

Studies on the Toxicity of Insect Growth Regulators against the Fall Webworm (*Hyphantria cunea* Drury) and the Rice Stem Borer (*Chilo suppressalis* Walker)

I. Comparisons of Insecticidal Activities against Various Instar Stages

미국흰불나방과 이화명나방에 대한 昆蟲 發育沮害劑의 毒性研究
I. 令期別 殺蟲力 效果 比較

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ABSTRACT The experiments were carried out to investigate the toxicological characteristics of Insect Growth Regulators (IGRs) such as chlorfluazuron, diflubenzuron, pyriproxyfen and tebufenozide against the various stages of instars of fall webworm (*Hyphantria cunea* Drury) and rice stem borer (*Chilo suppressalis* Walker). In fall webworm, the tolerance ratio (TR) with the 2nd~6th instars, as compared with LC₅₀ of the 1st instar, ranged 1.07~3.58, 1.13~6.06, 1.02~3.23 and 1.05~6.64, respectively. In the rice stem borer, the TR of the 3rd instar, as compared with LC₅₀ of the 1st instar, to chlorfluazuron, diflubenzuron, pyriproxyfen and tebufenozide were 2.86, 2.60, 19.80 and 15.30, respectively.

KEY WORDS IGR, fall webworm, rice stem borer, various instar stages

초 록 본 실험은 미국흰불나방(*Hyphantria cunea* Drury)과 이화명나방(*Chilo suppressalis* Walker)에 대한 chlorfluazuron, diflubenzuron, pyriproxyfen과 tebufenozide와 같은 IGRs의 독성효과를 조사하기 위해 수행되었다. 미국흰불나방에서, 1령충의 LC₅₀치(ppm) 기준 2령충~5령충의 내성비 범위는 chlorfluazuron, diflubenzuron, pyriproxyfen과 tebufenozide에서 각각 1.07~3.58, 1.13~6.06, 1.02~3.23, 1.05~6.64이었다. 이화명나방에서, 1령충에 대한 3령충의 LC₅₀치(ppm)의 내성비는 chlorfluazuron, diflubenzuron, pyriproxyfen과 tebufenozide에서 각각 2.86, 2.60, 19.80, 15.30이었다.

검색어 곤충발육저해제, 미국흰불나방, 이화명나방, 곤충영기

The fall webworm (*Hyphantria cunea* Drury) is a common polyphagous pest with a worldwide distribution in tropical and subtropical regions. It is one of the invasive pests from USA in 1958. The insect was known to have the 160 species of host trees with broad-leaf and it was the most notorious pest of forest, street trees and garden plants during 1970s in South Korea. The insect is still the major pest of street trees and garden plants.

The rice stem borer (*Chilo suppressalis* Walker),

a common pest of rice field in eastern Asia, had been a main pest in rice culture by 1980 in South Korea. Even at this point, the insect should be controlled with pesticides to minimize yield loss. Song *et al.* (1982) reported the multiplication and the occurrence of the insect at partial localities since 1990. Both the fall webworm and the rice stem borer are bivoltine insects in Korea.

A new class of insecticide, the main derivatives of benzoylphenyl ureas (BPUs), have recently shown

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promise in controlling insects of agricultural, forestal, medical and veterinary importance (Menn & Hollingworth 1985). By Awad & Mulla (1984), these novel materials generally referred to Insect Growth Regulators (IGRs) induce morphological and physical disorders and produce deleterious effects and lethal abnormalities in treated insects. Up to the date, the development of IGRs has been received a lot of attention as a potential measures for selective control of insect pests and as a relatively low toxic pesticide for environment in the world (Awad & Mulla 1984, Menn & Hollingworth 1985).

In reviews of research on diflubenzuron (Dimilin®), gypsy moth (Abdelmonem & Mumma 1981), codling moth (Hoying & Riedel 1980), oriental tobacco budworm (Lee 1985), spruce budworm (Retnakaran 1979) and soybean looper (Reed & Bass 1980) were tested against the different stages and instars of the insects to the IGR and on cyromazine, house fly (Awad & Mulla 1984a) and Australian sheep blow fly (Fiedel & McDonnell 1985), it was tested against the different stages and instars of the insects. In reviews of research on RH-5849, japanese beetle and fall armyworm (Monthean & Potter 1992), tobacco cutworm (Park *et al.* 1992) and tobacco hornworm (Wing 1988) were tested against the different stages and instars of the insects.

