EFFECT OF HEXAFLUMURON ON FEEDING RESPONSE AND REPRODUCTION OF ADULT BOLL WEEVIL

J. D. Lopez, Jr., M. A. Latheef and Shenghuan Li
USDA, ARS, SPARC, APMRU
College Station, TX

Abstract

Hexaflumuron was evaluated as a toxicant and reproduction inhibitor at 5, 10, 25, 50 and 100 ppm (ai weight:volume basis) in a mixture with 10% sucrose as a feeding stimulant for spring and fall pheromone trap-captured female boll weevils (BW), *Anthonomus grandis grandis* Boheman. Subsequent to feeding, the weevils were evaluated for fecundity and larval hatch of eggs. Compared to 10% sucrose alone, the gustatory response was significantly depressed at concentrations of 25 ppm and above for females captured in the spring. Similarly, gustatory response decreased significantly at concentrations of 5 ppm and above for females captured in the fall. Female weevils captured in the fall ingested significantly more of all test and control solutions than those captured in the spring. There was no evidence of toxicity that resulted in death of treated female BWs. Mean number of eggs per female were significantly lower for females fed all hexaflumuron concentrations compared to control for spring-captured weevils. In the fall, there was more variability in the mean number of eggs laid between treatments than in the spring. In the spring, percent larval hatch of eggs was significantly less at concentrations of 10 ppm and above compared to control while in the fall, larval hatch of eggs was more variable and differed significantly from control at concentrations of 25 ppm and above. The potential of hexaflumuron for use as a toxicant or reproduction inhibitor in a feeding stimulant/attracticide formulation against BW populations in pre- or post-eradication areawide suppression programs appears to be limited because of the inhibition of gustatory response compared to control at concentrations which significantly reduce fecundity and fertility.

Introduction

The development of control strategies complementary to the boll weevil (BW), *Anthonomus grandis grandis* Boheman, eradication program is essential for use in environmentally-sensitive areas and to suppress resurgence of weevil populations in post-eradication zones. One of these control technologies is aimed at suppression of adult weevils with the use of feeding attractants and stimulants. Success of this technology has already been achieved for control of the corn rootworm, *Diabrotica* spp., adult populations on corn through use of a semiochemical-based product containing an environmentally-compatible insecticide (Hoffmann et al. 1998). Also, efforts to develop similar adult control technologies for the corn earworm, *Helicoverpa zea* (Boddie) are currently being pursued (Joyce and Lingren 1998; Lopez et al. 1999).

Because of the hazards associated with widespread application of broad spectrum insecticides in BW pre- and post-eradication programs, there is a need for identifying selective chemicals to use as toxicants or reproduction inhibitors in a feeding-based control technology. Hexaflumuron has potential for this use. It is a benzoylphenylurea which inhibits chitin synthesis and mainly acts as a larvicide and ovicide (Retnakaran and Wright 1987). Mitlin et al. (1977) reported that female BWs treated with a similar benzoylphenylurea, diflubenzuron at 0.4% and mated with untreated males produced eggs which did not hatch. Moore et al. (1978) reported that larval hatch of eggs was reduced significantly when female weevils were dipped in a diflubenzuron solution and mated with untreated males. Haynes and Smith (1993) found that female weevils dipped in or fed a new benzoylphenylurea, CIBA-GEIGY's CGAA-184699 and mated with untreated males oviposited 95-99% less and larval hatch of eggs was reduced to zero. Marco et al. (1998) reported that topical application of hexaflumuron at 0.03 μg (ai) per adult weevil and foliar application at 31 μg (ai) per sugar beet leaf to which adult weevils were exposed, resulted in significant inhibition of larval hatch of eggs oviposited by sugar beet weevil, *Aubeonymus mariaefranciscae* Roudier.

We report here on a laboratory study to determine the effect of hexaflumuron on gustatory response of sex pheromone trap-captured female BWs when provided in a feeding stimulant solution. Subsequent to feeding, females were evaluated for fecundity and larval hatch of eggs. Our objective was to assess whether or not hexaflumuron could be used as a toxicant or reproduction inhibitor in the development of adult control technology using feeding attractants/stimulants for areawide suppression of the BW.

