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Author(s): Juan D. López, Jr., Mohamed A. Latheef and W. Clint Hoffmann
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Effect of Hexaflumuron on Feeding Response and Reproduction of Bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)[1]

Juan D. López, Jr., Mohamed A. Latheef, and W. Clint Hoffmann[2]

USDA-ARS, 2771 F&B Road, College Station, TX 77845

Abstract. Hexaflumuron (Consult® 100 EC, Dow AgroSciences) is an insect growth regulator that inhibits chitin synthesis. The efficacy of hexaflumuron mixed with 2.5-M sucrose (mg^{-l}) was evaluated for toxicity, proboscis extension, gestation, and reproduction of bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), in a laboratory. The intention was to determine whether or not hexaflumuron could be used as a toxicant in a feeding-stimulant mixture for suppression of bollworm. Newly emerged bollworm adults actively seek carbohydrates. For feral male bollworm captured in pheromone-baited traps, the lethal concentration (LC_{50}) values 24 and 48 hours after exposure were 295.6 and 96.03 mg^{-l}, respectively. These values were significantly different. Hexaflumuron at 100 mg^{-l} or more significantly depressed gustatory response of feral male and laboratory-reared male and female bollworms. There was no significant difference in proboscis-extension response between bollworms fed 1.0 and 2.5 M sucrose solutions. Percentage hatch of eggs oviposited by laboratory-reared females during 3 consecutive days was significantly less at concentrations of 5.0 mg^{-l} or greater of hexaflumuron when compared with females fed 2.5-M sucrose. However, when laboratory-reared males fed hexaflumuron were compared with males fed 2.5-M sucrose solution, percentage of eggs that hatched was not significantly less. This suggests that hexaflumuron acted as an ovicide. It is posited that hexaflumuron could be a useful toxicant in a feeding-stimulant mixture for suppression of bollworm.

Introduction

Bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is a destructive pest of agricultural crops throughout the world, primarily because of its long-range movement as an adaptive strategy (Farrow and Daly 1987). In North America, bollworm is the second most important pest species in cotton, *Gossypium hirsutum* L., and Fitt (1989) estimated that the Heliothine complex caused more than US $1 billion in damage despite use of insecticidal applications costing US $250 million for controlling these pests each year. Insecticidal control primarily targeting the immature stages of the insect could cause health issues for pest control applicators and consumers. Development of control strategy alternative to conventional insecticidal control procedures could lead to reduction in pesticide use, worker and environmental contamination, and energy consumption.

[1]Mention of a commercial or proprietary product does not constitute an endorsement for its use by the U. S. Department of Agriculture. USDA is an equal opportunity provider and employer.

[2]Corresponding author email: clint.hoffmann@ars.usda.gov

Feeding attractants and stimulants have received increasing attention for areawide management of bollworm, primarily because the adults must feed on floral nectaries and honeydew secretions from plants to reproduce (Lingren et al. 1998, Del Socorro et al. 2010, Gregg et al. 2010). This nocturnal behavior of the bollworm has potential for exploitation as a pest management strategy for suppression by an adult feeding approach. This approach entails the use of a feeding attractant and stimulant in combination with a toxicant that when ingested by the adult will reduce fecundity/fertility at sub-lethal dosages or kill the adult.

A toxicant that may have potential for use in adult control technology is hexaflumuron. Hexaflumuron is a benzoylphenylurea that inhibits chitin synthesis and mainly acts as an ovicide and larvicide (Retnakaran and Wright 1987). It has contact toxicity to adults of pink bollworm, *Pectinophora gossypiella* (Saunders) (El-Barkey et al. 2009), cotton leafworm, *Spodoptera littoralis* Boisduval (Hossain et al. 1996), and African armyworm, *Spodoptera exempta* (Walker) (Degheele et al. 1993). Exposure of adults to hexaflumuron caused fewer cotton leafworm eggs to hatch (Hossain et al. 1996). Feeding of newly eclosed adult beet armyworm, *Spodoptera exigua* (Hübner), on 10% honeywater containing 100 mg^{-1} of chlorfluazuron, an acylurea chitin synthesis inhibitor, resulted in only 16% hatch of eggs (Van Laecke et al. 1989).

One objective of this research was to determine the toxicity of hexaflumuron mixed with a feeding stimulant ingested by adult bollworm. Another objective was to assess the effects of lethal or sublethal concentrations of hexaflumuron on feeding response (both proboscis extension and gustatory) and reproduction of bollworm.

