

Survival of *Ostrinia nubilalis* (Hübner) After Exposure to *Bacillus thuringiensis* Berliner Encapsulated in Flour Matrices^{1, 2}

Michael R. McGuire³, Robert L. Gillespie⁴ and Baruch S. Shasha

Plant Polymer Research, USDA-ARS
National Center for Agricultural Utilization Research
1815 North University
Peoria, Illinois 61604 USA

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ABSTRACT Two types of pregelatinized corn flour were used to produce granules containing *Bacillus thuringiensis* Berliner subsp. *kurstaki* and various additives for control of the European corn borer, *Ostrinia nubilalis* (Hübner), in the whorl of corn plants. Laboratory-reared larvae were applied to corn whorls in the greenhouse and field, and a high natural infestation occurred at one field site (Champaign). In the greenhouse and at all three field sites, five of these formulations were just as effective as Dipel 10G, a commercially available *B. thuringiensis* product, for control of European corn borer larvae. In all greenhouse studies and at one of the three field sites (Champaign), the dose of *B. thuringiensis* could be reduced by as much as 75% when a phagostimulant was added to flour granules without significant loss of corn borer control. The phagostimulant dose response was not observed at the other two field sites in which larval infestations were relatively low. Flour type had no significant effect on European corn borer control under greenhouse and field conditions. Greenhouse evaluations provided results significantly similar to results from two of the field sites indicating the usefulness of the technique. The data presented highlight the versatility and potential for using novel formulation techniques for enhancing the efficacy of *B. thuringiensis*.

KEY WORDS Formulation, *Ostrinia nubilalis*, *Bacillus thuringiensis*, flour, granules.

Bacillus thuringiensis Berliner subsp. *kurstaki* has been used to control the European corn borer, *Ostrinia nubilalis* (Hübner), for approximately 20 years, but results with *B. thuringiensis* have often been variable when compared with synthetic insecticides (McWhorter et al. 1972, Lynch et al. 1977). Lynch et al. (1980) attributed the variability to unsatisfactory formulations. Dunkle and Shasha (1988, 1989) began an effort to reduce this variability by developing a cornstarch granule which encapsulated *B. thuringiensis* and a UV protectant for the pathogen. They found that the encapsulation of *B. thuringiensis* and a

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³ To whom reprint requests should be addressed.

⁴ Current address: Cooperative Extension, Washington State University, Courthouse, P. O. Box 37, Ephrata, WA 98823.

sunscreen in a starch granule resulted in increased residual activity of the pathogen when compared to unprotected spores and crystals (Dunkle and Shasha 1989).

Research suggests that lepidopterous larvae, including the European corn borer, may stop feeding on treated foliage before they receive a lethal dose of *B. thuringiensis* (Heimpel and Angus 1963, Dulmage and Martinez 1973, Johnson and Freedman 1981, Salma et al. 1981). These studies also indicate that an increase in the palatability of *B. thuringiensis* might reduce variability and improve the level of control for targeted pests. Palatability of this pathogen might be enhanced through formulation ingredients. In fact, Bartelt et al. (1990) demonstrated that *O. nubilalis* would preferentially feed on starch (Miragel) granules containing Coax (CCT Corp., Litchfield Park, AZ), a commercially available feeding stimulant composed of cottonseed flour, vegetable oil, and a disaccharide, but not on Miragel granules without Coax. McGuire et al. (1990) then demonstrated the utility of using Coax in Miragel formulations of *B. thuringiensis* under field conditions.

Recent developments in the area of encapsulation of microbial pesticides has led to extremely versatile formulation processes (McGuire and Shasha 1992). The encapsulation procedures can utilize pregelatinized flour, that may act as a feeding stimulant by itself (Gillespie et al. 1994), or be altered to incorporate substances such as plant tissue, salts, sugars, etc. The procedures are simple, require little processing, and as a concept offer significant potential for changing the way we think about formulation and application of pesticides. The granules produced by this process can adhere to plant foliage and keep the active agent viable for a longer period of time than conventional formulations of *B. thuringiensis* (McGuire et al. 1994). Conventional granular formulations are prepared by applying *B. thuringiensis* to the outer surface of corn grit, clay, or sand. Although Gillespie et al. (1994) demonstrated that corn borer larvae would feed on the granules in closed systems or on cotton leaves, the true test of the granules is in the field where myriad other factors operate in the corn borer habitat.

