MORTALITY OF BOLLWORM AND TOBACCO BUDWORM LARVAE EXPOSED TO MICROBIAL AND CHEMICAL INSECTICIDES IN TREATED BT AND NON-BT COTTON ASSAYS

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Abstract

Laboratory colonies of bollworm (*Helicoverpa zea* (Boddie)) and tobacco budworm (*Heliothis virescens* (F.)) were exposed to microbial and chemical insecticides on non-Bt (DP1441) and Bt (DP1321) cotton leaves in spray-table and field-plot experiments. The microbial insecticides included commercial formulations of *Bacillus thuringiensis* (Dipel®) and *Heliothis* (*Helicoverpa*) nuclear polyhedrosis virus (Elcar®, Gemstar®, and Heligen®). A range of field application rates was evaluated with each microbial and chemical insecticide against neonate and 3rd instar larvae, and residual activity was measured in a small plot field experiment. Trends in activity provided quantitative assessments of insecticidal and Bt cotton activity against the susceptible laboratory strains of insects studied. These data may be useful as future benchmark comparisons for resistance management and strategic optimization of the need to spray non-Bt and Bt cotton.

Introduction

Bt cotton expressing insecticidal toxins derived from *Bacillus thuringiensis* is widely adopted across the U.S. Cotton Belt, especially in areas of the southern U.S. where populations of insecticide-resistant tobacco budworm (*Heliothis virescens*) previously limited profitable production (Luttrell and Jackson 2012, Luttrell et al. 2015). Bollworm (*Helicoverpa zea*) is less susceptible to the toxins expressed in Bt cotton and supplemental sprays of insecticide are often applied to Bt cotton for control of bollworm. This results in additional costs, and it negates the positive ecological benefits potentially afforded by unsprayed Bt cotton (Luttrell et al. 2015).

The threat of insect resistance to Bt cotton has been an ongoing major issue for the cotton industry, biotech firms marketing the transgenic traits, and regulatory agencies since commercial deployment of Bollgard® cotton in 1996. We have been involved with studies to benchmark and monitor susceptibility of *H. virescens* and *H. zea* to the Bt toxins expressed in Bt cotton in laboratory assays of field-collected insects (Ali et al. 2004, 2005, 2006, 2007, 2009, Luttrell and Ali 2009), and we have more recently initiated field studies to evaluate the economic and environmental costs of over-spraying Bt cotton (Allen et al. 2012, 2013, 2014, Jackson et al. 2012, Little et al. 2014, Luttrell et al. 2012a, 2012b).

Current Bt cottons express multiple toxins, and the availability of purified or representative sources of toxin for insect assays is more restricted than that previously observed when we were conducting assays for insect susceptibility to the single-toxin Bt cottons. To address the need for a publicly-available method for monitoring insect susceptibility and to encourage quantitative comparisons of various insect management options on Bt and non-Bt cotton, the USDA ARS Southern Insect Management Research Unit (SIMRU) initiated research to measure *H. zea* and *H. virescens* response to different toxin sources. Reported here are experiments conducted in 2015 to measure the activity of microbial and chemical insecticides on non-Bt and Bt cotton leaves. This work was conducted with laboratory insects, which are known to be susceptible to Bt toxins. Additional research will be needed to characterize variability in field insects and relate results of the plant-based assays to previously-published studies of industry-provided insecticidal protein in diet-based assays.

Methods

All assays were conducted with laboratory colonies of *H. zea* and *H. virescens* maintained at the USDA ARS SIMRU facility in Stoneville, MS. These insects have been in laboratory culture for more than ten years and are considered to be susceptible to insecticides and Bt toxins. Leaf tissue from the upper three to five nodes of non-Bt (DP1441) and Bt (DP1321) cotton was obtained from field plots grown specifically for laboratory assays and isolated from insecticide sprays. Multiple planting dates were used to provide succulent cotton for the assays over the growing season. Crop phenological development ranged from first-flower to peak-flower, and assays were conducted from June through September. Two experiments included a range of application rates of Dipel® (*B. thuringiensis*), Gemstar® (*Heliothis* NPV), Karate Z® (lambda-cyhalothrin) and Prevathon® (chlorantraniliprole) applied to Bt and non-Bt cotton leaves and infested with neonate and 3rd instar bollworms. The application rates varied 27-fold with the upper rate at least as high as the label recommended rate (higher with the microbials). A third experiment was conducted to compare activity of different commercial products of *Heliothis* nuclear polyhedrosis virus to that of Dipel and Karate on non-Bt cotton.

