Comparative Effects of Insect Growth Regulators on Longevity and Mortality of Beet Armyworm (Lepidoptera: Noctuidae) Larvae[1, 2]

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ABSTRACT Laboratory bioassays demonstrated the toxic effects of three distinct types of insect growth regulators, diflubenzuron, fenoxycarb, and RH-5992, against 1- and 6-d-old larvae of the beet armyworm, *Spodoptera exigua* (Hübner). Diflubenzuron and RH-5992 were somewhat more active and provided faster knockdown of beet armyworm larvae than did fenoxycarb. RH-5992 and diflubenzuron were 12-21 and 3-5 times more effective, respectively, against beet armyworm larvae than fenoxycarb. Larvae exposed to fenoxycarb lived up to 34 d following treatment and continued to feed. Therefore, fenoxycarb in field settings may not be as acceptable for controlling larvae of the beet armyworm as are diflubenzuron and RH-5992.

KEY WORDS Insecta, *Spodoptera exigua,* diflubenzuron, fenoxycarb, RH-5992, bioassay, insect growth regulators.

Control of beet armyworm, *Spodoptera exigua* (Hübner), on cotton and horticultural crops in the southeastern United States continues to be difficult. Beet armyworm is a polyphagous pest found on many cultivated and wild host plants throughout tropical and subtropical regions of the world (Metcalf et al. 1962). It has been identified as one of the three most economically important species in the genus *Spodoptera* in North America (Tumlinson et al. 1990), and has been recognized as a cotton pest in the southeastern United States for many years (Smith 1989). Outbreaks occurred on cotton in dry years such as 1977, 1980, 1981, and 1988, and populations were difficult to control with insecticides in Alabama during 1984 and 1985 (Smith 1989). Beet armyworm resistance to chemical insecticides has been reported in numerous studies (Meinke and Ware 1978, Chaufaux and Ferron 1986, Yoshida and Parrella 1987, Delorme et al. 1988, Brewer and Trumble 1989, Brewer et al. 1990). Chemical insecticide resistance has forced integrated pest management (IPM) practitioners to explore alternative types of control materials for use against this pest.

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Benzoylphenylurea insect growth regulators (IGRs), such as diflubenzuron, have proven to be effective alternatives to chemical insecticides for controlling beet armyworm in many crop systems (Van Laecke 1988, Smith 1989, Van Laecke and Degheele 1991a, b). However, variable levels of beet armyworm tolerance to diflubenzuron have been reported (Van Laecke 1988). The addition of tribufos (DEF), a defoliant, and profenofos, an organophosphate insecticide, to diflubenzuron has been shown to enhance its toxicity against fourth instar beet armyworm (Van Laecke and Degheele 1991b). Studies also have shown that 500 ppm doses of diflubenzuron prevented 93.3% of third instar beet armyworm from developing into adults and that adult survivors had vestigial wings (Van Laecke et al. 1989). Thorough studies to determine the magnitude of effects of diflubenzuron on longevity and mortality of beet armyworm larvae of various ages have not been conducted, nor have studies to evaluate the effect of other types of IGRs on beet armyworm larvae. This study was conducted to evaluate the effects of three IGRs, diflubenzuron, fenoxycarb, and RH-5992, on the mortality and longevity of 1- and 6-d-old beet armyworm larvae.

Materials and Methods

Insect Growth Regulator Formulations. One percent active ingredient (AI) dilutions (10g/liter) of diflubenzuron (Dimilin 25 wettable powder [WP], Uniroyal Chemical Co., Middlebury, CT), fenoxycarb (Insegar 25 WP, Maag Agrochemicals, Vero Beach, FL), and RH-5992 (RH-5992 2 flowable [F], Rohm and Haas Company, Philadelphia, PA) were prepared in the laboratory with distilled water. Six serial dilutions ranging from 0.00001 to 1.0% were then prepared for each compound on each treatment date. Diflubenzuron inhibits synthesis of chitin and is classified as a benzoylphenylurea compound; fenoxycarb is a carbamate compound acting as a juvenile hormone analog; and RH-5992 is an agonist of the insect molting hormone, ecdysterone (Rohm and Haas Co. 1989).

