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Resistance in Sunflower and Interaction with *Bacillus thuringiensis* for Control of Banded Sunflower Moth (Lepidoptera: Tortricidae)

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ABSTRACT Four sunflower accessions were compared with a susceptible check, hybrid '894', in the greenhouse to determine their resistance to the banded sunflower moth, *Cochylis hospes* Walsingham, and their interaction with *Bacillus thuringiensis* Berliner variety *kurstaki.* Antibiosis, expressed as lower larval weights, was detected in all of the accessions. In addition to being antibiotic, sunflower accession Ames 3291 was antixenotic to banded sunflower moth oviposition and exhibited an additional impact on larval weight when *B. thuringiensis* was applied. By itself, *B. thuringiensis* provided better control of banded sunflower moth than the resistance tested. However, banded sunflower moth-resistant sunflower would be a good option when *B. thuringiensis* or another insecticide is not applied, and it may prevent the economic threshold from being reached.

KEY WORDS *Cochylis hospes, Helianthus annuus, Bacillus thuringiensis,* host plant resistance

THE BANDED SUNFLOWER moth, *Cochylis hospes* Walsingham, is an important economic pest of cultivated sunflower, *Helianthus annuus* L., in North America (Charlet et al. 1997). Early instars feed primarily on sunflower pollen and floral tissues, whereas 3rd and later instars feed on seeds and cause most of the economic damage to seeds (Rogers 1978, Charlet and Gross 1990). Chemical insecticides used for control of the banded sunflower moth can have adverse effects on natural enemies and pollinating insects (Rogers and Kreitner 1983). Consequently, interest has developed in using alternative strategies for management of insect pests of sunflower.

One alternative to chemical insecticides is host plant resistance, which can be a highly efficient component of insect pest management systems because it offers farmers an economical and ecologically reasonable means of suppressing insect pests (Smith 1989). The use of insect-resistant cultivars can result in population reduction of pest insects and fewer insecticide treatments and potentially can be integrated with biological control agents. In many cases, even moderately insect-resistant cultivars are useful in enhancing the effects of predators, parasites, and pathogens of pest insects on a variety of crops (Isenhour and Wiseman 1987, Kea et al.1978, Kartohardjono and Heinrichs 1984). Hare and Andreadis (1983) demonstrated that larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), were more susceptible to *Beauveria bassiana* when reared on less suitable solanaceous hosts. Hamm and Wiseman (1986) reported that the effect of nuclear polyhedrosis virus on fall armyworm, *Spodoptera frugiperda* (J. E. Smith), was enhanced when larvae were fed tissue from resistant maize lines. Richter et al. (1987) reported significantly different nuclear polyhedrosis virus LC_{50} values (median lethal concentration) for fall armyworm larvae fed tissue from different host plants. Bong et al. (1991) demonstrated that resistant maize, alone or in combination with cytoplasmic polyhedrosis virus, has a significant effect on the growth and development of *Helicoverpa zea* (Boddie).

Bacillus thuringiensis Berliner variety *kurstaki,* an entomopathogen, paralyzes the insect midgut and inhibits feeding in infected individuals (Falcon 1971). Commercial *B. thuringiensis* products have been used successfully to control lepidopterous insect pests on a variety of crops (Falcon 1971). There is little information available on the combined effects of mechanisms of sunflower resistance and *B. thuringiensis* on banded sunflower moth. The purpose of this study was to determine mechanisms of resistance in selected sunflower accessions and to study the interaction of plant resistance with *B. thuringiensis* for control of banded sunflower moth.

Materials and Methods

Four sunflower accessions (PI 172906, PI 195945, PI 480471, and Ames 3291) were obtained from the USDA–ARS Plant Introduction Station, Ames, IA. The accessions were chosen for testing because they exhibited resistance to banded sunflower moth in previous testing (NPGS 1998). Sunflower hybrid 894 was used as a susceptible check (hybrid 894 is a public domain hybrid that has been produced by a number of commercial sources). These accessions were grown in a greenhouse at North Dakota State University, Fargo, during the fall of 1996 and 1997. Plants were grown in 20-liter plastic pots filled with Sunshine mix #1 (Fisons Horticulture, Bellevue, WA). The recommended rate of a soluble fertilizer (20-19-18 N–P–K, Peat-Like Special; Peters Professional, Fogelsville, PA) and iron chelate micronutrient (Sprint 330; Ciba, Greensboro, NC) was applied 1 wk after transplanting. Natural light was supplemented with artificial lighting for a photoperiod of 16:8 (L:D) h, and the greenhouse was maintained at 22 ± 5°C. Plants were watered as needed.

