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Insecticide Resistance of the Green Rice Leafhopper, *Nephotettix cincticeps*, to the Systemic Insecticides Used for Seedling-Box Application

Jirapong Jairin[a,*] Nobuki Kojima[b] and Toru Nagata[c]

[a] Ubon Ratchathani Rice Research Center, P.O. Box 65, Muang, Ubon Ratchathani 34000,Thailand.
[b] Tsukuba International Centre, 3-6 Koyadai, Ibaraki 305-0074, Japan.
[c] Faculty of Agriculture, Ibaraki University, Ami, Ibaraki 300-0393, Japan.

* Corresponding author, E-mail: jirapongj@ricethailand.org

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ABSTRACT: The mortality and feeding inhibition of the green rice leafhopper (GLH), *Nephotettix cincticeps* Uhler, to four systemic insecticides, namely imidacloprid, cartap, propaphos and disulfoton, were determined by a parafilm method. The three local populations of GLH with different resistance levels to organophosphates (OPs) were compared with respect to LC_{50} and FI_{50} obtained by this testing method. Feeding inhibition at sublethal doses was most remarkable, with imidacloprid followed by cartap with no regard to resistance levels, while feeding inhibition by propaphos and disulfoton was observed only with the OPs resistant GLH. The residual period of imidacloprid granule (2%) and cartap granule (4%) applied to the seedling-box were determined in terms of mortality and feeding inhibition. Imidacloprid gave the longest residual period of lethal action and feeding inhibitory action. The GLH population was remarkably reduced in the imidacloprid treated plot compared to the untreated plot, while a reduction in population density was not observed in the cartap treated plot.

KEYWORDS: feeding inhibition, insecticide resistance, *Nephotettix cincticeps*, parafilm method, seedling-box application.

INTRODUCTION

Insecticide resistance is a problem in all insect groups that serve as vectors for causing plant diseases, including rice virus diseases. The green rice leafhopper (GLH), *Nephotettix cincticeps* Uhler, is considered to be one of the devastating insect pests that has adapted to a series of insecticides used for its control. GLH not only feed on rice plants, directly causing yield reduction, but they also transmit viruses which cause severe diseases of rice[6]. Pest control in Japan, as in most rice producing countries, has largely relied on insecticides. GLH has also long been controlled by insecticides since the introduction of synthetic insecticides after World War II. Extensive use of insecticide has contributed to the development of insecticide resistance in many field populations of this species[8]. Resistance of GLH to organophosphate insecticides first appeared in Shikoku Island in 1961[5]. Many GLH local populations resistant to carbamates and organophosphates (Ops) have been reported[4,13]. A similar situation in other groups of insecticides to GLH around Japan has continuously been reported[10,12]. To overcome the development of insecticide resistance, minimal use of insecticides is the most important point[12].

Mechanical transplanting is currently widely used in Japan. Granule insecticides are applied in the seedling-box before transplanting. The insecticides are embedded into the paddy soil near the root zone and easily absorbed by the rice plant. In this treatment, the systemic insecticides are imbibed orally by sucking insects through the plant tissues, so it is appropriate to estimate insecticide susceptibility through the same administration way as the actual application route of systemic insecticides. In addition, in this treatment, the insects may avoid contact with some systemic insecticides by behavioristic resistance, which is expected to be a more promising strategy for controlling virus diseases transmitted by the vector insects[9,18,19]. Considering this viewpoint, we developed a specific testing method to determine an insect's susceptibility to systemic insecticides which exert two functions, lethal action and feeding inhibitory action. In this experiment, the occurrences of insecticide resistance to these systemic insecticides were assessed and the residual period of the widely used systemic insecticide, as seedling-box application, was determined.