Bioassays. Neonate beet armyworm larvae were obtained from laboratory cultures maintained at the Insect Biology and Population Management Research Laboratory in Tifton, GA. The colony is maintained without addition of field-collected insects. These larvae were reared on diets similar to those described for fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Perkins 1979). The larvae were held in capped 30-ml plastic cups containing standard bean diet placed in an environmental cabinet at 24 ± 2°C, a photoperiod of 12:12 (L:D) h, and 50 ± 5% RH for either 1 or 6 d before exposure to the insecticide. This procedure allowed larvae to reach uniform size and age prior to treatment. After either 1 or 6 d, the larvae were removed from the cabinet and used for the bioassays. A single larva was placed in a 30-ml cup containing 10 ml of fresh bean diet that had been treated with 0.10 ml of either distilled water or one of the previously prepared IGR dilutions. Thirty 1-d-old and twenty 6-d-old larvae per dilution were exposed to treated diet at one time. Three replications were conducted for each treatment and larval age. The diet was air-dried for 2 h after the addition of water or IGR dilutions before a larva was placed into a cup of treated diet. Cups were then capped and returned to the environmental cabinet, and status of individual insects was checked each day. Life stage (larva,

pupa, adult), morphological deformities, and time of death were recorded for each insect. Larvae were considered dead if they did not respond to touch by a dissecting needle within two probes. The test was continued until all individuals had either died or become adults.

Statistical Analyses. Means and standard deviations were calculated for all data. Concentration-mortality regressions were estimated for larval mortality and for total (larvae + pupae) mortality by probit analyses (POLO-PC; LeOra Software 1987). Significance of the differences in lethal concentrations of the insecticides tested was based on non-overalap of 95% confidence limits. Confidence limits were calculated only when analysis did not indicate heterogeneity of data. Potency of diflubenzuron and RH-5992 relative to fenoxycarb was determined based on methods developed by Finney (1971). Mortality rates were determined by dividing the number of dead individuals by either the total number of larvae tested (larval and final mortality) or the number of surviving pupae (pupal mortality) per IGR concentration for each larval age category.

Results and Discussion

Bioassay with 1-D-Old Larvae. All IGRs tested were similarly efficacious against 1-d-old beet armyworm larvae (Table 1). LC_{50} values were numerically highest (0.00145% AI) for larval mortality following exposure to fenoxycarb. However, based on overlap of 95% confidence limits, no significant differences were observed in larval mortality among any of the three compounds tested. LC_{90} values for larval mortality, however, were distinctly different (Table 1). RH-5992 treatments caused 90% mortality at significantly ($P \leq 0.05$) lower concentrations than did fenoxycarb. Use of fenoxycarb did not result in levels of larval mortality, at similar concentrations, as high as mortalities caused by other tested IGRs. Larvae did not always die following exposure to fenoxycarb; they continued to feed and undergo additional molts. Eventually, individuals pupated and additional deaths occurred during this stage due to deformities. Relative potency of diflubenzuron and RH-5992 against 1-d-old beet armyworm larvae, compared with control by fenoxycarb, was 3.00 and 12.35, respectively, with the greater number indicating a greater degree of effectiveness (mortality). Final generation mortality (LC_{50}'s) was similar among all IGRs tested (Table 1). However, achieving 90% control was more difficult with fenoxycarb than with RH-5992; LC_{90}'s were significantly ($P \leq 0.05$) higher for fenoxycarb than for RH-5992. Diflubenzuron treatments resulted in lethal concentration estimates similar to both fenoxycarb and RH-5992.

Mortality of 1-d-old beet armyworm larvae ranged from 12.2 to 100% when larvae were exposed to diflubenzuron at concentrations from 0.0001 to 1.0% AI (Table 2). Larvae lived an average of 3.4 to 6.2 d after being exposed to the compound. Death occurred at the molt between first and second instar when rates of 0.01 to 1% AI were used. At lower concentrations, death occurred at molts between the first and second, or second and third instar. Some pupal mortality occurred, but the majority of the mortality occurred in metamorphic intermediates appearing as part larva and part pupa. Adult deformities ranged from 2.8 to 28.6% at progressive treatment rates from 0.00001 to 0.001% AI. These adults either lacked wings or had vestigial wings. Mortality of 1-d-old larvae

Table 1. Response of 1-d-old beet armyworm larvae to insect growth regulators.

Compound	n*	Stage of mortality**	Slope ± SE	LC_{50} (95% CL)*	LC_{90} (95% CL)*
Diflubenzuron	630	Larvae	2.012 ± 0.376	0.00091 (0.0003-0.0015)	0.00393 (0.00225-0.02057)
		Final mortality	2.254 ± 0.560	0.00055 (0.00026-0.00077)	0.00202 (0.00142-0.00434)
Fenoxycarb	630	Larvae	0.731 ± 0.090	0.00145 (0.00001-0.00709)	0.08260 (0.01668-11.74748)
		Final mortality	0.692 ± 0.081	0.00010 (0.00001-0.00037)	0.00726 (0.00206-0.05851)
RH-5992	630	Larvae	2.940 ± 0.382	0.00025 (0.00006-0.00061)	0.00068 (0.00030-0.00502)
		Final mortality	2.937 ± 0.447	0.00026 (0.00016-0.00036)	0.00071 (0.0005-0.00108)

* n indicates number of tested individuals per compound; CL, confidence limits. Values presented as % AI.

