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OVIPOSITION BY THE BANDED SUNFLOWER MOTH, *COCHYLIS HOSPES* (LEPIDOPTERA: COCHYLIDAE) IN RESPONSE TO *HELIANTHUS ANNUUS* POLLEN[1]

John F. Barker and Sharon Grugel[2]

ABSTRACT

Oviposition on an artificial substrate by the banded sunflower moth *Cochylis hospes* Walsingham was examined in response to sunflower pollen *(Helianthus annuus)* and sunflower pollen extract. Sunflower pollen in quantities as small as 0.2 mg significantly reduced oviposition on an artificial substrate relative to a control without pollen. Aqueous pollen extract applied to the artificial substrate significantly reduced oviposition with respect to the control substrate that was treated with solvent. Banded sunflower moths have gained some reproductive or competitive advantage by ovipositing on the bracts of sunflower and a deterrent effect of pollen could, at least in part, have had functional significance in the development of a behavioral preference for the bracts of the sunflower head as an oviposition site.

Sunflower *Helianthus annuus* L., pollen has been found to stimulate oviposition by the sunflower moth, *Homeosoma electellum* Hulst (Delisle et al. 1989) and its European counterpart, *Homeosoma nubulellum* (Metayer et al. 1993). The life cycles of the banded sunflower moth *Cochylis hospes* Walsingham and *H. electellum* in North America have been described by Westdal (1949, 1975) and Allen (1944) respectively. The larvae of both pest species infest the sunflower head and feed on the florets, pollen and seeds. A difference in behavior between the two species is expressed in the preference of *C. hospes* to oviposit on the bracts of bud stage sunflower heads (Westdal, 1949) while *H. electellum* prefers the open inflorescences for oviposition (DePew, 1983). Ovipositing C. *hospes* females must respond to different cues or respond differently to the same cues that *H. electellum* responds to. The objective of this study was to examine the ovipositional response of *C.hospes* to pollen.

MATERIALS AND METHODS

Insects: Adult banded sunflower moths were collected from a laboratory colony maintained at 27° ± 1° C, 50% relative humidity, and a 15:9 L:D cycle. Sunflower heads in the R2 bud stage (Schneiter and Miller, 1981) are routinely used as the oviposition substrate in the banded sunflower moth rearing procedure. Fifty female and 20 male moths were collected about 24 h after eclosion and placed in a small vented mating cage that was 15 cm in diameter by 7 cm deep. The moths were provisioned with 5% sucrose solution and

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held for two days to allow eggs in the ovaries to mature and insure mating. Preliminary experiments had shown that oviposition peaked around the 3rd day after eclosion. On the third day after eclosion, 50 female and 20 male moths were transferred from the mating cages for bioassay an hour before lights off to each of 3 larger oviposition cages, measuring 34 × 24 × 12 cm (L × W × H). The sides of the cage had been cut out and covered with a screen to allow for air circulation. Each of the latter oviposition cages was considered to be one replicate of the bioassay.

Sunflower and pollen: Pollen and R2 sunflower heads were from sunflower hybrid 894 which is a standard research sunflower in the northern Great Plains. Pollen was collected by brushing the pollen into an envelope with an artists camel hair brush and stored in tightly closed vials at –20° C.

Bioassay of pollen on an artificial substrate: It had been found in preliminary experiments that moistened floral foam could be used as an artificial oviposition substrate for C. *hospes*. The floral foam was cut into 1 cm2 by 6 cm long pieces. Each piece of floral foam was held upright by impaling it on a nail shaft fixed perpendicularly to a plexiglass base. In each of seven replicates, 50 female moths had a choice of ovipositing on a control piece of artificial substrate and 5 pieces of experimental substrate that had 0.2, 0.7, 1.5, 3.0, or 6.0 mg of pollen applied to the surface. Experimental and control substrates were distributed at random within the cage. The moths, were allowed to oviposit overnight and the next morning they were removed from the cage within 2 h after lights on. The number of eggs laid on the experimental substrate and its control were counted and expressed as an average mean ± SEM. The smallest quantity of pollen tested was 0.2 mg because smaller quantities became increasingly difficult to accurately weigh and apply to the substrate. Controls were identical but had no pollen applied to the substrate. After application of the pollen, each piece of experimental substrate and the control was moistened to saturation with water because moist foam was more acceptable as an oviposition site than dry foam.