MATERIALS AND METHODS

Insects

The GLH used in this study were collected from three locations in Japan: The Taniyama population (TNM) was collected from an area intensively treated by insecticide in Kagoshima city, Kagoshima prefecture in 1998. The Tsukuba population (TKB) was collected

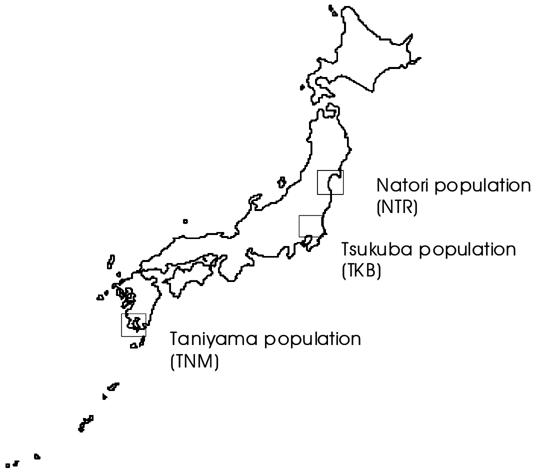

Fig 1. Location of the three areas in Japan, where the three strains of GLH were collected. The Taniyama population was collected from an area intensively treated by insecticide. The Natori population was collected from a less treated area. The Tsukuba population was collected from a rice field in Tsukuba.

from Ibaraki prefecture in 2000. The Natori population (NTR) was collected from a less treated area in Natori city, Miyagi prefecture in 1998 (Fig 1). The TNM and NTR strains were supplied by Kagoshima Prefectural Experiment Station and Miyagi Prefectural Experiment Station, respectively. The insect colonies were established from three local populations in a plastic cage (34×26×34 cm) and placed in a rearing room at 25 °C, and kept under light for 14 h and in the dark for 10 h at Tsukuba International Centre (TBIC). The insects were maintained on the rice variety Akitakomachi, which has no major resistance genes for GLH.

Insecticides

Four insecticides commonly used for seedling-box application in Japan were used in this study. Imidacloprid [1-((6-chloro-3-pyridinyl)methyl)-N-nitro-2-imidazolidinimine] is a systemic insecticide chemically related to the tobacco toxin, nicotine, acting on the nervous system. Cartap hydrochloride [S,S'-(2-(dimethylamino)-1,3-propanediyl) dicarbamothioate] is a nereistoxin analogue insecticide. Propaphos [4-methylthiophenyl dipropyl phosphate] is an organophosphorus insecticide commonly used against leafhopper and planthopper since 1986. Disulfoton [O,O-diethyl S-(2-(ethylthio)) ethyl phosphoro dithioate] is an aliphatic organothiophosphate insecticide which was first used in seedling-box application. Reduction in its efficacy was revealed in the later half of the 1960s in the Kyushu areas and it was replaced by propaphos and cartap.

Concentration-Mortality and -Feeding Inhibition Bioassays (Parafilm Method)

Cartap, propaphos, disulfoton and imidacloprid were of technical grade with purity greater than 99% and obtained from Wako Pure Chemical Industries, Ltd., Japan. The fifteen serials dilutions of insecticide concentrations (0-800 ppm) for each insecticide were prepared. Three insecticides, namely imidacloprid, cartap and propaphos, were administered to the insects as a solution diet containing 2.5% sucrose, whereas disulfoton was administered as a 2.5% sucrose solution containing Tween 60, through an artificial membrane of stretched thin parafilm[11]. A total of 5 male adults were anaesthetized with carbon dioxide and transferred into a plastic vial (3 cm in diameter and 3 cm in height). The vial was covered with stretched parafilm and a 0.3 ml droplet of the diet containing the log concentration of the insecticides was placed on the parafilm, then the droplet was sandwiched with another stretched film. The vials were placed at 25 °C in 14-h light and 10-h dark periods, over 60% RH. The experiment was conducted with 3 replicates. The mortality of the insects was recorded at 24 or 48 h after treatment. The insect mortality at each concentration of the insecticides was corrected by the mortality of the control sucrose solution according to Abbott[1]. Data were subjected to probit analysis for each concentration-mortality experiment[2] and LC_{50} values were calculated. After 48 h, the insects were removed and the weight of honeydew in each vial was determined using a 0.1-mg sensitivity electronic balance (ER-120A). The feeding rate (F) was calculated as follows: $F = W_1 - W_2$ (where W_1 is the first weight and W_2 is the reweighing after desiccation). The percentage of reduction in honeydew excretion was calculated on the basis of control and then converted into probit for calculating FI_{50} (50% feeding inhibition concentration as ppm).