Materials and Methods

Hexaflumuron (Consult® 100 EC containing 100 gm a.i/l (0.8344 lb/gal) (Dow AgroSciences, King's Lynn, Norfolk, England) dilutions were prepared (mg^{-1} a.i wt/vol) in 1.0- and 2.5-M sucrose (Sigma, St. Louis, MO) solutions. Male bollworms captured in pheromone-baited traps and laboratory-reared females and males were used. Feral males were captured using 75-50 Texas pheromone traps (Hartstack et al. 1979) baited with Zealure® (Hercon Environmental, Emigsville, PA). The traps were in an agricultural area (latitude 30.61º N and longitude 96.32º W) in the Brazos River Valley near College Station, TX. Only males trapped the previous night and provided with deionized water before testing were used. Laboratory-reared bollworm pupae were obtained from Jamie Whitten Delta States Research Center, USDA-ARS, Stoneville, MS. Pupae during emergence were sexed and placed separately without food in 3.8-liter glass jars. Only females and males that emerged the previous night before each study were used for testing.

A series of hexaflumuron concentrations prepared in 2.5-M sucrose solution and 2.5-M sucrose alone as a check were fed to male bollworms captured in sex pheromone traps. Groups of 10 males that had fed on the same concentration were placed without food in 0.92-liter glass jars and checked for mortality at 24 and 48 hours. A male was considered dead when it could not right itself when upside down.

Proboscis-extension response of male bollworms captured in sex-pheromone traps was evaluated under red light at night in an insectary. A series of sublethal concentrations of hexaflumuron prepared in 1.0- and 2.5-M sucrose solutions were placed in the wells of porcelain spot plates and the moths were allowed to contact

test solutions with their front tarsi. Responses of bollworm were compared with moths fed either a 1.0- or 2.5-M sucrose solution. The moths were observed for a positive proboscis-extension response that required contact of the test solution with the proboscis. A total of 10 replications was used, each consisting of the response of 10 males per test solution.

To determine gustatory response, solutions of hexaflumuron were weighed on an electronic balance (Model No. A-200DS, Denver Instrument Co., Denver, CO) before and after feeding of laboratory-reared females and males. Adults were mounted individually on a feeding apparatus (Fig. 1) and offered the test solutions contained in a disposable polystyrene microcentrifuge tube (0.5 ml). Check solutions exposed simultaneously to the same experimental conditions were used to account for loss by evaporation. The feeding apparatus used was described by Lopez and Lingren (1994) and Clemens (1996).

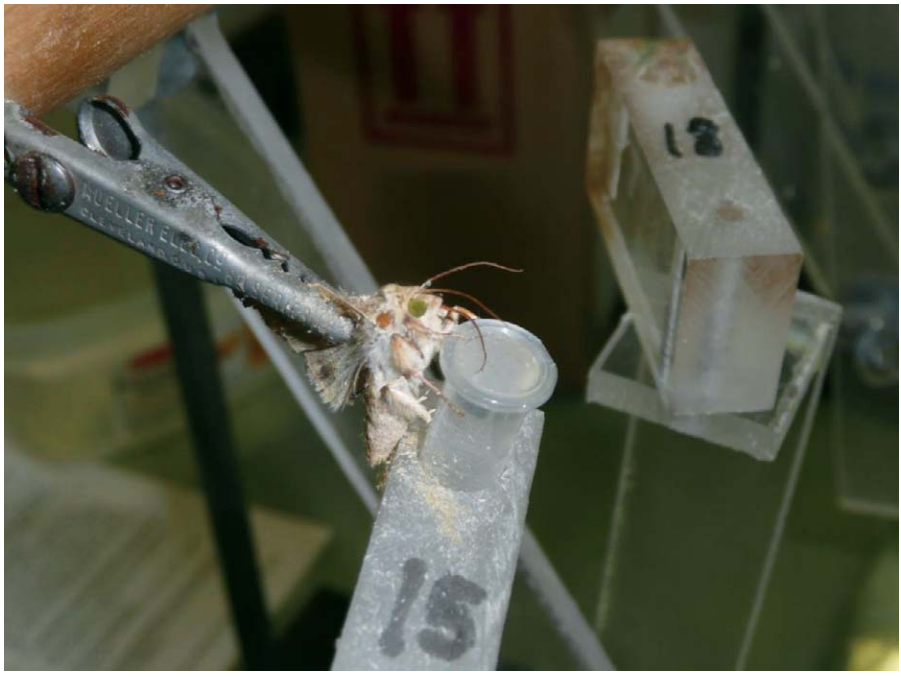

Fig. 1. Feeding apparatus used to study the gustatory response of *H. zea*.

