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Penetrations of flupyrazofos against *Plutella xylostella* (Lepidoptera:Yponomeutidae) and *Spodoptera exigua* (Lepidoptera:Noctuidae)

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Abstract : Tolerance mechanism to flupyrazofos was examined with *Plutella xylostella* (L.) and *Spodoptera exigua* by investigating the penetration rate of flupyrazofos into larvae body. On determining effective washing of ^{14}C-flupyrazofos, the washing volume to recover over 98% of ^{14}C-flupyrazofos was observed at three times (each time: 1 mL). To select a suitable solvent, the recovery rates of each solvent in 3rd instar larvae of DBM were above 98%, but the washing rates of acetone, hexane and ethyl-acetate were 85.1%, 67.2% and 68.4%, respectively. In the BAW larvae, although the recovery rates of each solvent were above 99%, the washing rates of acetone, hexane and ethyl-acetate were 83.5%, 65.9% and 71.7%, respectively. The PT_{50} values of ^{14}C-flupyrazofos were 0.731 h (44 min) in the DBM larva and 0.504 h (30 min) in the BAW larva. Radiocarbon in acetone washing (external fraction) decreased more quickly in the BAW larva than in the DBM larva, and amount of radiocarbon in larvae body increased more quickly with time in the DBM larva than in the BAW larva. In contrast, amount of radiocarbon in excreta increased more rapidly with time in the BAW larva than in the DBM larva.(Received October 31, 1999; accepted February 23, 2000)

Key wods : Flupyrazofos, penetration, *Plutella xylostella* (L.), *Spodoptera exigua.*

Introduction

Over the several decades, diamondback moth (DBM), *Plutella xylostella* (Linnaeus), and beet armyworm (BAW), *Spodoptera exigua* (Hübner), have become the most important insect pests of crops in the world. Although these species is believed to have originated in the south-southeast parts of Europe and Asia, its present status is in most parts of the world which is attributed to the extended cultivation of host plants and its superb migratory habit (Mikkola, 1970; Chu, 1986). Furthermore, continuous growing of host plants and favorable climatic conditions result in DBM and BAW attaining high population densities with over-lapping generations all the year round. If not managed properly from the early growth stage of the crop, these insects could cause a serious yield loss by excessive feeding on the leaves at larvae stage.

The control of these insects has been primarily dependent on continued or repeated applications of insecticides. Although insecticides have effectively controlled these pests, their extensive use for several decades has disrupted the control ability of natural enemies, and has led to outbreak of these pests, and development of resistance to various types of insecticides (Miyata *et al.*, 1986; Sun *et al.*, 1986; Lee *et al.*, 1993). These pests have also developed resistance against cartap, chitin inhibitors, and even against *Bacillus thuringiensis* Berliner (Hama, 1992). Although many insecticides have been registered for the control of these pests, new methods for the control are still in the process of development in Korea. Decreased efficacy and increasing concern on adverse effects of the earlier types of insecticides have brought about the need for the development of new types of selective alternatives or new methods of protection with reduced use of conventional insecticides.

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Therefore, considerable effort has been focused on the development of new products as commercial insecticides. It is believed that decreased absorption is an assistant for detoxifying enzymes to degrade larger amount of insecticide by extension of penetration time (Sawicki and Lord, 1970). Metcalf *et al.* (1967) reported that metabolites of nine carbamate insecticides were not different, but the speed of degradation was faster in the resistant house fly than the susceptible one. Similar results were also reported in the cattle tick and two-spotted spider mite (Smissaert *et al.*, 1970; Schnitzerling *et al*,. 1974). Kao *et al.* (1984), however, demonstrated the difference in amount of α-monoacid of malathion between the resistant and susceptible house fly.

Flupyrazofos, organophosphorus insecticide, has been developed by Korea Research Institute of Chemical Technology (Hwang, 1989)(Fig. 1). It could be an effective alternative against DBM because of its outstanding insecticidal activity. This insecticide has been recently investigated regarding its absorption, retention and vapor pressure and is of increasing importance in the control of DBM (Kim *et al.*, 1997; Yang *et al.*, 1997). Flupyrazofos is much more potent insecticide to DBM than BAW and being very specific to the DBM larvae (Lee *et al.*, 1997). However, little work has been done on the percutaneous penetration. In the laboratory study described herein, we dealt with percutaneous penetrations for the elucidation of characteristics of flupyrazofos to *Plutella xylostella* (L.) as a susceptible insect and *Spodoptera exigua* as a tolerance insect.

