ACTIVITY OF DIFFERENT FORMULATIONS OF *BACILLUS THURINGIENSIS* ON LEPIDOPTERA IN COTTON

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Abstract

A series of laboratory and field experiments were initiated in 1995 to determine if laboratory assays with different *Bacillus thuringiensis* (Bt) commercial products accurately predict field efficacy against tobacco budworm (*Heliothis virescens*) on cotton. Concentration-mortality studies were conducted with *H. virescens*, cotton bollworm (*Helicoverpa zea*), and beet armyworm (*Spodoptera exigua*)in diet incorporation, leaf disc, and terminal assays with 11 different commercial Bt's. Results were compared to field studies when 5 rates of each Bt were tested against high population densities of *H. virescens* and *H. zea*. Data from the field studies failed to show differences in Bt products or rates. As a result, LC_{50}'s from the different assays were converted to *H. virescens* units, *H. zea* units, and *S. exigua* units and compared to a spray table study using terminals with *H. zea*. When data were converted to *H. zea* units most of the assay procedures correlated with the spray table data. This illustrates the importance of establishing Bt potency and the importance of using the target species as the assay orginism. With cotton this species should be *H. virescens*, but more data is required.

Introduction

Bacillus thuringiensis (Bt) is an efficacious microbial insecticide for control-ling insect pests on several crops. However, the activity of different isolates containing different endotoxin proteins varies with the target pest species (Bell and Romine 1980) and perhaps the crop itself. This variation in activity and the inherent variation in mass producing a biological insecticide complicates efforts to standardize Bt activity. This problem was studied extensively two decades ago by Howard Dulmage and his colleagues at the USDA/ARS laboratories in Weslaco, TX. Their research led to the development of a standard assay procedure for estimating potency (Dulmage 1973), establishment of a reference standard for comparing all other isolates (Dulmage 1973), and a general spirit of cooperation among public and private groups developing Bt's as commercial microbial insecticides.

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During the 1980's, Bt was regarded as having little or no value for cotton insect control because of the high efficacy of pyrethroid insecticides. Over the past few years cotton growers have observed increased problems controlling pest populations, particularly the tobacco budworm (*Heliothis virescens*), because of resistance to the pyrethroid insecticides. As a result, interest in alternative control methods, particularly the use of Bt, has dramatically increased. This interest has created a measurable commercial market for Bt insecticides on cotton. Several industry groups including established firms and new biotechnology firms have targeted this cotton market for new Bt insecticides. Although commercialization of Bt cotton threatens the size of the commercial market for foliar applied Bt on cotton, there is still significant interest in developing microbial insecticides for use on fields planted to non-Bt cotton.

Unfortunately, cooperative efforts during the 1970's to standardize Bt activity have not been continued in the 1990's. As a result, there are currently no uniformly accepted assay procedures or reference standards for estimating potency or activity of Bt isolates against specific pests. Some industry groups utilize assays based on mortality of the cabbage looper (*Trichoplusia ni*) and assign potencies in international units as developed by Dulmage et al. (1976). Others have adopted assay procedures based on mortality of the beet armyworm (*Spodoptera exigua*), and a few industry groups report activity as "total endotoxin protein" or "total lepidoptera active protein". All of these different procedures have advantages and limitations, but the lack of conformity among the procedures and the different industry groups creates confusion for pest managers. This confusion is further complicated by a general lack of knowledge about the relationships between laboratory assay procedures and expected activity against target pests in the field.

With this lack of information, cotton growers, agricultural consultants, and extension specialists have no real basis to compare different Bt products. Although the development of commercial Bt products that control a wide range of lepidopteran pests is a major goal of industry groups interested in marketing Bt on a wide range of crops, the most important use of Bt on cotton is for control of the tobacco budworm which is resistant to most traditional insecticides (Graves et al. 1994, Plapp and Campanhola 1986, Luttrell et al. 1987, Leonard et al. 1988, Elzen et al. 1994, Wall 1994, Kanga et al. 1994). Other lepidopteran pests are either difficult to control with endotoxin proteins or can be easily controlled with traditional insecticides. Tobacco budworm is also very susceptible to endotoxin proteins (Tanada 1956, Hall and Andres 1959, McEwan et al. 1960, Hall et al. 1961).