Antixenosis. Greenhouse-grown sunflowers were used to test banded sunflower moth ovipositional preference in the greenhouse. Two free-choice trials were conducted using a randomized complete block design with 7–8 and 14–16 single plant replicates of each sunflower accession during the spring of 1997 and 1998, respectively. The experiments were initiated when plants were at the R3–4 growth stage (Schneiter and Miller 1981). Capitula (floral heads) with a portion of the stem were excised individually and transferred to an Erlenmeyer flask filled with 250 ml of distilled water. The capitula were randomly arranged in a circular fashion in a plastic cage (37 by 28 by 15 cm). Banded sunflower moth adults, reared in the laboratory according to procedures described by Barker (1988), were obtained from the USDA–ARS Biosciences Research Laboratory, Fargo, ND. A small plastic cup containing 10 male and 20 female adult banded sunflower moths (2 d old) was placed in the center of the cage. Females were allowed to oviposit for 24 h. Bracts from each capitulum were excised individually and examined in the laboratory under a microscope for banded sunflower moth eggs.

Antibiosis. Greenhouse-grown sunflower were used to test for antibiosis and interaction with *B. thuringiensis.* The experiments consisted of 4–6 single plant replicates per sunflower accession. A two-factor (2 × 5) split-plot arrangement was used in a randomized complete block design during the spring of 1996 and 1997. Main plot treatments were applications of *B. thuringiensis.* Subplot treatments were sunflower accessions. Banded sunflower moth eggs were prepared (Barker 1988) and applied at the rate of 75 eggs to each capitula at R5.1–5.3 growth stage according to methods of Charlet and Brewer (1995). After 72 h, 50% of the plants were sprayed with *B. thuringiensis* at the rate of 0.25 kg/ha (Javelin WG (wettable granules), 52,863 *Spodoptera* units per milligram; Sandoz, San Diego, CA), using a hand sprayer. The sprayer was calibrated to deliver 5 ml of the spray solution per capitula. At 3 wk after infestation, the capitula were excised and brought to the laboratory. All seeds from each capitula were examined for insect damage using a microscope. Numbers of live larvae and their weight were recorded.

Table 1. Number of banded sunflower moth eggs laid on sunflower capitula in greenhouse free-choice experiments

Sunflower accession	No. eggs laid per capitula[a]
PI 172906	72.0 ± 14.1a
PI 480471	50.2 ± 16.5ab
PI 195945	44.6 ± 5.6b
Hybrid 894	38.5 ± 6.4b
Ames 3291	12.5 ± 3.5c

[a] Means within a column followed by a common letter are not significantly different (LSD, $\alpha = 0.05$; ANOVA, $F = 11.1$; df = 4, 56; $P \leq 0.01$). Average of 2 greenhouse trials; 22 capitula were examined for each treatment.

Statistical Analysis. Residuals were analyzed to determine whether the data met assumptions of analysis of variance (ANOVA) (i.e., data were normal and variances homogeneous) (PROC Univariate; SAS Institute 1995). Variables not meeting the assumptions were transformed as log counts (Steel et al. 1997). Data were analyzed using the general linear model procedure (SAS Institute 1995). When the *F*-test for treatments was significant, means and least square means were compared using least significant difference (LSD) and *t*-tests, respectively ($P < 0.05$).

Results

Antixenosis. Fewest eggs were laid on Ames 3291 (Table 1). The rate of oviposition was greatest on PI 172906.

Antibiosis. For unsprayed sunflower accessions, fewer larvae were on PI 195945 and Ames 3291 than on PI 172906 and PI 480471 (Table 2). Fewest larvae were found on hybrid 894. There were no significant differences in numbers of larvae among the sunflower

Table 2. Number and weight of 3-wk-old banded sunflower moth larvae and number of damaged seeds on unsprayed and *B. thuringiensis* sprayed sunflower capitula in 2 greenhouse experiments

Sunflower accession	No. larvae[a]		wt. per larva, mg[b]		No. damaged seeds[c]	
	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed
PI 172906	67.6 ± 2.3aA	3.8 ± 0.5aB	11.8 ± 0.1bA	12.6 ± 0.9aA	69.9 ± 18.2bA	16.0 ± 5.4aB
PI 195945	15.4 ± 1.4cA	5.9 ± 0.8aB	10.0 ± 0.6cA	11.8 ± 0.7aA	48.7 ± 7.9bA	10.9 ± 3.7aB
PI 480471	22.7 ± 1.6bA	1.0 ± 0.0aB	11.9 ± 0.3bA	10.6 ± 0.2abA	4.39 ± 10.1bA	2.8 ± 0.2bB
Ames 3291	13.4 ± 1.2cA	2.6 ± 0.5aB	12.7 ± 0.5bA	6.1 ± 0.8bB	53.7 ± 12.4bA	13.7 ± 11.8aB
Hybrid 894	3.1 ± 0.5dA	1.8 ± 0.4aB	20.5 ± 2.0aA	9.8 ± 0.6bB	104.5 ± 12.7aA	16.5 ± 3.1aB

Means within a column followed by a common lowercase letter or within a row and variable followed by a common uppercase letter are not significantly different ($P > 0.05$, *t*-test).

