TOXICITY OF DELTA ENDOTOXIN FROM *BACILLUS THURINGIENSIS* SUBSPECIES AND ISOLATES AND BETA EXOTOXIN AGAINST BOLLWORM AND TOBACCO BUDWORM AND EFFICACY OF TRANSCONJUGATE OF DELTA ENDOTOXIN AGAINST TOBACCO BUDWORM IN A FIELD TEST

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Abstract

LC_{50}s of Dipel 2X, an endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*, (HD-1 isolate) strain and Design Novartis CGA 237218, a transconjugated endotoxin *B. thuringiensis* subsps. *kurstaki*/*aizawa*i hybrid strain were significantly different after 168 h. Strains were 15 and 28 fold more toxic against larvae of reference strains of tobacco budworm, *Heliothis virescens* (F.), than bollworm, *Heliocoverpa zea* (Boddie), respectively. Both strains had lower LC_{50} values against bollworm and tobacco budworm than exotoxin thuringiensin (Di Beta}. Mixture of Dipel 2X endotoxin-exotoxin did not exhibit synergism against either species. At 168 h slope values of gut-disrupting endotoxins and protein inhibitor exotoxin for the bollworm and tobacco budworm ranged from 0.18 to 0.84 and 0.40 to 0.91, respectively. Slopes were flat, indicating that actions were probably multiple and took place at different times during the 168 h. No significant difference in LD_{50}, as measured by μg Dipel/larva by oral treatment of the tobacco budworm was shown for a field collected strain and the same laboratory reference strain. In a field test 58% to 85% control of third to fifth stage tobacco budworm larvae was obtained 2 to 4 d post-treatment after 2 spray applications of Design at 2, 2.5 and 3 kg/ha to first-second stage larvae. Six days post-treatment 39% to 64% control was determined for third-fifth stage of larvae at the same rates. No trend was shown for rates. Larval populations in untreated check plots exceeded the economic threshold of 0.05 larvae/terminal on all sample dates.

Introduction

Use of transconjugates of *Bacillus thuringiensis* endotoxins on cotton will increase in the future because they will provide adequate control of larval populations of the tobacco budworm and offer another alternative for resistance management programs.

No information has been published on toxicity of the exotoxin, thuringiensin (Di Beta), in the laboratory against either the bollworm or tobacco budworm. We wanted to determine if exotoxin was more toxic than both endotoxins tested. No information has been published on comparative toxicity against either species of these lepidopterans by mixtures of endotoxin (Dipel 2X) - exotoxin in the laboratory. Toxicity of Design, a transconjugated endotoxin *B. thuringiensis* isolate, was also tested to determine if it might be equal to or more toxic than Dipel 2X, a standard endotoxin strain, to the bollworm than the tobacco budworm. A field test was conducted to determine efficacy of Design applied as a foliar spray for control of small larvae of the tobacco budworm.

Materials and Methods

Laboratory

Exotoxin thuringiensin (44% A.I. in Calcium) Technical Lot No. 25-552-BD, Abbott ABG-6162A (hereafter called exotoxin), as described by Anonymous (1989), and endotoxin, *B. thuringiensis* subsp. *kurstaki*, Dipel 2X with a potency of 32,000 international units (IU)/mg were obtained from Abbott Laboratories, North Chicago, IL. No potency value was determined for exotoxin. Transconjugated *B. thuringiensis* subsp. *kurstaki* (HD191) mutant D/*aizaiwa* (HD135) mutant R (Agree of CGA237218) with a potency of 25,000 IU/mg was obtained from Novartis, Greensboro, NC. Product potencies were determined by bioassay with standard insect strains according to standard procedures. Dipel, a wettable powder with a potency of 16,000 IU/mg, was used in oral treatments against tobacco budworm.

The bollworm strain was obtained from USDA-ARS Laboratory, Tifton, GA and was maintained without selection in the Weslaco laboratory for the past 5 years. Tobacco budworm colony has been maintained in the Brownsville and Weslaco for the past 20 years. No insects were added to either colony during this time.

Doses of *B. thuringiensis* endotoxin and exotoxin alone or in mixture in one ml distilled water were pipetted onto diet (Raulston and Shaver 1971) surface and allowed to dry. Diet was prepared within two days of use. Each 30 ml cup contained 8 to 10 ml of diet. One neonate larva of bollworm or tobacco budworm was placed in each cup. Doses were replicated three to five times with at least 30 neonate larvae/dose/cup/replication. Check cups had distilled water pipetted onto diet surface.

