

Dispersal of *Bacillus thuringiensis* Spores by Nonsusceptible Species of Stored-grain Beetles^{1,2}

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ABSTRACT

Rhyzopertha dominica (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *T. confusum* Jacquelin duVal, *Cryptolestes pusillus* (Schönherr), *Sitophilus granarius* (L.), and *S. oryzae* (L.) were not appreciably affected by a commercial formulation of *Bacillus thuringiensis* var. *kurstaki* on wheat or a flour-cornmeal mixture at 100 or 500 mg/kg. However, *R. dominica*, *S. oryzae*, *T. castaneum*, and *T. confusum* transferred viable spores from treated to untreated wheat by carrying them externally or passing them with their feces.

Formulations of *Bacillus thuringiensis* Berliner will control *Plodia interpunctella* (Hübner) and *Cadra cautella* (Walker) in stored grain (Kantack 1959, Godavaribai et al 1962, Burges 1964, van der Laan and Wassink 1964, McGaughey 1976). However, species of Coleoptera that infest stored grain are reported to be only slightly or not at all susceptible to *B. thuringiensis* (Steinhaus and Bell 1953, Shaikh and Morrison 1966, McGaughey 1976). Moreover, some stored-grain beetles are known to harbor several species of bacteria, apparently without ill effects, and also to transmit bacteria including *Salmonella montevideo* from contaminated to uncontaminated grain (Harein and de las Casas 1968, Crumrine et al. 1971, Husted et al. 1969, Schuster et al. 1972). The role of the beetles in transmitting *Salmonella* was considered minor because the concentration of inoculum necessary to achieve significant transmission is not likely to occur in stored grain. However, an application of *B. thuringiensis* that would be sufficient to control stored-grain moths would provide a concentrated inoculum that stored-grain beetles might disseminate. Studies were therefore made (1) to confirm the nonsusceptibility of selected stored-grain beetles, and (2) to determine whether these species could disseminate *B. thuringiensis* spores from treated grain to untreated commodities and to the surrounding environment.

Methods and Materials

The beetle species tested were: lesser grain borer, *Rhyzopertha dominica* (F.); sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *T. confusum* Jacquelin duVal; flat grain beetle, *Cryptolestes pusillus* (Schönherr); granary weevil, *Sitophilus granarius* (L.); and rice weevil, *S. oryzae* (L.).

Dipel®, a commercial formulation of *B. thuringiensis* var. *kurstaki* labelled as containing at least 25

billion viable spores/g and 16,000 International Units of Potency [*Trichoplusia ni* (Hübner)]/mg, was used to treat 2 media. Wheat of ca. 13% moisture content was treated with aqueous suspensions prepared in a tissue grinder in concentrations appropriate for use at 20 ml/kg of wheat. The suspensions were incorporated into the wheat by placing both in jars and then rolling the jars on a ball mill roller. A flour-cornmeal-brewers' yeast mixture (49.8:49.8:0.4) was treated with dry *B. thuringiensis* formulation added directly to the mixture in jars and then incorporated by tumbling the jars end over end on a Fisher-Kendall® dry materials mixer.

Effect of *B. thuringiensis* on Beetle Larvae

For the test of susceptibility of all beetle larvae except the granary weevil and rice weevil, wheat and the flour-cornmeal mixture were treated with doses of 100 or 500 mg of *B. thuringiensis* formulation/kg. Separate 1-pt mason jars containing 25 g of treated or untreated flour-cornmeal mixture were infested with eggs of the sawtoothed grain beetle (15 eggs/sample), red flour beetle (45), confused flour beetle (20), and flat grain beetle (50). These eggs were collected from 7- to 21-day-old beetles from the laboratory colonies in finely sifted flour, removed from the flour with a sieve, and counted by using a small aspirator and a dissecting microscope. Jars containing 100-g samples of treated or untreated wheat were infested with lesser grain borer eggs collected from laboratory colony adults by allowing them to oviposit between small pieces of heavy black paper fastened together so the eggs were placed only around the edges. Then the pieces of paper were separated and cut into segments bearing 20 eggs (counted by using a dissecting microscope) and inserted into each jar. All jars were held until adults emerged. Mortality was calculated from the difference in the numbers of eggs added and adults that emerged; percentages were corrected for mortality in the untreated samples.

Tests with the granary weevil and rice weevil were made by allowing 50 adults (7-21 days old) of each species from the laboratory colonies to lay eggs in 100-g samples of treated or untreated wheat. The adults were removed after 10 days, and the samples were held until the F₁ adults emerged. Mortality was calculated from the difference in the number of prog-

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eny that emerged from treated and untreated samples.

