Effects of Sublethal Doses of Two Insect Growth Regulators on *Helicoverpa zea* (Lepidoptera: Noctuidae) Reproduction[1, 2]

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ABSTRACT The effects of sublethal doses of two insect growth regulators on *Helicoverpa zea* (Boddie) reproduction were investigated. Adults which developed from larvae that fed on a pinto bean diet containing diflubenzuron (Dimilin) or an ecdysone agonist (RH 5992) were inbred and outcrossed with adults which developed from larvae that were fed on a normal pinto bean diet. Data were collected on fecundity, fertility, and sperm transfer. Sublethal doses of both compounds administered to *H. zea* larvae caused reduced fertility in surviving male imagos but had no effect on fecundity or fertility of the female imagos. Reduced fertility in treated males was largely caused by a lower incidence of sperm transfer. Implications of our results for *H. zea* population management are discussed.

KEY WORDS *Helicoverpa zea,* Diflubenzuron, insect growth regulator.

Insect growth regulators (IGRs) alter normal insect growth and eventually cause the death of the treated insect (Fronk 1978). In general, IGRs, which act as chitin synthesis inhibitors, ecdysone agonists, and/or juvenile hormone analogs, have been touted as excellent integrated control insecticides due to their specificity to a targeted pest, their relative nontoxicity to beneficial organisms, and their general safety to vertebrates, mollusks, and plants (Wilkinson et al. 1978, Deakle and Bradley 1982, Heller et al. 1992, Horn 1988, Rohm and Haas Co. 1989). These benefits would make IGRs attractive alternatives to neurotoxic chemical insecticides for managing some pest populations.

IGRs have recently been evaluated for management of corn earworm, *Helicoverpa zea* (Boddie) (Chandler et al. 1992a, b). Chitin synthesis inhibitors (diflubenzuron and teflubenzuron) were efficacious against 1- and 7-d-old *H. zea* larvae, but only provided useful levels of mortality at high rates ≥ 0.01% (AI) (Chandler et al. 1992b). RH-5992 (Rohm and Haas Co., Philadelphia, PA), an ecdysone agonist, was highly efficacious against 1- and 7-d-old larvae, providing > 90% mortality at concentrations > 0.001% (AI) (Chandler et al. 1992a). Both of these studies indicate that effective rates are sublethal to some of the treated larvae. We investigated the effects of sublethal doses of these IGRs on reproductive capabilities of *H. zea*.

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[2] All programs and services of the U. S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

Materials and Methods

IGR Formulations. One percent dilutions (10,000 mg/l) of diflubenzuron (Dimilin 2 flowable [F] Uniroyal Chemical Co., Middleberry, CT) and RH-5992 2F (Rohm and Haas Chemical Co.) were prepared in the laboratory using distilled water. Dilutions of 0.0001, 0.001, 0.01, and 0.1.% were then prepared for each compound on each treatment date.

Larval Treatment. Neonate *H. zea* that had been reared as described by Burton (1969) were obtained from a laboratory colony at the Insect Biology and Population Management Research Laboratory in Tifton, GA. A single larva was placed in a 30-ml cup containing fresh pinto bean diet in which 0.10 ml of either of the previously prepared IGR dilutions or distilled water (control) had been incorporated. Three-hundred sixty larvae were treated for each treatment dilution. The treated diet was airdried for 2 h before a larva was introduced into a cup of diet. Cups were then capped and held in a room at 28 ± 1°C and ≈60% RH. Percentage mortality was determined after moth emergence by dividing the number of individuals not emerging as moths by the total number of individuals treated.

Fertility and Fecundity. Adults (T) that developed from larvae that had fed on a pinto bean diet containing Dimilin (0.05 and 0.001% AI) and RH-5992 (0.01 and 0.001% AI) were inbred (T×T) or outcrossed (T×N, N×T) with adults (N) which had developed from larvae that fed on a normal pinto bean diet. Progeny from crosses from each IGR treatment were compared with progeny from control crosses (N×N). Individually-paired females from each cross (15 per cross) were allowed to lay eggs for 5 d. The number of eggs laid and the percentage of eggs hatching were recorded for each mated female from each cross. Because of high larval mortality in the treatment with 0.01% RH-5992, reciprocal crosses were not included in this study.