Serial dilutions (50%) of exotoxin at 1.56 to 10,000 μg/cup, endotoxin (Dipel 2X) and trans-conjugated endotoxin (Design) were tested at 0.0024 to 125 μg/cup were tested alone from 1990 to 1993.. Mixtures of endotoxin (Dipel 2X) - exotoxin were tested at 0.005 to 5.08 μg/cup (1.6:1 ratio endotoxin to exotoxin/concentration). LC_{50} values were converted to μg/cm2 by area of diet surface based on 6.15 cm2/cup. Tests were conducted from at Weslaco.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1258-1260 (1999)
National Cotton Council, Memphis TN

A field collected strain of tobacco budworm from Altimera, Tamaulipas, Mexico and the same laboratory reference strain were bioassayed by oral application for toxicity in 1971 at Brownsville.

Doses of 2.5, 5, 10, 20, 40, 80 and 120 μg Dipel wettable powder/μl deionized water were applied to the oral cavity of fifth stage larvae of the tobacco budworm with a topical applicator calibrated to deliver one microliter. Larvae which weighed 287 ± 144 mg (170 to 457 mg) were held upside down with mouth parts facing upwards and the microliter was placed inside the cavity on tissue of foregut. . Ten-X magnification was used to observe placement in oral cavity. Care was taken to determine that the larvae did not regurgitate the droplet when the larvae were placed on artificial diet following the treatment.

Cumulative mortalities of larvae were determined 24, 48, 72, 96 and 168 h post-treatment for all treatments. All mortalities were corrected for larval mortalities of check. IU were not used in dose calculations because exotoxin activity is not measured on an IU basis. Insects were held at 27 ± 3 °C and 50 to 70% RH to determine mortalities by all treatments. LC_{50}, as μg/cm2, slope ± standard error (SE) and 95% confidence interval (CI) was determined by Probit analysis (SAS 1988) after correction for check mortalities at each time of assay. When ratio of slope/SE was <1.96, t=∞. P<0.05 the regression was not significantly different from 0. When 95% CI overlapped LC_{50} values were not significantly different.

Total number of dead and live larvae were determined after 72hr in the oral treatment evaluations. LD_{50} values, slope ± SE and 95% confidence interval were determined by Probit analysis.

Field Test

In 1991 Acala HS-46 cultivar was grown in San Pedro de las Colonias, Coahuila, Mexico. Design, as a 3.8% water soluble suspension, was applied at 2.0, 2.5 and 3.0 kg/ha with a motorized backpack sprayer calibrated to deliver 310 l/ha. Plots were arranged in a randomized complete block design with 4 replicates. Plots were 80 m2 (12 rows 80 cm apart, 10 m wide x 80 m long).

The 1st and 2nd stadia larvae were counted. All 3rd stage and older larvae were removed from entire plants in all plots one d prior to the first application. The 3rd stadia and older larvae were placed on artificial diet and reared to pupation. Emerging adults were identified to species.

Two applications were made two days apart to better evaluate the efficacy of a short lived insecticide. Eggs, first and second instar larva (1 to 5.9 mm long) and third to fifth instar larvae (>6.0 mm) were then counted on 20 plant terminals (upper 7 nodes)1 d following the first application and 2, 4 and 6 d following the second application. Means were separated using t=3.182, P<0.05, df=3 by paired treatment (Steel and Torrie 1960) on each date for eggs, first-second and third-fifth stadia comparing each rate with the untreated check.

Results and Discussion

Laboratory

After 24 h post-treatment a non-significant regression or unrealistic LC_{50} values for both the bollworm and the tobacco budworm were determined for exotoxin and endotoxins alone and mixture of exotoxin and endotoxin (Dipel 2X) (Table 1). No significant differences in LC_{50} values were shown for bollworm by Dipel 2X 72 and 96 h posttreatment. LC_{50} values were significantly different for the tobacco budworm at these same h posttreatment. LC_{50} values for Dipel 2X and hybrid endotoxin against the tobacco budworm were significantly and 15 and 28 fold greater than LC_{50} values shown for the bollworm 168 h post-treatment. After 48 and 72 h LC_{50} values of Design were 18 and 29 fold greater and significantly different for tobacco budworm than for bollworm, respectively. There were no significant differences between LC_{50} values of the two species at these times for Dipel 2X. Design was more toxic to tobacco budworm in a shorter period of time than bollworm. LC_{50} of endotoxin or transconjugate of endotoxins was significantly more toxic than the exotoxin against tobacco budworm and bollworm. LC_{50} values of exotoxin after 168 h were >4 and >2 μg/cm2 for bollworm and tobacco budworm, respectively. Toxicity of endotoxin and exotoxin mixture was equal for both pest species. From 72 to 96 h endotoxin and transconjugate of endotoxins were significantly more toxic than the mixture.