Materials and Methods

Chemical

Hexaflumuron was supplied as Consult® 100 EC containing 100 gm ai/liter (0.8344 lb/gallon) by Dow AgroSciences, King's Lynn, Norfolk, England. We prepared test solutions of hexaflumuron at 5, 10, 25, 50 and 100 ppm (ai wt:vol) in 10% sucrose (Sigma, St. Louis, MO). The 10% sucrose solution alone served as the control. Test solutions were stored in a refrigerator and warmed to room temperature before each use.

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Test Insects

BW pheromone traps baited with 10 mg lure (Hercon®, ai: grandlure 1.2%, Hercon Environmental Corp., Emigsville, PA) were placed in field crop locations in the Brazos Valley near College Station, TX during the spring and fall of 1999. Traps were emptied daily except during weekends and adult weevils were placed in Ziplock® plastic bags and held in the laboratory for use in the studies. Weevils were sexed as they were removed from the Ziplock bags for testing. Only weevils captured the previous day were used.

Feeding Apparatus

The needles of 10 μl Hamilton® micro-liter syringes (Hamilton Co., Reno, NV) were cut off and the cut ends were ground into cone-shaped tips. Syringes were inserted perpendicularly into 1/16 in. diameter individual holes drilled into a Plexiglas® plate 14 X 4 in. (¼ in. thick) which was fastened at ca. 45° angle with screws to a 1 X 3 in. wooden plate. This plate sat on a 1½ X 5½ in. piece of lumber (conventionally known as 2 x 6). The cone-shaped tips of the syringes served as a platform for the weevils to rest while feeding. A total of six syringes inserted through the holes made in the Plexiglas plate formed the feeding apparatus.

Test Solutions and Gustatory Response

Solutions of hexaflumuron at 5, 10, 25, 50 and 100 ppm and 10% sucrose solution as the control were pipetted into the syringes. Female weevils were fed by picking up the individual weevil between the thumb and forefinger and inserting the snout of the beetle into the tips of the syringes. Weevils were fed to satiation. The amount of test solutions ingested was determined in μl as the difference between before and after feeding meniscus levels in the syringes. A total of 20 female BWs were fed on each concentration. After each test was completed, syringes were cleaned with 95% ethyl alcohol.

Reproduction Effects

After feeding with hexaflumuron, female weevils were held in petri dishes (4 X 1 in.) with 1/3-grown squares ≥ 6.5 and ≤ 9.0 mm diameter. Uninfested squares were collected from greenhouse- or field-grown cotton plants. Two females were paired with 2 males in each petri dish and fed every 2 days with 6 and 4 fresh squares, respectively, in the spring and fall. After exposure to the females, squares were incubated in 6 X 6 in. plastic bags kept in a laboratory maintained at 23.9 ± 2.0° C and RH ≥ 65%. To facilitate adequate mating and prevent excessive feeding and frass accumulation on the squares, males were removed and killed after 2 consecutive square changes. Treatments were evaluated for 30 to 32 days even when one female died, but evaluations were discontinued when both females died. The squares were dissected under a 30X stererozoom microscope, eggs and larvae were counted and eggs carefully removed from the squares. Eggs were held over a piece of moist paper toweling in a 25-ml plastic soufflé cup with a plastic lid and examined for larval hatch for up to 5 consecutive days. In the spring, the squares were incubated for 5 to 6 days before they were dissected while in the fall, the squares were dissected either the same day after removal from the petri dish or were incubated for 1 to 2 days before dissection.

Statistical Analysis

For analyses of fecundity, eggs and larvae were summed for each replicate of each treatment and divided by the number of females alive, regardless of number of squares used. To calculate percent larval hatch, the number of larvae that hatched for each replicate of each treatment was divided by the number of eggs summed for each group. Data were analyzed with PROC GLM procedure using SAS (1998). In tests with significant F-values at the 5% level, treatment means were separated with LSD at the 5% level. Unequal numbers of treatment observations were summed and the mean was used to determine the standard error of the mean in calculation of LSD values. Regression lines were fitted using Microsoft Excel, version 7.

Results and Discussion

Gustatory Response

The gustatory responses of overwintered BW females collected from pheromone traps in the spring were significantly different for some of the concentrations (Table 1). Compared to control, hexaflumuron significantly reduced gustatory response at concentrations of 25 ppm and above. There was no significant difference compared to control in gustatory response for concentrations between 5 and 10 ppm. The gustatory response of females at the 25 ppm concentration compared to control was significantly less than that at 50 and 100 ppm. There was no significant difference in gustatory response between the 50 and 100 ppm concentrations.