The purpose of the experiments reported in this paper are (1) to assess the effects of the various additives to the formulation on acceptance of the granules by European corn borer larvae, and (2) to determine if greenhouse tests with corn mimic tests done in the field in relation to responses by larvae to the various types of granules. Clearly, the development of a greenhouse test requiring 4-6 weeks to completion (including plant growing time) allows for far more experimentation than a single field test each growing season. However, the greenhouse tests have never been "calibrated" to any great extent that would allow us to assume that activity of granules in the greenhouse is related to activity in the field.

Materials and Methods

Granule composition and preparation. Two types of pregelatinized corn flours (Illinois Cereal Mills, Paris, IL) were used to formulate granules to test the effects of various additives and concentrations of *B. thuringiensis* on the survival of European corn borer larvae. Flour 961 is a fine powder which passes a 40-mesh screen but not 100 (+40-100) and, upon formulation, the powder

agglomerates into larger granules. Therefore, ingredients added during the mixing process are entrapped throughout the granule. Flour 980 is sold as a larger particle (+10-20 mesh). Upon formulation, no agglomeration of particles occurs. Instead, active agents are coated within a thin layer around the surface of the granule.

Granules were prepared for greenhouse studies using methods developed by Shasha and McGuire (1992). The following details are presented as a summary of these techniques.

Granules formulated with CaCl_2 . A CaCl_2 solution was prepared by dissolving 450 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 300 ml cold (5°C) water to yield 550 ml solution containing 0.818 g CaCl_2/ml . Seven ml of this precooled solution was mixed with 25 g of flour. If additives such as *B. thuringiensis* or Coax were included in the formulation, they replaced an equal weight of the flour. *B. thuringiensis* technical powder (70,000 IU/mg, Abbott Laboratories, North Chicago, IL, Lot number 48-019-V9) was used in all experiments. After thorough mixing, the resulting product was air dried. Flour 961 granules were then sieved to the desired particle size.

Granules formulated with molasses. Procedures similar to those above were used to prepare granules, except a diluted solution of unsulfured molasses (Nugget brand, Stockton, CA) (80 g molasses: 20 g water) was used in place of the CaCl_2 solution. Quantities of other ingredients remained the same.

Granules formulated with fresh plant leaves. Due to the nature of this process, only flour 961 was used to create these granules. Fresh leaves (5 g) from cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), or horseradish (*Armoracia rusticana* Gaertn.) were placed in the bottom of a 250-ml Waring blender jar. Twenty-five g flour or flour-*B. thuringiensis* mixture was layered on top and then homogenized. During this process, the blender was stopped, the mixture was hand mixed, and blending was resumed. The resulting product was a fairly uniform granule that required little drying.

Large scale formulation of granules for field studies. For field studies requiring larger amounts (> 20 kg) of granules, the procedure was modified for pilot scale production. A calcium chloride solution was prepared by dissolving 600 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 750 ml of cold water (5°C) to yield 1025 ml solution containing 0.585 g of CaCl_2/ml . To produce granules, 1000 g of flour 961 or 980 was mixed with 30 g of *B. thuringiensis* technical powder, followed by 350 ml of cooled (5°C) CaCl_2 solution. All the ingredients were mixed in a 7.75-liter double planetary mixer (Charles Ross and Son Co., Hauppauge, NY). A separatory funnel was used to slowly add (over a 5 min period) the cooled CaCl_2 solution to the mixture. The formulation was removed from the mixer, pressed through a 4-mesh screen, and dried. Granules containing Coax were prepared using the same procedure except 10% of the flour was substituted with Coax.