Considering the potential value of Bt for tobacco budworm control and the standardization problems described above, we initiated a project in 1995 to compare the relative

activity of a wide range of Bt isolates in different laboratory and field experiments. Relative results obtained in the different assay studies will eventually be compared to the results of field studies to determine: 1) if laboratory assays with different Bt's accurately predict field results, and 2) which assay procedure is the most accurate in predicting efficacy of Bt in field studies. The experiments will require several more years of study, but preliminary results are presented to encourage others to study the problem.

Materials and Methods

Several commercial formulations of Bt were chosen for use in these experiments. The formulations chosen for these experiments are products that are likely to be used by consultants and producers in Mississippi. The formulations chosen were: Dipel ESr, Dipel ES/NTr, Condorr, Condor XLr, Javelinr, SAN 420, Biocotr, Biocot XLr, MVPr, MVP IIr, and Designr. By comparing results from laboratory assays (diet incorporation, spray table with cotton terminals, and spray table with cotton leaf discs) to those obtained in field studies, projections of the field performance can be determined. Depending on the assay type, the test species used was *H. zea*, *H. virescens*, and/or *S. exigua*. For all liquid formulations of Bt the concentrations used were: 0.056, 0.167, 0.5, 1.5, and 4.5 pints/acre. For all dry formulations the concentrations were: 0.03, 0.01, 0.3, 1.0, and 3.0 pounds/acre. For use in the laboratory assays all Bt concentrations were reduced to serial dilutions equivalent to those used in the field study. There were a total of 57 treatments, including a pyrethroid treatment and an untreated check, in each replicate of the assays conducted. The same concentrations and formulations were used in all laboratory and field studies, and all treatments were included in every replicate to minimize experimental error. Results from laboratory assays were compared to those from the field study by correlation analyses. All experiments conducted during this research were arranged in a complete randomized block design.

Diet Incorporation Assay

The methods involved in the diet incorporation study were very similar to the standard methods of Dulmage et al. (1976). Large quantities of an artificial wheat-germ based diet (King et al. 1985) were obtained from the Gast Rearing Facility at Mississippi State University. The diet was allowed to cool to a temperature of approximately 45-50^{0}C. Following the cooling process, aliquots of the different formulations were incorporated into the diet with mini-blenders. The diet was then distributed into mylar trays containing 20 individual cells and allowed to solidify. Each cell was then infested with a single neonate larva and sealed with mylar. The larvae and cell trays were transported to a large walk-in environmental chamber and held for 7 days. At 7 days, mortality was determined by counting the number of cells still infested with live or dead larva. A total of 20 larvae were exposed to each replicate of each concentration. This study was replicated 4 times or until sufficient data were obtained to calculate dose-mortality regressions. Regressions were calculated by probit analysis using the POLO computer program.

Spray Table with Cotton Terminal Assay

All terminal spray table studies were conducted by treating cotton terminals (ca. the upper 30 cm of the plant) collected from an untreated cotton field in a computerized-spray chamber (To et al. 1995). Treatments were applied in the spray chamber equipped with a single TX-4 nozzle containing a 50-mesh screen. The total spray volume for each treatment application was 5 gallons per acre. The terminals to be infested were placed in water pics (Luttrell et al. 1987) and held on racks of 10 pics per rack under the center path of the nozzle. Following treatment application, the spray residues were allowed to dry and each terminal was infested with one neonate *H. zea* larva. The infested terminals were covered with ventilated cups and placed in a large temperature-controlled holding room for 2 days. After 2 days of exposure, mortality was determined by observing each terminal for the surviving larva. Mortality was recorded and surviving larva were transferred to artificial wheat-germ based diet (King et al. 1985) for subsequent observation at 7 and 14 days posttreatment. All treatments included 20 larvae and were replicated 4 times with a single replicate being treated on a given day. Data were corrected for missing larvae in the check and percentage data were transformed to arcsin prior to analysis. Data were studied by analysis of variance and means were separated by Student Newman Kuel's (SNK) test at an error rate of P=0.05.