On the other hand, Demark & Bennett (1989) evaluated the inhibition efficacy of the chlorfluazuron against the nymph of the german cockroach and compared it with inhibition activities against the 3rd and the 5th nymphal stages. Bae *et al.* (1992) evaluated the effect of the buprofezin against the brown planthopper (*Nilaparvata lugens* Stal) in the laboratory and pot trials, and the inhibition activities to the developmental nymphal stages were reported by Ahn *et al.* (1992)

However, the specific activities of the IGRs such as chlorfluazuron, diflubenzuron, pyriproxyfen and tebufenozide against the different stages of larval instars of the fall webworm and the rice stem borer have not yet been reported. Fortunately, the diflubenzuron 25% WP and the chlorfluazuron 5% WP were only recommended at the rate of 6,000 times dilution for fall webworm control (cited in insect pests and diseases of trees and shrubs p 143, published Forestry Research Institute Forestry Administration) Laboratory studies were conducted to assess the relative mortality activities the IGRs against the developmental larval instars of fall webworm and rice stem borer.

MATERIALS AND METHODS

The fall webworms (*Hyphantria cunea* Drury) collected as egg masses and the gregarious colonies from cherry tree at gardens of Woosung apartment in Cheongju City on May 25th in 1992, were continually reared with mulberry leaves in rearing boxes (34×20×53 cm: Philip Harris) of 28±2°C of temperature, 80~85% of relative humidity and 12:12 (L:D) of photoperiods. The adult rice stem borers (*Chilo suppressalis* Walker) collected from rice field with black light-traps at Cheongweon county, Chungbuk province, at early in the August of 1992, laid in a susceptible rice variety (Chucheong-byeo) and there the egg-laid plant was reared in the beaker (500 ml) which contains seedlings of the variety, after hatching from the egg-mass. The environmental conditions of all experiments carried out were the same as mentioned in advance.

The IGRs used in this experiment were obtained from the manufacturers and suppliers of agrochemicals in Korea (Table 1). The standard solution was

Table 1. Name and purity of chemicals used in the experiment

| Common name | Trade name | Chemical name | Purity (%) |
|----------------|------------|--|------------|
| Chlorfluazuron | Atabron | 1-[3,5-dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)phenyl]-3-(2,6-difluorobenzoyl)urea | 97.3 |
| Diflubenzuron | Dimilin | 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea | 91.4 |
| Pyriproxyfen | Sumilarv | 2-[[1-methyl-2-(phenoxyphenoxy)ethoxy]pyridine | 98.1 |
| Tebufenozide | Mimic | N-tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide | 95.0 |

Table 2. Comparisons of according to developmental larval instars of fall webworm (*Hyphantria cunea* Drury) in mortality effects of chlorfluazuron by the leaf dipping method

| Instar | N | LC ₅₀ (95%FL) ^a | LC ₉₅ | Slope±SE | TR ^b |
|--------|-----|---------------------------------------|------------------|-----------|-----------------|
| 1st | 250 | 6.98(5.1-9.0) | 62.93 | 1.72±0.21 | 1.00 |
| 2nd | 250 | 7.49(5.4-9.8) | 83.02 | 1.57±0.20 | 1.07 |
| 3rd | 250 | 7.78(5.1-10.9) | 163.06 | 1.24±0.18 | 1.11 |
| 4th | 250 | 8.24(6.1-10.8) | 90.90 | 1.58±0.19 | 1.18 |
| 5th | 350 | 23.56(19.4-28.6) | 136.04 | 2.16±0.21 | 3.38 |
| 6th | 350 | 25.01(20.5-30.3) | 130.26 | 2.30±0.25 | 3.58 |

^a95% fiducial limits(ppm); TR^b(Tolerance Ratio): LC₅₀ values of each instar/ LC₅₀ values of 1st instar

prepared to the 1% solution (W/V) with reagent acetone (Gr 99.5%) and the preparation of applying solution was made the mixture of acetone(diluted chemical) and distilled water (1:9 V/V) at 5-6 levels for the analysis of probits.