All treatments were replicated four times on different dates with 15 larvae per replicate. Spray-table and field applications were made with a TX-6 nozzle calibrated to deliver 6 gpa. Observations were made at different time intervals post exposure of larvae to the treated leaves, but surviving larvae were transferred to meridic diet after 5 days for subsequent observations. Resulting data on insect survival were studied via a factorial Analysis of Variance (AOV) in JMP10. Factors were insecticide treatments and type of plant tissue (Bt or non-Bt) for two studies, and insecticide treatment and species for the virus experiment.

Similar procedures were used for a field study designed to measure residual activity of Dipel, Gemstar, Karate, and Prevathon. Applications were made with a back-pack sprayer and leaves were collected at different time intervals over a 16-day period. All assays, experimental handling procedures and data analyses were the same as those for the spray-table assays.

Results and General Observations

Trends in insect mortality for *H. zea* and *H. virescens* exposed to the different microbial and chemical insecticides on Bt and non-Bt cotton are presented in Figure 1 (neonate *H. zea* exposed to Dipel, Gemstar, Karate, and Prevathon), Figure 2 (3rd instar *H. zea* exposed to Dipel, Gemstar, Karate, and Prevathon), and Figure 3 (neonate *H. zea* and *H. virescens* exposed to Dipel, Elcar, Gemstar, Heligen, and Karate). Residual activity of Dipel, Gemstar, Karate and Prevathon as measured by *H. zea* mortality on treated leaves at different times posttreatment is summarized in Figure 4.

In the study with neonate *H. zea* exposed to Dipel, Gemstar, Karate, and Prevathon (Figure 1), significant treatment by plant tissue interactions ($P<0.0001$) were detected at all posttreatment observations (5, 10, and 20 days). Although mean separation is not indicated on the figure, there was no survival of neonate *H. zea* exposed to any of the application rates of Karate or Prevathon, including those 1/27th that of the recommended high rate. Mortality of larvae on untreated Bt cotton at 5 days (68.3%) was less than that of larvae exposed to Karate or Prevathon on Bt and non-Bt cotton (100%) but greater than that of larvae exposed to non-Bt cotton treated with the lower two rates of Dipel (16.7 and 35%) and all rates of Gemstar except the 10 oz/a rate (48.3%). By pupation at 20 days posttreatment, mortality of larvae exposed to the highest rate of Gemstar on non-Bt cotton (98.3%) was comparable to that observed with Karate and Prevathon.