Greenhouse studies. ICI 8555 hybrid corn was planted in 17.5-cm diameter pots. Plants were thinned to one plant/pot in the V3 stage of development. At the V7 stage of development, granules were sprinkled over the whorl and upper two leaves of the plant. Immediately following the application of granules, 20 ± 2 neonate European corn borers (< 12 h post-eclosion) were placed on the leaves, using a fine bristle brush, just above the whorl. After one week the treatments were evaluated by dissecting each corn plant and counting the number of live larvae per plant.

Four experiments were designed to assess the effects of various additives, formulation procedures, and amount of *B. thuringiensis* per plant on survival of larvae. Bartelt et al. (1990) demonstrated that the effect of a feeding stimulant can be assessed with this technique. In the first experiment, granules prepared with flour 961 and the three plant leaves were compared with granules prepared with CaCl_2 or molasses. The concentration of *B. thuringiensis* was 800 IU/mg granule; 75 mg granules were placed in each whorl and granule size was +20-40. As controls, granules were prepared without *B. thuringiensis* and tested. In the second experiment, the granule size was changed (+16-20) and the *B. thuringiensis* concentration was set at 1600 IU/mg or 400 IU/mg. Again, formulations consisted of flour 961 with cotton, molasses, or CaCl_2 . In addition, granules formulated with CaCl_2 and Coax and a commercial product, Dipel 10G (1,600 IU/mg) (Abbott Laboratories, North Chicago, IL) were included for comparison purposes. In the next two experiments, granules made with flour 980 and molasses or CaCl_2 were used. In one experiment the amount of *B. thuringiensis* per granule was varied while in the other experiment, the amount of granules per whorl was altered.

Experiments with flour 961 were set up in a completely randomized design, and analysis of variance (ANOVA) was used to determine treatment effects. Where possible, contrasts were used to identify meaningful comparisons of treatments. Otherwise, a protected least significant difference (LSD) was calculated for comparison of individual means. Experiments conducted with flour 980 were completed over a 3 d period with each treatment tested on five plants each day (complete block). Again, contrasts and LSD were used to detect differences among means.

Field studies. Soil preparation, planting, weed control, and other cultivation techniques were done in accordance with practices that were standard for each location (Table 5). Plots were 15 m long by 4 rows wide (0.75 m centers), and the design of the experiments was a randomized complete block with 10 treatments (8 flour granule treatments, Dipel 10G, and an untreated control). There were six blocks at each study site. Neonate *O. nubilalis* were applied to the whorl of 30 plants located in the middle rows of each plot at a rate of approximately 40 larvae per plant (Ortega et al. 1980). Granules were applied over the rows in 18 cm bands, with a high clearance vehicle, fitted with metered applicators (Noble, Lincoln, NE) calibrated to deliver 11.2 kg/ha (10 lbs/a). Evaluation of the treatments was conducted about 6 wk later, after *O. nubilalis* pupated. The first 25 plants infested with larvae were split from base to tassel to measure the total length of vertical tunnels. ANOVA (Lund 1988) performed across the three sites indicated a significant site-by-treatment interaction, probably due to the difference in levels of larval establishment at the three sites. Therefore, the treatment effects were analyzed separately for the three sites. All means were compared with the least significant difference procedure (Lund 1988) so that all treatments could be compared to each other. Also, contrasts were used to determine differences among groups of flour formulations and to look for interactions among additives. In addition, to determine the relationship of greenhouse results to field results, granules used in the field were also tested under greenhouse conditions described above.

Results

Greenhouse studies. In the first experiment, granules made with flour 961 and different formulation methods and additives reduced European corn borer larval survival when compared with the untreated control (Table 1) ($F = 21.1$, $df = 10,50$, $P < 0.001$). Specifically, larvae exposed to granules formulated with *B. thuringiensis* and molasses, fresh plant tissue, or CaCl_2 survived less well than those exposed to control granules (Table 1). However, European corn borer larvae placed on plants treated with granules formulated with corn, horseradish, or molasses, without *B. thuringiensis* had significantly higher mortality than those placed on other control plants.