Three tests were used to determine the effects of hexaflumuron on reproduction of the bollworm. In tests 1 and 2, hexaflumuron concentrations evaluated were 1, 10, 100, and 1,000 mg^{-l} and 2.5, 5.0, 10.0, 25.0, and 50.0 mg^{-l}, respectively. Females were fed hexaflumuron, and were compared with females fed 2.5-M sucrose solution. All females were paired with males. In test 3, laboratory-reared males were fed hexaflumuron at 2.5, 5.0, 10, 25, and 50 mg^{-l} and check males were fed 2.5-M sucrose solution, and were paired with females. In

each test, females and males within 24 hours after emergence were fed test concentrations. The amount of test solutions ingested by each sex in one half hour was determined as described previously for gustatory response. At the end of each test, moths were provided with 10% sucrose solution and placed in a 0.92-liter glass jar. Paper towel cut into circular strips was used to close the mouth of the jar, and a piece of paper towel strip suspended inside the top of the jar served as a climbing, resting, and ovipositional substrate. Moths were moved to clean jars after the 2nd day and each day thereafter for 3 days. Paper towel containing a sample of as many as 30 eggs was collected from each jar and placed in a sealed plastic cup. Eggs were checked for the number of larvae that hatched 2 days after collection and for 3 consecutive days. At the end of each test or in the middle of the test if the females died or were in copula, females were dissected to determine the number of spermatophores in the bursa copulatrix. Eggs from nonmated females were not used to determine the number of larvae that hatched from the eggs.

All data were analyzed using the PROC GLM procedure of SAS (2002). Means with significant *F*-values were separated with Tukey's Studentized Range (HSD) Test with $P = 0.05$, except for one occasion when means were separated at $P = 0.1$ (see results). Lethal concentration (LC) values were computed using the PROC PROBIT procedure with LACKFIT option. The significance of LC values were separated based upon the lack of overlap at the 95% upper and lower confidence limits.

Results and Discussion

Toxicity. Hexaflumuron was toxic to male bollworms captured in pheromone-baited traps (Table 1). The LC_{50} of 295.60 mg^{-l} at 24 hours was significantly different from that of 96.03 mg^{-l} at 48 hours. This significant difference between the LC_{50} values at 24 and 48 hours indicated hexaflumuron is a relatively slow-acting insecticide. Compared with other insecticides that have been evaluated in the same way (Clemens 1996, Lopez et al. 1997), hexaflumuron has relatively low oral toxicity. Based on the LC_{10} values of 82.9 and 24.9 mg^{-l} at 24 and 48 hours, respectively, sublethal concentrations probably are less than 25 mg^{-l}.

Table 1. Toxicity of Hexaflumuron (mg^{-l}) Mixed in 2.5 M Sucrose to Male Bollworm Captured in Pheromone-baited Traps, and Expressed as 24- and 48-hour Response in Lethal Concentration (LC) Values

	Number of hours of feeding	
Probit statistics	24	48
Slope (± SD)	3.98 ± 0.36	3.74 ± 0.53
χ^2	5.95 (4)ns	8.78 (4)ns
LC_{10}[1]	82.9a	24.86b
95% CLs	(66.13-98.42)	(8.95-39.81)
LC_{50} [1]	295.6a	96.03b
95% CLs	(259.0-346.6)	(71.33-122.53)
LC_{90}[1]	1,054a	370.83a
95% CLs	(797.82-1,552)	(248.86-861.14)

[1]LC_{x0}s followed by the same lowercase letter in each row are not significantly different based upon lack of overlap in 95% confidence limits. ns = not significant at $P = 5\%$ (SAS 2002).

Proboscis-Extension Response. Sublethal concentrations of hexaflumuron were mixed in 1- and 2.5-M sucrose solutions, and their effects vis-à-vis proboscis-extension response of males captured in sex pheromone traps were evaluated. Molarity of sucrose solutions did not significantly influence extension of the proboscis (F = 0.03; df = 1, 108; P > 0.05). Hexaflumuron, however, did significantly influence proboscis-extension response of bollworm (F = 2.41; df = 5, 108; P < 0.05). There was no significant interaction between hexaflumuron and molarity of sucrose solutions (F = 0.63; df = 5, 108; P > 0.05). Therefore, the proboscis-extension response of bollworm males for 1- and 2.5-M sucrose solutions was combined (Fig. 2). The proboscis-extension response was significantly less at 50 mg^{-l}. Because proboscis extension is the initial response necessary for feeding, these results indicated that hexaflumuron could be used at sublethal concentrations of 25 mg^{-l} or less in the field without interference with initiation of feeding.

Gustatory Response. The gustatory response of laboratory-reared female bollworms was significantly less compared with 2.5-M sucrose at 100 mg^{-l} or greater (F = 44.5; df = 5, 99; P < 0.001). Gustatory response was not significantly less at concentrations to 10 mg^{-l} (Fig. 3). There was no significant difference in gustation between 1,000 and 10,000 mg^{-l}. Although the inhibitory effect of hexaflumuron on gustation is for laboratory-reared females, it is likely the inhibition of gustatory response also influenced the LC values for the male bollworms captured in pheromone-baited traps. Also, there was no significant difference in bollworm gustation between 2.5, 5, 10, 25, and 50 mg^{-l} concentrations of hexaflumuron (F = 2.33; df = 5, 54; P > 0.05; Fig. 4).