Similar to overwintered females, the gustatory response of BWs collected from pheromone traps in the fall showed significant differences between concentrations. (Table 1). However, contradictory to the results with overwintered weevils, hexaflumuron significantly reduced gustatory response compared to control at concentrations of 5 ppm and above for the fall-captured weevils. There was no significant difference in ingestion of hexaflumuron for concentrations between 5, 10 and 25 ppm. There was also no significant difference in amount ingested for hexaflumuron concentrations between 25 and 50 ppm. The BWs ingested the least amount of hexaflumuron at 100 ppm. The gustatory responses at 50 and 100 ppm were not significantly different from each other.

The gustatory response to hexaflumuron and control sugar solutions of the overwintered BW females collected in the spring was significantly lower from that of those collected in the fall (average = 2.75 vs. 2.11 μl). No evidence of hexaflumuron toxicity resulting in the death of treated female weevils was observed for both the spring and fall populations.

It is obvious that hexaflumuron inhibited gustatory response of pheromone trap-captured female BWs at low concentrations. Feeding deterrence induced by hexaflumuron at concentrations > 62.5 ppm was reported earlier for the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Su and Scheffrahn 1993). This is a major concern in considering its potential for use in feeding-based control technology. A major consideration would be the concentration at which it has reproductive or toxic effects. Higher gustatory response of fall-captured weevils compared to spring-captured weevils may probably reflect the lower amount of foraging resources present in the fall relative to the spring, especially during 1999 when there was a wet spring and dry fall.

Fecundity

Regardless of concentration, the number of eggs laid by spring-captured female BWs increased initially with time, peaked on or about the 18th day after feeding and declined thereafter in a curvilinear fashion, except for 50 ppm for which the curve appeared to flatten out (Fig. 1). For fall-captured weevils, a similar curvilinear relationship was observed for hexaflumuron concentrations at 50 and 100 ppm. The shapes of the curves for other treatments were more variable (Fig. 2). In the spring, the number of eggs deposited by females compared to control decreased significantly for all concentrations of hexaflumuron (Table 2). The numbers of eggs oviposited at 5, 10 and 25 ppm treatments were comparable. Similarly, numbers of eggs deposited at 50 and 100 ppm were comparable. However, number of eggs laid at 50 ppm was significantly less than that at 25 ppm. In the fall, there was more variability in number of eggs laid between treatments than in the spring. Numbers of eggs deposited in the control were not significantly different from those at 25 and 50 ppm, but control female laid significantly more eggs than at 5 and 10 ppm. The number of eggs deposited at 100 ppm was the lowest and was significantly different from control.

Fertility

The relationship between days after feeding BW females with hexaflumuron and percent larval hatch of eggs for the spring- and fall-captured BWs is shown in Figures 3 and 4. It is apparent that compared to control percent larval hatch of eggs decreased soon after ingesting hexaflumuron for both spring- and fall-captured BWs, but the effect of hexaflumuron was not sustained for more than ca. 2 weeks when thereafter larval hatch of eggs started to increase considerably. Percent larval hatch of eggs oviposited by the females captured in the spring was significantly different between treatments (Table 2). There was no significant difference in percent larval hatch of eggs between control and 5 ppm. Percent larval hatch of eggs was significantly different from control at concentrations of 10 ppm and above. Percent larval hatch of eggs was not significantly different between 10 and 25 ppm or between 50 and 100 ppm. Percent larval hatch of eggs for the control was very high at 95%. Larvae that did not hatch from eggs oviposited by treated females appeared to be fully developed and alive inside the eggs, but were unable to hatch.

In the fall, percent larval hatch of eggs was significantly less at concentrations of 25 ppm and above compared to control (Table 2). Percent larval hatch of eggs at 50 ppm was the lowest but was not significantly different from that at 100 ppm. Percent larval hatch of eggs at 25 and 100 ppm was comparable, but percent larval hatch of eggs at 50 ppm was significantly less than at 25 ppm. Percent larval hatch of eggs for control averaged 88%.

Although significant inhibitory effects on fecundity and fertility of female BWs that fed on various concentrations of hexaflumuron are obvious, there is also considerable overlap between the concentrations that deter feeding and those that have adverse effects on reproduction. It is likely that the feeding deterrent effects of the higher hexaflumuron concentrations evaluated also probably had a reductive effect on inhibition of reproduction of the BW.