Residual Period of Systemic Insecticides Applied as Seedling-Box Treatment

One hundred grams of pre-germinated seed of Akitakomachi was sown in a seedling-box (30×60×2.5 cm). The granular insecticides, imidacloprid (Admire 2% Granular) and cartap (Padan 4% Granular), were applied to the seedling-box prior to transplanting at the recommended rate for controlling GLH. The treated rice seedlings were transplanted into a rice field with a transplanting machine. The control plants were left untreated with insecticides. The experimental plots (4×12 m) were immediately surrounded by a plastic

Table 1. Toxicity of four systemic insecticides to adult male GLH after 48 h by parafilm method.

Insecticide	LC_{50} (ppm)					
	TNM	Slope*	TKB	Slope	NTR	Slope
imidacloprid	0.03	0.62	0.08	0.56	0.08	2.04
cartap	9.86	1.49	4.44	2.54	2.64	1.43
propaphos	200.53	0.90	4.69	0.62	0.18	1.61
disulfoton	529.02	0.78	21.98	0.61	0.81	0.49

*Slope of regression line.
TKB, TNM and NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively.

fence after transplanting. The experimental plots were arranged in a randomized complete block design with 2 replications. Seven days after transplanting (DAT), the rice plants were removed to plastic pots and taken to the laboratory at 7 day-intervals. Eight hills were sampled from each plot. Only one tiller from each hill was selected for testing with a bromocresol green-treated filter paper in a plastic cup using a procedure modified from Pathak and Heinrichs[14]. Four tillers from each hill were selected for the parafilm sachet method[15] at 70 DAT. Plants were infested with 5 newly emerged adult males of GLH. The mortality was recorded after 24 h and 48 h and the feeding rate was recorded after 24 h, at the regulated temperature. The feeding rate was measured by honeydew production area on filter paper treated with 0.04 w/v % bromocresol green solution (Wako Pure Chemical Industries, Ltd., Japan) in the plastic cup at 7-63 DAT and in parafilm sachet at 70-84 DAT. The numbers of leafhoppers in the treated plots were counted. Sticky boards (18×25 cm) were used to collect the insects from 50 hills in each treated plot. The data was analyzed with ANOVA.

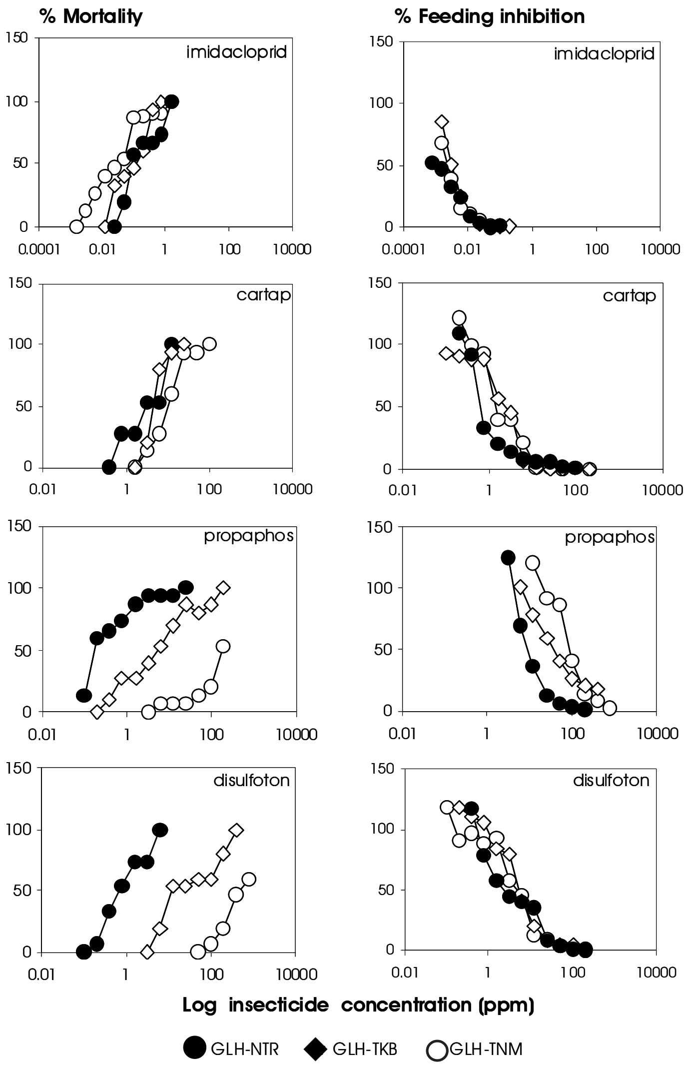

Fig 2. Concentration-mortality and -feeding rate relationship in male adult GLH.
Legends: ● NTR, ◆ TKB, ○ TNM

