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Susceptibility of Embryonic and Postembryonic Developmental Stages of *Riptortus clavatus*(Hemiptera : Alydidae) to Diflubenzuron

톱다리개미허리노린재의 배자발육에 미치는 Diflubenzuron의 영향

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ABSTRACT Laboratory studies were done to evaluate the effects of diflubenzuron on embryonic and postembryonic development of *Riptortus clavatus* Thunberg. Diflubenzuron prevented egg hatch ; younger eggs(0~12 h old) were 2 times as susceptible as older(48-60 h old) eggs, but embryos of both younger and older eggs developed normally. Susceptibility of nymphs to diflubenzuron decreased with each successive molt. Compared with the first instar, relative tolerance to diflubenzuron was 1.5 times for the second instar, 18.2 times for the third instar, 39.4 times for the fourth instar and 42.4 times for the fifth instar. Even as low concentrations, diflubenzuron prevented significant numbers of third instar nymphs from developing to fourth and fifth instar nymphs or adults. Weight, longevity and fecundity of adults surviving treatment in the final (fifth) instar were also adversely affected.

KEY WORDS Bean bug, *Riptortus clavatus*, diflubenzuron, ovicidal effect

초 록 키틴생합성저해제인 diflubenzuron을 톱다리개미허리노린재(*Riptortus clavatus* Thunberg)에 미량국소처리하였을때 충태 및 영기에 따른 약제의 감수성차이와, 종령약충 처리후 우화율 및 우화성충에 미치는 영향을 조사하였다.

Diflubenzuron은 살란효과를 보였으며 산란후 12시간내의 알은 산란후 48~60시간의 알보다 감수성이 높았으나, 알의 나이에 관계없이 처리된 알의 배는 정상적으로 발육하였다. 영기별 감수성은 영기가 진행될수록 낮아져 1령약충이 2령에 비하여 1.5배, 3령약충에 비하여 18.2배, 4령약충에 비하여 39.4배, 5령약충에 비하여 42.4배 높은 감수성을 나타내었다. 종령약충에 처리하였을때 우화율과 우화성충의 체중, 수명 및 산란율은 현저히 감소하였다.

검 색 어 톱다리개미허리노린재, diflubenzuron, 살란효과, 약제감수성

The economic importance of a bean bug, *Riptortus clavatus* (Thunberg), in the cultivation of beans in Korea has increased in recent years (Koh et al. 1984, Kim et al. 1988). Its control is presently based on repeated application of broad-spectrum, organophosphorus insecticides. However, the increasingly adverse effects of organophosphorus insecticides necessitate development of effective yet more selective insecticides for controlling this insect.

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Benzoylphenyl urea (BPU) insecticides such as diflubenzuron may be a suitable alternative. They act directly within the integument to block the terminal polymerization stage in chitin biosynthesis in both larvae and adults (Hajjar & Casida 1978), but their exact mode of action has not been defined. BPUs have become increasingly important in integrated pest management for the control of various insect pests(e.g., Westigard 1979, Elliott & Anderson 1982, Broadbent & Pree 1984) because of their insecticidal toxicity, lack of phytotoxicity, low mammalian toxicity, and little or no adverse effects on natural enemies. Their delayed action and narrow spectrum of effectiveness will, however, be one of the limiting factors in the future use and further development of these chemicals.

In a previous paper (Kim et al. 1992), we established that diflubenzuron affected adversely ovarian development of *R. clavatus* adults, suggesting that significant reduction in the fecundity of adults treated with diflubenzuron may be the result of inhibition of ovarian development. The effects of the insecticide on the other stages of this insect have not been reported. Therefore, we did laboratory studies to investigate the ovicidal effects, nymphal stadial susceptibility of diflubenzuron, and effects on the developmental characteristics of survivors of treatment as last instar nymphs.

MATERIALS AND METHODS

Insects. Adult *R. clavatus* were initially obtained from fields in Daejeon, Korea in October 1986. They were reared without exposure to any insecticide on bean(*Glycine max* [Merrill]) seedlings (3-4 wk after germination) in plastic cages(40 by 40 by 55 cm) maintained at 26±1 ℃, 50-60% RH, and a photoperiod of 16 : 8 (L : D) in the laboratory at the Korea Research Institute of Chemical Technology. To facilitate daily separation of eggs and nymphs of the same age, groups of 20 pairs were placed with three plastic pots(6 by 10 cm), each with four plants (3 wk after germination) held within plastic cages (30 by 26 by 20 cm) for 24 h. The adults were then removed from the cages. Eggs and nymphs of defined ages were used for the experiments.

Chemical. Diflubenzuron(90% AI) was supplied by Youngil Chemical Co., Seoul, Korea.

Effect on Egg Hatch. To determine the ovicidal effect of diflubenzuron, batches of eggs either 0-12 or 48-60 h-old were treated topically with solutions of diflubenzuron in acetone. Treated eggs were held at 25±1℃, 50-60% RH, and a photoperiod of 16 : 8(L : D). Controls were treated with acetone only. Numbers of hatched eggs were counted daily until eclosion was completed.