Sperm Transfer. Males which had developed from larvae that fed on a pinto bean diet containing Dimilin (0.01% AI) ($n = 23$) and RH-5992 (0.005% AI) ($n=21$) were outcrossed with females which had developed from larvae that had fed on an untreated pinto bean diet. Females from these crosses were compared with females that had mated with males which had developed from untreated larvae. The spermatheca and the bursa copulatrix with spermatophores were removed from each mated female and examined for motile eupyrene and apyrene sperm.

Statistical Analysis. Mortality data were analyzed by probit analysis (POLO-PC; LeOra Software 1987). Fecundity and egg hatching data were analyzed as replicated means by the General Linear Model Procedure, and significantly different means were separated by the LSD procedure (SAS Institute 1985), $P < 0.05$. Females with motile eupyrene sperm in their spermatheca were assigned a value of "1" and females with no motile eupyrene sperm were assigned a value of "0". Mean values for treated and control crosses were compared using the unequal variance - unequal n t-test.

Results and Discussion

Helicoverpa zea larvae were more susceptible to RH-5992 than Dimilin (x^2 = 55.25, df = 2, $P < 0.0001$); however, the slope of the response curve was not different (x^2 = 1.9, df = 1, $P > 0.167$) (Fig. 1). Our data corroborate the findings of Chandler et al. (1992a, b). Although the two IGRs have different modes of action, the ultimate larval response to each compound as shown by adult emergence was similar for the range of concentrations tested.

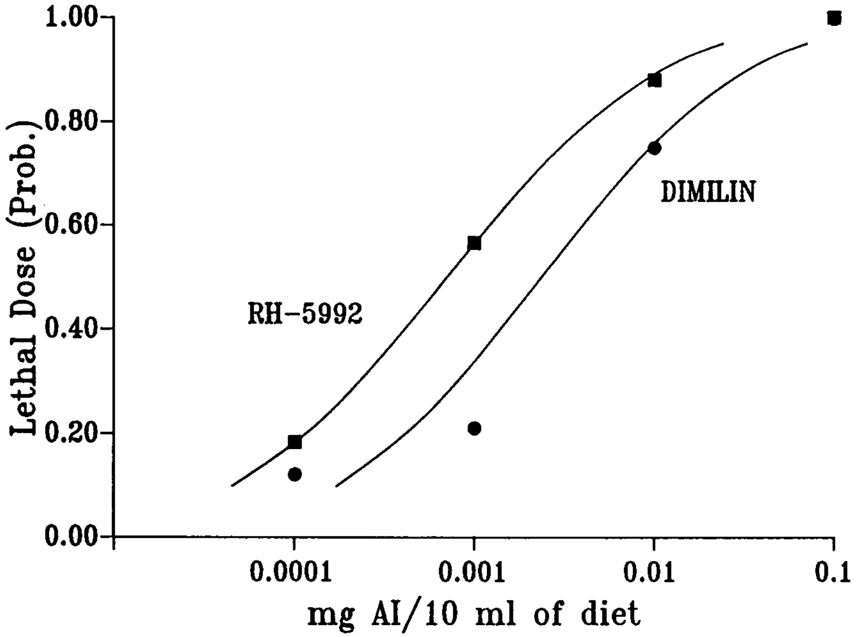

Fig. 1. Regression lines derived from the probability of lethal dose for *H. zea* larvae plotted against the concentration of two insect growth regulators within the pinto bean diet. Probit equations are as follows: Dimilin, y = 7.96 + 1.123 x; RH - 5992, y = 8.38 + 1.07 x.

The number of eggs laid by *H. zea* females was not significantly ($P > 0.05$) affected by the sex of the larva receiving the treatment, the type of IGR treatment, or the IGR concentration (Tables 1, 2). However, the percentage of eggs that hatched was affected by each of these factors. Percentage of the eggs hatching was significantly ($P < 0.05$) higher in the control crosses than in crosses involving treated males. The difference in the percentage of eggs hatching in crosses involving control males and treated males from treated larvae increased as the concentration of each IGR increased. RH-5992 (0.01% concentration) produced the greatest difference in the percentage of eggs

Table 1. Fertility and fecundity of *Helicoverpa zea* reared on a normal pinto bean diet or on a pinto bean diet containing Dimilin.

