Insecticide Resistance of the Green Leafhopper, *Nephotettix cincticeps*, to Systemic Insecticides by "Parafilm Test": a Simple Method to Evaluate the Efficiency of the Insecticide

Jirapong Jairin[1/] Nobuki Kojima[2/] Toru Nagata[3/]

ABSTRACT

The insecticide susceptibility of the green leafhopper, *Nephotettix cincticeps* to systemic insecticides, imidacloprid, cartap, propaphos and disulfoton, were determined by a newly developed testing method, the"parafilm test" using an artificial membrane to give an artificial diet composed of 2.5 % sucrose and series dilution of insecticides. This test can measure both mortality (LC_{50}) and feeding inhibition (FI_{50}). When the three local populations of green leafhopper with different resistance levels to organophosphates and carbamates were compared with LC_{50} and FI_{50} obtained by this testing method. Feeding inhibition at sublethal doses was found to be the most remarkable with imidacloprid, followed by cartap without regarding to resistance levels, while feeding inhibition by propaphos and disulfoton was observed only with the resistant green leafhopper.

Key words: feeding inhibition, insecticide resistance, *Nephotettix cincticeps*, parafilm test, systemic insecticide

INTRODUCTION

Insecticide resistance is a problem in all insect groups that serve as vectors for causing plant diseases, including rice virus diseases. The green leafhopper (GLH), *Nephotettix cincticeps* (Uhler) is considered to be the most devastating insect pests in Asia. They do not only feed on rice plants, directly causing yield reduction, but they also transmit viruses which cause severe infection of rice. Pest control has largely relied on insecticides the in most rice

[1/] Ubon Ratchathani Rice Research Centre, P.O. Box 65, Muang district, Ubon Ratchathani province, 34000

[2/] Tsukuba International Centre, 3-6 Koyadai, Ibaraki, Japan 305-0074

[3/] Faculty of Agriculture, Ibaraki University, Ami, Ibaraki, Japan 300-0393

growing countries. GLH have also long been controlled by insecticides since the introduction of synthetic insecticides after the World War II. Extensive use of insecticides has contributed to the development of insecticide resistance in many field populations of this species (Ghorpade, 1990 and 1993; Hirai, 1994; Kato *et al.*, 1999; Nagata *et al.*, 1979 and 2000). To overcome the development of insecticide resistance, minimal use of insecticides is the most important practical term (Nomura *et al.*, 1999).

Granule insecticides are applied in the rice field and embedded into the paddy soil near the root zone. In this treatment the systemic insecticides are imbibed orally by sucking insects and it is appropriate to estimate insecticide susceptibility through the same administration pathway as the actual application route of systemic insecticides. In addition, in this treatment, the insects may avoid contact with the chemical by behavioristic resistance, which is expected to be more promising strategy for controlling virus diseases, transmitted by the vector insects (Kono *et al.*, 1975; Nagata *et al.*, 2000; Sogawa, 1971; Widiarta *et al.*, 1997; Yedaly, 2000). Then a specific testing method has been developed to determine an insect's susceptibility to systemic insecticides which exert two functions namely lethal action and feeding inhibitory action. In this experiment, the occurrences of insecticide resistance to these systemic insecticides were assessed.

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 Natori population (NTR) was collected from a less treated area in Natori city, Miyagi prefecture in 1998. Both populations were supplied by Kagoshima Prefectural Experiment Station and Miyagi Prefectural Experiment Station, respectively. The Tsukuba population (TKB) was additionally collected from Ibaraki prefecture in 2000. The insect colonies were established from three local populations in a plastic cage (34x26x34 cm) and placed in a rearing room at 25°C, light 14 hrs dark 10 hrs 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 were used

imidacloprid (pyridyl-methylamine) is a relatively new systemic insecticide and is chemically related to the tobacco toxin, nicotine which acts on the nervous system. It was first registered for the use as a pesticide in the U.S.A in 1994; cartap hydrochloride, a nereistoxin analogue insecticide; propaphos is an organophosphorus insecticide commonly used against leafhopper and planthopper since 1986; and disulfoton, as an aliphatic organothiophosphate insecticide.

Concentration-mortality and -feeding inhibition bioassays (Parafilm test)

The serials of insecticide concentrations for each insecticide were prepared (Table 1). 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 containing Tween 60, through an artificial membrane of stretched thin parafilm (Nagata and Hayakawa, 1998; Yedaly, 2000). A total of five male adults were anaesthetized with carbon dioxide and transferred into a plastic vial (3-cm diametre x 3-cm 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, light 14 hrs dark 10 hrs, over 60% of relative humidity. The experiment was conducted with three replications. The mortality of the insects was recorded at 24 hrs or 48 hrs after treatment. The insect mortality of each concentration for all the insecticides was corrected by the mortality of the control sucrose solution according to Abbott (1925). Data were subjected to probit analysis for each concentration-mortality experiment (Bliss, 1935) and 50 % mortality values were calculated. After 48 hrs, 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 (50% feeding inhibition concentration as ppm FI_{50}).

