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Honey Bees (Hymenoptera: Apidae) as Vectors of *Bacillus thuringiensis* for Control of Banded Sunflower Moth (Lepidoptera: Tortricidae)

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ABSTRACT A study was conducted in 1996 and 1997 to determine if honey bees, *Apis mellifera* L., could vector *Bacillus thuringiensis* Berliner variety *kurstaki* from hives equipped with a pathogen applicator to sunflower capitula and if the amount of *B. thuringiensis* deposited on the capitula would be sufficient to control the banded sunflower moth, *Cochylis hospes* Walsingham. The study demonstrated that honey bees became contaminated with *B. thuringiensis* as they exited hives equipped with filled pathogen applicators and deposited enough *B. thuringiensis* on the capitula to cause banded sunflower moth larval mortality. When 2 methods of applying *B. thuringiensis* were compared, the honey bee vectoring method gave better or equivalent control of the banded sunflower moth larvae than manual sprays, resulting in higher seed yields than manual sprays. The presence of honey bees also increased seed set which contributed to greater yield.

KEY WORDS *Helianthus annuus*, *Cochylis hospes*, *Apis mellifera*, *Bacillus thuringiensis*

BOTH SUNFLOWER PRODUCTION and beekeeping are important agricultural industries in North Dakota, where 48% of the nation's sunflower and 10% of the nation's honey is produced (NDASS 1997). The banded sunflower moth, *Cochylis hospes* Walsingham, is a major economic pest of sunflower in North Dakota (Charlet et al. 1987). Honey bees, *Apis mellifera* L., are the primary pollinators of sunflower (Sosa 1988), and in North Dakota, sunflower is an important foraging crop for honey bees. However, the use of chemical pesticides to control pest insects in sunflower, such as the banded sunflower moth, can have an adverse effect on honey bees and honey production, predators, and parasites. Thus, interest has developed in using alternative strategies for insect pest management that will provide effective pest insect control, but that will not harm honey bees. One such alternative is the entomopathogen *Bacillus thuringiensis* Berliner variety *kurstaki* which is registered for use on sunflower for control banded sunflower moth. Despite being non-toxic to honey bees, predators, and parasites and not being an environmental contaminant, its use in pest control is limited because it is susceptible to inactivation by sunlight (West 1984) and has a limited host range. Efforts to improve these qualities have not been entirely successful, although improvements have been made in the application of foliar-applied *B. thuringiensis* products (Young and Yearian 1986).

Honey bees have been studied as vectors of microbial biological control agents. They have been tested as vectors of fungi antagonistic to gray mold of strawberry (Peng et al. 1992) and of bacteria antagonistic to fire blossom blight of apple and pear (Thomson et al. 1993). Honey bees also have been tested as vectors of

Heliothis nuclear polyhedrosis virus (HNPV) against *Helicoverpa zea* (Boddie) on crimson clover flowers (Gross et al. 1994). The purpose of this study was to determine if honey bees could vector *B. thuringiensis* from hives equipped with a pathogen applicator to sunflower heads (capitula), and if the amount of *B. thuringiensis* deposited on the capitula was sufficient to control banded sunflower moth larvae.

Materials and Methods

Pathogen Applicator. The honey bee pathogen applicator (Fig. 1) was slightly modified from Gross et al. (1994). Instead of transparent Plexiglas, the applicator was constructed of black Plexiglas (0.3 cm thick) and was 6.0 cm high by 20.3 cm wide. A removable pathogen tray (1.9 cm wide, 20.3 cm long) can be inserted from either side of the applicator. The applicator was designed to be inserted in the front center of a modified bottom board of a honey bee hive. When in use, the pathogen tray was filled with a dust formulation of *B. thuringiensis*. Honey bees exiting the hive passed through the tray and became contaminated with *B. thuringiensis*. Returning honey bees entered through a separate pathway and did not pass through the applicator. When the applicator was not in use, it was removed and replaced with a wooden block insert. This allowed the hive to be converted back to a standard configuration with a common entrance and exit for the honey bees.