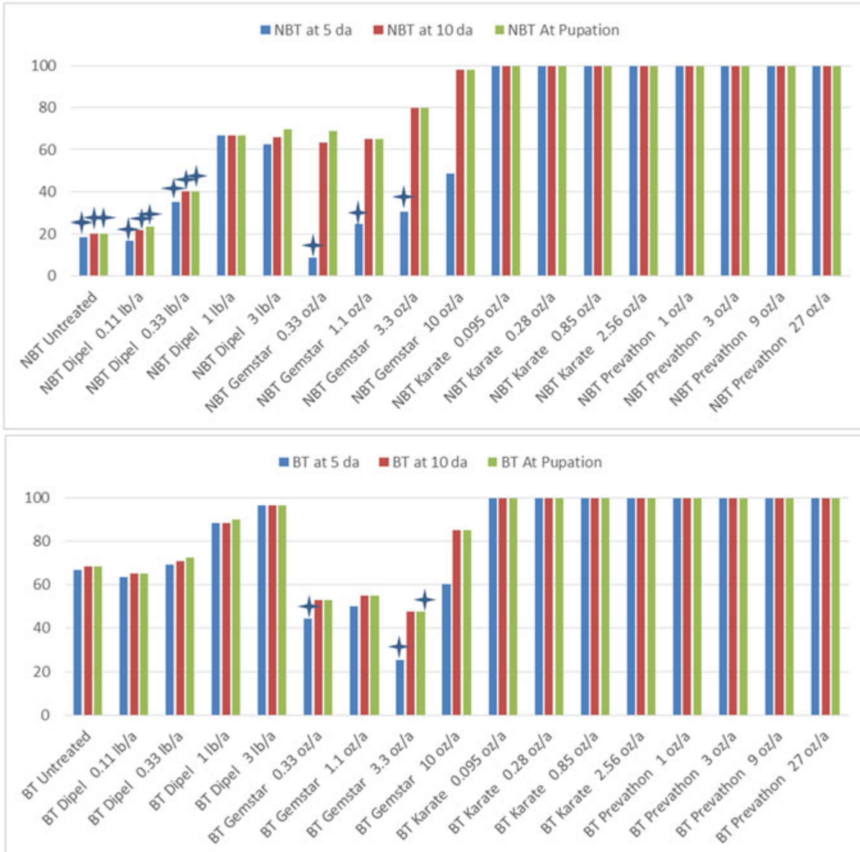

Figure 1. Mortality of neonate *H. zea* larvae exposed to microbial and chemical insecticides on non-Bt (NBT) and Bt (BT) cotton. ✦ indicates mortality significantly less than that observed for untreated Bt cotton.

With 3rd instar *H. zea* exposed to Dipel, Gemstar, Karate, and Prevathon (Figure 2), all treatments on Bt cotton at 5 days posttreatment, including untreated Bt cotton, had higher mortality (75 to 100%) than that observed for the untreated non-Bt cotton (21%). All treatments applied to non-Bt cotton with the exception of the two lower rates of Gemstar (0.11 and 1.1 oz/acre) had higher mortality (50 to 100%) than that observed for insects on untreated non-Bt cotton. All treatments had greater cumulative mortality at pupation (20 days posttreatment) greater than that observed for untreated non-Bt cotton (31%). Treatments with less cumulative mortality at pupation than that observed for untreated Bt cotton were non-Bt cotton treated with the two lower rates of Gemstar (0.33 and 1.0 oz/acre) applied to Bt cotton. All treatments applied to Bt cotton, except the three lower rates of Dipel (0.11, 0.33 and 1.0 lb/acre), had cumulative mortality at 20 days posttreatment equal to Karate and Prevathon treatments and greater than that of untreated Bt cotton (83.3%).