** Larval mortality indicates number of individuals dying as larvae; final mortality evaluated after adult eclosion of survivors.

treated with RH-5992 was similar to that observed with diflubenzuron (Table 2). Larval death, however, occurred somewhat faster, ranging from 1.6 to 3.1 d after treatments were applied. Most deaths occurred during the first instar and mimicked symptoms observed following death caused by diflubenzuron. These larvae began to molt sooner than normal, thus resulting in premature death. Pupal mortality and deformities were observed following exposure to RH-5992. Deformities consisted of metamorphic larva-pupa intermediates. Percentage of deformed adults ranged from 4.5 to 7.9% and occurred only when the two lowest rates of RH-5992 were used (Table 2). Deformities consisted of vestigial wings.

One-d-old beet armyworm larvae exposed to fenoxycarb responded differently than did larvae exposed to diflubenzuron or RH-5992. As indicated in Table 1, larval mortality caused by fenoxycarb did not occur at rates similar to larvae treated with the other IGRs. Mortality of larvae treated with fenoxycarb ranged from 16.7 to 100% at rates from 0.00001 to 1.0% AI (Table 2), and larval death occurred from 7.1 to 33.6 d after treatment. Larval death from fenoxycarb took approximately 10 times as long as death resulting from diflubenzuron and RH-5992. Time to larval death increased as fenoxycarb concentrations were increased, and insects continued to feed and molt prior to death. Pupal mortality ranged from 34.7 to 100% with the deaths due to metamorphic deformities (larval-pupal intermediates) (Table 2). Percentage of deformities in adults was highest (67%) following exposure to fenoxycarb at 0.01% AI. Adult deformities consisted of incomplete wing formation.

Bioassay with 6-D-Old Larvae. RH-5992 was significantly ($P \leq 0.05$) more effective than the other two IGRs tested in killing 6-d-old beet armyworm larvae, based on non-overlap of LC_{50}'s (Table 3). Diflubenzuron and fenoxycarb were equally effective against 6-d-old beet armyworm larvae despite the fact that LC_{50}'s for fenoxycarb were ≈4 times larger than LC_{50}'s for diflubenzuron. LC_{90} values for larval mortality were similar ($P > 0.05$) between RH-5992 and diflubenzuron, although fenoxycarb required approximately 16 times greater concentration than diflubenzuron to give 90% mortality (Table 3). Relative potencies of diflubenzuron and RH-5992 compared with fenoxycarb were 5.3 and 21.45, respectively. As was noted for 1-d-old larvae, fenoxycarb did not effectively kill greater than 50% of 6-d-old larvae. However, surviving larvae may die as pupae before adult eclosion. LC_{50}'s for final mortality of all three tested compounds were similar ($P > 0.05$) (Table 3). However, the LC_{90} value for fenoxycarb was significantly ($P \leq 0.05$) higher than values for the other two compounds, indicating that 6-d-old beet armyworm larvae tolerated fenoxycarb more than the other IGR's.

In comparing the LC_{50}'s between 1- and 6-d-old larvae, it should be noted that numerical values were lower for both larval and final mortality with 6-d-old larvae after exposure to diflubenzuron and RH-5992 (Tables 1 & 3). Reasons for this unexpected occurrence are unknown, but these results indicated that it was easier to kill 50% of 6-d-old larvae with these two compounds than 1-d-old larvae. These trends were not obvious when comparing LC_{90}'s between the compounds.

Mortality of 6-d-old larvae ranged from 10 to 100% following their exposure to diflubenzuron at rates of 0.00001 to 1.0% AI (Table 4). Greatest mortality occurred with the three highest concentrations. Larval death occurred from 2.3

Table 2. Mortality and longevity of 1-d-old beet armyworm larvae following treatment with insect growth regulators.