Sunflower Plot. A field plot (133.3 by 133.3 m²) at the North Dakota State University Research Site near Prosper, ND, was planted in late May in 1996 and 1997 to sunflower hybrid '894' at the rate of 20 kg seed per

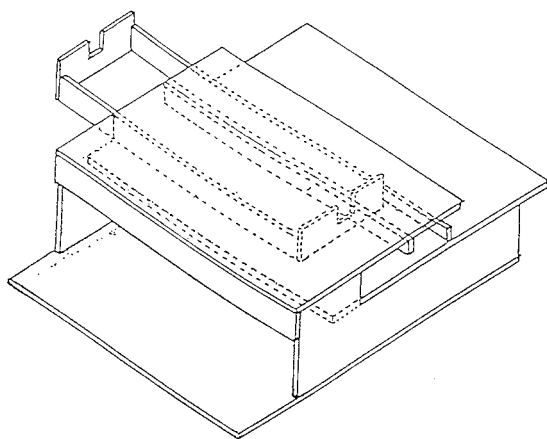


Fig. 1. Honey bee pathogen applicator with tray.

hectare with 76.2 cm between rows. The plants were thinned at the 2-leaf stage to be ≈ 25 –30 cm apart within the rows. The plot was subdivided into 4 blocks (66.7 by 66.7 m²) separated by alleys 3.5–4.5 m wide. Standard cultivation practices were used to maintain the sunflower plants for experimentation.

Honey Bee Vectoring Trials. One week before the experiments began, a honey bee hive (obtained from Don Nelson, a commercial beekeeper) with 2–3 full-size supers and a pathogen applicator (Fig. 2) was placed in the center of each of the 4 blocks. The

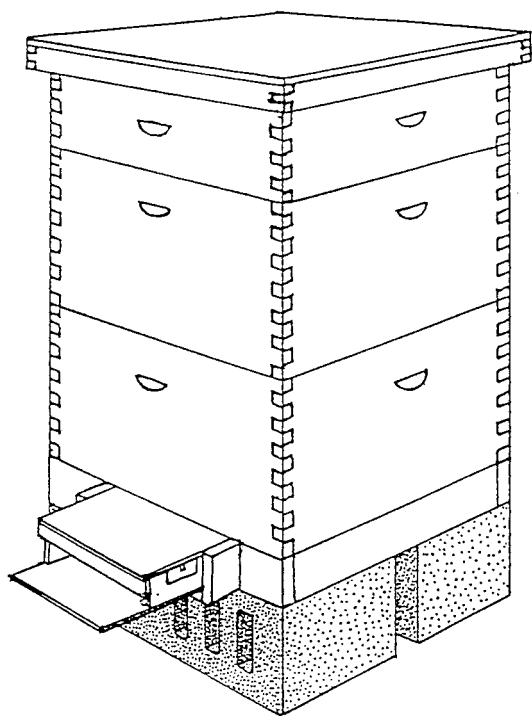


Fig. 2. Honey bee hive with a pathogen applicator in an operating position.

pathogen applicator was inserted to allow the honey bees time to adjust to the applicator before vectoring trials began. Vectoring trials took place at early blooming stage (R5.1–5.3, Schneider and Miller 1981) during the 2nd week of August both years. The vectoring trials consisted of filling the pathogen trays of the applicators with 50 g of Dipel 2 \times WP, a *B. thuringiensis* product (Chemical and Agricultural Products Division, Abbot, North Chicago, IL), and allowing honey bees to pass through the pathogen tray as they exited the hive. Two days later, the vectoring trial was repeated. Each trial period ran from 1000 to 1700 hours (CDT).