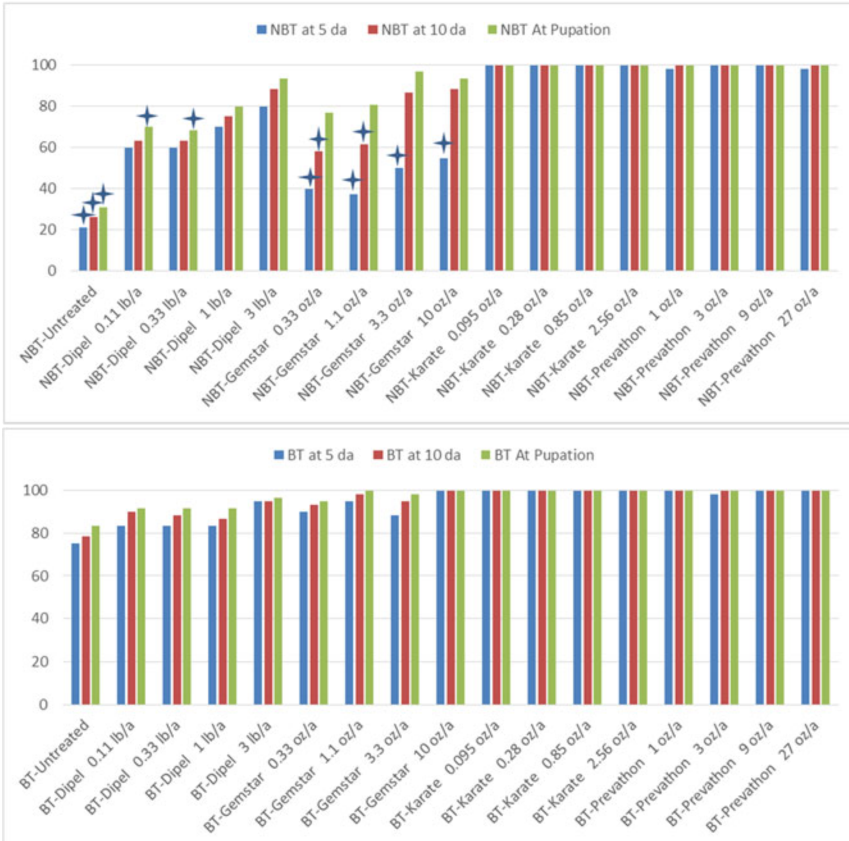

Figure 2. Mortality of 3rd instar *H. zea* larvae exposed to microbial and chemical insecticides on non-Bt (NBT) and Bt (BT) cotton. ✦ indicates mortality significantly less than that observed for untreated Bt cotton.

In the study comparing activity of Dipel and Karate against neonate *H. zea* and *H. virescens* to different commercial formulations of *Heliothis* nuclear polyhedrosis virus on non-Bt cotton (Figure 3), there were no significant interactions between treatments and species. At the 5 day posttreatment observation, treatment ($p<0.0001$) and species ($P=0.0251$) effects were significant. At all other observation times, only treatment effects were significant ($P<0.0001$). With *H. zea* observations at 5 days, treatments with higher mortality than untreated non-Bt cotton included all three rates of Dipel (0.11, 0.33 and 1 lb/acre), the two higher rates of Gemstar (3.3 and 10 oz/acre), the highest rate of Heligen (2 oz/acre), and both rates of Karate (0.00256 and 0.0256 oz/acre) which were 1/1000th and 1/100th of the recommended rate. With *H. virescens* observations at 5 days posttreatment, the three rates of Dipel tested (0.11, 0.22, and 1 lb/acre) and the highest rates of Karate tested (0.256 oz/acre or 1/10th the recommended rate) had more mortality than the untreated non-Bt cotton. By 14 days posttreatment, all treatments except the lowest rate of Elcar (2.2 gm/acre) had higher cumulative mortality than the untreated non-Bt cotton infested with *H. zea*. Similar trends were observed with *H. virescens*, but few virus treatments were significantly different than that of *H. virescens* exposed to untreated non-Bt cotton (36.1% mortality).