Table 1. Effect of *B. thuringiensis* entrapped in flour granules (+20-40 mesh size) on survival of European corn borer larvae placed on corn grown in the greenhouse.

Formulation Flour-method	Concentration*	Mean $\pm$ SEM Larvae per Plant**
961 Horseradish	800	0.8 ± 0.37
961 Horseradish	0	3.8 ± 0.9
961 Corn	800	0.6 ± 0.24
961 Corn	0	5.2 ± 0.6
961 Cotton	800	1.2 ± 0.3
961 Cotton	0	11.7 ± 1.1
961 Molasses	800	0.8 ± 0.3
961 Molasses	0	6.0 ± 1.6
961 CaCl_2	800	1.5 ± 0.3
961 CaCl_2	0	10.3 ± 1.7
Untreated	0	15.0 ± 2.0
Protected LSD $P < 0.05$		2.986

* IU *B. thuringiensis* per mg granule; 75 mg granules placed in each whorl.

** 20 ± 3 larvae placed in each whorl. 5 plants tested for each formulation.

In the second experiment, the effects of formulation method and concentration of *B. thuringiensis* were evaluated (Table 2). Although not all granules without *B. thuringiensis* were evaluated, larval survival on plants treated with molasses or cotton granules without *B. thuringiensis* was similar to that on untreated plants, in contrast to the first experiment. Comparison of individual means indicated that Dipel 10G, molasses with 1600 IU/mg and molasses with 400 IU/mg were equally effective. Granules prepared with other methods or ingredients were less effective, but all granules with *B. thuringiensis* killed more larvae than control granules. Contrasts on means with flour granules demonstrated that granules made with 1600 IU/mg were more efficacious than granules made with 400 IU/mg ($F = 6.67$; $df = 1,141$; $P < 0.05$). In pairwise comparisons of granules made with both concentrations of *B. thuringiensis*, those made with molasses were significantly more effective than all others ($P < 0.05$). In order of effectiveness, the granules were ranked molasses > cotton = CaCl_2 + Coax > CaCl_2 ($P < 0.05$). Similarly, granules prepared with CaCl_2 were less effective than granules lacking CaCl_2 ($F = 5.56$; $df = 1,141$; $P < 0.05$).

Table 2. The effect of *B. thuringiensis* concentration and flour 961 granule formulation (+16-20 mesh size) on survival of European corn borer larvae placed in corn grown in the greenhouse.

Formulation Flour-method	Concentration*	Mean $\pm$ SEM Larvae per Plant**
961 Molasses	1600	0.6 $\pm$ 0.26
961 Molasses	400	1.5 $\pm$ 0.56
961 Molasses	0	10.3 $\pm$ 0.79
961 Cotton	1600	2.7 $\pm$ 0.77
961 Cotton	400	3.0 $\pm$ 0.65
961 Cotton	0	10.4 $\pm$ 0.57
961 CaCl_2 , Coax	1600	2.4 $\pm$ 0.61
961 CaCl_2 , Coax	400	3.3 $\pm$ 0.45
961 CaCl_2	1600	3.6 $\pm$ 0.84
961 CaCl_2	400	6.0 $\pm$ 0.94
Dipel 10G	1600	0.6 $\pm$ 0.23
Untreated	0	10.9 $\pm$ 0.54
Protected LSD $P < 0.05$		2.436

* IU *B. thuringiensis* per mg granule; 75 mg granules placed in each whorl.