Similar to proboscis-extension response, we wanted to determine whether or not gustatory response was influenced by molarity of sucrose solutions. Gustatory response was evaluated at 10, 25, and 50 mg^{-l} using 1- and 2.5-M sucrose solutions. Gustatory response was significantly influenced not only by molarity of sucrose solutions (F = 56.86; df = 1, 108; P < 0.001), but also by concentrations of

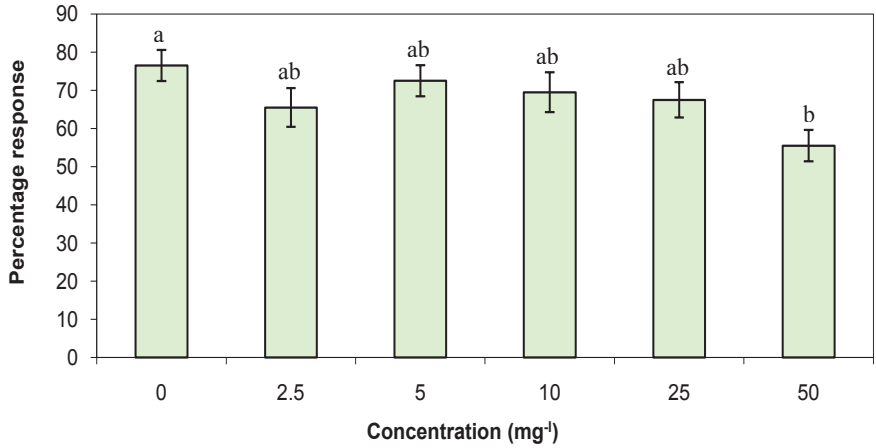

Fig. 2. Mean proboscis-extension response (± SD) of female bollworm fed hexaflumuron (n = 100) (the same letters on a column indicate no difference (P > 0.05) between treatments (HSD test)).

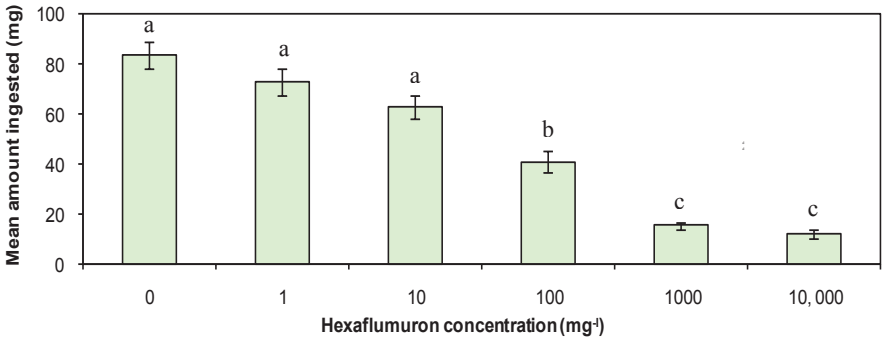

Fig. 3. Mean amount of hexaflumuron (± SD) consumed by laboratory-reared bollworm females (the same letters on a column indicate no difference (P > 0.05) between treatments (HSD test)).

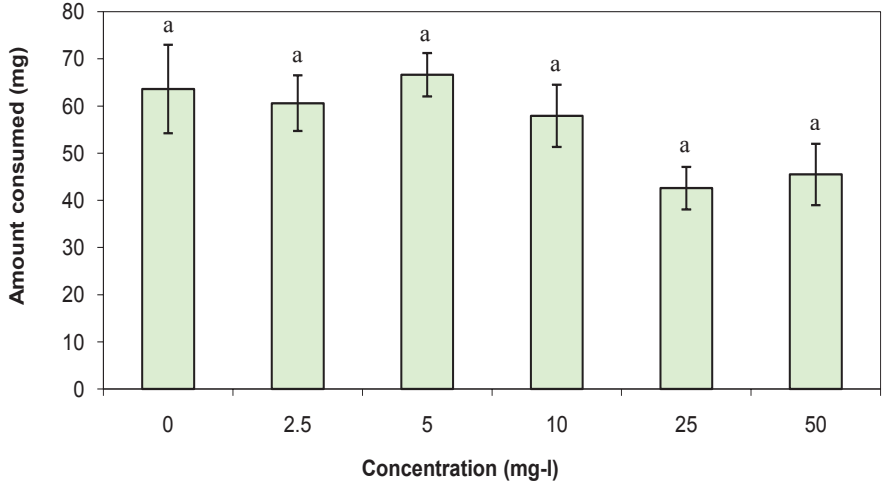

Fig. 4. Mean amount of hexaflumuron (± SD) consumed by laboratory-reared bollworm females (the same letters on a column indicate no difference (P > 0.05) between treatments (HSD test)).

