CONTROL OF *HELIOTHIS VIRESCENS* ON FLUE-CURED TOBACCO USING *BACILLUS THURINGIENSIS* AND A COTTONSEED FLOUR FEEDING STIMULANT[1,2]

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Isolates of *Bacillus thuringiensis* var. *kurstaki* in combination with a feeding stimulant (Coax®-a cottonseed flour adjuvant) were tested against the tobacco budworm (*Heliothis virescens* (Fabricius)) on flue-cured tobacco. Coax®, used as an adjuvant with *B. thuringiensis*, gave slight or no improvement in control of budworms established before treatment. In only one of three tests did it significantly enhance the control of budworm infestations established after application.

INTRODUCTION

The *Heliothis* complex (primarily the tobacco budworm, *H. virescens* (Fabricius)) is an important problem on flue-cured tobacco in the southeastern United States. This complex is important because the larvae are destructive and difficult to control once they enter the terminal bud. Particularly in hot, dry weather, larvae tend to remain in the tightly closed bud where they are protected from pesticide contact (12). Under such conditions in North Carolina, farmers using conventional insecticides and application techniques often achieve only 50-60% control. If infestations are heavy, this may not be adequate to protect the crop from significant loss.

During the 1960's workers began to demonstrate the effectiveness of various plant extracts as feeding stimulants for insect pests of cotton (5, 9, 10, 14). This discovery raised the question of whether feeding stimulants could be used to increase the ingestion of microbial insecticides by pests (and thus increase the efficacy of the insecticide). Several investigations have now shown that feeding stimulants can increase the effectiveness of *Bacillus thuringiensis* Berliner (*B.t.*) and/or nuclear polyhedrosis virus against *Heliothis* spp. in cotton (2,3, 4, 8, 15, 16). In many of these investigations, cottonseed flour and cottonseed oil were demonstrated to be two of the more effective adjuvants.

In order to determine the effect of a commercially produced cottonseed flour adjuvant (Coax® , Traders Oil Mill Co.) on efficacy of *Bacillus thuringiensis* var. *kurstaki* against budworms on tobacco, five tests were carried out at several locations in North Carolina during 1980. Two basic hypotheses were tested: 1) Coax®, when added to *B.t.*, increases the efficacy of *B.t.* in the control of an established infestation of tobacco budworms in flue-cured tobacco and 2) when added to *B.t.*, the adjuvant increases the efficacy of *B.t.* in the control of budworms hatching after treatment application.

MATERIALS AND METHODS

Tests were conducted against artificial infestations of tobacco budworms using two techniques. In one technique, larvae were placed in the bud area and allowed three to four days to become established before treatment. This simulated a natural infestation already established at the time of treatment. In a 2nd technique, larvae were placed on the plant after treatment. These larvae were placed on the 4th or 5th leaf below the bud; ca. 50% were placed on the upper surface of the leaf and 50% on the lower surface (see Neunzig, 8). This technique simulated a hatch of budworms after treatment. For all tests, a fine brush or probe was used to transfer larvae from artificial diet to the plant. Establishment is apparently improved by allowing neonate larvae to feed for 24 hours on artificial diet before transfer to tobacco (W. J. Mistric, Jr., Personal Communication).

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Tests 1 to 3: Budworms were obtained from a laboratory culture maintained at NC State University, Raleigh, NC (wild material introduced annually). After hatching, neonate larvae were placed on artificial diet for one day prior to use. Treatments included *B.t.* var. *kurstaki* 16,000 IUP/mg (Dipel® WP, Abbott Laboratories) at 0.56 and 1.12 kg/ha; a cottonseed flour adjuvant (Coax® , Traders Oil Mill Co.) at 3.36 kg/ha; combinations of *B.t.* at 0.56 kg/ha with 1.68 and 3.36 kg/ha of the cottonseed flour adjuvant; acephate 75% SP (Orthene Tobacco Insect Spray® , Chevron Chemical Co.) at 1.12 kg/ha; and a control.