** 20 $\pm$ 3 larvae placed in each whorl. 5 plants tested for each formulation.

In the third experiment, granules made with flour 980 and either CaCl_2 or molasses were tested (Table 3). Survival on plants treated with either control granule was not significantly different from survival on untreated plants. However, all granules containing *B. thuringiensis* killed significantly more larvae than granules without *B. thuringiensis*. In this experiment, contrasts ($\text{df} = 1$) demonstrated no significant differences in survival between molasses and CaCl_2 across both concentrations of *B. thuringiensis* ($F = 1.24$; $\text{df} = 1,28$; $P > 0.1$) nor between concentrations of *B. thuringiensis* across both formulations ($F = 1.24$; $\text{df} = 1,28$; $P > 0.1$). Similarly, no significant concentration $\times$ formulation interaction was observed ($F = 0.138$; $\text{df} = 1,28$; $P > 0.05$).

Table 3. The effect of *B. thuringiensis* concentration and flour 980 granule formulation (+10-20 mesh size) on survival of European corn borer larvae placed in corn grown in the greenhouse.

Formulation Flour-method	Concentration*	Mean $\pm$ SEM Larvae per Plant**
980 Molasses	800	1.8 ± 0.4
980 Molasses	100	2.8 ± 0.9
980 Molasses	0	8.6 ± 0.7
980 CaCl_2	800	2.8 ± 1.0
980 CaCl_2	100	4.8 ± 0.9
980 CaCl_2	0	11.0 ± 2.7
Untreated	0	10.4 ± 1.5
Protected LSD $P < 0.05$		3.899

* IU *B. thuringiensis* per mg granule; 150 mg granules placed in each whorl.

** 20 ± 3 larvae placed in each whorl. 15 plants tested for each formulation.

In the fourth experiment (Table 4), the amount of granules placed in each whorl was varied. Granules formulated with molasses and CaCl_2 were used to assess the effect of placing 75 or 150 mg granules in each whorl on larval survival. As above, no significant differences were observed with respect to formulations ($F = 0.004$; $\text{df} = 1,35$; $P > 0.1$), and no significant interaction occurred ($F = 0.22$; $\text{df} = 1,35$; $P > 0.1$). However, granules made with molasses and no *B. thuringiensis* were just as effective as granules made with molasses and *B. thuringiensis*.

Field studies. At all three field sites, there were significant differences among treatments in the amount of tunneling by European corn borer larvae

Table 4. The effect of reducing the amount of granules in the whorl of corn on European corn borer larvae exposed to *B. thuringiensis* entrapped in flour granules (+10-20 mesh size).

Formulation Flour-method	Concentration*	Mg/Whorl	Mean $\pm$ SEM Larvae per Plant**
980 Molasses	800	75	1.4 $\pm$ 0.9
980 Molasses	800	150	0.6 $\pm$ 0.4
980 Molasses	0	75	4.6 $\pm$ 0.5
980 CaCl ₂	800	75	1.6 $\pm$ 0.4
980 CaCl ₂	800	150	2.2 $\pm$ 0.4
980 CaCl ₂	0	75	13.8 $\pm$ 4.4
Untreated	0	0	15.6 $\pm$ 1.4
Protected LSD $P < 0.05$			4.327

* IU *B. thuringiensis* per mg granule.

** 20 $\pm$ 3 larvae placed in each whorl. 15 plants tested for each formulation.

[df = 9,45; $P < 0.001$; $F = 9.55$ (Metamora); $F = 6.20$ (Ankeny); $F = 7.35$ (Champaign)]. Similarly, larval survival in greenhouse studies with the same granules was significantly different among treatments ($F = 32.43$; df = 9,98; $P < 0.001$) (Table 6). At all three field sites and in the greenhouse, very few differences in mean survival occurred among plants treated with *B. thuringiensis*. In all cases except one (Champaign Flour 980-1600 IU/mg without Coax), granules formulated with 1600 IU/mg performed as well as the commercial product Dipel 10G, which is also formulated at 1600 IU/mg. In many instances, granules formulated with 400 IU/mg were as effective as those formulated with 1600 IU/mg. However, contrasts (df = 1) of means from granules made with flour (Table 7) indicated a significant *B. thuringiensis* concentration effect at Metamora and Champaign and in the greenhouse. A significant effect of Coax also was observed in the greenhouse and at Champaign where overall infestation levels were higher than the other two field sites. The Flour 980-1600, no Coax treatment at Champaign is apparently responsible for the significant flour $\times$ dose interaction.