hexaflumuron (F = 19.93; df = 2, 108; P < 0.001). However, gustatory response was not significantly influenced by gender (F = 1.48; df = 1, 108; P > 0.05). There was a significant three-way interaction between hexaflumuron, molarity, and gender (F = 3.09; df = 2, 108; P < 0.05). Fig. 5 shows that females fed significantly less on 1.5-M hexaflumuron at 50 mg^{-l} when compared with 25 mg^{-l} or females fed 1.5-M sucrose solution alone. Similarly, males fed significantly less on hexaflumuron at 50 mg^{-l}. Gustatory response at 25 and 50 mg^{-l} was comparable. These results were similar to those obtained for 2.5-M sucrose solution.

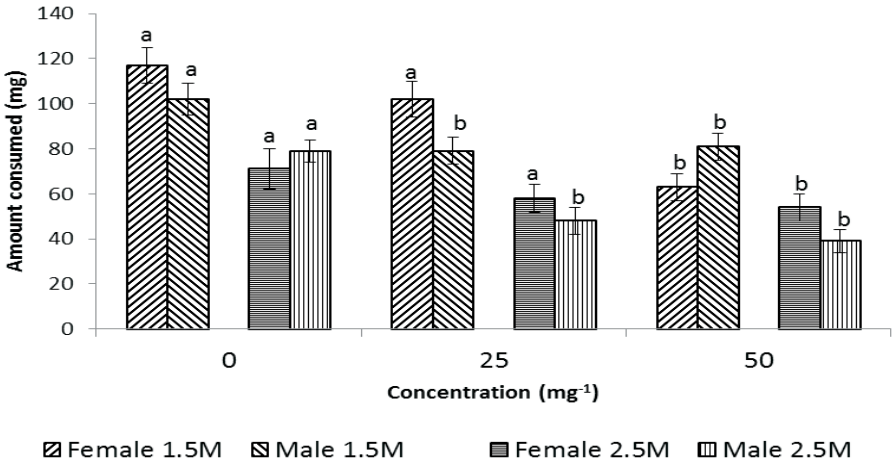

Fig. 5. Mean amount of hexaflumuron (± SD) mixed in 1- and 2.5-M sucrose solutions consumed by bollworm females and males (the same lowercase letter within each sex within the same molar sugar solution was not significantly different (HSD test, $P > 0.05$)).

Egg Hatch. In test 1, there was a significant reduction in percentage hatch of eggs when hexaflumuron at 1, 10, 100, and 1,000 mg^{-l} in 2.5-M sucrose solution was fed to females for 3 days ($F = 38.77$; df = 4, 69; $P < 0.0001$ for Day 1; $F = 63.06$; df = 4, 69; $P < 0.0001$ for Day 2; $F = 76.01$; df = 4, 65; $P < 0.0001$ for Day 3). The percentage of eggs that hatched was significantly less at concentrations of hexaflumuron as low as 1 mg^{-l} (Table 2). Data were not obtained for 10,000 mg^{-l} because females died soon after ingestion. The largest decrease in percentage of eggs that hatched was observed at 1,000 $mg^{-l,}$ which was not significantly different from 10 or 100 mg^{-l}. It is likely that gustatory-response inhibition at hexaflumuron concentrations of 100 mg^{-l} or greater influenced the reduced effects on larval hatch of eggs at the 100 and 1,000 mg^{-l} concentrations. In test 2, female bollworms were fed hexaflumuron at 2.5, 5, 10, 25, and 50 mg^{-l}, and the percentage of eggs that hatched was significantly less for all 3 days of testing when compared with bollworms fed 2.5-M sucrose alone ($F = 14.5$; df = 5, 80; $P < 0.0001$ for Day 1; $F = 29.8$; df = 5, 82; $P < 0.0001$ for Day 2; $F = 49.1$; df = 5, 84; $P < 0.0001$ for Day 3, Table 3). When male bollworms were fed hexaflumuron at 2.5, 5, 10, 25, and 50 mg^{-l}, and compared with males fed 2.5-M sucrose solution only (Test 3, Table 4), there was no significant difference in the percentage of eggs that hatched during all 3 days of observations ($F = 0.51$; df = 5, 28; $P > 0.05$ for Day 1; $F = 0.49$; df = 5, 28; $P > 0.05$ for Day 2; $F = 0.34$; df = 5, 27; $P > 0.05$ for Day 3). This suggests that feeding male bollworm hexaflumuron did not influence the percentage of eggs that hatched. Similar result was found for sugarbeet leaf weevil, *Aubeonymus mariaefranciscae* Roudier by Marco et al. (1998) who reported that no egg hatch inhibition was observed when male weevils treated with hexaflumuron were mated with nontreated females. The predominant effect on the percentage of eggs that hatched after feeding female bollworms with hexaflumuron indicates hexaflumuron

probably acted as an ovicide, confirming previous reports (Retnakaran and Wright 1987, Horowitz et al. 1992, Hossain et al. 1996).