[a] Means are significantly different for number of larvae per capitula 3 wk after artificial infestation with 75 banded sunflower moth eggs (ANOVA, treatment: $F = 768.8$; df = 1, 1; $P = 0.02$; accession: $F = 63.8$; df = 4, 4; $P < 0.01$; interaction: $F = 57.8$; df = 4, 4; $P < 0.01$).

[b] Mean larval weights were significantly different (ANOVA, treatment: $F = 14.8$; df = 1, 1; $P = 0.16$; accession: $F = 6.01$; df = 4, 4; $P = 0.05$; interaction: $F = 10.8$, df = 4, 4; $P = 0.02$).

[c] Mean numbers of damaged seeds were significantly different (ANOVA, treatment: $F = 26.8$; df = 1, 1; $P = 0.12$; accession: $F = 8.3$; df = 4, 4; $P = 0.03$; interaction: $F = 1.2$; df = 4, 4; $P = 0.43$).

accessions tested and hybrid 894 when they were sprayed with *B. thuringiensis.* Larval survival was lower on sunflower sprayed with *B. thuringiensis* than on unsprayed sunflower. The interaction between sunflower accessions and *B. thuringiensis* was significant.

When tested without *B. thuringiensis,* larval weight was lower on the test accessions than on hybrid '894' (Table 2). Among the accessions tested, larval weight was lowest on PI 195945. When larval weights between sprayed and unsprayed sunflower were compared, only accession Ames 3291 and hybrid 894 differed. The interaction between sunflower accessions and *B. thuringiensis* was significant.

Seed Damage. Without *B. thuringiensis,* fewer insect-damaged seeds were found on the accessions tested than on hybrid 894 (Table 2). However, when sunflower capitula were sprayed with *B. thuringiensis,* there were fewer insect-damaged seeds on PI 480471 than on the other sunflowers tested. For all sunflower tested, fewer insect damaged seeds were found on *B. thuringiensis* sprayed than on unsprayed capitula.

Discussion

Laboratory-reared banded sunflower moth females did not prefer Ames 3291 for oviposition. Therefore, the antixenosis trait of this accession could be of value in the development of resistance against adults of the banded sunflower moth.

Although few larvae were found on hybrid 894 that had not been sprayed with *B. thuringiensis,* the larvae that were collected weighed more than those on the other accessions tested (Table 2). Because hybrid 894 is susceptible, the larvae on it probably had developed faster and most had probably dropped off the plants before larval collections were made at 3 wk.

Antibiosis can be expressed in a number of ways, including low weight and reduced survival of larvae. Based on the weights of larvae reared on the unsprayed sunflower, all of the accessions tested were antibiotic compared with the susceptible hybrid 894 (Table 2). Because hybrid 894 was a susceptible check, it was expected that larvae would grow normally when *B. thuringiensis* was not present and would be large compared with larvae on antibiotic accessions. It also was expected that application of *B. thuringiensis* to hybrid 894 would result in larvae of low weight.

However, the response of larvae on *B. thuringiensis*-treated Ames 3219 was not expected. There was an additive response between the antibiosis of Ames 3291 and *B. thuringiensis.* The ratio of larval weights on treated and untreated Ames 3291 and susceptible hybrid 894 were equivalent (0.48) and different from the ratios of the other accessions (1). This suggests that the antibiosis mechanism in Ames 3291 differs from that in the other antibiotic sunflower.

B. thuringiensis provided better control of the banded sunflower moth than the resistance of the accessions. However, in situations where an economically important infestation of banded sunflower moths is not detected, natural resistance might provide sufficient control to avoid economic damage. Plant resistance is a good option for producers because it can be combined with *B. thuringiensis* or another insecticide. The partial protection offered by resistant sunflower would be most beneficial if it were based on the resistance traits found in Ames 3291 because of the additive effect of the resistance of this accession with *B. thuringiensis,* and because the combination of antixenosis and antibiosis in that accession could reduce the banded sunflower moth population and help prevent the economic threshold from being reached.

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