We do feel that based on the detection of not overwhelming but significant inhibitory effects on gustation and reproduction using our experimental techniques, we can evaluate other chemicals for their potential use in feeding-based adult control technology for the BW.

Conclusion

The potential of hexaflumuron for use as a toxicant in a feeding stimulant/attracticide formulation appears to be limited because of the inhibitory effect of the chemical on gustatory response at concentrations that will greatly reduce fecundity and fertility and contribute to control of BW populations in areawide suppression programs.

Disclaimer

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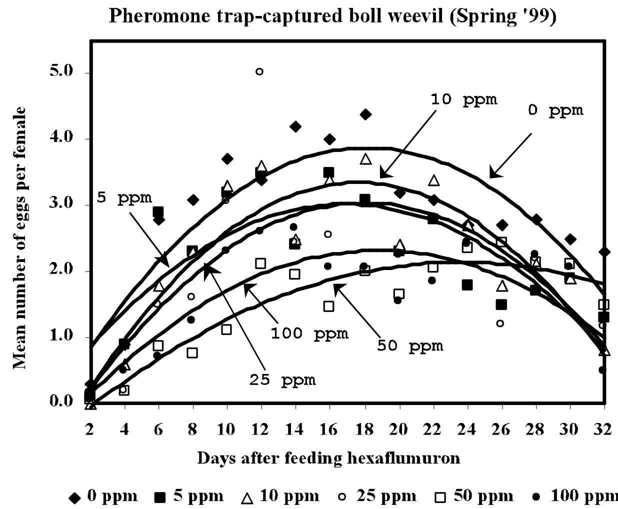

Figure 1. Relationship between number of eggs deposited by female and days after feeding hexaflumuron. Regression equations were:
$Y_{0\ ppm} = -0.0116X^2 + 0.421X + 0.063$; $R^2 = 0.7542$
$Y_{5\ ppm} = -0.0097X^2 + 0.3279X + 0.2604$; $R^2 = 0.6169$
$Y_{10\ ppm} = -0.0127X^2 + 0.4476X + 0.5991$; $R^2 = 0.819$
$Y_{25\ ppm} = -0.0111X^2 + 0.398X + 0.5291$; $R^2 = 0.553$
$Y_{50\ ppm} = -0.0047X^2 + 0.222X + 0.4679$; $R^2 = 0.8571$
$Y_{100\ ppm} = -0.0077X^2 + 0.2889X + 0.3946$; $R^2 = 0.6578$,
where Y = mean number of eggs/aand X = days after feeding hexaflumuron.

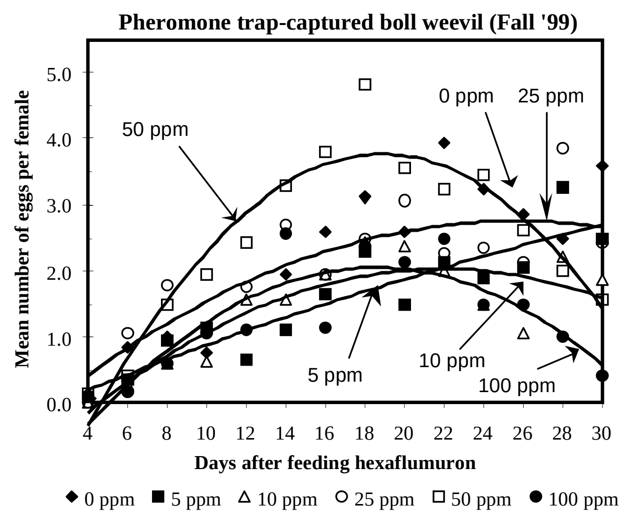

Figure 2. Relationship between number of eggs deposited by female and days after feeding hexaflumuron. Regression equations were:
$Y_{0\ ppm} = -0.0054X^2 + 0.3077X - 1.169$; $R^2 = 0.8391$
$Y_{5\ ppm} = -0.0009X^2 + 0.1274X - 0.3054$; $R^2 = 0.8329$
$Y_{10\ ppm} = -0.0068X^2 + 0.2988X - 1.2374$; $R^2 = 0.7632$
$Y_{25\ ppm} = -0.005X^2 + 0.2569X - 0.5289$; $R^2 = 0.6911$
$Y_{50\ ppm} = -0.0186X^2 + 0.7027X - 2.8631$; $R^2 = 0.9066$

Y_100 ppm = $-0.0113X^2 + 0.4191X - 1.8332$; $R^2 = 0.7542$, where Y = mean number of eggs/aand X = days after feeding hexaflumuron.