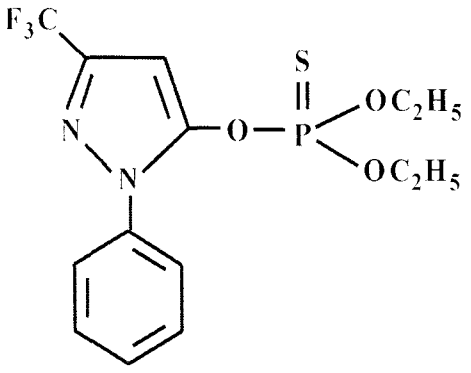

Fig. 1 Chemical structure of flupyrazofos.

Materials and Methods

Insects

Diamondback moth, *Plutella xylostella* (Linnaeus), and Beet armyworm, *Spodoptera exigua* (Hübner), were used in this study. DBM was originally obtained from Korea Research Institute of Chemical Technology (KRICT) at Daeheon, Korea. The insects have been maintained at NIAST, Suwon, Korea, since 1996. DBM larvae were reared on 6- to 9-day-old rape seedling in a cage (40 cm×40 cm×45 cm). BAW was initially collected from welsh onion field at Suwon, Korea in September 1994. These were reared on artificial diet (Shorey, 1965) in a plastic cage (22 cm×16 cm×9 cm). Rearing conditions for both insects species were 25±1℃, 50-60% RH, and photoperiod of 16:8 (L:D) through 25th generations in the laboratory. Sucrose solution (20%) was provided for adult food source. Both species have been in continuous culture without exposure to insecticides since their introduction into the laboratory.

Chemicals

^{14}C-Labelled flupyrazofos, *O,O*-diethyl *O*-1-[U-^{14}C] phenyl-3-trifluoromethyl pyrazol-5-yl phosphorothioate (purity: 99%, specific radioactivity: 185.7 MBq (5.02 mCi/mmol), was provided by KRICT. Acetone, hexane and ethyl-acetate of optima grade were purchased from Kanto Pure Chem. (Tokyo, Japan).

Determination of washing solvent and washing volumes

^{14}C-Flupyrazofos was freshly prepared in optima grade acetone. Doses were adjusted to levels that cause no mortality. A droplet containing 4,000 dpm (equivalent to 0.1 μL of ^{14}C-flupyrazofos solution) for the 3rd instar of DBM and 20,000 dpm (equivalent to 0.5 μL of ^{14}C-flupyrazofos solution) for the 4th instar of BAW were applied topically to the dorsal plate of each insect species with a micro-topical applicator. The treated larvae were then immediately placed into a funnel (ϕ7 cm) stopped with cotton, and 1 mL of solvent was poured over the larvae five times. The solvents used in this study were acetone, hexane and ethyl-acetate. The radioactivity of the solvents was determined by liquid scintillation counter (Beckmann, LS6000). Three replicates of 20 larvae each were tested.

Penetration and excretion rate of flupyrazofos

^{14}C-Flupyrazofos was treated as in previous section. The control was treated with acetone only. The insects were sampled at 0.5, 1, 2, 4 and 8 h after treatment for both species. Tests consisted of three replicate of 10 larvae each.

On each sampling time, treated larvae were held in 20 ml scintillation vial before washing. The insects were washed three times in 1 mL aliquot of acetone, and these extract were combined. The bodies after washing were freeze-dried at -50℃ by Vacuum Tray Freeze Dryer (Ilshin Lab Co., Ltd., TD 5070 APR) for 72 h, and oxidized by biological oxidizer (Packad 306). The samples were macerated in ethyl-acetate (10 mL) and methanol (10 mL) mixture with a glass hand mixer (50 mL capacity) in ice water. The crude extract was sonicated by ultra sonicator (Bransonic, Danbury, USA) for 10 min. The percentage of concentration-time profile for the two species were compared with the rate of surface washing fraction, body fraction and excreted fraction.