Table 2. Mean Percentage Hatch of Eggs Deposited by Female Bollworm Fed During a 3-day Period Various Concentrations of Hexaflumuron Paired with Males Fed 2.5-M Sucrose Solution (Test 1)

Concentration	Mean % ± SD of eggs that hatched[1]			
(mg^{-1})	Day 1	Day 2	Day 3	Total
0	81.6 ± 2.4a	85.2 ± 2.4a	82.6 ± 3.0a	82.6 ± 1.8a
1	41.9 ± 8.4b	56.5 ± 6.6b	40.1 ± 8.9b	45 ± 7.2b
10	8.5 ± 3.9c	8.6 ± 5.8c	0 ± 0c	5.8 ± 2.5c
100	11.6 ± 6.0c	4.4 ± 4.2c	1.2 ± 1.2c	5.6 ± 2.7c
1,000	1.6 ± 1.6c	0 ± 0c	0 ± 0c	0 ± 0c

[1]Means followed by the same lowercase letter in a column are not significantly different according to Tukey's HSD test (P = 5%).

Table 3. Mean Percentage Hatch of Eggs Deposited by Female Bollworm Fed During a 3-day Period Various Concentrations of Hexaflumuron Paired with Males Fed 2.5-M Sucrose Solution (Test 2)

Concentration	Mean % ± SD of eggs that hatched[1]			
(mg^{-1})	Day 1	Day 2	Day 3	Total
0	75.8 ± 8.5a	79.4 ± 5.3a	84.3 ± 6.1a	80.8 ± 5.8a
2.5	72.4 ± 6.2a	69.7 ± 7.5a	70.0 ± 8.2b	69.8 ± 7b
5	28.7 ± 6.7b	10.2 ± 4.2b	0 ± 0c	13.5 ± 3.3b
10	20.4 ± 7.5bc	6.7 ± 5.1b	0.2 ± 0.2c	7.2 ± 3.2b
25	3.1 ± 1.6c	0.3 ± 0.3b	0 ± 0c	1.4 ± 0.7b
50	18.6 ± 6.1bc	5.5 ± 4.8b	4.5 ± 4.5c	9.8 ± 4.7b

[1]Means followed by the same lowercase letter in a column are not significantly different (P > 0.05) according to Tukey's HSD test.

Table 4. Mean Percentage Hatch of Eggs when Male Bollworm Was Fed Various Concentrations of Hexaflumuron and Paired with Females Fed 2.5-M Sucrose Solution (Test 3)

Concentration	Mean larval hatch (%) ± SD[1]			
(mg^{-1})	Day 1	Day 2	Day 3	Total
0	71.9 ± 15.2a	72.9 ± 10.1a	70.2 ± 17.9a	69.7 ± 14.9a
2.5	66.7 ± 14.5a	70.1 ± 15.3a	76.3 ± 16.3a	71.1 ± 14.5a
5	78.7 ± 8.2a	82.3 ± 12.4a	86.0 ± 8.6a	82.2 ± 9.5a
10	85.9 ± 3.3a	81.2 ± 5.1a	83.7 ± 3.9a	83.8 ± 3.3a
25	82.4 ± 3.8a	88.5 ± 1.6a	84.3 ± 6.9a	85.1 ± 3.9a
50	79.9 ± 7.8a	81.9 ± 4.8b	74.0 ± 3.5a	80.1 ± 5.1a

[1]Means followed by the same lowercase letter in a column are not significantly different (P > 0.05) according to Tukey's HSD test.

Fecundity. When females were fed hexaflumron at 2.5, 5, 10, 25, and 50 mg^{-l} and paired with males, there was no significant difference in the total number of eggs deposited during a 3-day period (F = 0.83; df = 5, 27; P > 0.05; Fig. 6). There was a significant difference in the number of eggs deposited during a 3-day period when males fed hexaflumuron were paired with females (F = 3.80; df = 5, 18; P < 0.05). Moths fed 2.5-M sucrose solution deposited significantly fewer eggs compared with moths fed hexaflumuron at 10 mg^{-l} (Fig. 7). Greater concentration of hexaflumuron other than 10 mg^{-l} did not significantly influence bollworm fecundity.