Spray Table with Cotton Leaf Disc Assay

All leaf disc studies were conducted by treating cotton leaf discs in a computerized-spray chamber (To et al. 1995). Initially, leaves were collected from the upper canopy of untreated cotton plants and leaf discs (circle 1" in diameter) were excised from the leaves in the laboratory using a specially designed leaf punch. Treatments, application procedures, spray volumes, and data analyses were essentially the same as those described in the spray table study involving cotton terminals. The leaf disc assays were conducted by planting 5 neonate larvae on each excised leaf disc held in a 1" diameter petri dish. To prevent dessication of the leaf disc a moistened filter paper disc was placed between the leaf tissue and the petri dish. Following treatment appli-cation, the spray residue was allowed to dry on the leaf disc. All the petri dishes containing treated leaf discs were held under the laboratory conditions and mortality observations described for the spray table study. Surviving larvae were transferred to diet at 2 days posttreatment and subsequent obser-vations were made at 7 days posttreatment. The study was replicated 4 times.

Field Study

All field studies were located in cotton fields at the Plant Science Research Farm, Mississippi State University. Plots

were a single row approximately 10 feet long. Within the plots, at least 20 plants containing dominant terminals were infested with 10 neonate *H. zea* and *H. virescens* larvae per plant using the bazooka technique as described by Davis et al. (1989) and Jenkins et al. (1982). After 24 hours of time to allow larval establishment, treatments were applied using a CO_2 powered back-pack sprayer equipped with 2 TX-6 nozzles per row. A spray volume of 8.9 gallons per acre and 50-mesh screens were used. Treatments were replicated 4 times in a randomized complete block design and observations were made 2 and 9 days posttreat-ment. Data analyses were essentially be the same as those described for the spray table study involving cotton terminals and leaf discs. All assay pro-cedures previously described were compared to the field study to determine which assay most accurately predicted field performance.

Results and Discussion

The original objective of this experiment was to determine which assay method most accurately predicted field results by correlating results obtained with each assay method to those obtained in a field test. The data obtained from the field test at 2 and 9 days posttreatment indicated that the percentage of terminals infested with *H. virescens* larvae and terminal damage (Table 1) were similar to those infested with *H. zea* (Table 2). At 2 days posttreatment the percentage of terminals infested with *H. virescens* and *H. zea* in plots treated with Bt insecticides were numerically higher than those in the plots treated with a pyrethroid but numerically less than those in the untreated check plots. The same is true for the percentage of terminals still infested at 9 days posttreatment for both species (Tables 1 and 2). Due to overwhelming natural infestations of *H. virescens* and *H. zea* larvae in the field, statistical differences among the treatments were not recorded for the field studies. Although the data show expected trends, differences between treatments were not detected and additional studies will be required (Tables 1 and 2).

As a result, we decided to use data collected at 7 days posttreatment for the cotton terminal assay with *H. zea* as a reference of field performance. Thus, the purpose of the test became determining how well the different assays predicted *H. zea* mortality on cotton. Although our primary interest was tobacco budworm, the variable field data were not adequate and spray table studies with tobacco budworm on terminals have not been conducted. Correlations were made by comparing all results of assay methods with each species expressed as LC_{50}'s to the LC_{50} of *H. zea* exposed to terminals treated with different formulations of Bt. Before comparing the diet incorpor-ation and leaf disc assays to the *H. zea* terminal assay, data were converted to product weight units, published billion international units (BIU), *H. zea*, *H. virescens*, and *S. exigua* units. The *H. virescens*, *H. zea*, and *S. exigua* units were calculated by standardizing all assays with results of the diet incorporation assay. For example, the LC_{50} of each Bt formulation in the diet incorporation assay was divided by the LC_{50} of all other assay methods.