Leaf dipping method was used to evaluate the effect of IGRs on the fall webworm. Mulberry leaf disk (φ 9 cm) was dipped in the treated solution for 30 sec. After the excess solution on the surface was absorbed by filter paper, the treated disk was dried in the shaded lot of laboratory. Then, it was put in the petri-dish (φ 9 cm) and the testing instars younger than 1 day after hatching or molting according to developmental instars of infested on the treated leaf within petri-dish. Untreated mulberry leaf disk was supplied at every 24 hours into the same size of petri-dish after 24 hours feeding of treated disk. The number of tested instars per each concentrations of IGRs was infested with 5 replications, 10 instars per replication in 1~2 instars and the other instars increased the replications without any reduction in the number of treated instars per concentration. The efficacy was counted with the appearance of stable mortality at 4 days after treatment (DAT). For evaluation of the rice stem borer, rice stem dipping method was used. A bundle of Chucheongbyeon bound with 6 stems (5 cm long) at 60 days after transplanting, was dipped into the solution for 30 sec. After the excess solution on the bundles was absorbed by cottons, the treated bundle was dried at the shade in the laboratory. Then, these were put in the beaker (500 ml) and the instars of 1 day old after hatching and 1 day old after molting from 2nd instars were infested on treated bundles. The number of tested instars per concentration was

infested with 5 replications, 10 instars per replication/beakers without distinguish the stage of instars. The dead instar was counted at the 6th days after infestations. The LC₅₀ was calculated by probit analysis with computer.

RESULTS AND DISCUSSIONS

The mortality effects of chlorfluazuron by developmental larval stages of fall webworm (*Hyphantria cunea* drury) in the leaf dipping method were shown in Table 2.

In Table 2, the LC₅₀ values of chlorfluazuron by developmental larval stages of fall webworm were increased and the values of TR in 5th and 6th instars as compared with that of 1st~4th instars were increased 3.38 and 3.58 times, respectively.

In chlorfluazuron against the nymphal german cockroach (*Blattella germanica* L.) reported by Demark & Bennett (1989), low diet concentration of chlorfluazuron to kill the 2nd instars increased 2 times as compared with that of the 5th instars and the mortality ratio of the 5th instars as compared with 2nd instar was. Although it was not agreed with tested insects, the experimental results of the fall webworm showed the correspondent tendency with results of remarks.

The mortality effects of the diflubenzuron in accordance with developmental larval stages of the fall webworm in the leaf dipping method were shown in Table 3

In Table 3, the LC₅₀ values of diflubenzuron against the developmental larval instars of the fall webworm increased gradually and the TR values of 4th and 5th instars increased progressively, and as com-

Table 3. Comparisons of mortality effects of diflubenzuron according to developmental larval instars of fall webworm (*Hyphantria cunea* Drury) in mortality effects of diflubenzuron by the leaf dipping method

| Instar | N | LC ₅₀ (95%FL) ^a | LC ₉₅ | Slope±SE | TR ^b |
|--------|-----|---------------------------------------|------------------|-----------|-----------------|
| 1st | 250 | 5.78(4.4-7.2) | 29.29 | 2.33±0.28 | 1.00 |
| 2nd | 250 | 6.54(4.9-8.3) | 37.55 | 2.17±0.27 | 1.13 |
| 3rd | 300 | 9.13(6.7-12.0) | 90.09 | 1.65±0.20 | 1.58 |
| 4th | 300 | 10.24(7.6-13.3) | 107.32 | 1.61±0.18 | 1.77 |
| 5th | 300 | 19.40(14.3-26.3) | 371.67 | 1.28±0.14 | 3.36 |
| 6th | 300 | 35.02(25.6-48.0) | 607.05 | 1.32±0.15 | 6.06 |