***Bacillus thuringiensis* on Honey Bees.** This study tested if honey bees became contaminated by *B. thuringiensis* as they exited hives with *B. thuringiensis*-filled pathogen trays. Treatments were as follows: (1) honey bees were captured as they exited hives with filled pathogen trays; (2) honey bees were captured on sunflower heads within the 1st 2 h of the vectoring trial; (3) honey bees were captured as they exited hives with the pathogen trays empty; and (4) a water control was used for subsequent bioassays. For each treatment on each vectoring trial, 30 honey bees were captured randomly and placed individually in snap cap vials (10 by 25 mm) containing 5 ml of distilled water. On the following day, the vials with the captured honey bees were individually shaken on a vortex machine for 30 s, and the wash solutions from the 30 samples per treatment were bulked. The solutions were used as possible sources of *B. thuringiensis* to treat artificial diet (Barker 1988) for bioassay of banded sunflower moth mortality.

Banded sunflower moth larvae were obtained from the USDA–ARS Biosciences Research Laboratory, Fargo, ND. Individual diet cups (30 ml) were filled with 4 ml of diet. After the diet had solidified and cooled, aliquots (150 μ l) of the bulked wash solutions were evenly applied to the surface of the artificial diet. Controls were treated with 150 μ l of distilled water. Three replications of 30 cups each were evaluated per treatment and control. After the treated diet surface had air-dried, a single neonate banded sunflower moth larva was placed in the center of each cup using a camel's hair brush and the cup covered with a plastic lid. The cups were placed in a growth chamber with a photoperiod of 14:10 (L:D) h and a temperature of $28.5 \pm 1^\circ\text{C}$. Larval mortality was determined after 3 d by examining larvae under a microscope.

***Bacillus thuringiensis* on Sunflower Capitula.** This study was designed to compare the relative potency of *B. thuringiensis* deposited on sunflower heads by honey bees to spray application of *B. thuringiensis* for the banded sunflower moth mortality. Treatments were as follows: (1) capitula exposed to contaminated honey bees; (2) capitula before exposure to contaminated honey bees sprayed with an aqueous solution of Dipel 2 \times WP at the recommended rate; (3) uncontaminated capitula (bagged to prevent exposure to contaminated honey bees and not sprayed); and (4) a water control for subsequent bioassays. Thirty capitula per treatment were randomly collected. Capit-

ula were placed in a plastic bag, brought to the laboratory, and frozen. On the following day, all the florets from the sampled capitula were excised, placed in beakers containing 100 ml of distilled water, and rinsed twice. Each wash solution was shaken on a vortex machine for 30 s, and the wash solutions from the 30 capitula per treatment were bulked. The wash solutions from each treatment, and distilled water as a control, were used to treat diet (Barker 1988) for bioassay against the banded sunflower moth larvae. The bioassay was conducted as described earlier.

Comparison of *B. thuringiensis* Application Methods. This study tested the efficacy of honey bees as vectors of *B. thuringiensis* compared with manual sprays of *B. thuringiensis* in controlling banded sunflower moth on sunflower. Treatments were as follows: (1) honey bee vectoring of *B. thuringiensis*; (2) hand-spraying of *B. thuringiensis*; and (3) control (untreated). Sampling sites radiated outward 7.6, 15.2, and 22.8 m from the center of each block in each of the 4 cardinal directions. When the sunflower plants were just beginning to reach R5.1 (bloom stage), 3 sunflower heads were randomly selected at each sampling site, and treatments were randomly assigned to the selected plants. The selected heads between R5.3 and R5.5 were infested with 50 banded sunflower moth eggs to augment natural banded sunflower moth field populations using the methods of Charlet and Brewer (1995).

One day before the initial vectoring trial, plants to be manually sprayed were treated with the recommended rate of Dipel 2x WP, a *B. thuringiensis* product, using a hand-operated sprayer. The hand-sprayed and control plants were covered with plastic mesh Delnet pollination bags (50.8 by 50.8 cm) (Applied Extrusion Technologies, Middletown, DE) to exclude bees. At physiological maturity (R9), the selected capitula were individually hand-harvested, oven-dried, hand-threshed, and cleaned. Random samples of 100 seeds from each capitula were used to determine the percentage of banded sunflower moth damaged seeds (using the methods of Peng and Brewer [1995]) and seed set by examining each seed in the lab under a microscope for the presence of exit holes and damaged kernels. A 2nd random sample of 100 seeds per capitula was used to measure seed weight in grams. Seed oil concentration was determined by nuclear magnetic resonance (Oxford 4000 NMR Analyzer, MN) in a random sample of seeds (30 ml). The procedures for preparations and analysis of sunflower by NMR have been described by Granlund and Zimmerman (1975). The seed yield (grams per head) was calculated for each treatment.