		$\bar{x}$ ± S. E. *	
Dimilin (% concentration)	Crosses** (female × male)	% Egg Hatch	No. of eggs laid by female
–	N × N	36.7 ± 4.1 a	1321 ± 138 a
0.05%	T × N	29.7 ± 4.3 ab	1189 ± 132 a
0.05%	N × T	14.9 ± 4.2 c	1221 ± 133 a
0.05%	T × T	21.1 ± 4.3 bc	1426 ± 133 a
–	N × N	45.8 ± 4.7 a	1570 ± 164 a
0.001%	T × N	40.3 ± 4.7 a	1437 ± 151 a
0.001%	N × T	27.8 ± 4.4 b	1676 ± 164 a
0.001%	T × T	28.0 ± 4.6 b	1827 ± 160 a

* Means within a column (for each concentration) not followed by the same letter are significantly different Least Squares Means procedure ($P < 0.05$; SAS Institute [1985]).

** N, larval stage reared on normal diet; T, larval stage reared on diet containing Dimilin.

hatching in N × N and T × T crosses. Percentage of eggs hatching in females from treated larvae alone was not reduced by either IGR compound.

Male sterility could have resulted from the failure of sperm to be transferred, malformed or misplaced spermatophores, nonmotile sperm, the absence of eupyrene sperm, or other factors. Because the reduction in the percentage of eggs hatching was a result of treatment of males with both IGRs, we compared the nature of the sterility resulting from the treatments. We chose a larval-adult mortality rate of 80% in an attempt to induce a high incidence of sublethal effects in the treated individuals but still have an adequate number of insects with which to conduct the test. Therefore, it was necessary to use a different concentration of each IGR (Dimilin - 0.01% AI; RH-5992 - 0.005% AI). These IGR concentrations produced an observed larval-adult mortality of 81.7% and 82% for Dimilin and RH-5992, respectively. The mean percentage of females with eupyrene sperm in their spermathecae after mating with treated males was significantly ($P < 0.05$) less than the mean percentage of females with eupyrene sperm in their spermathecae after mating with untreated males (Table 3). All sperm found in the spermathecae were motile. Females that had no sperm in the spermathecae also had no sperm in their bursa copulatrix and no sperm in the spermatophore transferred to the bursa copulatrix. These

Table 2. Fertility and fecundity of *Helicoverpa zea* reared on a normal pinto bean diet or on a pinto bean diet containing RH-5992.

		$\bar{x}$ ± S. E. *	
RH-5992 (% concentration)	Crosses** (female X male)	% Egg Hatch	No. of eggs laid by female
–	N X N	42.7 ± 7.1 a	1290 ± 184 a
0.01%	T X T	2.4 ± 10.1 b	1623 ± 232 a
–	N X N	44.2 ± 6.0 a	1608 ± 203 a
0.001%	T X N	37.6 ± 5.6 ab	1838 ± 192 a
0.001%	N X T	23.2 ± 6.0 c	1435 ± 202 a
0.001%	T X T	29.7 ± 5.8 bc	1566 ± 203 a

* Means within a column (for each concentration) not followed by the same letter are significantly different Least Squares Means procedure ($P < 0.05$; SAS Institute [1985]).

** N, larval stage reared on normal diet; T, larval stage reared on diet containing RH-5992.

observations indicated that both IGRs induced sterility in males by impairing their ability to transfer sperm.

The effect of Dimilin and RH-5992 on *H. zea* fertility was similar to the effects of other IGRs on other insect species. Redfern et al. (1980) found that the sterility effect of diflubenzuron and its analogs on the large milkweed bug, *Oncopeltus fasciatus* (Dallas), was greater for males than females. Treated *O. fasciatus* males produced motile sperm but were unable to transfer sperm to females during copulation. Charttoraj and Dwivedi (1980) reported that penfluron also induced sterility in *Spodoptera litura* (Boised) and that penfluron was more effective on males than females. Similarly, Khan and Srivastava (1989) found that sublethal doses of diamino-furyl-s-triazine (AI3-22641) administered to *Euproctis icilia* Stoll larvae (24-h-old last instars) produced sterility in adults. Again, sterility was more pronounced in *E. icilia* males than females.