Slopes of endotoxins and exotoxins ranged <1 at all times. Slopes of both endotoxins were greater for tobacco budworm than bollworm, but slope for exotoxin was greater for bollworm than tobacco budworm. Perhaps actions of endotoxins which cause ion-pores in gut epithelium are additive each 24 h period from 24 to 168 h. Slopes of endotoxin, exotoxin and mixture were -0.041± 0.09. 0.089± 0.05, and 0.14 ± 0.07, respectively. They did not differ significantly from 0 (data not shown in table) for the bollworm after 24 h. Slopes of endotoxin, exotoxin and mixture were -0.43±0.4, 0.13±0.1 and -0.04±0.07, respectively. They did not differ significantly from 0 (data not shown in table) for the tobacco budworm after 24 h. Slopes of endotoxin and exotoxin were -0.082±0.1 and 0.054±0.1, respectively. They did not differ significantly from 0 (data not shown in table) for the tobacco budworm after 48 h.

For the endotoxin, exotoxin, the mixture and the transconjugate we tested 553, 697, 505, and 683 bollworm larvae, respectively. For the same sequence of four preparations we tested 355, 283, 755 and 1135 tobacco budworm larvae, respectively.

For the oral treatment of fifth stage tobacco budworm larvae, slope ± SE, LD_{50} as μg/larvae and 95% CI for the field collected strain was 595, 0.41±0.1, 14,806 and 1,373.27-7.1 x 10^7, respectively. The same results in the same sequence for the laboratory strain were 591, 0.67±0.2, 5,262.3 and 961.8-6.2 x 10^{10} respectively. LD_{50} values differed 2.7 fold but toxicity was equal for the two strains. They had overlapping 95% CI values.

Slopes of regression by oral or diet surface treatment were equally flat indicating the toxicity is not great between doses.

Field Test

Third-fifth stage larvae were removed from plots prior to the first application of Design and all reared adults were tobacco budworm. One d after the first application there was a 29% reduction of egg populations for all rates compared to the untreated check;. After two, four and six d 4%, 11% and 0% reduction in egg populations was determined, respectively. Egg populations in the check were 2.9/terminal one day after the first application and 1.5, 2.5 and 1.7 eggs/terminal on two, four and six d after the second application, respectively. The two sprays of Design showed little to no effect on populations of eggs (data not shown in table).

Counts of first and second stage larvae ranged from 1.1 to 2.0/terminal in treated and untreated plots in pretreatment counts (Table 2); no significant difference in populations was shown. In treated plots one day after the first application an average of 3.5 first and second stage larvae/plant were found. An average of 0.9, 0.9 and 0.5 larvae of the same size/terminal were found two, four and six d post-treatment of both applications, respectively (data not shown in table). Percentage control of third and fifth instar larvae by hybrid endotoxin was 55% to 79% one day after first application. First and second instar larvae were controlled. This was reflected in the reduction of the third and fifth instar larval counts. All rates of Design provided larval control. They were about equally effective against the tobacco budworm.

We estimate that a mean of 110,000 plants of cotton/ha were planted; therefore 0.1 larva/plant is 11,000 larvae/ha. If the economic threshold is assumed to be 0.05 larvae of all sizes (5,500 larvae/ha) then all populations in the untreated check were equal to or exceeded this population on 60% of the sample dates. The greatest larval population in the check was 1,260,000 larvae/ha and it occurred on day 192. All third to fifth stage larval populations exceeded the designated economic threshold in the untreated check. These are high, naturally occurring populations for this single pest for the eight days the two applications were evaluated. Design provided adequate control of a field population of tobacco budworm with two applications made two days apart.

Conclusion

With this diet surface bioassay technique Dipel and Design were 15 and 28 fold more toxic to larvae of laboratory reference strains of tobacco budworm than bollworm, respectively. There was no significant difference in LD_{50} values by oral treatment to 5^{th} instar larvae of tobacco budworm of a field collected population and the same reference strain. In the field test two applications resulted in adequate control.

Acknowledgment

Thanks are extended to Novartis, Inc., Mexico, D.F., Mexico for the results of the field test.