Test 1 was conducted on commercially grown Speight G-28 transplanted May 5 in Wake Co., NC. Plots were 0.010 ha (150 plants), consisting of four rows of tobacco each and were separated on each side by a vacant 5th row and on the ends by 4.88 m vacant alleys. Seven treatments (**Table 1**) were arranged in a randomized complete block design with three replications. Twenty-six days after transplant, 40 plants/plot were infested by placing larvae in the bud. After three days, the number of established larvae was determined and treatments applied. All treatments were applied using a tractor-mounted sprayer with three hollow cone HB-8 nozzles positioned ca. 30 cm over each row. Delivery rate of the spray was 187 liters/ha at 4.2 kg/cm^2. After four days, plants were checked for budworm larvae and an additional 30 plants/plot were infested by placing larvae below the bud. The effect of treatment on this post-treatment infestation was evaluated after four days.

Test 2 was conducted on commercially grown McNair 944 transplanted May 15 in Granville Co., NC. Plots were as in Test 1. Forty-six days after transplant (June 30), larvae were placed in the buds of 40 plants/plot. After three days, larval establishment was determined and the infested portion of each plot was treated in a manner similar to Test 1. However, solid cone TG-2 nozzles were used and 299 liters/ha of spray were applied at 3.5 kg/cm^2. Budworm survival was evaluated four days after treatment. On July 7, the remaining untreated tobacco in each plot was treated using the same technique as the earlier treatment. About 24 hours later, 30 plants/plot were infected, and ca. 96 hours after treatment an additional 30 plants/plot were infested. Infestations were made by placing larvae on leaves below the bud. Larval survival was evaluated three days after each infestation. A short, heavy rain fell three hours after the first post-treament infestation.

In Test 3, McNair 944 planted at the Central Crops Research Station, Clayton, NC, was cut back in mid-August and one sucker was allowed to regrow. Plots consisted of two rows containing ca. 90 plants. Three treatments (**Table 1**) were arranged in a randomized complete block design with three replications. Treatments were made with a CO_2 pressurized backpack sprayer with a three-nozzle boom. Materials were applied the morning of Sept. 15 at 3.5 kg/cm^2, 150 liters/ha, through solid cone CE-2 nozzles. Budworms were placed below the bud area on ca. 90 plants 24 hours after treatment. A moderate natural infestation was present at this time, and all naturally occurring larvae were removed. An evaluation of survival was made three days after infestation. Wild larvae that hatched after the artificial infestation as well as the artificially placed larvae were counted. A light rain fell one day after the infestation and temperatures were cool.

In Tests 1 to 3, budworms surviving through the test period were compared to the pretreatment count (for pretreatment infestation) or the number of larvae placed (for post-treatment infestation) to determine the percent decline in numbers (**Table 1**). These percentages were transformed to an arc sine, and a two-way analysis of variance and Duncan's new multiple range test used to compare treatments. Original units are presented

Table 1. Effectiveness of *Bacillus thuringiensis* var. *kurstaki* and a cottonseed flour adjuvant, Coax®, against artificial infestations of tobacco budworms on flue-cured tobacco, 1980, Tests 1 to 3.

	Average percent decline					
	Test 1[a]		Test 2[a]			Test 3[b]
Treatment, rate	Infested pre-treat.	Infested 96-hrs. post-treat.	Infested pre-treat.	Infested 24 hrs. post-treat.	Infested 96 hrs. post-treat.	Infested 24 hrs. post-treat.
Untreated check	13.7 b	86.0 c	20.4 d	89.0a	78.7 b	31.4a
Dipel WP, 0.56 kg/ha	42.5a	96.3ab	40.2 bc	95.1a	82.2ab	51.2 b
Dipel WP, 1.12 kg/ha	46.7a	89.4 bc	50.5ab	95.1a	81.1ab	--
Dipel WP, 0.56 kg/ha + Coax 1.68 kg/ha	34.9a	90.0 bc	59.4a	93.0a	89.7ab	--
Dipel WP, 0.56 kg/ha + Coax 3.36 kg/ha	44.9a	90.6 bc	48.3ab	98.4a	94.6a	74.7 c
Coax 3.36 kg/ha	10.5 b	87.9 bc	24.5 cd	90.3a	75.4 b	--
Orthene Tobacco Insect Spray (75SP), 1.12 kg/ha	35.5a	100.0a	58.3ab	98.5a	83.0ab	--
Overall effect of treatment (Probability of >F)	.0049	.0057	.0008	.4389	.1685	.0006

[a] Averages followed by a common letter are not significantly different at the 5% level (Duncan's new multiple range test).