Results

Determination of washing solvent and washing volumes

Three solvents were tested to determine the most suitable solvent and the proper volumes of solvent for washing ^{14}C-flupyrazofos off the body of the 4th instar of BAW. Over 96% of ^{14}C-flupyrazofos was recovered, when the BAW larvae were washed two times with each 1 mL of solvent, regardless of solvents used (Table 1). Over 99% of ^{14}C-flupyrazofos could be recovered with acetone by washing three times (each time: 1 mL). Washing recovery rates of ^{14}C-flupyrazofos were 99.5%, 98.9% and 98.7% when the BAW larvae were washed three times (each time: 1 mL) with acetone, hexane and ethylacetate, respectively. 3 mL for washing were enough to recover >99% of ^{14}C-flupyrazofos.

Table 2 shows the recovery of ^{14}C-flupyrazofos, treated on the dorsal plate of the 3rd instar of DBM, by washing the DBM larvae with 3 mL (1 mL each time) of each solvent. The recovery of each solvent were >98%. However, body washing rates were different for each solvent. The rate of each solvent was 85.1% (acetone), 67.2% (hexane) and 68.4% (ethyl acetate). The remainings in the DBM body after washing with each solvent were 14.4% with acetone, 31.7% with hexane and 30.4% with ethyl acetate. Similarly, the recovery rates of each solvent in 4th instar of BAW were >99% and highest body washing rate was observed with acetone (Table 3). These results indicate that acetone is the most suitable solvent for washing ^{14}C-flupyrazofos of the DBM and BAW larva.

Penetrations and excretion rate of flupyrazofos

Table 4 shows the PT_{50} of ^{14}C-flupyrazofos into the 3rd instar of DBM and the 4th instar of BAW. The PT_{50} values were 0.731 h (44 min) into 3rd instar larva of DBM and 0.504 h (30 min) on the 4th instar

Table 1. Recovery of ^{14}C-flupyrazofos after washing the 4th instar of BAW in different solvents

Washing volume (1 mL)	Relative rate (%) of recovered flupyrazofos		
	Acetone	Hexane	Ethyl acetate
1st washing	76.9±4.6[a)]	74.2±8.2	70.7±9.2
2nd washing	21.6±5.3	22.4±7.6	26.0±5.8
3rd washing	1.0±0.4	2.3±1.1	2.0±1.0
4th washing	0.3±0.2	0.7±0.5	0.8±0.6
5th washing	0.2±0.2	0.4±0.3	0.5±0.3
Total (3 mL)	100	100	100

[a)]Standard deviation from triplicate.

Table 2. Mass balance of flupyrazofos in washings and body of the 3rd instar of DBM

Fraction	Recovery (%)		
	Acetone	Hexane	Ethyl acetate
Solvent	85.1±11.1[a)]	67.2±9.3	68.4±7.8
Larvae	14.4±1.3	31.7±3.5	30.4±2.6
Recovery (%)	99.5±6.8	98.9±4.2	98.8±5.3

[a)]Standard deviation from triplicate.

of BAW. The changes in recovery rates were different between the 3rd instar of DBM and 4th instar of BAW. The recovery rates of ^{14}C-flupyrazofos decreased more quickly in the 4th instar of BAW than in the 3rd instar of DBM (Table 4). These results explain that flupyrazofos could penetrate more quickly into the 4th instar BAW larva than into the 3rd instar of DBM. The penetration process approached the equilibrium for both insect species at 4 h after treatment.

Fig. 2~4 show distribution of ^{14}C-flupyrazofos in washing, body and excreted fraction of the 3rd instar of DBM and the 4th instar of BAW. In washing fraction, relative amount of washed radiocarbon decreased more quickly with exposure time in the 4th instar of BAW than in the 3rd instar of DBM. The penetration process approached equilibration (10%) for

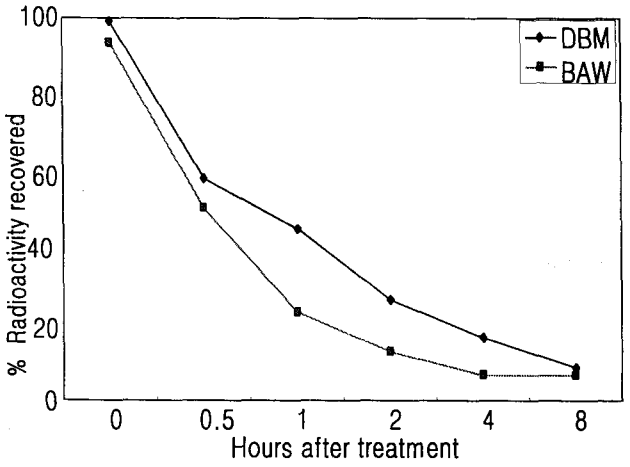

Fig. 2. Distribution of ^{14}C-flupyrazofos in acetone washing (external fraction) of the 3rd instar of DBM and the 4th instar of BAW.