Statistical Analysis. PROC univariate, residual analysis was used to check if the data met assumptions of analysis of variance (ANOVA). The arcsine square-root transformation for percentage of damaged seeds and seed set was used for analysis (Steel et al. 1997). All values were analyzed using the general linear model procedure (SAS Institute 1995). When the *F* test for treatments was significant ($P < 0.05$), means

Table 1. Mean percentage mortality of banded sunflower moth larvae on diet treated with wash water from *B. thuringiensis*-contaminated and uncontaminated honey bees, Prosper, ND

Source of wash water	% mortality (mean \pm SE)	
	1996	1997
Contaminated bees exiting hives	98.7 \pm 1.1a	90.0 \pm 1.1a
Contaminated bees captured on capitula	80.8 \pm 3.6b	66.8 \pm 1.4b
Uncontaminated bees exiting hives	18.3 \pm 2.7c	9.4 \pm 0.6c
Control (water)	15.0 \pm 1.5c	7.2 \pm 0.5c

Means followed by the same letter in a column are not significantly different ($P \leq 0.05\%$, LSD).

were compared by using multiple *t*-tests or least significance difference (LSD).

Results

***Bacillus thuringiensis* on Honey Bees.** In both years, wash solutions rinsed from honey bees captured as they exited hives with filled pathogen trays or from honey bees captured on capitula induced significantly higher banded sunflower moth larval mortality (1996: $F = 261.52$; $df = 3, 15$; $P = 0.0001$; and 1997: $F = 353.92$; $df = 3, 9$; $P = 0.0001$) compared with the wash solutions from uncontaminated honey bees or control (Table 1). There was no significant difference in larval mortality between the wash solutions from uncontaminated honey bees and water as a control.

***Bacillus thuringiensis* on Capitula.** For both years, the wash solutions from capitula exposed to contaminated honey bees or from capitula manually sprayed with *B. thuringiensis* induced significantly higher larval mortality (1996: $F = 42.70$; $df = 3, 15$; $P = 0.0001$; and 1997: $F = 740.39$; $df = 3, 9$; $P = 0.0001$) than wash solutions from uncontaminated capitula or water controls (Table 2). There was no difference in banded sunflower moth larval mortality between wash solutions from uncontaminated capitula and water as a control.

Comparison of *B. thuringiensis* Application Methods. Application methods significantly affected the percentage of damaged seeds in both years (1996: $F = 140.98$; $df = 2, 36$; $P = 0.0001$; and 1997: $F = 116.10$; $df = 2, 36$; $P = 0.0001$). The honey bee vectoring method produced results equal to or better than a single *B. thuringiensis* spray application in both years (Table 3). Allowing honey bee access to the plants also resulted in a greater percentage of seed set (filled seeds) in

Table 2. Mean percentage mortality of banded sunflower moth larvae on diet treated with wash water from *B. thuringiensis*-contaminated and uncontaminated capitula, Prosper, ND

Source of wash water	% mortality (mean \pm SE)	
	1996	1997
Capitula contaminated by bees	86.7 \pm 3.3a	87.5 \pm 1.5a
Capitula sprayed with <i>B. thuringiensis</i>	58.3 \pm 5.3b	68.2 \pm 1.3b
Capitula unsprayed	8.3 \pm 1.7c	10.1 \pm 0.7c
Control (water)	8.3 \pm 2.4c	8.5 \pm 0.6c

Means followed by the same letter in a column are not significantly different ($P \leq 0.05\%$, LSD).