^a95% fiducial limits (ppm); TR^b (Tolerance Ratio); LC₅₀ values of each instar/LC₅₀ values of 1st instar

Table 4. Comparisons of mortality effects of pyriproxyfen according to developmental larval instars of fall webworm (*Hyphantria cunea* Drury) in mortality effects of pyriproxyfen by the leaf dipping method

| Instar | N | LC ₅₀ (95%FL) ^a | LC ₉₅ | Slope±SE | TR ^b |
|--------|-----|---------------------------------------|------------------|-----------|-----------------|
| 1st | 420 | 30.66(25.0-37.3) | 253.91 | 1.79±0.16 | 1.00 |
| 2nd | 420 | 31.17(21.0-46.2) | 284.62 | 1.71±0.22 | 1.02 |
| 3rd | 300 | 35.94(26.7-48.4) | 522.93 | 1.41±0.14 | 1.17 |
| 4th | 350 | 40.33(31.5-51.0) | 346.39 | 1.76±0.16 | 1.32 |
| 5th | 400 | 88.28(68.8-113.8) | 1240.35 | 1.43±0.11 | 2.88 |
| 6th | 400 | 99.10(73.8-132.5) | 1885.21 | 1.29±0.12 | 3.23 |

^a95% fiducial limits (ppm), TR^b (Tolerance Ratio). LC₅₀ values of each instar/LC₅₀ values of 1st instar

pared with those of 1st~4th instars, it was 3.36 and 6.06 times, respectively.

The diflubenzuron was studied on various insects by many researchers. New IGR (EL-494) structures closely similar to diflubenzuron except for the bromophenyl-methylpyrazine moiety and the dichloro substitution were reported by Retnakaran (1979). The comparisons of EC₅₀ values with EL-494 in according to developmental larval instars of spruce budworm (*Choristoneura fumiferana*) by diet tests were 0.205 ppm for the 3rd instar, 0.249 for the 4th, 0.287 for the 5th and 0.486 for the 6th. The TR values of EL-494 to the 4th, 5th and 6th instars of the spruce budworm as compared with that of the 3rd instar were gradually increased by the stage of instars, i.e., 1.2, 1.4 and 2.3 times, respectively. In comparison of mortality effects on diet surface treated with diflubenzuron against the developmental stages of soybean looper (*Pseudoplusia includens* Walker) by Reed & Bass (1980). LD₅₀ values for the 5th instars were significantly greater than that of similar values for 1st and 3rd instars and significantly less than LD₅₀ values for 6th instars. Abdel-

monem & Mumma (1981) reported that the EC₅₀ values of mortality inhibition with diflubenzuron to the 3rd and the 4th instars of gypsy moth (*Lymantria dispar*) by continuous feeding on artificial diet method showed 0.052 ppm and 0.009, respectively. The EC₅₀ values of diflubenzuron to the 3rd, 4th, 5th and 6th instars of tobacco budworm (*Helioverpa assulta* Guenee) by continuous feeding on artificial diet treatment were 16.2, 21.3, 20.4 and 27.6 ppm, respectively (Lee 1985).

In comparisons, Retnakaran (1979) and Reed & Bass (1980) were in concord with our results, but Abdelmonem & Mumma (1981) and Lee (1985) did not compare EC₅₀ values because of the different feeding amounts in accordance with developmental instars by continuous feeding on artificial diet method.

The mortality effects of pyriproxyfen in conformity with developmental larval stages of the fall webworm by the leaf dipping method were shown in Table 4.