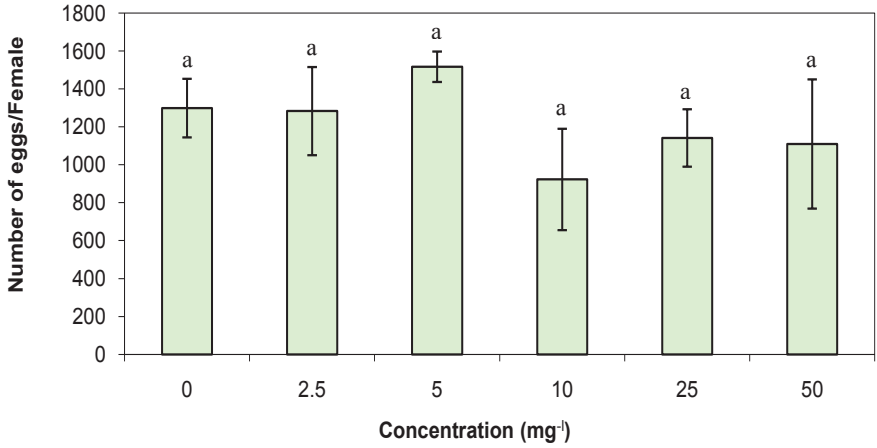

Fig. 6. Mean number of eggs (± SD) per female bollworm fed hexaflumuron and paired with males fed 2.5-M sucrose solution (the same letters on a column indicate no significant difference (P > 0.05) between treatments (HSD test)).

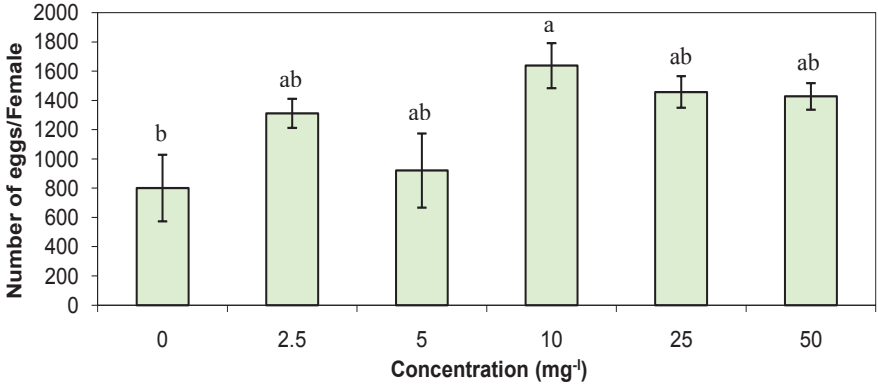

Fig. 7. Mean number of eggs (± SD) deposited by laboratory-reared female bollworms during a 3-day period when males were fed hexaflumuron (the same letters on a column indicate no significant difference (P > 0.05) between treatments (HSD test)).

Mating Frequency. When females fed hexaflumuron at 1, 10, 100, and 1,000 mg^{-l} were compared with females fed 2.5-M sucrose solution, mating frequency varied significantly between treatments (F = 2.38; df = 2, 70; P < 0.1). Mating frequency was significantly less at 10 mg^{-l}; however, there was no consistent trend in mating frequency relative to hexflumuron concentration (Fig. 8). When hexaflumuron at 2.5, 5.0, 10.0, 25.0, and 50.0 mg^{-l} was fed to females, and compared with those fed 2.5-M sucrose solution, there was no significant influence

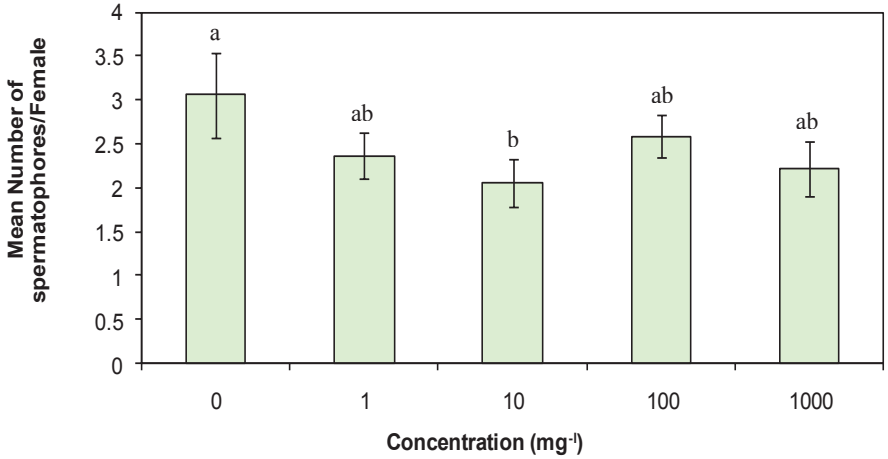

Fig. 8. Mean number of spermatophores/female (± SD) fed hexaflumuron and paired with males fed 2.5-M sucrose solution (the same letters on a column indicate no significant difference (P > 0.05) between treatments (HSD test)).

in mating frequency (F = 0.85; df = 5, 86; P > 0.05; Fig. 9). When males were fed hexaflumuron at 2.5, 5, 10, 25, and 50 mg^{-l} and compared with males fed 2.5-M sucrose solution, there was no significant difference in mating frequency (F = 0.40 df = 5, 30; P > 0.05; Fig. 10).

