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Note

Mechanisms of Low Susceptibility to Imidacloprid in a Laboratory Strain of the Small Brown Planthopper, *Laodelphax striatellus* FALLÉN

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INTRODUCTION

Imidacloprid, 1-[(6-chloro-3-pyridyl)methyl]-*N*-nitro-2-imidazolidinimine belongs to a new class of insecticides, which shows high insecticidal activity against sucking insects especially leaf and planthoppers[1]. Electrophysiological and biochemical studies on the mode of action of imidacloprid showed that it acts as an agonist on the nicotinic acetylcholine receptor in post synaptic membranes.[2-5] Due to its unique mode of action, resistance against imidacloprid has not been observed on any field population of the small brown planthopper, *Laodelphax striatellus* FALLÉN.[6] However, our laboratory strain (N-strain) of the small brown planthopper reared under pressure of malathion and propoxur showed relatively low sensitivity to imidacloprid.[7]

In order to elucidate the underlying mechanisms confirming low susceptibility of N-strain of the small brown planthopper to imidacloprid, cuticular penetration and possible metabolic detoxication system under the influence of synergists were studied.

MATERIAL AND METHODS

1. Insects

The small brown planthopper, *Laodelphax striatellus* was used in this study. Experiments were performed with 2-5 days old female adults reared on rice seedlings in our laboratory at 25°C. S-strain was collected in Tokyo area, and has been reared with no pressure of any insecticide since 1960. N-strain was collected in Nagasaki pref. in 1972, and has been reared under continuous pressure of 500 ppm malathion plus 500 ppm propoxur mixture sprayed weekly. Calculated resistance ratios for disulfoton, propoxur, ethofenprox and imidacloprid were 110, 472, 6.1 and 18.1 respectively in N-strain of the small brown planthopper in the susceptibility study.[7]

2. Chemicals

Imidacloprid (purity 95%) was synthesized at Yuki Research Center (Nihon Bayer Agrochem K.K., Yuki, Japan). PBO (piperonylbutoxide), DEF (tributylphosportrithioate) and TCBE (trichlorobenzene ester) as synergists were also synthesized at Yuki Research Center. ^{14}C-imidacloprid was provided by Bayer AG (Leverkusen, Germany), and specific activity was 111 μCi/mg. Stock solutions of the chemicals were prepared in acetone.

3. Methods

3.1 Topical application

Stock solutions of imidacloprid and/or synergists (0.2 μl) were topically applied on the pronotum of the insects with a topical applicator (Burkard Manufacturing Co., Ltd., Herts, England) under light anesthesia with CO_2. Ten insects were kept in a Petri dish (90 mm in diameter) on wet filter paper. The mortality was determined 24 h after application and the LD_{50} value was calculated by probit analysis. The doses of PBO, DEF and TCBE tested were 40, 2 and 20 ng/insect, respectively. None of the synergists showed insecticidal activity at the dose used.

3.2 Penetration

Radiolabeled imidacloprid (20 dpm/insect) was topically applied to the insects as described above. One hundred heads of female adults were used for one replication. The treated insects were kept in a vial (20 ml, Packard, Switzerland) for 0-12 hr. Then they were transferred to a funnel with glass wool and rinsed three times with 3 ml acetone. The rinsed insects were combusted with a sample oxidizer (ASC-113, Aloca, Tokyo, Japan) and ^{14}C was collected as CO_2. The radioactivities of ^{14}C-CO_2 samples were counted using a scintillation counter (LC5801, Beckman, CA, U.S.A.). The acetone solution of the external rinse was concentrated to 500 μl and 5 ml of the scintillation cocktail (Ready Organic, Beckman) was added. The samples of the external rinse were counted with the scintillation counter. The vials used to keep the insects were washed with 5 ml of the scintillation cocktail and counted for radioactivity as well.

RESULTS

1. Studies with Synergists

Table 1 displays the synergistic effect of PBO, DEF and TCBE on susceptibility of N-strain of the small brown planthopper to imidacloprid. PBO, known as a microsomal oxidase inhibitor, DEF, known as an aliesterase inhibitor and TCBE known as a specific inhibitor of microsomal demethylation did not provide synergistic effects.

2. Penetration Studies

The penetration rates of imidacloprid in S- and N-strain of the small brown planthopper treated with 0.1 ng/insect (LD_{50}

Table 1 Effect of synergists on the efficacy of imidacloprid in N-strain of the small brown planthopper.