In Table 4, the LC₅₀ values of pyriproxyfen against the developmental larval instars of the fall webworm

Table 5. Comparisons of mortality effects of tebufenozide according to developmental larval instars of fall webworm (*Hyphantria cunea* Drury) in mortality effects of tebufenozide by the leaf dipping method

| Instar | N | LC ₅₀ (95%FL) ^a | LC ₉₅ | Slope±SE | TR ^b |
|--------|-----|---------------------------------------|------------------|-----------|-----------------|
| 1st | 250 | 5.98(3.5-10.3) | 24.04 | 2.72±0.63 | 1.00 |
| 2nd | 250 | 6.29(3.5-11.4) | 27.00 | 2.60±0.63 | 1.05 |
| 3rd | 250 | 6.09(4.3-8.0) | 50.86 | 1.78±0.27 | 1.02 |
| 4th | 250 | 6.05(3.9-8.4) | 88.77 | 1.41±0.21 | 1.01 |
| 5th | 400 | 39.49(31.4-50.4) | 509.01 | 1.48±0.13 | 6.60 |
| 6th | 250 | 39.73(29.2-53.8) | 508.60 | 1.49±0.15 | 6.64 |

^a95% fiducial limits (ppm). TR^b (Tolerance Ratio). LC₅₀ values of each instar/LC₅₀ values of 1st instar

increased gradually and the values of TR in the 5th and the 6th instars as compared with that of the 1st instar were 2.88 and 3.23 times, respectively. The LC₅₀ values of pyriproxyfen to the differential instars of the fall webworm showed little by little increasing tendency as abovementioned it. However, the LC₅₀ values of pyriproxyfen to the larval instars of the fall webworm showed in low values as compared with that of the other IGRs tested. On the other sides, the pyriproxyfen was shown to be the feasible IGRs for the controls of housefly (*Musca domestica*) and mosquito (*Culex pippiene pallens*) reported by Kim *et al.* (1993).

The mortality effects of tebufenozide according to developmental larval stages of fall webworm by the leaf dipping method were shown in Table 5.

The tebufenozide was also known as the RH-5992, one of the hydrazide compounds similar to RH-5849, a nonsteroidal ecdysone agonist. In Table 5, the LC₅₀ values of tebufenozide against the developmental larval instars of the fall webworm were gradually increased and the values of TR in the 5th and the 6th instars showed rapid increase as compared with that of the 1st~4th instars, and showed the 6.60 and 6.64 times as compared with that of 1st instar, respectively. Unfortunately, these results were not compared with other reporters until now. The RH-5849, one of similar compound of tebufenozide, was reported by several researchers; Monthean & Potter (1992), Park *et al.* (1992), Tateishi & Takeda (1993) and Wing (1988), against the larvae of insects. The LC₅₀ values of RH-5849 against the 1st, 2nd, 3rd, 4th and 5th instars of tobacco budworm (*Spodoptera litura*) by cabbage leaf dip-

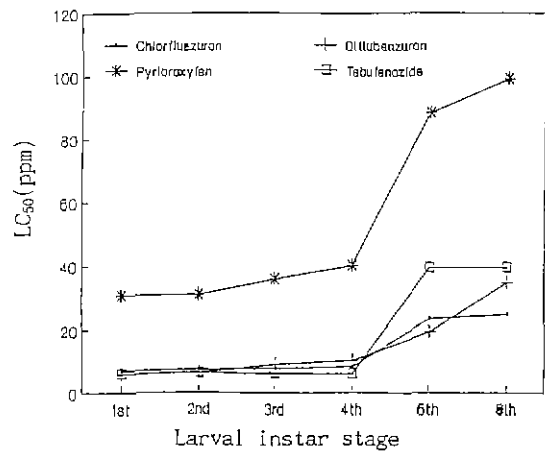


Fig. 1. Differences of susceptibility in each larval instar stage of fall webworm (*Hyphantria cunea* Drury) to insect growth regulators (IGRs)

ping method reported by Park *et al.* (1992), were 19.1, 17.6, 23.6, 22.1 and 15.5 ppm, respectively and he also reported that the feeding rate(%) decreased rapidly for 48 hours after leaf dipping treatment at higher concentrations and the larval weight also did not increase at hours after treatment as compared with that of untreated control. The results of tobacco budworm experiment with RH-5849 were not coincide with the results of ours. There are two reasons for this discrepancy. One is the different feeding times: 48 hours for RH-5849 and 24 hours for tebufenozide. The other is the estimation of the influences with decreases of feeding rate and larval weight. In feeding conditions reported by Guyer & Neumann (1988), ecdysis comparisons of LC₅₀ (ppm in diet) of chlorfluazuron and diflubenzu-