Compound	Rate (% solution)*	n**	% larval mortality	Avg. larval age at death (d after treatment) ± SD	% pupal mortality	% pupae deformed	Avg. age at pupation (d after treatment) ± SD	% adults deformed	Avg. age at adult eclosion (d after treatment) ± SD	Total % mortality
Diflubenzuron	1.0	90	100.0	3.4 ± 1.8	–	–	–	–	–	100.0
	0.1	90	100.0	3.7 ± 1.9	–	–	–	–	–	100.0
	0.01	90	98.9	4.6 ± 3.5	100.0	100.0	16.0 ± 0.0	–	–	100.0
	0.001	90	55.6	5.5 ± 5.4	47.5	40.3	16.0 ± 2.5	28.6	24.4 ± 2.5	76.7
	0.0001	90	13.3	6.2 ± 5.8	11.5	10.3	13.4 ± 1.9	15.9	21.4 ± 2.0	23.3
	0.00001	90	12.2	5.4 ± 5.9	8.9	5.1	12.8 ± 1.8	2.8	20.6 ± 1.8	20.0
Fenoxycarb	1.0	90	100.0	33.6 ± 14.9	–	–	–	–	–	100.0
	0.1	90	98.9	29.5 ± 12.6	100.0	100.0	31.0 ± 0.0	–	–	100.0
	0.01	89	57.3	24.0 ± 11.9	84.2	84.2	25.0 ± 5.6	66.7	28.8 ± 2.5	92.1
	0.001	90	48.9	18.8 ± 11.3	52.2	39.1	20.0 ± 5.4	27.3	27.7 ± 2.4	75.6
	0.0001	91	26.4	10.6 ± 9.0	35.8	29.9	17.0 ± 2.8	14.0	25.7 ± 2.1	52.7
	0.00001	90	16.7	7.1 ± 9.2	34.7	26.7	14.7 ± 1.8	8.2	23.2 ± 2.0	45.6
RH-5992	1.0	90	100.0	1.7 ± 0.7	–	–	–	–	–	100.0
	0.1	90	100.0	1.6 ± 0.6	–	–	–	–	–	100.0
	0.01	90	100.0	2.1 ± 1.4	–	–	–	–	–	100.0
	0.001	90	96.7	2.7 ± 1.7	0.0	0.0	18.3 ± 1.5	0.0	26.7 ± 1.2	96.7
	0.0001	90	23.3	2.7 ± 1.2	8.7	4.3	13.0 ± 1.6	7.9	20.9 ± 2.0	30.0
	0.00001	90	22.2	3.1 ± 3.0	5.7	4.3	12.7 ± 1.5	4.5	20.4 ± 1.6	26.7
Untreated	–	90	3.3	1.0 ± 0.0	12.6	8.0	13.0 ± 1.6	2.6	20.9 ± 1.7	15.6

* 1% solution = 10 g/liter = 10,000 ppm.
** Number of individuals at start of bioassay.

Table 3. Response of 6-d-old beet armyworm larvae to insect growth regulators.

Compound	n*	Stage of mortality**	Slope ± SE	LC_{50} (95% CL)*	LC_{90} (95% CL)*
Diflubenzuron	420	Larvae	1.170 ± 0.153	0.00026 (0.00015-0.00043)	0.00329 (0.00188-0.0074)
		Final mortality	1.212 ± 0.162	0.00008 (0.00005-0.00014)	0.00095 (0.00054-0.00215)
Fenoxycarb	418	Larvae	0.778 ± 0.084	0.00119 (0.00009-0.0061)	0.05276 (0.00953-4.5985)
		Final mortality	0.728 ± 0.088	0.00012 (0.00005-0.00024)	0.00690 (0.00314-0.02032)
RH-5992	419	Larvae	1.035 ± 0.120	0.00006 (0.00001-0.0002)	0.00109 (0.00032-0.02178)
		Final mortality	1.063 ± 0.158	0.00004 (0-0.00012)	0.00057 (0.00017-0.00184)

* n indicates number of tested individuals per compound; CL, confidence limits. Values presented as % AI.
** Larval mortality indicates number of individuals dying as larvae; final mortality evaluated after adult eclosion of survivors.

Table 4. Mortality and longevity of 6-d-old beet armyworm larvae following treatment with insect growth regulators.