[b] Averages followed by a common letter are not significantly different at the 1% level (Duncan's new multiple range test).

in the table.

Tests 4 and 5: Budworms were obtained from the culture maintained at the Oxford Tobacco Research Station, Oxford, NC (ca. 200 generations in continuous culture). After hatching, larvae were fed on artificial diet for two days prior to placement in the field.

For Test 4, ca. 3,200 Speight G-70 plants grown at the Oxford Tobacco Research Station were infested ca. six weeks after transplanting by placing a larva in the bud area of each plant. After three days, 150 plots of 20 plants were marked off in a completely randomized design. In each plot, eight successfully infested plants were marked for examination after treatment. Four isolates of *B.t.* var. *kurstaki* were tested (HD-1, HD-241, HD-244, HD-263, Abbott Laboratories), each at three rates (0.14, 0.28 and 0.56 kg/ha). Each of these treatments plus 2.2 kg/ha of Coax® were applied to five plots. Five additional plots received the *B.t.* treatments without the adjuvant. Plots were sprayed on June 24 with a CO_2 powered backpack sprayer with an adjustable nozzle at a rate of 234 liters/ha with a pressure of 4.2 kg/cm^2. A heavy rain (ca. six cm in two days) fell starting two hours after the spraying was completed. This prevented examination of the plots until six days after treatment. Plants were examined again for budworms 13 days after spraying, and they were subjectively rated from 0 (lowest) to 7 for budworm damage.

About one week after completion of Test 4, all the plants were cut off and removed from the field. One sucker was allowed to grow per plant, and this new growth was used in Test 5. This tobacco was artificially infested with tobacco budworms on Aug. 4 and examined for successful establishment after three days. At that time, 100 plots in 10 blocks were marked off in a randomized complete block design. Each plot contained 25-45 plants, 15 of which had been successfully infested and marked for later examination. The plots were sprayed one day later. Analysis of variance from test 4 showed that the rate of application of the *B.t.* isolates did not affect efficacy. Apparently, all treatment levels were above the minimum needed to detect rate effects. This has also been reported elsewhere (1, 6, 11). Therefore, only one rate of *B.t.* (0.14 kg/ha) was applied in Test 5. Application techniques were the same as for Test 4. Plots were examined for budworms 3, 6 and 10 days after treatment. Damage ratings were made at 10 days.

The numbers of budworms and the average damage ratings were transformed to $\sqrt{X + 0.5}$ prior to analysis of variance (ANOVA). The ANOVA took into consideration the factorial design of Tests 4 and 5, and sources of variation were partitioned so that pertinent interactions among the factors could be analyzed. After ANOVA, Duncan's new multiple range test was applied to the transformed data sets, but mean values are presented in original units in **Table 2.**

RESULTS AND DISCUSSION

The hypothesis that Coax® when added to *B.t.* increases the control of an established infestation of budworms was examined in Tests 1, 2, 4 and 5. Weather conditions at the time of Test 4 appear to have adversely affected budworm survival. This may have reduced the usefulness of the test. Nonetheless, control with all insecticide treatments was relatively good when compared to the untreated check. No significant effect was seen with the addition of Coax® . In Tests 1, 2 and 5, budworm survival in untreated plots was good. Weather during these tests was hot and dry and larvae were protected in tightly closed buds. Control with *B.t.* alone in these tests was poor

Table 2. Effectiveness of 4 isolates of *Bacillus thuringiensis* var. *kurstaki* and a cottonseed flour Coax®, against artificial infestations of tobacco budworms on flue-cured tobacco at Oxford, N. C., 1980, Tests 4 and 5.