To determine if the greenhouse tests provided results similar to those obtained in the field, Spearman rank correlations were done separately for the greenhouse and each individual field site. Correlations of 0.78 ($P < 0.008$) and 0.58 ($P < 0.08$) were calculated for the greenhouse vs Metamora and greenhouse vs Ankeny comparisons, respectively. At Champaign, where the Flour 980-1600, no Coax treatment appeared anomalous, the correlation was 0.462 ($P = 0.18$).

Table 5. Flour granule evaluation site information, 1992.

Characteristics	Site		
	Metamora, IL	Champaign, IL	Ankey, IA
Corn hybrid	Funk 4385	Mixed	ICI 8555
Planting date	April 12	June 10	April 30
Plant growth stage at infestation	V ₈	V ₁₀	V ₈
Date larvae applied	June 18	Aug. 6	June 18
Granule application	June 25	Aug. 13	June 23
Evaluation date	Aug. 28-Sept. 1	Sept. 28-29	Sept. 22

Approximately 40 larvae were applied to each plant (Ortega et al. 1980). Larvae were supplied for all three sites by the Corn Insects Research Unit, USDA-ARS, Ames, IA.

Discussion

Encapsulation of *B. thuringiensis* in renewable matrices such as cornstarch and flour has been studied for several years. Data presented here demonstrate the versatility and usefulness of these products for controlling European corn borer in field corn. Granules formulated with relatively inexpensive ingredients are as effective as commercially available products, but the advantage gained by adding substances to increase feeding (Bartelt et al. 1990), resist washoff (McGuire and Shasha 1992), or screen sunlight (Dunkle and Shasha 1989) make the flour-encapsulated formulations unique.

Granules made with CaCl₂, molasses, or fresh plant tissue represent new methods for preparing formulations of *B. thuringiensis*. These methods are relatively simple and require little drying, grinding, or sizing. Data from greenhouse and field tests demonstrated that European corn borer larvae will feed on the granules within the whorl of a corn plant and that *B. thuringiensis* remains effective after formulation.

In the greenhouse, certain formulations performed better than others; i. e., granules prepared with molasses and flour 961 killed more larvae in corn plant whorls than did granules made with CaCl₂ or plant leaves. Similarly, Coax, a commercially available feeding stimulant, had a significant positive effect when added to granules made with CaCl₂. However, response by insects did not differ when granules made with flour 980 and CaCl₂ were compared with flour 980 and molasses. The apparent flour type × formulation interaction may be due to the mechanism of formulation. Granules made with flour 961 form a matrix entrapping active agents throughout, whereas active agents are more or less coated onto the surface of flour 980 particles. Feeding preference may not be as important for flour 980 granules because *B. thuringiensis* may be soaked off the granules and onto foliage. Larvae may then obtain a lethal dose by feeding on contaminated leaves in the whorl.

In the field, data demonstrate the utility of these formulations for controlling European corn borer larvae. Although establishment of larvae was relatively

Table 6. Response by *O. nubilalis* larvae following application of different formulations of *B. thuringiensis* to corn under greenhouse and field conditions.