Compound	Rate (% solution)*	n**	% larval mortality	Avg. larval age at death (d after treatment) ± SD	% pupal mortality	% pupae deformed	Avg. age at pupation (d after treatment) ± SD	% adults deformed	Avg. age at adult eclosion (d after treatment) ± SD	Total % mortality
Diflubenzuron	1.0	60	100.0	2.5 ± 1.4	–	–	–	–	–	100.0
	0.1	60	100.0	2.3 ± 1.0	–	–	–	–	–	100.0
	0.01	60	98.3	2.7 ± 1.5	100.0	100.0	6.0 ± 0.0	–	–	100.0
	0.001	60	73.3	6.1 ± 2.7	62.5	50.0	7.4 ± 1.2	33.3	15.7 ± 2.9	90.0
	0.0001	60	30.0	9.7 ± 2.9	38.1	31.0	7.5 ± 1.7	15.4	15.2 ± 1.9	56.7
	0.00001	60	10.0	8.5 ± 1.4	11.1	11.1	6.9 ± 1.1	2.1	13.7 ± 1.2	20.0
Fenocycarb	1.0	60	100.0	22.1 ± 10.6	–	–	–	–	–	100.0
	0.1	60	100.0	21.8 ± 9.1	–	–	–	–	–	100.0
	0.01	59	66.1	16.9 ± 5.9	80.0	65.0	16.2 ± 3.5	75.0	25.3 ± 3.3	93.2
	0.001	59	39.0	11.9 ± 4.5	52.8	41.7	11.1 ± 2.1	52.9	21.0 ± 2.5	72.2
	0.0001	60	31.7	9.7 ± 2.0	24.4	17.1	9.8 ± 1.4	19.4	19.2 ± 1.7	48.3
	0.00001	60	8.3	9.2 ± 4.8	27.3	27.3	7.7 ± 1.2	15.0	16.3 ± 1.8	33.3
RH-5992	1.0	60	100.0	2.9 ± 0.9	–	–	–	–	–	100.0
	0.1	60	100.0	3.5 ± 1.0	–	–	–	–	–	100.0
	0.01	60	96.7	4.6 ± 2.7	100.0	50.0	–	–	–	100.0
	0.001	60	98.3	4.9 ± 2.1	100.0	100.0	–	–	–	100.0
	0.0001	60	48.3	4.8 ± 1.8	9.7	6.5	6.9 ± 1.5	7.1	14.6 ± 1.8	53.3
	0.00001	59	25.4	6.2 ± 2.1	25.0	18.2	6.9 ± 3.0	12.1	14.1 ± 1.5	44.1
Untreated	–	60	1.7	9.0 ± 0.0	5.1	3.4	6.4 ± 0.8	3.6	13.7 ± 1.1	6.7

* 1% solution = 10 g/liter = 10,000 ppm.
** Number of individuals at start of bioassay.

to 9.7 d after exposure to diflubenzuron, depending upon concentration, with higher concentrations giving shorter times to cause mortality. Length of time for larval death to occur with diflubenzuron was between 2 and 6 d for 1- and 6-d-old larvae, respectively (Table 2 and 4). Some pupal deformities and mortality resulted with 6-d-old larvae, with all deformities consisting of metamorphic (larval-pupal) intermediates (Table 4). Pupation of 6-d-old larvae occurred within 6 to 7 d after being exposed to diflubenzuron, and adult eclosion occurred from 14 to 15 d after treatment; these times of life stage development were similar to observations with untreated larvae. Adult deformities included wing loss or the appearance of vestigial wings. Larvae exposed to the three high rates of RH-5992 took longer to die than did larvae exposed to the three high rates of diflubenzuron (Table 4). Larval mortality resulting from RH-5992 occurred within 2.9 to 4.6 d, while mortality resulting from diflubenzuron occurred within 2.3 to 2.7 d. As with diflubenzuron, some metamorphic larval-pupal deformities resulted following exposure to RH-5992. Adult deformities consisted primarily of vestigial wings.

Six-d-old beet armyworm larvae exposed to fenoxycarb lived longer than did larvae treated with other IGRs tested (Table 4). Larvae lived from 9.2 to 22.1 d following exposure to 0.00001 to 1.0% AI dilutions of fenoxycarb. Larvae continued feeding on treated diet before they pupated. Pupal mortality reached 80% when larvae were exposed to the 0.01 AI concentration of fenoxycarb. Most pupal mortality resulted in insects deformed as metamorphic larval-pupal intermediates. In most instances, time to pupation after exposure to fenoxycarb was longer than with untreated larvae (Table 4). Adult deformities (vestigial wings) ranged from 15 to 75% following exposure to 0.00001 to 0.01% AI concentrations of fenoxycarb (Table 4).

These data show that all three IGRs tested provided levels of mortality that may be adequate for control of both 1- and 6-d-old beet armyworm larvae. In general, RH-5992 and diflubenzuron provided quicker kill of larvae than did fenoxycarb and would be more useful against field populations of this insect pest. Larvae treated with fenoxycarb continued to feed longer than larvae treated with the other IGRs; such feeding could be detrimental in actual field situations. Diflubenzuron and Rh-5992 provided somewhat better kill of 6-d-old larvae than of 1-d-old larvae based on LC_{50} values. Differences were not as apparent when LC_{90} values were compared. Since beet armyworm resistance to diflubenzuron has been documented in Europe (Van Laecke 1988) and may be found in other areas, RH-5992 may be of greater value for managing this pest in the field.

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