Table 6. Comparisons of mortality effects with several IGRs against the 1st and 3rd larvae of rice stem borer (*Chilo suppressalis* Walker) in the rice stem dipping method

| Treated IGRs | Instar | N | LC ₅₀ (95%FL) ^a | LC ₉₅ | Slope±SE | TR ^b |
|----------------|--------|-----|---------------------------------------|------------------|-----------|-----------------|
| Chlorfluazuron | 1st | 360 | 32.02(22.4-43.1) | 375.95 | 1.54±0.17 | 1.00 |
| | 3rd | 300 | 91.66(73.4-112.2) | 510.74 | 2.21±0.22 | 2.86 |
| Diflubenzuron | 1st | 360 | 163.23(126-207) | 1680.91 | 1.62±0.18 | 1.00 |
| | 3rd | 300 | 424.48(336-514) | 2040.91 | 2.41±0.32 | 2.60 |
| Pyriproxyfen | 1st | 360 | 35.94(26.7-48.4) | 522.93 | 1.41±0.14 | 1.00 |
| | 3rd | 300 | 711.69(321-1596) | 6024.72 | 1.77±0.35 | 19.80 |
| Tebufenozide | 1st | 360 | 2.31(0.4-12.6) | 207.27 | 0.84±0.16 | 1.00 |
| | 3rd | 300 | 35.38(24.4-49.4) | 893.55 | 1.17±0.12 | 15.30 |

^a95% fiducial limits(ppm); TR^b (Tolerance Ratio); LC₅₀ values of each instar/LC₅₀ values of 1st instar

ron against the penultimate instars of *Stodoptera littoralis* were to 0.05, 10 and 1.0, 180 for continuous feeding and feeding for 6 hours, respectively. Wing (1988) also reported that the ED₅₀ values of RH-5849 against the larvae of tobacco hornworm (*Manduca sexta*) showed the 30~670 times activities as compared with that of 20-hydroxyecdysone and Monthean & Potter (1992) reported that the RH-5849 was effective enough against the Japanese beetle (*Popillia japonica*) grubs at the concentrations as low as 1 ppm in soil and increased the mortality at 30 and 100 ppm at 3 days after treatment against the fall armyworm (*Spodoptera frugiperda*). In the view point of the results with RH-5849 mentioned above, the efficacies of tebufenozide (RH-5992) will be expanded into broad ranges of major insect pests.

In summary of the inhibition effects with IGRs against the developmental larval instars of the fall webworm (Fig 1), the LC₅₀ values of IGRs increased gradually in accordance with developmental larval instars of the fall webworm and the mortality of IGRs on larval stages from the 1st to the 4th instars showed good efficacy at low concentrations of chlorfluazuron, diflubenzuron and tebufenozide compounds except the pyriproxyfen. Although, the efficacy in the 5th and the 6th larvae of the fall webworm was slightly higher than that of the 1st~4th instars, these three IGRs seemed to be a feasible developmental pesticides to control the fall webworm and other insects.

The mortality effects of IGRs against the rice stem

borer (*Chilo suppressalis* Walker) larvae by the rice stem dipping method were shown in Table 6.

LC₅₀ values of IGRs against the 1st and the 3rd larvae of the rice stem borer ranged 32.02~91.66 ppm with chlorfluazuron, 163.23~424.48 with diflubenzuron, 35.94~711.69 with pyriproxyfen and 2.31~35.38 with tebufenozide. Even though, the level of TR increased at the 3rd larvae of the rice stem borer. The tebufenozide (RH-5992) showed the feasibility as developmental pesticides to control the rice stem borer. The potentials for pest controls in agriculture and forestry area needed to be evaluated. Actually, the possibilities were studied to the widen spectrums of their uses. In these points, the utilization of IGRs aimed the selective, of which agents affected various kinds of insect pests with minimum detrimental effects on the human or on the pest's natural enemies for the integrated pest management programs.

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