Formulation	Additive	Concentration*	Mean (SEM)			
			Larval Survival**	Greenhouse	Metamora	Champaign
Flour 961	Coax	1600	2.5 (0.4) a	7.67 (2.08) a	26.50 (7.84) a	11.85 (4.59) ab
Flour 961	Coax	400	2.5 (0.5) a	17.17 (4.65) ab	60.67 (11.55) abcd	12.70 (3.17) ab
Flour 961	None	1600	3.3 (0.5) bc	15.67 (5.91) ab	40.83 (6.47) ab	9.31 (2.29) ab
Flour 961	None	400	4.0 (0.6) bc	20.00 (4.01) ab	81.50 (7.99) cd	12.28 (2.77) ab
Flour 980	Coax	1600	2.3 (0.6) a	13.67 (2.00) ab	40.33 (4.76) ab	12.70 (2.87) ab
Flour 980	Coax	400	2.1 (0.4) a	32.67 (2.12) bc	54.17 (13.33) abc	16.93 (3.15) b
Flour 980	None	1600	1.5 (0.3) a	6.00 (2.26) a	90.00 (15.01) d	9.74 (2.77) ab
Flour 980	None	400	4.6 (0.5) c	43.88 (10.62) c	70.17 (9.21) bcd	17.78 (3.39) b
Dipel 10G		1600	1.1 (0.4) a	5.83 (2.32) a	30.17 (5.38) a	5.50 (2.02) a
Untreated		0	11.7 (1.0) d	86.33 (20.10) d	133.80 (18.37) e	38.95 (5.70) c

* IU *B. thuringiensis* /mg of dry granule.
** 20 ± 2 larvae placed on corn whorls treated with formulation. Seven days later, plants were dissected and live larvae counted. N=15 plants/treatment.
† Means (SEM) within a column followed by the same letter are not significantly different [*P* < 0.05; protected least significant difference (Lund 1988)].
Plot = 25 plants.

Table 7. Mean squares (df = 1) of treatment and interaction contrasts from field plots and greenhouse plants treated with flour encapsulated *B. thuringiensis*.

Comparison	Site			
	Greenhouse	Metamora	Champaign	Ankeny
Flour 961 v. Flour 980	30.4	954.1	1463.0	90.9
400 IU/mg vs 1600 IU/mg	126.0**	3745.0	3658.0**	194.1
Coax vs No Coax	145.0**	154.1	7778.0**	19.4
Interactions				
Coax × Flour	5.0	40.3	652.7	0.5
Flour × Dose	45.4	1387.0	5023.0**	53.8
Dose × Coax	145.0**	140.1	513.5	26.3
Coax × Flour × Dose	70.0	432.0	1271.0	10.5
Error Ms (df = 45 field)	26.7	384.5	877.4	82.0
(df = 18 greenhouse)				

* *B. thuringiensis* formulated at 400 IU/mg or 1600 IU/mg of granule dry weight.

** $P < 0.05$.

low at two field sites (approximately 2-3 larvae per infested but untreated plant at Ankeny and Metamora), results suggest that the flour granules were as effective as a commercial granule. While Coax did not seem to increase granule efficacy at these two sites, at Champaign, where the infestation was higher, an increase in control was observed with granules containing Coax. This higher level of infestation may have been due to the late timing of the experiment. After several attempts in June to use earlier planted corn were thwarted by rain, the experiment at Champaign was finally initiated on a mixed planting of late corn in July. This late planting, which was still in whorl stage in August, acted as a trap crop for naturally-occurring second generation corn borer moths. Plants that were not infested by hand were also heavily infested. Potentially, these data may be interpreted as an attempt to control corn borers in whorl stage corn under more natural conditions; i. e., infestation over a several week period. In this case, certain flour granules did very well in minimizing damage.

The greenhouse test, in most instances, yielded results that were typical of those in the field. Although the greenhouse test examined larval survival over a short period of time and field tests lasted through six weeks of larval development, the relative results were similar. While final testing is still necessary in the field to fully document the effects of any new formulation, the knowledge that greenhouse tests reflect, on a relative basis, what happens in the field, is important.

The economics of these formulations make them especially attractive for encapsulation. The ingredients are in surplus and the flours generally sell for less than \$0.44 per Kg as compared with Miragel (Staley, Inc., Decatur, IL), a pregelatinized starch which sells for approximately \$1.32 per Kg. The other ingredients used in our experiments are also inexpensive, and the versatility of this approach allows for incorporation of as many or as few active agents as desired. Obviously, this versatility opens up many new approaches and applications for insect control using granules. Only through thorough selection and screening of formulations for each specific application will the potential of these formulations be realized.