Effect on Nymphal Susceptibility. The appropriate concentrations of diflubenzuron in 0.8 μl of acetone were applied topically to the thoracic dorsa of nymphs in stadia 1-5. The response of nymphs at the time of molting and the nymphs that died or molted abnormally were recorded daily. The latter were also counted as dead.

Effect on Longevity and Reproduction. To examine the response of adults surviving treatment as nymphs, 0.01 and 0.001 μg diflubenzuron in 0.8 μl of acetone was applied topically to the thoracic dorsa of fifth instar nymphs. After treatment, groups of 20 pairs were placed with five pots, each with four plants(3 wk after emergence), in the plastic cages described above. The cages were then held for 60 d at 26±1℃, 50-60% RH, and a photoperiod of 16 : 8(L : D). If necessary, older bean plants were replaced by new ones. Emergence rate, weight of female and male adults, longevity and fecundity of adults were recorded.

Statistical Analysis. Data(emergence rate, weight, adult longevity, and fecundity) were analyzed by analysis of variance(ANOVA). Means were compared and separated by Fisher's protected least square difference at $P<0.01$ (SAS Institute 1986).

Table 1. The toxicity of diflubenzuron to *Riptortus clavatus* eggs

Egg age(h)	n	Slope±SE	EC_{50}, μg/egg(95% CL)	χ^2
0-12	60	2.25±0.26	0.11(0.090-0.134)	1.97
48-60	60	2.95±0.22	0.25(0.198-0.336)	2.23

RESULTS

Effect on Egg Hatch. When diflubenzuron was applied topically to *R. clavatus* eggs of two different ages, younger eggs were more susceptible to diflubenzuron than older ones(EC_{50} of 0.11 μg per 0-5 h-old egg, versus 0.25 μg per 48-60 h-old egg)(Table 1). Irrespective of egg age at the time of treatment, the embryos developed fully, but death was caused by that larvae were unable to exit through the chorion. Even nymphs that hatched from the treated eggs failed to feed and died prematurely.

Nymphal Stadial Susceptibility. Table 2 shows the failure rate in the first molt of *R. clavatus* when they were treated with diflubenzuron, beginning at different nymphal instars. Failure of molting included all abnormal individuals, such as those that died before molt or that molted partially. Most nymphs that had undergone partial ecdysis did not feed and died before the next molt. Bean bug nymphs became more tolerant to diflubenzuron with each successive molt. Compared with the first instar, relative tolerance was 1.5-fold for the second, 18.2-fold for the third, 39.4-fold for the fourth and 42.4-fold for the fifth instar. However, when the EC_{50} per unit body weight of nymphs was estimated, first, second and fifth instars were about equally susceptible. In contrast, the fourth and especially the third instar nymphs were much more tolerant to diflubenzuron.

Table 2. Susceptibility of nymphal instars of *Riptortus clavatus* to diflubenzuron

Instar	n	Stage of molting failure	Slope±SE	$EC_{50}(\times 10^{-3})$ μg/bug	$EC_{50}(\times 10^{-3})$ 95% CL	$EC_{50}(\times 10^{-3})$ μg/mg wt insect	χ^2
1	50	into 2nd	4.12±0.43	0.33(1.0)[a]	0.25-0.42	0.24	2.13
2	50	into 3rd	1.98±0.38	0.49(1.5)	0.38-0.69	0.19	4.07
3	50	into 4th	3.06±0.31	6.0 (18.2)	5-8	1.58	3.01
4	50	into 5th	1.91±0.27	13.0 (39.4)	10-18	0.99	2.84
5	50	into adult	2.33±0.32	14.0 (42.4)	10-18	0.22	1.77

[a] Letters in parentheses denote the susceptibility ratio compared with the first instar.

Because the insensitivity of the third instar compared with other instars was so drastic, we also investigated the fate of successive developmental stages after treatment as third instars with diflubenzuron (Table 3). With 0.0128 μg per nymph, mortality due to failure of the third instar to molt was 87%. The survivors failed to molt to the fifth instar. With 0.0016 μg per nymph, only 3% of the nymphs developed into adults. Even at 0.0005 μg per nymph, failure of the third instar to molt to the adult stage was 50%.