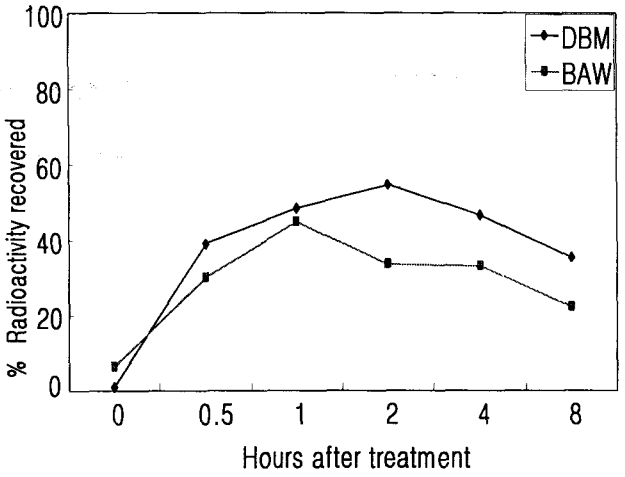

Fig. 3. Distribution of ^{14}C-flupyrazofos in body of the 3rd instar of DBM and the 4th instar of BAW.

both species at 8 h (Fig. 2). Accumulation of absorbed radioactivity was maximum at 1 h (38%) for BAW and 2 h (55%) for DBM, and began to decline slightly. Higher percentage of internal accumulation was observed in DBM body than in BAW body (Fig. 3).

The radioactivity recovered from the incubation vials provided an estimate of metabolic elimination. Relative amount of radiocarbon in excreted fraction increased more quickly with time in the 4th instar of BAW than in the 3rd instar of DBM(Fig. 4). The continuous increase of radioactivity from the incubation vials is in agreement with the decreased accumulation in internal fraction after the maximum peaks. These results could explain, in part, higher tolerance of BAW than DBM by the facts that flupyrazofos was excreted more quickly in BAW than in DBM.

Table 3. Mass balance of flupyrazofos in washings and body of the 3rd instar of BAW

Fraction	Recovery (%)		
	Acetone	Hexane	Ethyl acetate
Solvent	83.5±9.8[a)]	65.9±7.6	71.7±9.1
Larvae	16.2±4.3	33.2±5.2	27.5±7.9
Recovery (%)	99.7±6.4	99.1±7.2	99.2±8.3

[a)]Standard deviation from triplicate.

Table 4. Penetration time (PT) of flupyrazofos into 3rd instar of DBM and 4th instar of BAW

Insects	Regression Equation	PT_{50}[a)](hrs)	r^2
DBM	Y = 5.1717 + 1.3733X	0.731	0.990
BAW	Y = 5.5884 + 1.4212X	0.504	0.949

[a)]50% penetration time.

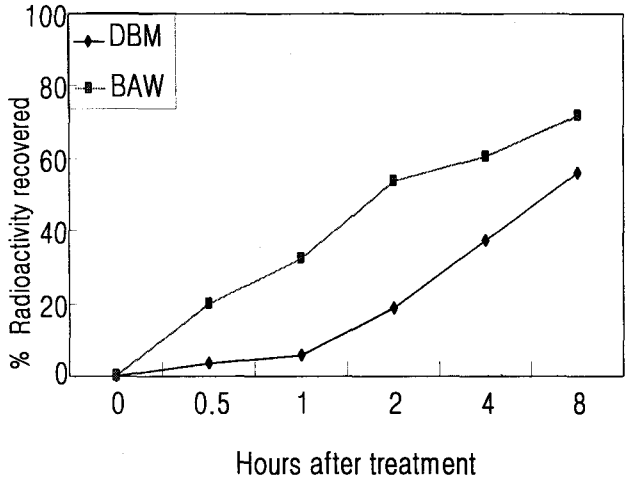

Fig. 4. Distribution of ^{14}C-flupyrazofos in excreta of the 3rd instar of DBM and the 4th instar of BAW.