RESULTS

Concentration-Mortality and -Feeding Inhibition Bioassays (Parafilm Method)

Insecticide Susceptibility

The LC_{50} values of imidacloprid, cartap, disulfoton and popaphos to GLH populations are shown in Table 1. There were large variations between LC_{50} values of insecticides in each insect strain. The LC_{50} of most insecticides for the NTR strain was the smallest and those for the TNM strain was significantly larger than that for all strains except the LC_{50} value of imidacloprid. The order of resistance level of GLH to cartap, disulfoton and popaphos was, TNM>TKB>NTR, but the order was different for imidacloprid (Table 1, Fig. 2). The LC_{50} values of disulfoton, propaphos and cartap for the TNM strain were 650, 1,000 and 3 times larger than for the NTR strain, while those for the TKB strain were 14, 20 and 2 times larger than for the NTR strain, respectively. The TNM strain showed resistance to propaphos and disulfoton (LC_{50} values 200 and 529 ppm), while these insecticides remained effective for the NTR strain (LC_{50} values 0.18 and 0.81 ppm).

From the parafilm method, imdacloprid was shown to be more effective against four insect strains than cartap, propaphos or disulfoton. The insecticide susceptibilities to imidacloprid were not different between any of the insect strains. The LC_{50} of NTR and TKB were 2 times larger than that of TNM. Imidacloprid achieved 100% corrected mortality at the concentrations 1.56, 0.78 and 1.56 ppm for TNM, TKB, and NTR, respectively (Fig. 2).

Feeding Inhibition

The FI_{50} values of imidacloprid, cartap, disulfoton and popaphos to GLH are shown in Table 2. Identically to the lethal concentrations, the FI_{50} value for the TNM strain was significantly larger than those for all strains except the FI_{50} value of imidacloprid. The FI_{50} values for NTR were the smallest (Fig. 3). Though the LC_{50} value of propaphos for TNM was larger than NTR by 1,000 times, the FI_{50} values still remained similar to each other at low concentrations (5.4 and 1.2 ppm).

Table 2. Feeding inhibition of four systemic insecticides in adult male GLH after 48 h by parafilm method.

Insecticide	FI_{50} (ppm)					
	TNM	Slope*	TKB	Slope	NTR	Slope
imidacloprid	0.002	1.08	0.003	1.23	0.001	0.35
cartap	2.728	1.11	1.849	1.31	0.675	3.64
propaphos	5.434	1.28	4.873	0.87	1.224	0.42
disulfoton	93.188	1.59	35.184	0.61	9.966	0.81

*Slope of regression line.
TKB, TNM and NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively.

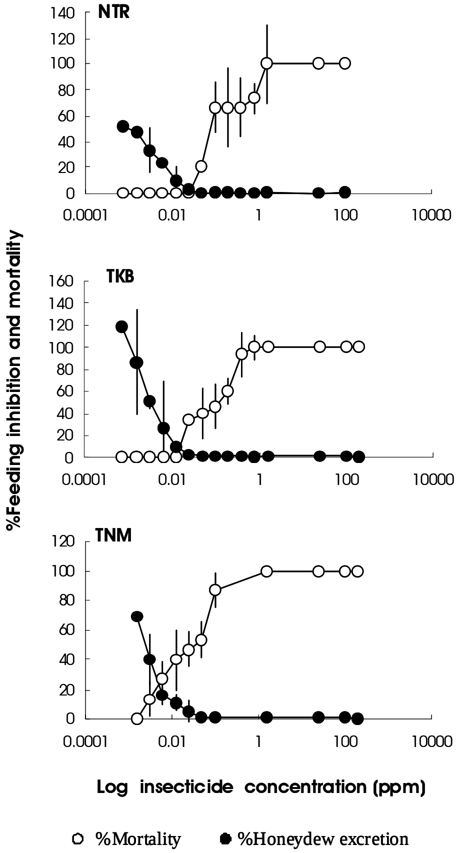

Fig 3. Relationship between %feeding inhibition and mortality of GLH treated with imidacloprid using the parafilm method. NTR, TKB and TNM refer to Table 1. Vertical bars indicate standard deviation.
Legends: ○ %Mortality
● %Honeydew excretion