Tests with the confused flour beetle were replicated twice. Tests with all other species were replicated 5 times.

Effect of B. thuringiensis on Adult Beetles and Progeny

Fifty adults of each species were confined on 100-g samples of treated (100 or 500 mg/kg) or untreated wheat (rice weevil, granary weevil, and lesser grain borer), or on 25-g samples of treated or untreated flour-cornmeal mixture (sawtoothed grain beetle, red flour beetle, confused flour beetle, and flat grain beetle). Each sample was examined after ca. 7, 15, and 25 days, and percentage mortality was determined each time. The adults were removed from the samples after the final examination (ca. 25 days), and the samples were held until the F₁ adults emerged. Percentage reduction in progeny was calculated from the differences in numbers of adults that emerged from the treated and untreated samples. The tests were replicated 5 times.

Dispersal of B. thuringiensis Spores by Adult Beetles

The 1st test was to determine whether lesser grain borers, rice weevils, confused flour beetles, or red flour beetles could transfer *B. thuringiensis* spores from treated to untreated wheat. Two 10-cc samples of treated (50 mg/kg) wheat were placed in opposite quadrants of 4-section, 100×15-mm disposable petri dishes. Untreated wheat (10-cc samples) was placed in the remaining quadrants. Four of these dishes, plus 1 additional dish with untreated grain in all quadrants, were used for each species. Fifty insects were placed in each dish. After 5 days, the insects were removed, a portion of the untreated grain from each dish was washed by shaking vigorously in distilled water for 60 sec, and dilutions of the wash water were plated on ½-strength Difco® nutrient agar (3 plates/dilution). (Preliminary trials had been made to estimate the appropriate dilutions and amounts of wheat and wash water to be used on samples infested by each species.) Also, a sample of uninfested treated wheat and composite samples of treated wheat from each set of infested dishes were washed and plated. The plates were incubated at 25°C and 60% RH, and colonies were counted after 48 h.

In the 2nd test, ca. 500 adults were confined on separate 500-g samples of treated (50 mg/kg) and untreated wheat in 1-qt mason jars. After 5 days, each group of insects was removed with a sterile aspirator, washed by shaking vigorously in 25 ml of distilled water for 60 sec, and dried with a gentle stream of air. Then each group of washed and dried insects was placed in a clean inverted mason jar (no food provided) over a screen and feces were collected in a dish for 5 days. The insects in each group were counted before being discarded. In this test, dilutions of the water used to wash each group of insects were plated on ½-strength nutrient agar to estimate the number of spores carried externally by

each insect. Also, a sample of the feces from each group of insects was weighed and suspended in 6 ml of distilled water by using a tissue grinder. Then duplicate dilutions were prepared, 1 set for plating directly on ½-strength nutrient agar, and the other for plating after heat treatment at 65°C for 30 min to kill vegetative cells.

Finally, representative plates prepared from the washed wheat, washed insects, and feces were incubated for an additional 48 h and wet mounts prepared from typical colonies were examined microscopically to confirm identification of the bacterium. The presence of crystals and spores was considered confirmation of *B. thuringiensis*. Few colonies of extraneous species of bacteria occurred, and they were clearly distinguishable on the basis of colony morphology.

Results

Effect of B. thuringiensis on Beetle Larvae

Of the 7 species tested, only the lesser grain borer larvae showed any susceptibility to *B. thuringiensis*. However, mortality was so low (12% at a dose of 500 mg/kg and 7% at 100 mg/kg) that it was not economically significant. Mortality of the other 6 species was ≤3%. (The avg numbers of F₁ adults of granary weevils and rice weevils that emerged from the untreated samples were 225 and 1823, respectively.)

Effect of B. thuringiensis on Adult Beetles and Progeny

The susceptibility of adults of all 7 beetle species likewise was low. The highest mortalities were 10% among lesser grain borers exposed for 29 days to wheat treated at 500 mg/kg, and 8% among granary weevils exposed for 25 days on wheat treated at 100 mg/kg. Among the other species, adult mortalities were ≤2%. These levels would probably not be economically significant.