	Test 4[b]			Test 5[b]			
	No. budworms/ 8 plants Days post-treat.		Avg. damage[c] rating 13 days	No. budworms/ 15 plants Days post-treat.			Avg. damage[c] rating 10 days
Treatment[a/]	6	13	post-treat	3	6	10	post-treat.
HD-1 + Coax	0.6a	0.0a	0.8a	8.6a	6.2a	2.9ab	2.7a
HD-1	0.3a	0.2a	1.0ab	10.2ab	7.8a	4.1 bc	3.0abc
HD-241 + Coax	1.1a	0.3a	1.2 b	8.9a	6.3a	3.2abc	3.5 c
HD-241	0.8a	0.1a	1.0ab	8.7a	6.2a	3.1ab	3.0abc
HD-244 + Coax	0.7a	0.1a	1.0ab	8.6a	5.8a	3.3abc	3.2abc
HD-244	0.7a	0.1a	0.9ab	9.3a	7.5a	4.8 c	3.0abc
HD-263 + Coax	0.6a	0.1a	1.0ab	8.3a	6.4a	2.4a	2.7ab
HD-263	0.6a	0.0a	1.0ab	9.3a	6.5a	3.8abc	3.1abc
Coax	3.3 b	0.3a	1.6 c	12.9 c	10.7 b	4.3 bc	3.6 c
Untreated	2.8 b	0.2a	1.8 c	11.8 bc	9.8 b	3.9 bc	3.3 bc
Combined isolates[d]	0.6	0.1	1.0	9.4	7.0	4.0**	3.0
Combined isolates[d] + Coax	0.8	0.1	1.0	8.6	6.2	3.0**	3.0

[a]All B.t. isolates contained 21.4 billion international units per kilogram in a wettable powder formulation. Test 4 data combined for 3 rates of application (0.14, 0.28 and 0.56 kg/ha); Test 5, 0.15 kg/ha only.

[b]Within columns, means followed by a common letter are not significantly different at the 5% level (based on analysis of variance and Duncan's new multiple range test).

[c]Rated 0 (no damage) to 7 (plant topped).

[d]Average number of budworms in B.t. treated plots only. Average values for plots with or without Coax were compared using a pooled Student's test. (**Means differ significantly at 1% level.)

(generally 20-30% compared to the untreated check). This is well below levels of control with *B.t.* previously reported (7, 11). It is, however, similar to control reported by Mistric and Smith (12) under apparently similar weather conditions. It is under such circumstances that enhancement of control would be most valuable. However, the addition of the adjuvant increased the efficacy of *B.t.* only slightly, if at all. In Test 2, the addition of 1.68 kg/ha of the adjuvant did significantly (5% level) increase mortality, but the increase was not significant with the addition of 3.36 kg/ha (**Table 1**). In Test 5, mortality was greater for most isolates with the addition of the cottonseed flour, but differences were small and not statistically significant (**Table 2**). No difference in damage ratings was noted between treatments with and without the adjuvant in Tests 4 and 5 (**Table 2**).

The second hypothesis (increased control of larvae hatching after treatment) was examined in Tests 1 to 3 (**Table 1**). In Test 1, high mortality (including the untreated check) may have resulted from the placement of larvae on the plants on the morning of a very hot, dry day. Despite this mortality, however, the overall effect of treatment was significant. *B.t.* at 0.56 kg/ha and acephate produced significantly higher mortality than no treatment. The addition of the adjuvant did not increase mortality over that of *B.t.* alone. Mortality in the untreated check was also high in Test 2, particularly for the 24-hour post-treatment infestation which was followed by a severe thunderstorm with high wind and heavy rain. The overall effect of treatment was not significant in either the 24-hour or 96-hour post-treatment infestations. However, the addition of 3.36 kg/ha of the adjuvant to *B.t.* did result in slightly higher (but not significant) mortalities than the use of *B.t.* alone, particularly for the 96-hour infestation. Natural mortality was much lower in Test 3 (31%) than in Tests 1 and 2 (79-89%). *B.t.* alone provided a highly significant (1% level) increase in mortality compared to the untreated check. The addition of 3.36 kg/ha of the adjuvant further increased the level of control to a highly significant degree (**Table 1**). Our results in Test 3 are supported by tests in cotton (4) in which Coax® increased the efficacy of pathogens against *Heliothis* spp. Johnson (8) also evaluated Coax® in combination with pathogens, including *B.t.*, on cotton. Compared to a pathogen alone, measurements of *Heliothis* activity were lower in treatments including Coax® , but in only one case was the difference significant. In these tests on cotton, insecticides plus adjuvants were applied several times and presumably were active against larvae before they reached protected feeding sites. This parallels our tests against developing infestations (second hypothesis).