The feeding rates of TNM, TKB and NTR for imidacloprid were reduced to more than 90% of the control at the concentrations 0.02, 0.01 and 0.01 ppm, respectively. This result indicated that imidacloprid and cartap showed characteristics as an antifeedant for all GLH strains, and propaphos and disulfoton showed characteristics as an antifeedant only for the TNM strain. The LC_{50}/FI_{50} ratios of TNM, TKB and NTR colonies were ca 15, 27 and 80 for imidacloprid, while those for cartap were ca 4, 2 and 4 respectively.

Relationship between the feeding inhibition and mortality

The relationship between the feeding inhibition and the mortality curve of each insect strain is shown in Figs. 3-6. According to this the feeding inhibition occurred at concentrations substantially lower than the lethal concentrations, and imidacloprid and cartap showed a pronounced characteristic as an antifeedant

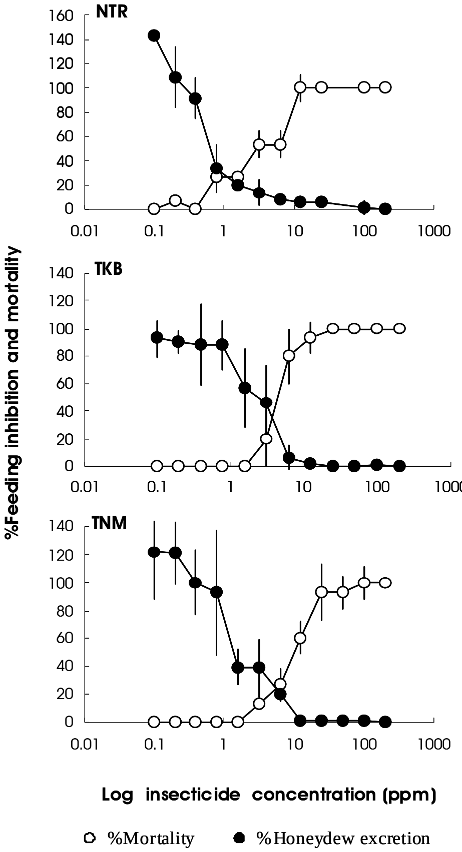

Fig 4. Relationship between %feeding inhibition and mortality of GLH and treated with cartap using the parafilm method. NTR, TKB and TNM refer to Table 1. Vertical bars indicate standard deviation.
Legends: ○ %Mortality
● %Honeydew excretion

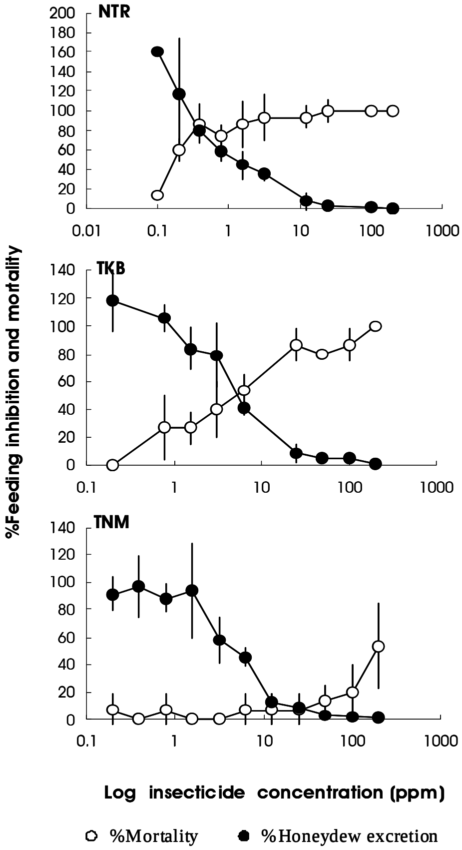

Fig 5. Relationship between %feeding inhibition and mortality of GLH and treated with propaphos using the parafilm method. NTR, TKB and TNM refer to Table 1. Vertical bars indicate standard deviation.
Legends: ○ %Mortality
● %Honeydew excretion