Progeny reduction was low (≤13%), and for all species it did not relate directly to the mortality of the respective adults or larvae in the previous tests. Thus, the number of lesser grain borer progeny was not reduced by either dose, although low mortality levels among adults and larvae were noted before; and the numbers of red flour beetle, confused flour beetle, and flat grain beetle progeny were reduced as much as 13%, while adults and larvae of those 3 species were unaffected before. The numbers of rice weevil and sawtoothed grain beetle progeny were not reduced, and the number of granary weevil progeny was reduced by only ca. 4%. (The avg numbers of F₁ adults that emerged from the untreated samples were: lesser grain borer 1812, granary weevil 2363, sawtoothed grain beetle 282, red flour beetle 236, confused flour beetle 283, rice weevil 3836, and flat grain beetle 465.)

Dispersal of B. thuringiensis Spores by Adult Beetles

The rice weevils transferred the most *B. thuringiensis* spores from treated to untreated wheat (aver-

age for 4 dishes was 24260 spores/g, $s_{\bar{x}} = 2304$), followed by confused flour beetles (11721 spores/g, $s_{\bar{x}} = 687$), red flour beetles (9578 spores/g, $s_{\bar{x}} = 1142$), and lesser grain borers (4142 spores/g, $s_{\bar{x}} = 480$). The lower level of spore transmission by the lesser grain borers probably reflects less movement about the dish. There was no evidence that beetle activity resulted in the movement of wheat kernels between quadrants of the dishes. The avg spore count for 5 composite samples of treated wheat was 6.56×10^6 /g. The background spore level in untreated infested wheat was ≤ 118 spores/g.

The numbers of spores washed from the bodies of the 4 species that had fed for 5 days in treated wheat also differed: rice weevils (1321 spores/insect) and lesser grain borers (1201 spores/insect) carried many more spores than did the confused flour beetles (266 spores/insect) or the red flour beetles (441 spores/insect). The number of spores per insect for all species after feeding for 5 days in untreated wheat was ≤ 0.7 .

The rice weevils, lesser grain borers, confused flour beetles, and red flour beetles were also capable of disseminating viable *B. thuringiensis* spores in their feces (Table 1). Moreover, the spore counts in feces from lesser grain borers, rice weevils, and confused flour beetles were reduced less than 25% by heat treatment of the dilutions, indicating less than 25% vegetative cells. The lack of a reduction for red flour beetles indicates that vegetative cells were not passed with its feces.

The values in Table 1 (number of spores/mg feces) were difficult to determine accurately because of contamination by body fragments. However, when most of the debris was removed, the avg amount of feces deposited by groups (1 from treated wheat and 1 from untreated wheat) of 500 insects of each species were: lesser grain borer ca. 2.2 mg, rice weevil ca. 14.2 mg, confused flour beetle ca. 12.0 mg, and red flour beetle ca. 11.4 mg. From these estimates, the confused flour beetles could be expected to distribute larger total numbers of spores in their feces than any of the other species, which did not differ greatly from each other in this respect.

Table 1.—Spores passed with feces of insects after feeding for 5 days in treated and untreated wheat.

Insect	No. spores/mg of feces after feeding on indicated wheat*			
	Untreated		Treated	
	No heat	Heat	No heat	Heat
Lesser grain borer	73	400	5041	4399
Rice weevil	2	0	1233	985
Confused flour beetle	0	9	8226	7061
Red flour beetle	2	0	532	536

* Duplicate dilutions were plated with and without heat treatment at 65°C for 30 min.

Discussion

Although *B. thuringiensis* applied to grain to control susceptible species of moths probably would not provide significant control of the species of beetles used in our tests, it might retard population increases of some species. The capability of these beetle species to disseminate viable *B. thuringiensis* spores to untreated grain or to the surrounding environment is significant but difficult to assess. We evaluated only the dissemination of spores. Though crystals would undoubtedly be carried externally by the beetles, the presence of intact crystals in feces is not known. Nevertheless, it is doubtful that enough spores or crystals would be transferred by beetles to untreated grain to affect infestations of any but the most susceptible species of stored-product Lepidoptera. *C. cautella* is highly susceptible to *B. thuringiensis* (McGaughey 1976), and Hagstrum and Sharp (1975) found that in stored citrus pulp containing *B. thuringiensis*, apparently not from deliberate treatment, as much as 9% incidence of diseased *C. cautella* larvae occurred during a storage season. In view of this, *B. thuringiensis* disseminated by beetles might significantly affect populations of *C. cautella*.

Perhaps a more significant effect of this dissemination would be the introduction of spores into processed commodities or the environment. Therefore, for use on stored grain, formulations that are free of spores or that contain spores that will not germinate might be preferred. Formulations containing killed spores may be as toxic to stored-product Lepidoptera as those containing live spores (McGaughey 1975).

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