ARTIFICIAL PINK BOLLWORM EGG INFESTATIONS AND LARVAL SURVIVAL IN NuCOTN 33b AND DELTAPINE COTTON CULTIVARS IN ARIZONA

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

Abstract

NuCOTN 33b (Bt cotton) (Monsanto Company, St Louis, MO) carrying the gene for the *Bacillus thuringiensis var. kurstaki* (Berliner) insect toxic protein is a new advance in technology for pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), control. To investigate grower concern for reduced efficacy in late-season because of breakdown or non-expression of the toxic protein, we compared the susceptibility of Bt and Deltapine 5415 (Monsanto Company, St Louis, MO) (non-Bt) cotton bolls to PBW at periodic intervals during the first and second cotton fruiting cycles. We placed >200 PBW eggs per boll on the inside surface of a bract of susceptible immature cotton bolls. The artificially infested bolls were later harvested and examined for evidence of PBW infestation. High percentages of both Bt and non-Bt cotton bolls had numerous larval entrance holes in the carpel walls of the bolls. Less than 1% of the Bt cotton bolls and over 70% of the non-Bt cotton bolls were found with living PBW larvae. Bt cotton bolls of the late-season second fruiting cycle were as resistant to PBW infestation as Bt cotton bolls of the first fruiting cycle.

Introduction

Early field research with cotton breeding lines carrying the gene regulating production of the insecticidal toxin associated with *Bacillus thuringiensis var. kurstaki* (Berliner) indicated a high degree of efficacy against pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) larvae (Wilson et al. 1992). These results were confirmed with more advanced breeding lines (Flint et al. 1995, Watson 1995). Additional genetic refinement resulted in the development of an agronomically acceptable Bt cotton which was first commercially produced for lint in Arizona in 1996 (Flint and Parks 1999). Significant acreages of Bt cotton were also grown in Arizona as early as 1994 for seed production (Ellsworth, personal comm.). Growers have readily accepted the new technology and 60 to 70% of the cotton acreage in Arizona was planted to Bt cotton in 1997 and 1998, respectively (Patin et al. 1999). The potential for development of PBW resistance to Bt cotton has been recognized (Bartlett 1995, Watson 1995) and intensive resistance management and monitoring programs developed (Watson 1995, Simmons et al. 1998, Patin et al. 1999). Additional concern has been expressed regarding the temporal distribution and level of the insect toxic protein in plants over the growing season. Ellsworth et al. (1995a) cautioned against late-season exposure of Bt cotton to PBW and other pests because of the risk of breakdown or lack of expression of the toxic protein in senescent cotton. Greenplate et al. (1998) reported that the CryIAc Bt toxic protein decreased in fruiting forms from 10 to 15 µg/g (fresh weight, fw) at 40 days after planting (DAP) to 1 to 2 µg/g fw at 120 DAP. Levels of the toxin in foliage were 20 and 5 µg/g fw at 40 and 120 DAP, respectively. Evaluations of experimentally grown Bt cotton in 1994 and 1995 and of commercially grown Bt cotton in 1995, 1996, and 1997 did not reveal significant differences in PBW susceptibility to cotton bolls associated with advancing stages of plant growth and crop maturity (Ellsworth et al. 1995a and b, 1996, Flint and Parks 1999).

The objectives of our studies in 1999 were to further evaluate the susceptibility to PBW of Bt (NuCOTN 33b) and non-Bt (Deltapine 5415) cotton bolls developing on fruiting branches at increasingly higher nodes on the cotton plant main stem reflecting increasing levels of phenological plant development.

Materials and Methods

Parental Deltapine 5415 and its transformed Bt cotton, Bollgard NuCOTN 33b (Delta and Pine Land Co., Scott, MS), cotton plots were 16 rows wide by 60 feet long and were grown at the Western Cotton Research Laboratory, Phoenix, AZ. Plots were arranged in a split-plot design with four replications.

Beginning on 7 July 1999 and weekly thereafter until 11 August 1999 (end of the first fruiting cycle, plant nodes 8 to 20), 30 flowers were tagged in each plot. Three weeks following each flower tagging (21 day old bolls), we stapled 2.54 cm^2 pieces of paper oviposition substrate (each piece with more than 200 PBW eggs) to a bract of each boll that developed from a tagged flower. We also artificially infested 21 to 28 day old immature green cotton bolls with PBW eggs on 28 September and 25 October (second fruiting cycle, plant node numbers >25). All PBW eggs and larvae used for artificially infesting cotton bolls were from a laboratory colony maintained at the Western Cotton Research Laboratory and reared on artificial diet (Bartlett and Wolf 1985). Bolls artificially infested with PBW eggs were picked one week later. The picked bolls were placed in screen-ventilated plastic sweater boxes (Fye 1976) and held in the laboratory (≈ 26 to 27°C) for three weeks. All larvae exited from the bolls and found as larvae, pupae or adults in the

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boxes were recorded. Also, each boll was examined with the aid of a microscope and all PBW 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.