for all GLH strains. The feeding inhibition of propaphos occurred only for the TNM strain but it occurred at higher concentrations than imidacloprid or cartap. The corrected mortality for the TNM strain was 50% at 200 ppm of propaphos, but the feeding rate was reduced to 50% at 5.4 ppm. The antifeedant characteristics of propaphos on the TKB and NTR strains were not obvious. In the NTR strain, a 50% reduction in feeding rate occurred at lower concentration than the 50% corrected mortality in imidacloprid and cartap, but not in propaphos and disulfoton at 48 h. For disulfoton, feeding inhibition occurred in TNM at the high concentration (93 ppm). Identically to that occurring in propaphos, no antifeedant characteristic occurred in TKB or NTR strains.

Residual Period of the Systemic Insecticides

Toxicity of Insecticides

The toxicity of two systemic insecticides, imidacloprid (Admire granule) and cartap (Padan granule), to GLH, applied in a seedling-box (50g/box; the recommended rate to control GLH) before transplanting is presented in Fig. 7. Of the two insecticides, imidacloprid had persistence against most insect strains, with more than 80% mortality during the first two weeks, while cartap was less effective. With cartap the mortality was less than 20%, even by the first week after transplanting. The mortality of most insect strains on imidacloprid-treated plants dropped below 50% at the heading stage. There was no significant correlation between insect mortality on cartap-treated plants and the days after treatment (DAT), but a significant correlation was recognized on imidacloprid-treated plants (Fig. 7).

Effect of Insecticides on Feeding Rate

Feeding rate as indicated by area of honeydew excretion on filter paper was measured and analyzed.

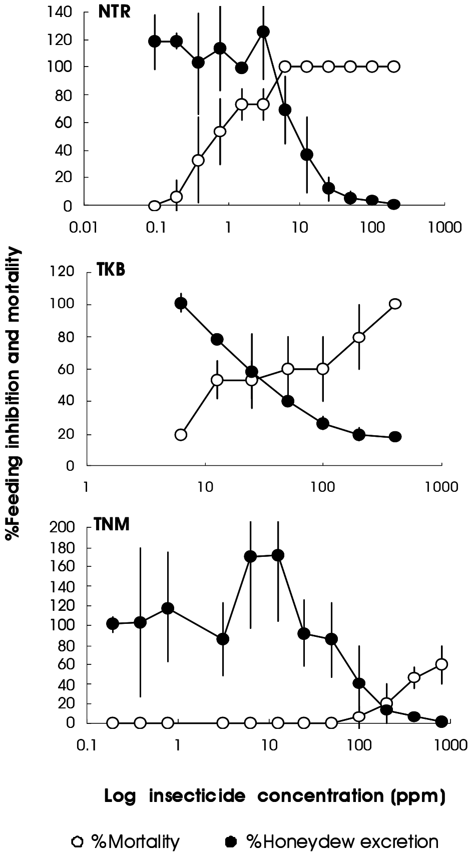

Fig 6. Relationship between %feeding inhibition and mortality of GLH and treated with disulfoton using the parafilm method. NTR, TKB and TNM refer to Table 1. Vertical bars indicate standard deviation.
Legends: ○ %Mortality
● %Honeydew excretion