Discussion

Determination of solvent for washing the insect body which treated with insecticides is very important because insecticides are generally not soluble in aqueous solutions (Matsmura, 1975) and they are partitioned between solvent and insect body surface (washing is a phenomenon occurring by different solubility of insecticides between in the solvent and in the insect integument, wax and cuticle). Therefore, it is expected that the recovery rate of insecticides should be different depending on the properties of each solvent. If the polarity of the solvent is low, the washing efficiency is decreased. But it is also possible that some highly polar solvent can wash metabolites of insecticides included in the insect cuticle, which has become more polar by metabolic reactions in the integument (Kennaugh *et al.*, 1993) because some solvent can damage the skin (Guthrie, 1980a; Guthrie, 1980b). For washing insecticides from the integuments of insects, it seems that less polar solvents (hexane, ethyl acetate) are unsuitable. In case of flupyrazofos, acetone was the most suitable for washing the DBM and BAW larvae.

Although flupyrazofos was penetrated very faster in BAW larva than DBM larva, toxicity induced with the penetration of flupyrazofos was not higher in BAW larva than DBM larva(Lee *et al*,. 1997). This indicated that tolerance mechanism of the BAW larva is not associated with penetration rate. It is well known that reduced penetration of insecticides is one of the resistance mechanisms in resistance insects. Shono (1989) reported that one of the resistance mechanisms of NAIDM housefly to diazinon was the decreased penetration rate. Kono *et al.* (1989) observed that penetration of methyl parathion was significantly less in resistant strain than in susceptible strain during 12 h after topical application, also suggesting the reduced penetration as a resistance mechanism in the tobacco budworm, *Heliothis assulta* (Guenée). Bull and Pryor (1990) and Saito *et al.* (1992) also suggest the reduced penetration as one of the resistance mechanism. Nevertheless, this is not the major resistance mechanism for the BAW larva. Some other reports showed different results. Ahn *et al.* (1988) reported that penetration rates of ^{14}C-permethrin were very similar in the two reference strains and Yu(1991) reported that there was no difference in the rate of cuticular penetration of carbaryl in both strains of houseflies. As shown above, contribution of the reduced penetration to resistance mechanism seems to be greatly different depending on insect species and insecticides. In flupyrazofos-tolerant BAW larva, it seems that tolerant mechanism is not related with penetration rate of flupyrazofos.

Radioactivity in the body of larvae was slightly higher in DBM than in BAW. These results were closely related to the excretion of flupyrazofos by metabolism. The radioactivity adhering to the vial in which DBM and BAW larvae were kept can be from the excreta, and this radioactivity was much higher in the tolerant BAW larvae than in the susceptible DBM larvae. This may indicate that tolerance come from the faster elimination rate in BAW and opposite DBM.

As the amounts of radiocarbon in the larva body increase, excretion of flupyrazofos metabolites is reduced. The reason why the radioactivity in DBM is higher than that of BAW is that simply the excretion rate is slower in DBM than that of in BAW.

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배추좀나방과 파밤나방에 대한 flupyrazofos의 체벽 투과량

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요약 : 배추좀나방과 파밤나방에 대한 flupyrazofos의 작용기작을 구명하기 위하여 한국화학연구소에서 분양을 받아 실내에서 19세대 누대 사육한 배추좀나방과 포장에서 채집하여 실내에서 인공사료를 이용하여 25세대 누대 사육한 파밤나방을 대상으로 ^{14}C-flupyrazofos를 처리하여 ^{14}C-flupyrazofos를 검출하기에 적합한 용매와 두종간의 체벽 투과량을 조사하였다. 배추좀나방과 파밤나방 충체에 있는 ^{14}C-flupyrazofos의 각 용매별 검출율은 hexane과 ethyl-acetate보다 acetone을 사용할 때 가장 높았다. flupyrazofos의 반수침투시간(PT_{50})은 배추좀나방에서는 0.731시간(44분)이었고, 파밤나방에서는 0.504시간(30분)으로 나타나 배추좀나방보다 파밤나방에서 침투시간이 다소 빨랐고, 배설량도 배추좀나방보다 파밤나방에서 많았으며 배설되는 시간도 빨랐다.

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