Results and Discussion

Cotton flowering decreased dramatically during the week of 9 August 1999 signaling the end of the first fruiting cycle. Entrance holes in bolls from first instar PBW larvae that hatched from eggs artificially attached to bracts of Bt and DPL 5415 cottons occurred on 80 to 100 percent of the Bt bolls examined (Fig. 1A) and 65 to 100% of the DPL 5415 bolls examined.

Percentages of Bt cotton bolls with live larvae were 0, 3.3, 0.8, 1.3, 0, 0, and 0 for bolls infested with PBW eggs on 20 July, 4 August, 18 August, 25 August, 1 and 28 September, and 25 October, respectively. Percentages of DPL 5415 cotton bolls with live PBW larvae were 36.0, 80.1, 84.6, 92.6, 65.6, 81.4, and 65.0, respectively for the same sampling dates (Fig. 1B).

Thus, under extreme, artificially induced, PBW infestation pressure, Bt cotton bolls during the entire first fruiting cycle and on two late-season sampling dates during the second fruiting cycle exhibited a high degree (<1% infested bolls) of PBW resistance (near immunity) compared with non-Bt cotton (>70%). The few live PBW (6 pupae, 1 adult, 1 first instar larva) found in Bt cotton could have occurred in bolls from plants originating from non-Bt cotton seed that occurs as a contaminant in Bt seed lots (Flint and Parks 1999). It is likely that reduced concentrations of the Bt toxic protein occurred in late-season bolls in our studies as has been already shown (Ellsworth 1995a, Greenplate et al. 1998). The first and last cotton bolls artificially infested with PBW eggs occurred on plants 83 and 180 days after planting, respectively. These numbers of days were about midpoint and 60 days longer than the CryIAc degradation from 10 to 15 µg/g fw to 1 to µg/g fw in cotton fruiting forms (Greenplate et al. 1998). We do not know the level of the Bt toxic protein occurring in the cotton bolls in our studies. However, the level expressed in bolls on plants as long as 180 days after planting was lethal to PBW larvae.

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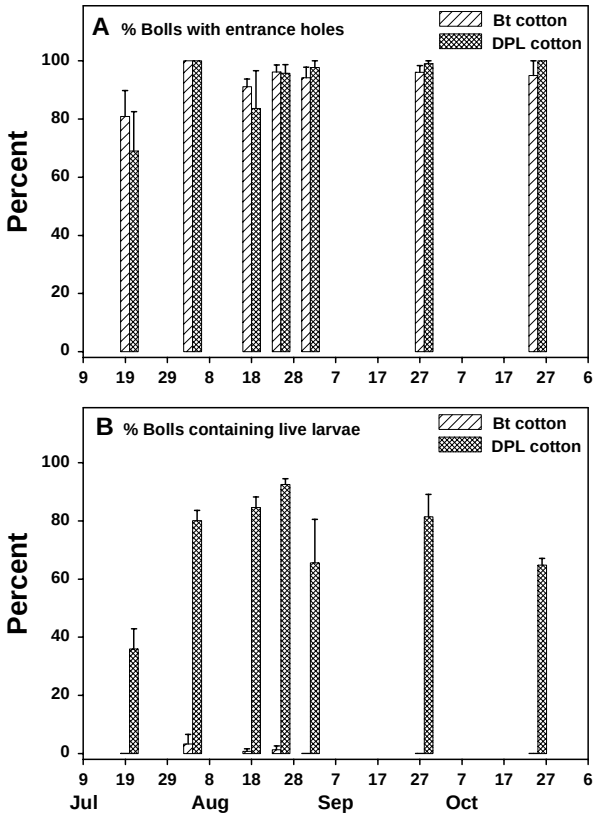

Figure 1. Mean (± S. E.) percentages of NuCOTN 33b and DPL 5415 cotton bolls with pink bollworm larval entrance holes (A) and bolls containing live larvae (B) following artificial infestations with >200 eggs per boll on each sampling date.