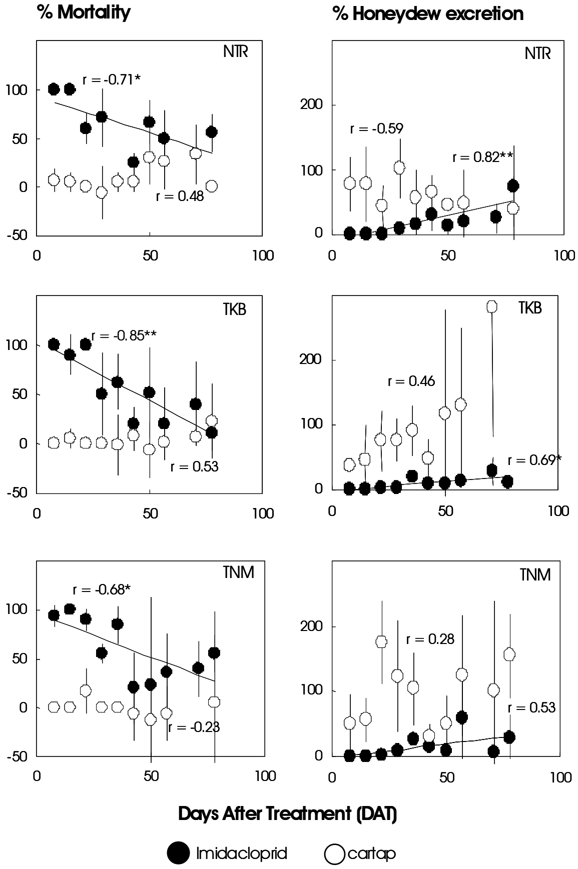

Fig 7. Effects of two systemic insecticides, imidacloprid and cartap, applied in a seedling-box before transplanting, on mortality and feeding rate of GLH. NTR, TKB and TNM refer to Table 1. Vertical bars indicate standard deviation.
Legends: ● imidacloprid
○ cartap

On imidacloprid- and cartap-treated plants, the feeding rates of most insect strains were lower than those of the control. Increase in feeding rate was slow on plants treated with imidacloprid. A significant correlation between the feeding rate and the DAT was recognized in imidacloprid-treated plots for all insect strains, but was not found in cartap-treated plots. Imidacloprid was still affecting the feeding rate of the insect until the flowering stage (80 DAT) (Fig. 7).

Effect of Seedling-Box Application on Insect's Population in the Field

We surveyed the population of the GLH in the experimental field plots at 85 DAT using a sticky board. The small brown planthopper (*Laodelphax striatellus*), white backed planthopper (*Sogatella furcifera*) and GLH were found. The nymphal density of both planthopper and GLH in the cartap plot and the untreated plots were higher than in the imidacloprid plot, indicating that the insect populations were rebuilt in the cartap and untreated plots. There was no significant difference in number of GLH between the cartap and the untreated plots. Most of the insects found in the imidacloprid plot were adults. There were a small number of nymphs in the imidacloprid plot (Fig.8).

Discussion

Better understanding of the effects of insecticides on insect pests and the rice plant may provide an insight into the evaluation of effects of insecticides and strategies to control or manage resistant insect pests. Various methods have been developed and used to evaluate the susceptibility of GLH to insecticides. Conventional topical application or the AChE sensitivity method are widely used to evaluate the toxicity of insecticides to GLH, but there have been few studies on