Based on our tests, the adjuvant Coax® appears to have little effect on the efficacy of *B.t.* against established budworm infestations in tobacco. Only Test 3 supports the hypothesis that the adjuvant increases the efficacy of *B.t.* against a developing infestation. Thus, the value of the adjuvant in field use appears to be limited at best. If growers apply pesticides to control an obvious, established budworm infestation (as is usually the case in North Carolina), the addition of the adjuvant to *B.t.* would not be justified. If applications are timed by use of pheromone trap catches to precede a large proportion of hatch or if growers make regular preventive applications, the use of Coax® in tobacco could under some circumstances increase control. However, additional research is needed to determine the conditions under which such use would be cost effective.

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LITERATURE CITED

1. Allen, N., W.S. Kinard, and C.S. Creighton. *Bacillus thuringiensis* controls the tobacco budworm. **Tob. Sci.** 5:58-62. 1961.
2. Bell, M.R., and R.F. Kanavel. Field tests of a nuclear polyhedrosis virus in a bait formulation for control of pink bollworm and *Heliothis* spp. in cotton in Arizona. **J. Econ. Entomol.** 70:625-629. 1977.
3. Bell, M.R., and R.F. Kanavel. Tobacco budworm: development of a spray adjuvant to increase effectiveness of a nuclear polyhedrosis virus. **J. Econ. Entomol.** 71:350-352. 1978.
4. Bell, M.R., and C.L. Romine. Tobacco budworm field evaluation of microbial control in cotton using *Bacillus thuringiensis* and a nuclear polyhedrosis virus with a feeding adjuvant. **J. Econ. Entomol.** 72:427-430. 1980.
5. Guerra, A.A., and T.N. Shaver. Feeding stimulants from plants for larvae of the tobacco budworm and bollworm. **J. Econ. Entomol.** 62:98-100. 1969.
6. Jackson, D.M., and W.J. Mistric, Jr. Effectiveness of four *Bacillus thuringiensis* var. *kurstaki* isolates on tobacco budworms and hornworms on flue-cured tobacco. **Tob. Sci.** 26:21-24. 1982.
7. Johnson, A.W. *Bacillus thuringiensis* and tobacco budworm control on flue-cured tobacco. **J. Econ. Entomol.** 67:755-759. 1974.
8. Johnson, D.R. Suppression of *Heliothis* spp. on cotton using *Bacillus thuringiensis, Baculovirus heliothis* and two feeding adjuvants. **J. Econ. Entomol.** 75:207-210. 1982.
9. Maxwell, F.G., J.N. Jenkins, J.C. Keller, and W.C. Parrott. An arrestant and feeding stimulant for the boll weevil in water extracts of cotton plant parts. **J. Econ. Entomol.** 56:449-454. 1963.
10. McMillian, W.W., and K.J. Starks. Feeding response of some noctuid larvae (Lepidoptera) to plant extracts. **Ann. Entomol. Soc. Am.** 59:516-519. 1966.
11. Mistric, Jr., W.J., and F.D. Smith. Tobacco budworm: control of flue-cured tobacco with certain microbial pesticides. **J. Econ. Entomol.** 66:979-982. 1973.
12. Mistric, Jr., W.J., and F.D. Smith. Tobacco budworm on flue-cured tobacco: percentage control with insecticides abnormally low in early 1970. **J. Econ. Entomol.** 67:99-101. 1974.
13. Neunzig, H.H. The biology of the tobacco budworm and the corn earworm in North Carolina with particular reference to tobacco as a host. Tech. Bull. No. 196, North Carolina Agricultural Experiment Station, North Carolina State University, Raleigh, N.C. 1969.
14. Parrott, W.L., T.N. Shaver, and J.C. Keller. A feeding stimulant for pink bollworm larvae in water extracts of cotton. **J. Econ. Entomol.** 61:1766-1767. 1968.
15. Smith, D.B., D.L. Hostetter, and R.E. Pinnell. Laboratory formulation comparisons for a bacterial (*Bacillus thuringiensis*) and a viral (*Baculovirus heliothis*) insecticide. **J. Econ. Entomol.** 73:18-21. 1980.
16. Yearian, W.C., R.G. Luttrell, A.L. Stacy, and S.Y. Young. Efficacy of *Bacillus thuringiensis* and *Baculovirus heliothis*-chlordimeform spray mixtures against *Heliothis* spp. on cotton. **J. Ga. Entomol. Soc.** 15:260-271. 1980.