Bt	5.0 ± 0.0	4.2 ± 0.3	1.9 ± 0.2
6			
DPL 5415	4.9 ± 0.1	4.0 ± 0.3	3.0 ± 0.5
Bt	1.9 ± 0.5	3.4 ± 0.5	0.0 ± 0.0
9			
DPL 5415	4.2 ± 0.2	2.7 ± 0.6	2.1 ± 0.0
Bt	0.0 ± 0.0	2.1 ± 0.5	0.0 ± 0.0

[a] Five first instar larvae 10 replications, 50 of each species, in each case, released on DPL 5415 or Bt leaves picked from field plots.