Strain	LD_{50} (μg/g insect)			
	Imidacloprid alone	+PBO	+DEF	+TCBE
N	2.17 (1.71–2.74)	1.11 (0.59–2.27)	1.06 (0.61–1.30)	1.19 (0.94–1.49)
S	0.12 (0.10–0.15)	—	—	—

The parentheses indicated 95% fiducial limit. The doses of PBO, DEF and TCBE tested were 40, 2 and 20 ng/insect, respectively.

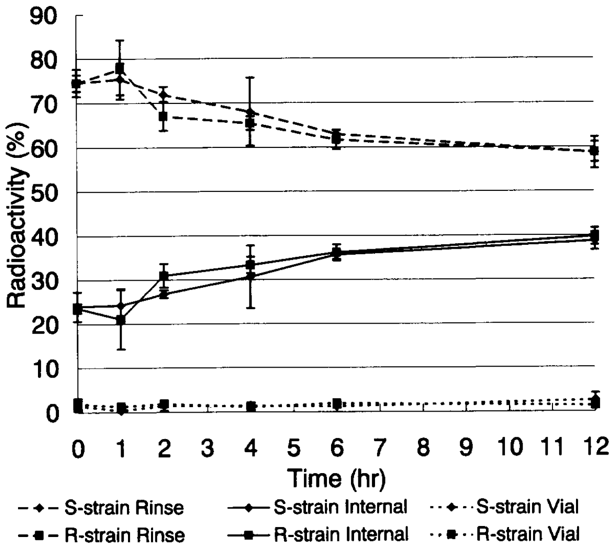

Fig. 1 Penetration rate of imidacloprid in S- and N-strain of the small brown plathopper.

of the susceptible strain) were shown in Fig. 1. In this study, 20% of imidacloprid penetrated into the body 1 hr after treatment, and then the amount of imidacloprid in the body was increased with the passage of time. There was no difference in the penetration rate between S- and N-strain.

DISCUSSION

Imidacloprid belongs to a new group of insecticides which is highly active against sucking pest insects. Because of its mode of action, imidacloprid is also highly effective against aphids, whiteflies, leafhoppers and planthoppers show resistance to conventional chemicals. However, relatively low susceptibility to imidacloprid was found in our laboratory strain (N-strain) of the small brown planthopper which showed high resistance to organophosphates and carbamates.

Synergists are good tools to investigate the possible underlying mechanisms confirming resistance in insects. Using PBO as a synergist, Hama[8] demonstrated that the increase of activity of mixed function oxidase confers propoxur resistance in the small brown planthopper. However, none of the synergists used in our study showed clear synergistic activity with imidacloprid in the small brown planthopper.

In some cases, reduced penetration contributes to resistance against insecticides. Noppun *et al.*[9] described that a phenthoate-resistant strain of the diamondback moth showed a rather low penetration rate compared with a susceptible strain. In a pyrethroid resistant strain of the house fly, *Musca domestica* L., Ahn *et al.*[10] described reduced penetration as a contributing factor as well. However, no difference in penetration rate was observed between S- and N-strain of the small brown planthopper after topical application of imidacloprid.

More detailed biochemical and toxicological studies are desired to clarify the low susceptibility of N-strain of the small brown planthopper toward imidacloprid.

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要 約

ヒメトビウンカ室内飼育系統におけるイミダクロプリドに対する低感受性機構

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イミダクロプリドに対し低感受性を示したヒメトビウンカ室内飼育系統における皮膚の透過性および協力効果について検討した. 皮膚の透過性について, 感受性系統 (S 系統) とイミダクロプリドに対し低感受性を示した室内飼育系統 (N 系統) との間に差は認められなかった. ミクロゾーム酸化酵素阻害剤である PBO, エステラーゼの阻害剤である DEF, ミクロゾームの脱メチル化阻害剤である TCBE を使用して, N 系統における協力効果について検討を行なったが, 協力効果を示す協力剤は認められなかった. N 系統でのイミダクロプリドに対する低感受性機構を明らかにするには, さらに詳細な生化学的, 毒物学的な研究が必要である. また, これらの研究は, イミダクロプリドの抵抗性対策を検討するうえで非常に有効であろう.