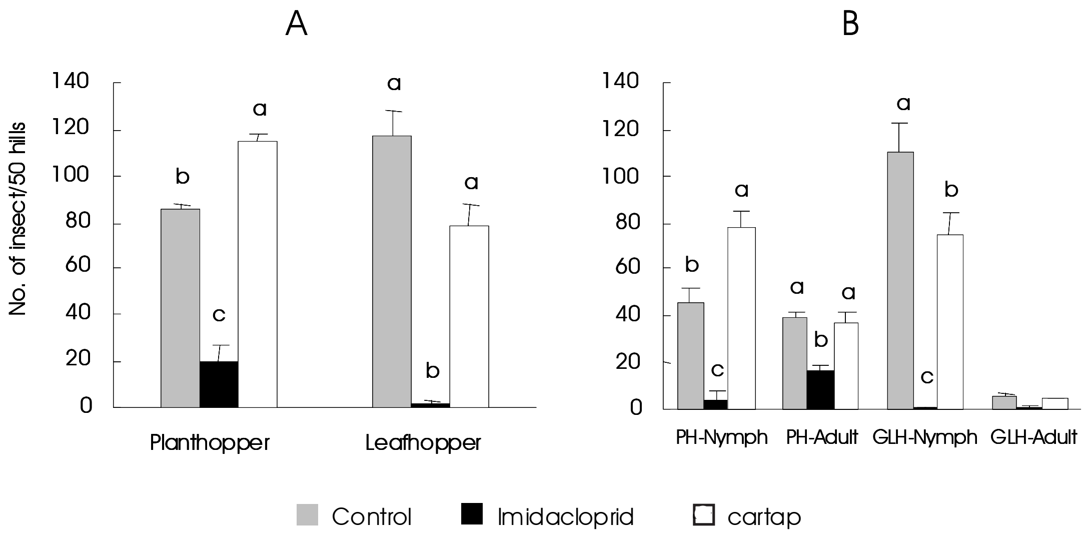

Fig 8. Population density of planthoppers (PH) and GLH (A) and number of nymphs and adults of PH and GLH (B) in the rice field plots at 85 days after transplanting. Values are expressed as means±SE. The bars with the same letter are not significantly different (P>0.05).
Legends: Control
Imidacloprid
cartap

how to evaluate both the feeding rate and the toxicity of systemic insecticides.

Based on the results of the parafilm method in this study, cartap was effective for all insect strains, TNM, TKB, and NTR. However, in field tests the mortality and honeydew excretion on rice plants collected from cartap-treated plots were not different from the untreated plots even 1 or 2 weeks after treatment. Because of the low effects of cartap (applied at 50g of Padan 4% granular per seedling-box) residue on rice plants, the feeding rate and mortality of the insects were not affected, and the GLH was able to build up their population in the cartap-treated plots.

Imiacloprid showed pronounced characteristics both in mortality and feeding inhibition at low concentrations for all insect strains. The LC_{50} and FI_{50} were smaller than cartap. 90% mortality was observed for all insect strains until 21 DAT and imidacloprid also caused reduction of feeding after the lethal effect diminished. Like other insecticides in neonicotinoids class, imidacloprid acts on the nervous system and molecular target sites is the insect nicotinic acetylcholine receptor[21]. The sublethal neurotoxicant can affect insect behaviour. The effects of sublethal doses of the insecticide were occurred when insufficient molecules to cause death reached the sites of insecticidal action[3]. It may acts as feeding deterrent like the resistant variety interfering nutrient absorption from the plant tissues.

Because of the long persistence of imidacloprid in the soil, its half-life was 107 days-1 year in US or 36-43 days in India[3,17]. At the recommended rate, 200g/ha of imidacloprid by seedling-box application, the residue in rice plant (0.01 ppm) was 65 DAT[7]. The present study also showed that the residue effect of imidacloprid on the rice plant could suppress the feeding activity of GLH and this effectiveness remained until the flowering stage of Akitakomachi at TBIC.

The LC_{50} values of propaphos and disulfoton for the TNM strain collected from an intensively treated area in southern Japan exceeded 200 and 500 ppm, respectively. The TKB strain showed moderate resistance to propaphos and disulfoton when compared with NTR strain collected from a less treated area in northern Japan. The slopes of regression lines of propaphos for NTR (1.61) were twice as steep as those for TNM (0.90) and TKB (0.62), but for disulfoton the slopes of regression lines for the TNM (0.78) and TKB (0.61) were higher than those for NTR (0.49). However we found that the feeding inhibitions of propaphos and disulfoton were strong with the OPs resistance strain, TNM, while it did not occur in TKB and NTR strains.

Antifeedant activity of systemic insecticides at sublethal doses has been considered to be an important characteristic for protecting treated plants from virus disease by suppression or interference of feeding of the vector insects. Feeding inhibition by a systemic insecticide can be evaluated by the intersection point between two curves, percentage mortality and percentage feeding inhibition, based on those in control

(Figs. 3-6). The intersection point indicated the property of a systemic insecticide as an antifeedant when tested by parafilm method. A low intersection point (where the feeding inhibition occurred at sublethal concentrations) is considered to show a strong antifeedant characteristic. In contrast, an insecticide giving a higher intersection point (the feeding inhibition occurs at or higher than lethal concentrations) is considered to give no feeding inhibitory action[11].

When we consider antifeedant activity of some insecticides as a strategy for preventing virus diseases, we should be careful about the negative function of the insecticides at sublethal concentration. Sublethal doses of some insecticides can stimulate reproductive activity of insect pests or increase the susceptibility of plants to insects[16,20]. With this consideration in mind, we have to be concerned about the effect of the insecticide residue on the rice plant on the insects after treatment, especially when insecticide is applied to a seedling-box before transplanting.

Acknowledgments

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