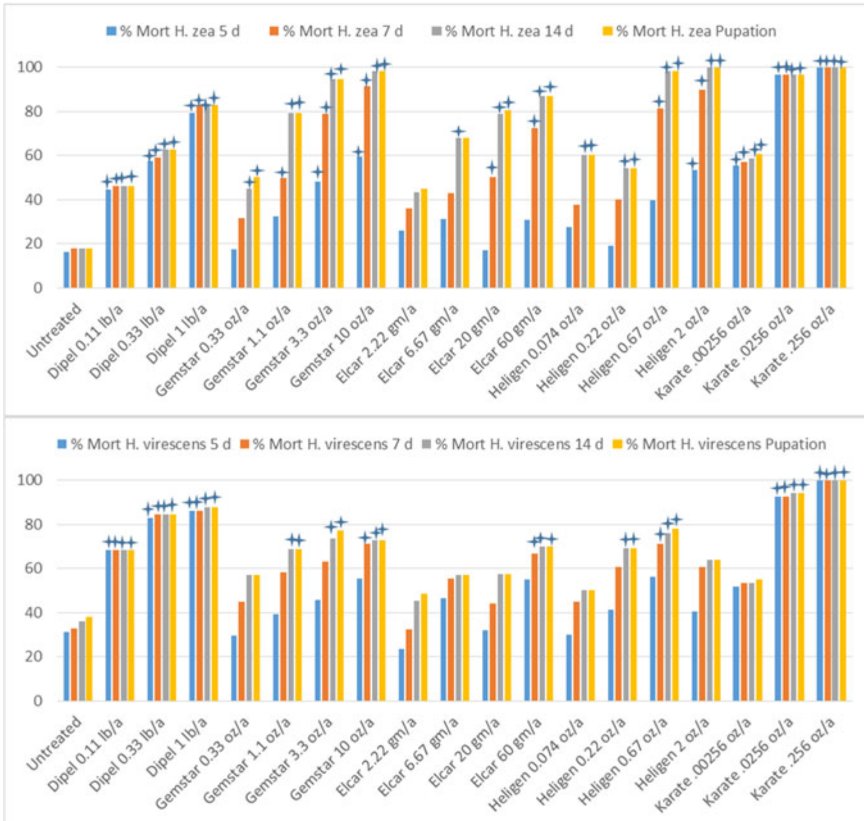

Figure 3. Mortality of neonate *H. zea* and *H. virescens* exposed to microbial insecticides (Dipel, Gemstar, Elcar, and Heligen) and compared to a pyrethroid (Karate) insecticide on non-Bt cotton. indicates significantly higher mortality than that observed for untreated non-Bt.

The recommended rate of Prevathon (27 oz/acre) showed no reduction in residual activity on cotton over the 16-day test period. The reduced rate of Prevathon (0.27 oz/acre) 1/100th the recommended rate showed a significant reduction at the 16-day observation. The full rate of Karate (2.56 oz/acre) showed reduced activity at the 16-day observation, and the reduced 1/100th recommended rate (0.0256 oz/acre) showed reduced activity at 1-day posttreatment. The Dipel (1 lb/acre) and Gemstar (10 oz/acre) treatments had reduced residual activity after 1-day of exposure to the field environment (Figure 4).

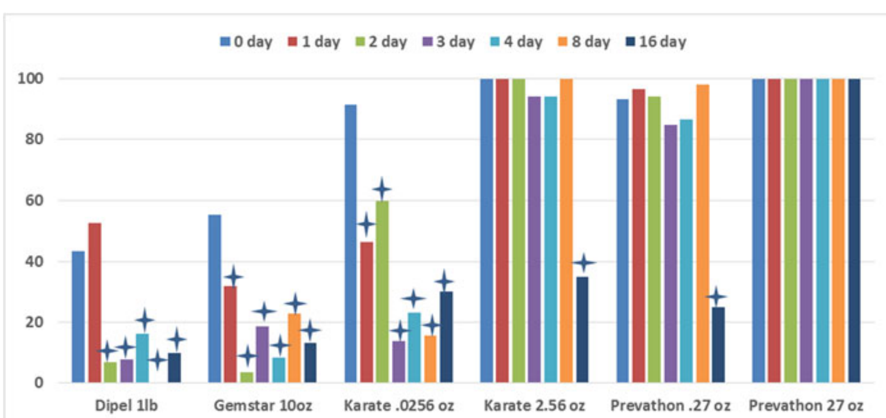

Figure 4. Mortality of neonate *H. zea* exposed for 7 days to leaves collected from non-Bt cotton plots treated with Dipel, Gemstar, Karate and Prevathon 0, 1, 2, 3, 4, 8, and 16 days posttreatment. indicates significantly reduced mortality over that observed at 0 hr posttreatment.

Collectively, these data, especially the significant mortality at reduced application rates, illustrate the high insecticidal activity of Karate, Prevathon and Bt cotton against laboratory-susceptible strains of *H. zea* and *H. virescens*. Additional studies with field-collected strains may provide different results and possible indications of reduced susceptibility in field populations. Plant-based assays may also be an option for tracking resistance evolution to multiple Bt insecticidal traits expressed in the newer Bt transgenic technologies. Critical comparisons of treatment effects at short periods posttreatment provide insight into insect control and plant protection capacity of different treatments. Comparisons at pupation, or estimates of generation survival, provide insight into treatment effects on resistance selection. These types of quantitative estimates of insect mortality may be useful in estimating the value of various insect management options and the threat of future resistance problems. We used similar procedures to document pyrethroid resistance in *H. virescens* (Luttrell et al. 1987), explore strategies for deploying pyrethroids against pyrethroid-resistant insects (Luttrell et al. 1991), and quantitatively estimate expected field-mortality from commercial formulations of *Bacillus thuringiensis* (Luttrell et al. 1998).

Pesticide Disclaimer

This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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