Bioassay of pollen extract on an artificial substrate: In each of six replicates, 50 female moths had a choice of ovipositing on pieces of control and experimental artificial substrates that were placed at random within each cage. Experimental substrates were treated with pollen extract and the controls were treated with solvent. The moths were allowed to oviposit overnight and the experiment was terminated as described above for bioassay of pollen. Extracts were prepared by weighing out 500 mg of pollen into each of four 50 ml centrifuge tubes and 20 ml of water or hexane solvent was added. The tube and contents were agitated on a vortex mixer for 1 min and then allowed to set for 10 min at 4 C before centrifugation at 7000 rpm for 10 min. The extract (supernatant) was poured through a Whatman #4 filter into a beaker containing pieces of the foam oviposition substrate to saturate them with extract. Hexane was allowed to evaporate from the experimental and control substrates for 30 minutes because of toxicity to the insects. Moisture as added to the hexane treated and control substrates before placing in the test cage.

Statistical methods: Data were analyzed with ANOVA, SNK (Student-Newman Keuls) method for separation of significant means and Mann-Whitney Rank Sum Test using Jandel Scientific Sigmastat software. Data presented in the tables were the raw data means ± SEM.

RESULTS AND DISCUSSION

Bioassay of pollen on an artificial substrate: An artificial substrate can be useful in testing the effect of individual host plant components on the

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oviposition behavior of an insect. In choice experiments, banded sunflower moths laid significantly more eggs on the control substrate than on each of the experimental substrates treated with 0.2 mg to 6 mg of pollen ($F = 12.2$; $df = 5,36$; $p = 0.001$) (Table 1). The data suggested that pollen in isolation from other sunflower components had deterrent properties although it did not completely block oviposition on the artificial substrate.

Bioassay of pollen extracts on an artificial substrate: Support for a pollen component with deterrent properties was obtained with aqueous pollen extracts which significantly reduced oviposition on the artificial substrate compared to the control that was treated with solvent ($p=0.015$) (Table2). Oviposition in response to an extract prepared with hexane did not have deterrent properties although the pollen residue from the hexane extract was still deterrrent. The ratio of eggs laid on the substrate treated with hexane compared to the control was 1.9 : 1. Banded sunflower moths have gained some reproductive or survival advantage by laying their eggs on sunflower bracts. In the adaptation of the banded sunflower moth to sunflower, cues from the water soluble factor were apparently strong enough to mask the effects of hexane soluble components that are present in pollen. The oviposition behavior of the banded sunflower moth is likely to involve a combination of cues from the bracts of the host plant such as attractants, texture, and moisture that interact with pollen deterrency to re-inforce oviposition on the bracts. The presence in pollen of a water soluble component that inhibited banded sunflower moth oviposition has potential for the development of a control strategy for this pest insect if it can be identified and then amplified in sunflower bracts to discourage oviposition and reproduction of this species.

Table 1 Oviposition by the banded sunflower moth on an artificial substrate in the presence of pollen.

Treatment	# of eggs
H_20 control	584.8 ± 98.4a
0.2 mg pollen	234.8 ± 46.4b
0.7 mg pollen	108.8 ± 22.8b
1.5 mg pollen	112.8 ± 24.4b
3.0 mg pollen	161.4 ± 33.2b
6.0 mg pollen	159.6 ± 34.2b

Means with same letter superscripts are not significantly different ($p = 0.001$ SNK mean separation. Number of replicates = 7

Table 2. Effect of aqueous pollen extracts on oviposition by the banded sunflower moth.

Treatment	# of eggs
Control	363.7 ± 86.3a
Aqueous extract of pollen	8.5.0 ± 16.4b

a,bSignificantly different by the Mann-Whitney Rank ßSum Test ($p = 0.015$)

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