Table 3. Efficacy of *B. thuringiensis* application methods on mean percentage of damaged seeds, seed set, and yield parameters under field conditions, Prosper, ND

Method	Mean \pm SE	
	1996	1997
% damaged seeds		
Bee-vectored	12.1 \pm 0.2b	12.2 \pm 0.4c
Sprayed	12.5 \pm 0.2b	13.2 \pm 0.4b
Control	21.1 \pm 0.2a	22.3 \pm 0.4a
% seed set		
Bee-vectored	78.5 \pm 0.2a	78.2 \pm 0.3a
Sprayed	74.4 \pm 0.2b	74.5 \pm 0.3b
Control	74.5 \pm 0.2b	74.6 \pm 0.3b
% seed oil content		
Bee-vectored	40.9 \pm 0.4a	40.7 \pm 0.5a
Sprayed	40.3 \pm 0.6ab	40.2 \pm 0.5ab
Control	38.9 \pm 0.4b	39.0 \pm 0.6b
Seed wt, g/100 seeds		
Bee-vectored	5.2 \pm 0.1a	5.2 \pm 0.1a
Sprayed	5.1 \pm 0.1a	5.1 \pm 0.1a
Control	5.1 \pm 0.1a	5.2 \pm 0.1a
Seed yield, g/plant		
Bee-vectored	61.4 \pm 0.9a	60.0 \pm 1.3a
Sprayed	58.3 \pm 0.9b	57.4 \pm 1.2a
Control	51.7 \pm 0.9c	51.3 \pm 1.3b

Means followed by the same common in a column letter are not significantly different ($P \leq 0.05\%$, LSD or multiple *t*-tests).

both years (1996: $F = 84.03$; $df = 2, 36$; $P = 0.0001$; and 1997: $F = 50.94$; $df = 2, 36$; $P = 0.0001$) compared with the treatments where honey bees were excluded (Table 3). The effect of the application method on seed oil content also was significant in both years (1996: $F = 8.36$; $df = 2, 24$; $P = 0.0018$; and 1997: $F = 4.12$; $df = 2, 24$; $P = 0.0289$) (Table 3).

Application methods did not result in significant differences in seed weight in either year (Table 3). However, there were significant differences in seed yield both years (1996: $F = 24.62$; $df = 2, 36$; $P = 0.0001$; and 1997: $F = 11.98$; $df = 2, 36$; $P = 0.0001$). Seed yield was significantly higher on capitula exposed to contaminated honey bees than on controls in 1996 and 1997, and in 1996 yield of exposed capitula exceeded that of manually sprayed capitula (Table 3).

There were no significant differences in seed damage, seed set, seed oil content, and seed weight and yield caused by main effects (distance and date) and 1st-order interaction (distance by date, distance by method, date by method) and 2nd-order interaction (distance by date by method).

To measure if the efficiency of the honey bees decreased with distance from the hives, data from plants exposed to honey bee vectors were analyzed separately. None of the parameters measured was significant.

Discussion

Based on the tests of wash solutions from honey bees captured on capitula, *B. thuringiensis* did adhere to the honey bees. Gross et al. (1994) demonstrated

that honey bees acquired *Heliothis* nuclear polyhedrosis virus (HNPV) on their bodies and were successful in disseminating the HNPV to crimson clover flowers when they were allowed to exit through pathogen trays filled with the HNPV. Honey bees deposited sufficient HNPV on crimson clover flowers to significantly increase mortality of *H. zea* larvae (Gross et al. 1994). Peng et al. (1992) reported that the head, mouthparts, antennae, legs, and setae of honey bees became covered with *Gliocladium roseum* Link, a biocontrol agent for gray mold of strawberry, as they exited hives with pathogen applicators.