When mortality of *H. zea* exposed to terminals treated with different formulations of Bt was compared to the other assay methods using product weight as the potency procedure the only assay that showed a statistically significant correlation was the leaf disc assay using *H. virescens* as the test species (Table 3). When BIU's were used the only statistically significant correlation was the diet incorporation assay using *H. virescens* as the test species (Table 3). When the data were converted to *H. zea* units, all assay methods, except those with *S. exigua*, showed statistically significant relationships to mortality of *H. zea* on terminals. (Table 4). This dramatic improvement illustrates the importance of developing a uniform assay method for product comparison and the importance of assaying the target species. When data were expressed in *H. virescens* and *S. exigua* units the only assay method that correlated with H. zea mortality on cotton was the leaf disc assay when *H. virescens* was used as the test species.

Preliminary data from this study indicate that current assay methods do not always correlate well with mortality expected from Bt in the field. These problems are further increased due to inconsistencies in the expression of potencies on current product labels and the relatively flat dose-mortality response of tobacco budworm to Bt's on cotton. There appears to be few differences among ratio, but the cost of different products and different rates are important considerations for growers. Based on these preliminary observations, standardization of Bt needs to be based on the targeted species. On cotton this species would be tobacco budworm, but we need more data to confirm these preliminary observations.

References Cited

Bell, M R. and C. L. Romine. 1980. Tobacco budworm field evaluation of microbial control in cotton using *Bacillus thuringiensis* and a nuclear polyhedrosis virus with a feeding adjuvant. J. Econ. Entomol. 73:427.

Davis, F. M., W. P. Williams, and B. R. Wiseman. 1989. Methods used to screen maize for and to determine mechanisms of resistance to the southwestern corn borer and fall armyworm. pp. 101-108. In Toward Insect Resistant Maize for the Third World; Proceeding of the Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. Mexico, D. F.: CIMMYT.

Dulmage, H. T. 1973. *B. thuringiensis* U.S. assay standard. Report of the adoption of a primary U.S. reference standard for assay of formulations containing the delta-endotoxin of *Bacillus thuringiensis*. Bull. Entomol. Soc. Am. 19:200-202.

Dulmage, H. T., A. J. Martinez, and T. Pena. 1976. Bioassay of *Bacillus thuringiensis* (Berliner) delta-endotoxin using the tobacco budworm. USDA Tech. Bull. No. 1528.

Elzen, G. W., S. H. Martin, B. R. Leonard, and J. B. Graves. 1994. Inheritance stability and reversion of insecticide resistance in tobacco budworm (Lepidoptera: Noctuidae) field populations. J. Econ. Entomol. 87:551-558.

Graves, J. B., B. R. Leonard, E. Burris, S. Micinski, S. H. Martin, C. A. White and J. L. Baldwin. 1994. Status of insecticide resistance in tobacco budworm and bollworm in louisiana. pp. 769-774. In D. J. Herber [ed.], Proceedings, 1994 Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

Hall, I. M. and Lloyd A. Andres. 1959. Field evaluation of commercially produced *Bacillus thuringiensis* (Berliner) use for control of lepidopterous larvae on crucifers. J. Econ. Entomol. 52:887-890.

Hall, I. M., R. L. Hale, H. H. Shorey, and K. Y. Awakawa. 1961. Evaluation of chemical and microbial materials for control of the cabbage looper. J. Econ. Entomol. 54:141-146.

Jenkins, L. N., W. L. Parrott, J. C. McCarty, Jr., and W. H. White. 1982. Breeding cotton for resistance to the tobacco budworm: techniques to achieve uniform field infestations. Crop Science 22:400-404.