Although several IGRs induced various levels of sterility in *H. zea* adults treated as larvae, the method of sterility induction is unknown. However, because each chemical compound had a greater effect on males than females, the inability of treated males to transfer sperm during copulation may have been a common effect. An understanding of the mechanism responsible for preventing the transfer of sperm may result in improved treatment efficacy.

Table 3. Percentage of female *Helicoverpa zea* with eupyrene sperm in their spermathecae after mating with males that were reared on a normal pinto bean diet or a pinto bean diet containing an insect growth regulator (IGR).

IGR (dose)	Mean % (± S. E.) of female with eupyene sperm*		d.f.	t*	P
	Normal female × Treated male	Normal female × Normal male			
Dimilin (0.01)	43.48 ± 10.6%	84.09 ± 5.6%	65	-3.75	0.0004
RH-5992 (0.005)	61.90 ± 10.9%	84.09 ± 5.6%	63	-2.02	0.048

* Females with motile eupyrene sperm in their spermatheca were assigned a value of "1" and females with no motile eupyrene sperm were assigned a value of "0". Mean values for treated and control crosses were compared using the unequal variance - unequal n t-test.

** Variances are equal, Probability for $F > 0.05$.

The higher susceptibility of 1-d-old versus 7-d-old *H. zea* larvae to diflubenzuron (Chandler et al. 1992b) and RH-5992 (Chandler et al. 1992a) may suggest a novel approach for controlling *H. zea*. Initial populations of *H. zea* often infest field crops at levels below economic thresholds, thus not requiring control. However, subsequent populations may reach economic thresholds and trigger the need for using chemicals for their control. If there is an overlapping of generations, larvae of different ages are found in the field. IGRs applied at a dosage that would control an economic infestation of early instars may be a sublethal and sterilizing rate for larger larvae from an earlier infestation. Such a dosage rate may provide greater control of pest populations than a dosage high enough to kill all late instars because the infestation of the late instar larvae was not economic, and because male larvae surviving the IGR treatment would enter the next *H. zea* generation as sterile moths. Also, a lower dosage may conserve more natural enemies. Field tests of these ideas would be warranted.

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References Cited

Burton, R. L. 1969. Mass rearing of the corn earworm in the laboratory. USDA-ARS Series 33-134.

Chandler, L. D., S. D. Pair and W. E. Harrison. 1992a. RH-5992, a new insect growth regulator active against corn earworm and fall armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 85: 1099-1103.

1992b. Effects of selected insect growth regulators on longevity and mortality of corn earworm and fall armyworm larvae (Lepidoptera: Noctuidae). J. Econ. Entomol. 85: 1972-1978.

Charttoraj, A. N. and S. Dwivedi. 1980. Effect of penfluron on the sterility and mortality of *Spodoptera litura* (Boised). Comp. Physiol. Ecol. 5 (4): 310-313.

Deakle, J. P. and J. R. Bradley, Jr. 1982. Effects of early season applications of diflubenzuron and azinphosmethyl on population levels of certain arthropods in cotton fields. J. Ga. Entomol. Soc. 17: 200-204.

Fronk, W. D. 1978. Chemical control, Pp. 209-240. *In* R. E. Pfadt (ed.), Fundamentals of applied entomology. MacMillan, NY.

Heller, J. J., H. Mattioda, E. Klein and A. Sagenmüller. 1992. Field Evaluation of RH-5992 on lepidopterous pests in Europe. pp. 59-65. Brighton Crop Protection Conference - Pests and Diseases - 1992.

Horn, D. J. 1988. Ecological approach to pest management. The Guilford Press, NY.

Khan, M. M. and B. B. L. Srivastava. 1989. Biological effect of insect growth inhibitor, diamino-furyl-s-triazine (AI3-22641) on development and reproductive potential of *Euproctis icilia* Stoll. Annals Agric. Sci., Fac. Agric., Ain Shams Univ., Cairo, Egypt, 34: 1215-1226.

LeOra Software, Inc. 1987. POLO: a user's guide to Probit Or LOgit analysis. Berkeley, CA..

Redfern, R. E., A. B. Demilo and A. B. Brokovec. 1980. Large milkweed bug: effects of diflubenzuron and its analogues on reproduction. J. Econ. Entomol. 73: 682-683.