Honey bees also were effective in contaminating sunflower capitula with *B. thuringiensis*, and the amount of *B. thuringiensis* deposited on the capitula was sufficient to result in high larval mortality. The honey bee vectoring method resulted in a level of protection from banded sunflower moth damage equal to or superior to a single spray application of *B. thuringiensis*. In addition, the percentage seed set increased on capitula exposed to honey bees. This agrees with Freund and Furgala (1982) and Robinson (1983), who demonstrated that sunflower cultivars open to honey bee pollination have a higher seed set than those not exposed to honey bee pollination. Another aspect of sunflower yield is percentage seed oil content. The honey bee vectoring method resulted in a significantly increased seed oil content compared with the control, but not to the manual spray. Schelotto and Pereyres (1977) demonstrated that seeds from bee-pollinated sunflower have a higher oil content than self-pollinated seeds. In this study, the slight increase in oil content in seeds from plants contaminated with *B. thuringiensis* by honey bees was probably a result of the lower percentage of damaged seeds. Undamaged seeds have a higher kernel/hull ratio, and because most oil is in the kernel, undamaged seeds have a higher oil percentage based on weight. We expected to see a weight difference, because Parker (1981) found that seed weight was higher for bee-visited than not visited sunflower. However, none of the treatments increased 100 seed weight compared with the control.

The honey bee vectoring method significantly increased yields compared with the controls and, in one year, even to the sprayed sunflower. The increased seed yields seen in this study are probably caused by a combination of honey bee pollinator activity which increased seed set and *B. thuringiensis*-induced mortality of banded sunflower moth larvae, which resulted in fewer damaged seeds and florets. Langridge and Goodman (1981) showed that by themselves, honey bees can increase sunflower seed set by 11% and can increase oil content. In this study, seed set increased by $\approx 4\%$ in plants exposed to honey bees. Seed oil content in bee-vectored plants did not differ from that of sprayed plants.

There was a trend toward increasing seed damage and decreasing seed set with distance from hives, but the differences were not significant. This may be because the distances tested were too small to show effects. Langridge and Goodman (1981) reported no significant differences in seed set caused by honey

bees at 13 and 90 m from the hive, but there was a significant drop in seed set at 120 m from the hives.

An additional benefit of having honey bees in the sunflower fields was an increased seed set. Increased seed set could be caused either by honey bee pollination resulting in more fertilized seeds or fewer florets being destroyed by banded sunflower moth larval feeding because of the control provided by *B. thuringiensis*. However, in the latter case, manually sprayed plants also should have an increased seed set, but this did not happen.

Honey bees as vectors of *B. thuringiensis* can be an alternative to chemical insecticide sprays and can be used successfully to control the banded sunflower moth. In addition, to vectoring *B. thuringiensis* for control of the banded sunflower moth, honey bees also may help sunflower production by increasing seed set and oil content. Other pests found on flowering sunflower capitula or other flowering plants foraged by honey bees may be amenable to similar control methods if the pathogen or toxin used does not harm honey bees, if the honey bees can act as a vector, and if the material is effective in controlling the pests on blooming sunflower.

The benefit of honey bee pollination to sunflower growers may be underestimated. In addition to their potential use for pest control, honey bee activity can result in a significantly higher seed set (4%), and seed oil content (1%). Overall, there was an increase in seed yield of 10 g per plant in bee-visited plants compared with control plants. Currently in the United States, sunflower is selling for \$0.26/kg and is typically planted at a rate of 44,460 plants per hectare. Under these parameters and our test conditions, sunflower exposed to bees carrying *B. thuringiensis* would be valued at \$714/ha; sunflower sprayed with *B. thuringiensis* would be worth \$677/ha, and sunflower not treated would be worth \$603/ha, compared with no treatment. The gain from spraying with *B. thuringiensis* would be \$74/ha and from using honey bees to vector *B. thuringiensis* would be \$111/ha. The value of honey bees to sunflower production can be significant. Sunflower growers can increase seed yield, crop value, and perhaps seed oil content by using honey bees to pollinate their oilseed sunflower fields and to vector *B. thuringiensis* for control of banded sunflower moth larvae.

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