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References Cited

- Bartelt, R. J., M. R. McGuire and D. A. Black.** 1990. Feeding stimulants for the European corn borer (Lepidoptera: Pyralidae): additives to a starch-based formulation for *Bacillus thuringiensis* Berliner. *Environ. Entomol.* 19: 182-189.
- Dulmage, H. T. and E. Martinez.** 1973. The effects of continuous exposure of low concentrations of the δ -endotoxin of *Bacillus thuringiensis* on the development of the tobacco budworm *Heliothis virescens*. *J. Invertebr. Pathol.* 22: 14-22.
- Dunkle, R. L. and B. S. Shasha.** 1988. Starch-encapsulated *Bacillus thuringiensis*: a potential new method for increasing environmental stability of entomopathogens. *Environ. Entomol.* 17: 120-126.
1989. Response of starch-encapsulated *Bacillus thuringiensis* containing ultraviolet (UV) screens to sunlight. *Environ. Entomol.* 18: 1035-1041.
- Gillespie, R. L., M. R. McGuire and B. S. Shasha.** 1994. Palatability of flour granular formulations to European corn borer larvae (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 87: 452-457.
- Heimpel, A. M. and T. A. Angus.** 1963. Disease caused by certain spore forming bacteria. In E. A. Steinhouse [ed], *Insect Pathology: an Advanced Treatise*. Vol. 2., Academic Press, New York, pp. 21-73.
- Johnson, D. E. and B. Freedman.** 1981. Toxicity of *Bacillus thuringiensis* Spo-Cr⁺ mutants for the European corn borer *Ostrinia nubilalis*. *Appl. Environ. Microbiol.* 42: 385-387.
- Lund, R. L.** 1988. MSUSTAT statistical analysis package, vers. 4.1. Research and Development Institute, Bozeman, MT.
- Lynch, R. E., L. C. Lewis, E. C. Berry and J. F. Robinson.** 1977. European corn borer control with *Bacillus thuringiensis* standardized as corn borer international units. *J. Invertebr. Pathol.* 30: 169-174.
- Lynch, R. E., L. C. Lewis and E. C. Berry.** 1980. Application efficacy and field persistence of *Bacillus thuringiensis* when applied to corn for European corn borer control. *J. Econ. Entomol.* 73: 4-7.
- McGuire, M. R. and B. S. Shasha.** 1992. Adherent starch granules for encapsulation of insect control agents. *J. Econ. Entomol.* 85: 1425-1433.

- McGuire, M. R., B. S. Shasha, L. C. Lewis, R. J. Bartelt and K. Kinney.** 1990. Field evaluation of granular starch formulations of *Bacillus thuringiensis* against *Ostrinia nubilalis* (Lepidoptera: Pyralidae). J. Econ. Entomol. 83: 2207-2210.
- McGuire, M. R., B. S. Shasha, L. C. Lewis and T. C. Nelsen.** 1994. Residual activity of granular starch encapsulated *Bacillus thuringiensis*. J. Econ. Entomol. 87: 631-637.
- McWhorter, G. M., E. C. Berry and L. C. Lewis.** 1972. Control of the European corn borer with two varieties of *Bacillus thuringiensis*. J. Econ. Entomol. 65: 1414-1417.
- Ortega, A., S. K. Vasal, J. Mihm and C. Hershey.** 1980. Breeding for insect resistance to maize, Pp. 371-419. In F. G. Maxwell & P. R. Jennings (ed), Breeding plants resistant to insects. Wiley, New York.
- Salma, H. S., M. S. Foda, A. El-Sharaby, M. Matter and M. Khalafallah.** 1981. Development of some Lepidopterous cotton pests as affected by exposure to sublethal levels of endotoxin of *Bacillus thuringiensis* for different periods. J. Invertebr. Pathol. 38: 220-229.
- Shasha, B. S. and M. R. McGuire.** 1992. Adherent starch granules. U. S. Patent Application S/N 07/913,565.
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