Rohm and Haas Company. 1989. RH-5992 Insect Growth Regulator. Technical Information Bulletin. AG-2255.

SAS Institute. 1985. SAS user's guide: statistics, version 5 ed. Cary, NC.

Wilkinson, J. D., K. D. Biever, C. M. Ignoffo, W. J. Pons, R. K. Morrison and R. S. Seay. 1978. Evaluation of diflubenzuron formulations on selected insect parasitoids and predators. J. Ga. Entomol. Soc. 13: 227-236.

CORRECTION: In the article, "Residual Efficacy of Cyfluthrin Applied Alone or in Combination with Piperonyl Butoxide or Piperonyl Butoxide + Chloripyrifos-methyl as Protectants of Stored Corn," by Frank H. Arthur and appeared in volume 29, issue 2, pages 276-287, an error occurred that resulted in the appearance of an incorrect Figure 2. The correct figure appears below.

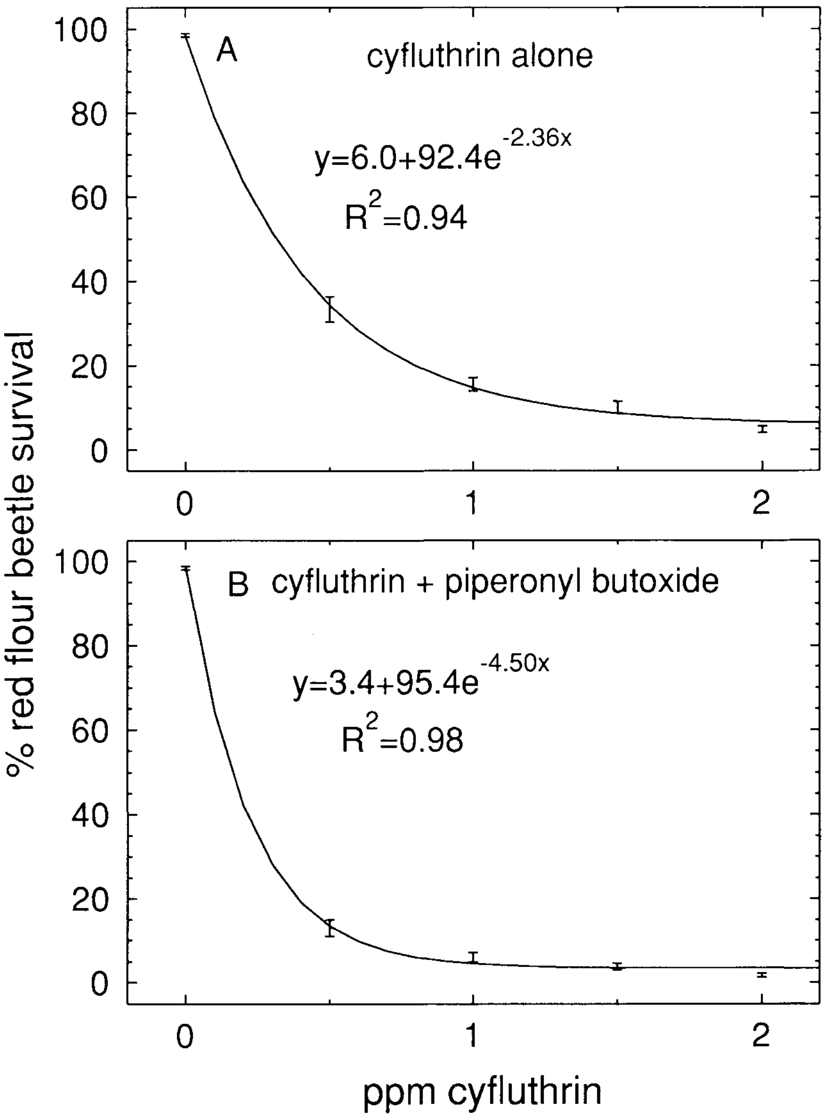

Fig. 2. Equations for predicted red flour beetle survival on corn treated with 0.0, 0.5, 1.0, 1.5, and 2.0 ppm cyfluthrin (A) and equations for predicted red flour beetle survival on corn treated with each rate of cyfluthrin + 8.0 ppm piperonyl butoxide (B). y = % survival, x = concentration.