Table 3. The fate of successive developmental stages after treatment as third instars with diflubenzuron

Concentration, μg/nymph	n	Cummulative mortality due to molting failure in instar, %		
		4	5	Adult
0.0128	30	87	100	–
0.0064	30	73	90	100
0.0032	30	47	77	100
0.0016	30	27	47	97
0.0008	30	13	23	67
0.0004	30	10	10	50

Effects on Longevity and Reproduction. Table 4 shows the effects on the biological characteristics of adults that survived topical application of duflubenzuron as fifth instar nymphs. Diflubenzuron was applied at 0.001 and 0.01 μg per nymph, these concentrations corresponded with the approximate EC_5 and EC_{50} for the fifth instar nymph, respectively. At 0.01 μg per nymph, the insecticide caused moderate, but significant reduction of body weight and very pronounced reduction of longevity of the emerging adults, which they appeared morphologically normal. Oviposition of the emerging adults was also greatly affected ; the untreated female adults deposited($\bar{X}\pm SD$) 140±9.2 eggs per female, but no eggs were laid by females surviving treatment. Similar results were obtained when fifth instars were treated with doses as low as 0.001 μg nymph. However, body weight of males was not reduced significantly.

Table 4. Effects of diflubenzuron on emergence rate, weight, longevity, and oviposition of *Riptorius clavatus* adults surviving treatment with diflubenzuron at the fifth instar

Dosage, μg/bug	n	Emergence (%)	Weight($\bar{X}\pm SD$), mg		Adult ♀ longevity, Days±SD	No. eggs ($\bar{X}\pm SD$) laid/♀
			♀	♂		
0.01	60	71.4	39.1±4.6*	33.5±6.5*	11.4± 3.5*	0
0.001	64	84.4	42.5±4.2*	40.8±8.9	13.5± 4.7*	0
0.	84	92.9	51.8±4.6	42.5±5.7	42.0±10.8	140.0±9.4

*Significantly different from control($P<0.01$; Fisher's protected least significant difference test [SAS Institute 1986]).

DISCUSSION

Diflubenzuron shows strong ovicidal activity against *R. clavatus*. Although the exact mode of its ovicidal action remains unknown, the insecticide interferes with chitin biosynthesis. Consequently, the treated embryos cannot leave the chorion because the cuticle lacks sufficient rigidity(Grosscurt 1978, Elliott & Anderson 1982). In our study, embryos were fully developed in the eggs of *R. clavatus* treated with diflubenzuron, but larvae could not exit through the chorion. Therefore, we conclude that the inhibitory effects on egg hatch might be caused by weakness of the cuticle.

Ovicidal effect of diflubenzuron declined with age of *R. clavatus* eggs, as previously reported for eggs of cotton leafworm(*Spodoptera littoralis* [Boisduval])(Ascher & Nemny 1974), codling moth(*Cydia pomonella*[L.])(Hoying & Riedl 1980, Elliott & Anderson 1982), oriental fruit moth(*Grapholitha molesta*[Busck]) and the obliquebanded leafroller(*Choristoneura rosaceana* [Harris])(Broadbent & Pree 1984). This differential sensitivity of eggs to diflubenzuron may be caused by changes in permeability of the chorion of their eggs. The amount of diflubenzuron penetrating into younger eggs and reaching the site of action is probably higher than in older eggs, although no data are available to confirm this hypothesis.

R. clavatus nymphs became more insensitive to diflubenzuron with each successive molt. Instars 1-2 were most affected, whereas the insensitivity of the later stages increased drastically. This effect has been noted previously with the soybean looper(*Pseudoplusia includens* [Walker])(Reed & Bass 1980) and the oriental tobacco budworm(*Heliothis assulta* [Guenee]) (Lee 1985). Contrary to our findings, susceptibility to diflubenzuron declined with age of spruce budworm(*Choristoneura fumiferana* [Clemens]) larvae(Granett & Retnakaran 1977) and gypsy moth(*Lymantria dispar* [L.]) larvae (Abdelmonem & Mumma 1981). The insensitivity of developmental stages to an insecticide may be due to an increase in body weight, a decrease in penetration, or biochemical and physiological changes of insect itself (Smith & Salkeld 1966). While no date are availbale to confirm the importance of second factor, at least the first factor does not seem plusible because the value of EC_{50} per mg weight of fifth instar is not different from that of first instar(Table 2). The most plausible factor seems to be the third one. In their studies with another chitin synthesis inhibitor penfluron belonging to the same chemical group as diflubenzuron, Abdelmonem et al. (1980) reported that lack of feeding in gypsy moth may result from the reduction in chitin synthesis. Such a reduction would weaken the mouthparts, or cause incomplete clearance of the foregut and the hindgut at the molt. Diflubenzuron showed antifeedant activity which ultimately resulted in death of the insects. Based on our results and these earlier reports, differential nymphal susceptibility might be explained by changs in biochemical or physiological processes which resulted in chitin synthesis inhibition.

Diflubenzuron prevented egg production in adults that survived treatment as nymphs. This effect was also reported in the spruce budworm adults surviving the larval treatment with insect growth regulators such as penfluron (Brushwein & Granett 1977) and chlorfluazuron(Madore et al. 1983). The suppression of oviposition may be mainly due to inhibition of ovarian development, although other biological factors such as reduced longevity(Table 4) may be involved in the phenomenon. Kim et al. (1992) reported that diflubenzuron had significant adverse effects on ovarian development of 0-3 h-old female adults. These effects resulted in reduced egg production.

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