References

Anonymous. 1989. DiBeta Technical Bulletin prepared by Abbott Laboratories, Chemical and Agricultural Products Division, North Chicago, IL, 5pp.

Beegle, C.C., H. T. Dulmage, D. A. Wolfenbarger and E. Martinez. 1981. Persistence of *Bacillus thuringiensis* insecticidal activity on cotton foliage. Environ. Entomol. 10:400-401.

SAS Institute. 1988. Technical P-179. Release 6.03. SAS Institute, Inc. 255pp.

Steel, R. G. D. and J. H. Torrie. 1960 Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York, NY. 481 Pp.

Table 1. Toxicity of neonate bollworm and tobacco budworm with endotoxin *Bacillus thuringiensis* and Beta exotoxin [Dibeta] in laboratory bioassays. 1990-1991.

Post-treatment Hours	Slope ± SE	LD_{50} as μg/cm2	95% Confidence Interval
Bollworm			
B.t. subsp. *kurstaki* [Dipel 2X]			
48	0.28±0.07	2.13	0.87-12.17
72	0.51±0.07	0.22	0.13-0.35
96	0.57±0.07	0.15	0.089-0.24
168	0.61±0.08	0.11	0.063-0.78
Thuringiensin			
48	0.95±0.4	$2.5x10^{6}$	$3747.8\text{-}1.0x10^{35}$
72	0.18±0.04	302.76	54.2-12342.8
96	0.23±0.038	34.34	12.3-181.0
168	0.84±0.062	4.88	3.52-6.81
B.t. subsp. *kurstaki* (Dipel] + Thuringiensin			
48	0.21±0.07	74.4	$3.2\text{-}3.25x10^{7}$
72	0.17±0.06	23.3	$1.06\text{-}6.3x10^{9}$
96	0.16±0.06	4.8	$0.37\text{-}2.5x10^{8}$
168	0.18±0.06	0.055	0.013-0.79
Transconjugate B.t. subsps. *Kurstaki/aizawai*			
24	0.24±0.07	$4.3x10^{8}$	$1.68x10^{5}\text{-}3.37x10^{18}$
48	0.57±0.06	6.76	4.41-10.57
72	0.84±0.06	0.88	0.63-1.22
96	0.85±0.06	0.53	0.38-0.74
168	0.8±0.06	0.34	0.23-0.48
Tobacco budworm			
B.t. subsp. *kurstaki* [Dipel 2X]			
72	0.63±0.1	0.15	0.07-0.26
96	0.96±0.13	0.06	0.028-0.099
168	0.9±0.19	0.0076	0.00057-0.023
Thuringiensin			
72	0.18±0.06	1709.27	$142.6\text{-}1.1x10^{8}$
96	0.26±0.06	32.19	10.06-21.5
168	0.4±0.05	2.95	1.56-5.33
B.t. subsp. *kurstaki* [Dipel 2X] + Thuringiensin			
48	0.14±0.06	$1.35x10^{4}$	$35.3\text{-}1.4x10^{35}$
72	0.26±0.06	2.52	0.62-58.33
96	0.4±0.06	0.19	0.1-0.43
168	0.47±0.06	0.039	0.024-0.063
Transconjugate B.t. subsps. *Kurstaki/aizawai*			
24	0.21±0.04	4631.22	$7668.1\text{-}5.5x10^{10}$
48	0.42±0.04	1.96	0.85-495.0
72	0.57±0.06	0.05	0.021-0.12
96	0.71±0.1	0.018	0.0072-0.044
168	0.91±0.13	0.012	0.0057-0.024

Table 2. Efficacy of Design against tobacco budworm in cotton. San Pedro, Coah., 1991.

Percentage Control			
		Larval Instar	
Rate Kg/ha	Eggs	1st - 2nd	3rd - 5th
1 Day Posttreatment, 1st Application - Sept. 8			
2	12	42	74
2.5	34	54	78
3	12	51	56
check[a/]	(2.9)	(3.5)	(1.1)
2 Days Posttreatment, 2nd Application - Sept. 10			
2	0	67	59
2.5	13	56	86
3	0	36	76
check[a/]	(1.5)	(0.9)	(1.3)
4 Days Posttreatment, 2nd t Application - Sept. 10			
2	0	68	73
2.5	15	65	79
3	19	60	70
check[a/]	(2.5)	(0.9)	(1.5)
6 Days Posttreatment, 2nd Application - Sept. 10			
2	0	40	75
2.5	0	80	85
3	0	67	78
check[a/]	(1.7)	(0.5)	(2.9)

[a/] Mean/Terminal