Kanga, L. H. B., F. W. Plapp, Jr., M. L. Wall, G. W. Elzen and Juan Lopez. 1994. Resistance monitoring and mechanisms in the tobacco budworm to organophosphates, carbamates, and cyclodiene insecticides. pp. 810-815. In D. J. Herber [ed.], Proceedings, 1994 Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

King, E G., G. G. Hartley, D. F. Martin, and M. L. Laster. 1985. Large-scale rearing of a sterile backcross of the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 78:1166-1172.

Leonard, B. R., J. R. Graves, and A. M. Pavloff. 1988. Variation of resistance in field populations of tobacco budworm (Lepidoptera: Noctuidae) to selected insecticides. J. Econ. Entomol. 81:1521-1528.

Luttrell, R. G., R. J. Roush, A. Ali, J. S. Reid and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80:985-989.

McEwan, F. L., E. H. Glass, A. C. Davis, and C. M. Splittstoasser. 1960. Field tests with *Bacillus thuringiensis* (Berliner) for control of four lepidopterous pests. J. Insect Pathol. 2:152-164.

Plapp, F. W. and C. Campanhola. 1986. Synergism of pyrethroids by chlordimeform against susceptible and resistant *Heliothis*. pp. 167-169. In D. J. Herber [ed.], Proceedings, 1994 Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

Tanada, Y. 1956. Microbial control of some lepidopterous pests of crucifers. J. Econ. Entomol. 49:320-329.

To, S. D. Filip, D. B. Smith, R. G. Luttrell, and J. T. Reed. 1995. Development and evaluation of an automated spray chamber. J. Entomol. Sci. 30:130-139.

Wall, M. L. 1994. Status of Insecticide Resistance in Tobacco Budworm and Bollworm in Louisiana. pp. 775-777. In D. J. Herber [ed.], Proceedings, 1994 Beltwide Cotton Production Research Conference, National Cotton Council, Memphis, TN.

Table 1. Survival of *Heliothis virescens* larvae and damage per 20 terminals in cotton small plots at 2 and 9 days posttreatment.

	2 days posttreatment		9 days posttreatment	
Treatment	% terminals with larvae	% terminals with damage	% terminals with larvae	% terminals with damage
All Bt's combined	53.25	46.81	20.98	32.16
Pyrethroid	45.00	55.00	22.50	31.30
Untreated Check	63.10	36.90	28.80	30.60

Table 2, Survival of *Helicoverpa zea* larvae and damage per 20 terminals in cotton small plots at 2 and 9 days posttreatment.

	2 days posttreatment		9 days posttreatment	
Treatment	% terminals with larvae	% terminals with damage	% terminals with larvae	% terminals with damage
All Bt's combined	57.78	42.27	14.90	30.81
Pyrethroid	47.50	52.50	26.30	27.50
Untreated Check	81.30	18.80	34.40	27.50

Table 3. Correlation coefficients (r) describing relationships between different assay methods and mortality of *Helicoverpa zea* exposed to terminals treated with different formulations of *Bacillus thuringiensis*.

Assay method	Product weight	Published BIU
diet inc. - CBW	-.037	-.551
diet inc. - TBW	.142	-.852*
diet inc. - BAW	.099	-.516
leaf disc - CBW	.258	-.310
leaf disc - TBW	-.796*	0.55
leaf disc - BAW	-.100	-.253

Table 4. Correlation coefficients (r) describing relationships between different assay methods and mortality of *Helicoverpa zea* exposed to terminals treated with different formulations of *Bacillus thuringiensis*.

Assay method	*H. zea* units	*H. virescens* units	*S. exigua* units
diet inc. - CBW	.900*	.136	.053
diet inc. - TBW	.913*	.419	.244
diet inc. - BAW	.909*	.176	.263
leaf disc - CBW	.872*	.591	.175
leaf disc - TBW	.962*	.856*	.866*
leaf disc - BAW	-.081	-.158	-.016