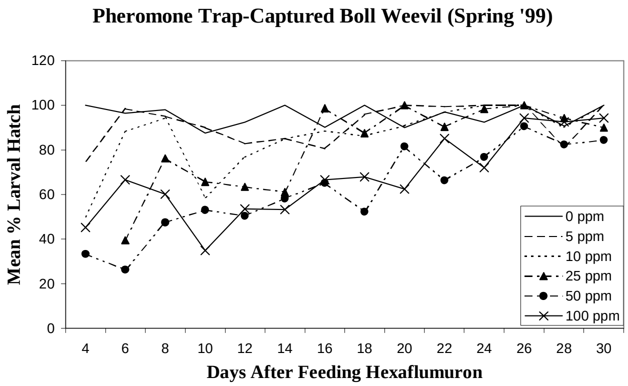

Figure 3. Relationship between percent larval hatch of eggs and days after feeding on various concentrations (ppm ai wt:vol) of hexaflumuron in 10% sucrose or 10% sucrose alone for female BWs collected in pheromone traps during spring 1999.

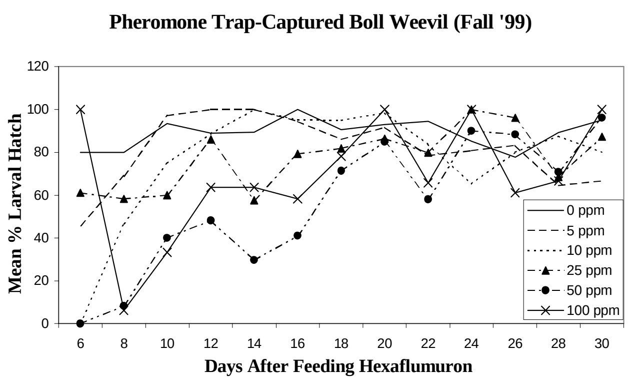

Figure 4. Relationship between percent larval hatch of eggs and days after feeding on various concentrations (ppm ai wt:vol) of hexaflumuron in 10% sucrose or 10% sucrose alone for female BWs collected in pheromone traps during fall 1999.

Table 1. Gustatory response (μl) of pheromone trap-captured females to various concentrations (ppm ai wt:vol) of hexaflumuron mixed with 10% sucrose compared to control (10% sucrose solution) alone.

Conc. of hexaflumuron (ppm)	Mean ± SE (μl) ingested / a	
	Spring (N = 20)	Fall (N = 20)
0	3.0±0.2a	4.3±0.3a
5	2.8±0.2a	3.1±0.2b
10	2.5±0.3ab	3.2±0.3b
25	2.1±0.2b	2.6±0.3bc
50	1.1±0.2c	2.0±0.3cd
100	1.0±0.2c	1.3±0.2d

Means within each column followed by the same lower case letter were not significantly different (LSD = 5% level).

Table 2. Mean number of eggs per female and mean percent larval hatch of eggs deposited every two days by pheromone trap-captured female boll weevil fed various concentrations (ppm ai wt:vol) of hexaflumuron mixed with 10% sucrose compared to control (10% sucrose) alone.

Conc.of hexaflumuron (ppm)	Mean ± SE number of eggs/a		Mean ± SE % larval hatch	
	spring	fall	spring	fall
0	3.1±0.2a	2.1±0.2a	95.1±1.5a	88.1±3.0a
5	2.3±0.2b	1.5±0.2bc	92.4±2.1a	84.4±3.2ab
10	2.3±0.2b	1.4±0.2c	87.3±2.7b	82.4±4.6ab
25	2.1±0.2b	2.0±0.3ab	82.7±3.3b	77.0±4.5bc
50	1.5±0.1c	2.5±0.3a	66.2±4.0c	58.3±4.8d
100	1.7±0.2c	1.2±0.2c	69.6±4.2c	67.1±5.9cd

Means within each column followed by the same lower case letter were not significantly different (LSD = 5% level).