Conclusions

Data presented here show hexaflumuron has potential for use as a reproduction inhibitor in an adult control system using feeding attractants/stimulants. Although issues for the use of hexaflumuron were identified during the registration process and it was not registered for use on field crops, the authors evaluated an alternate control approach targeting bollworm adults when hexaflumuron was mixed with a feeding stimulant to determine potential use that might overcome the registration issues. Hexaflumuron caused dramatic reductions of hatch of eggs oviposited by females that ingested as little as 1 mg^{-l}. Significant reduction in proboscis-extension response occurred at 50 mg^{-l}, and gustatory response was depressed significantly at concentrations as great as 100 mg^{-l}. It seems 25 mg^{-l} is the optimum concentration at which significant reduction occurred in the percentage of eggs that hatched. Sublethal concentration of hexaflumuron probably is in this

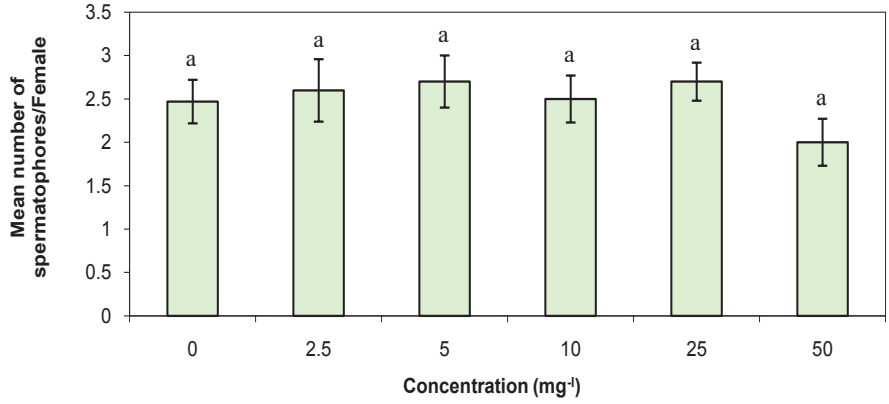

Fig. 9. Mean number of spermatophores/female (± SD) when female bollworms were fed hexaflumuron and paired with males fed 2.5-M sucrose solution (the same letters on a column indicate no significant difference ($P > 0.05$) between treatments (HSD test)).

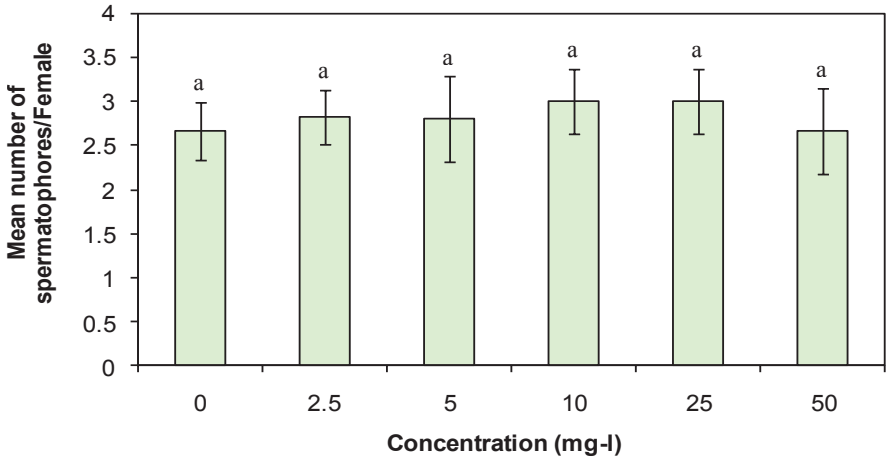

Fig. 10. Mean number of spermatophores/female (± SD) when bollworm males fed hexaflmuron and paired with males fed 2.5-M sucrose solution (the same letters on a column indicate no difference ($P > 0.05$) between treatments (HSD test)).

range. Although hexaflumuron is not labeled for use on agricultural crops in the U.S., a 21-day preharvest interval has been suggested in China (Huang et al. 2010). The methodology reported in this study should be applicable to control of bollworms as pests of many agricultural crops.

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