Table 1. The insect strains and insecticides concentrations for parafilm test

	Insect strain	Insecticide	Concentration (ppm)*
Green Leaf Hopper (GLH),			
Nephotettix cincticeps	Taniyama population	Imidacloprid	0-200**
	(TNM)	Cartap	0-200*
		Propaphos	0-200*
		Disulfoton	0-800***
	Tsukuba population	Imidacloprid	0-200**
	(TKB)	Cartap	0-200*
		Propaphos	0-200*
		Disulfoton	0-800***
	Natori population	Imidacloprid	0-200**
	(NTR)	Cartap	0-200*
		Propaphos	0-200*
		Disulfoton	0-800***

* Insecticide concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195, 0.098 and 0 ppm

** Insecticide concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195, 0.098, 0.049, 0.024, 0.012, 0.006, 0.003, 0.001 and 0 ppm

*** Insecticide concentrations: 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195, 0.098 and 0 ppm

RESULTS AND DISCUSSION

Concentration-mortality and feeding inhibition bioassays (parafilm test)

Insecticide susceptibility

There were large variations between LC_{50} values of insecticides in each insect strain (Table 2). The LC_{50} of most insecticides for the NTR strain was the smallest and for the TNM strain was significantly larger than for all strains except the LC_{50} value of imidacloprid. The order of resistance level of GLH to cartap, disulfoton and propaphos was TNM>TKB>NTR, but the order was different form imidacloprid (Table 2, Figure 1). The LC_{50} values of disulfoton, propaphos and cartap for the TNM strain were 650, 1,000 and three times as large as those for the NTR strain, while those for the TKB strain were 14, 20 and two times larger than for the NTR strain respectively. The TNM strain

showed resistance to propaphos and disulfoton (LC_{50} values of 200, 529 ppm), while these insecticides remained effective for the NTR strain (LC_{50} values 0.18, 0.81 ppm).

From the parafilm test, imdacloprid was shown to be more effective against three 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 two times larger than that of TNM.

Table 2. Toxicity of four systemic insecticides to adult male GLH after 48 hrs by parafilm test

Insecticide	LC_{50} (ppm)					
	GLH-TNM	Slope*	GLH-TKB	Slope*	GLH-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 lines

GLH-TKB, GLH-TNM and GLH-NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively

Table 3. Feeding inhibition of four systemic insecticides in adult male GLH after 48 hrs by parafilm test

Insecticide	FI_{50} (ppm)					
	GLH-TNM	Slope*	GLH-TKB	Slope*	GLH-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 lines

GLH-TKB, GLH-TNM and GLH-NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively

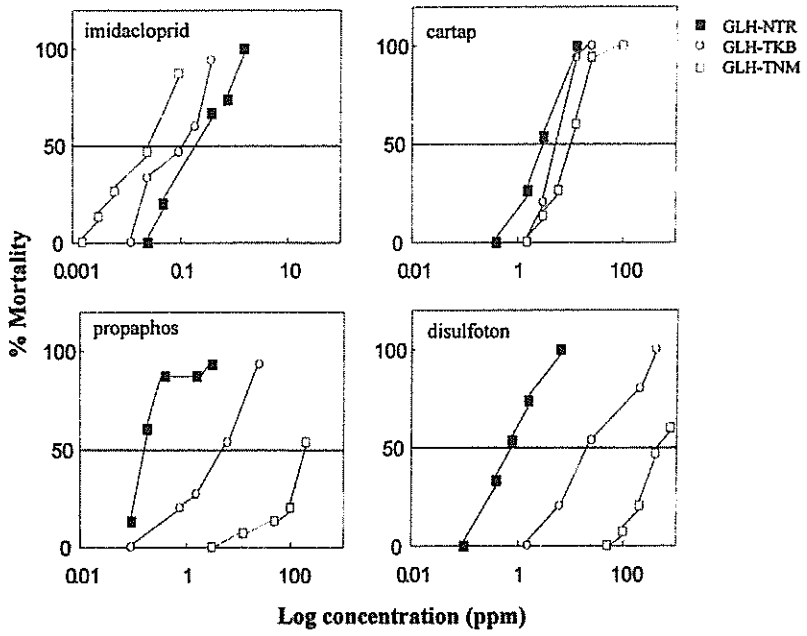

Figure 1. Concentration-mortality and -feeding rate relationship in male adult GLH. GLH-NTR, GLH-TKB, GLH-TNM and GLH-NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively

Feeding inhibition

Identically to the lethal concentrations, that value for the TNM strain was significantly larger than those for all strains except the FI_{50} value of imidacloprid (Table 3). The FI_{50} values for NTR were the smallest. 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, 1.2 ppm). The feeding rates of TNM, TKB and NTR for imidacloprid were reduced to more than 90% of the control at the concentrations of 0.02, 0.01 and 0.01 ppm, respectively (Figure 1). This result indicated that imidacloprid and cartap showed characteristics as an antifeedant for all GLH strains, while propaphos and disulfoton showed characteristics as an antifeedant only for the TNM strain.

Relationship between the feeding inhibition and mortality

According to this the feeding inhibition concentrations substantially occurred at lower than the lethal concentrations, and imidacloprid and cartap showed a pronounced characteristic as an antifeedant 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 (Figures 2 and 3). 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. (Tables 2 and 3) 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 hrs. For disulfoton, feeding inhibition occurred in TNM at the high concentration (93 ppm) (Table 3). Identically to that occurring in propaphos, no antifeedant characteristic occurred in TKB or NTR strains.

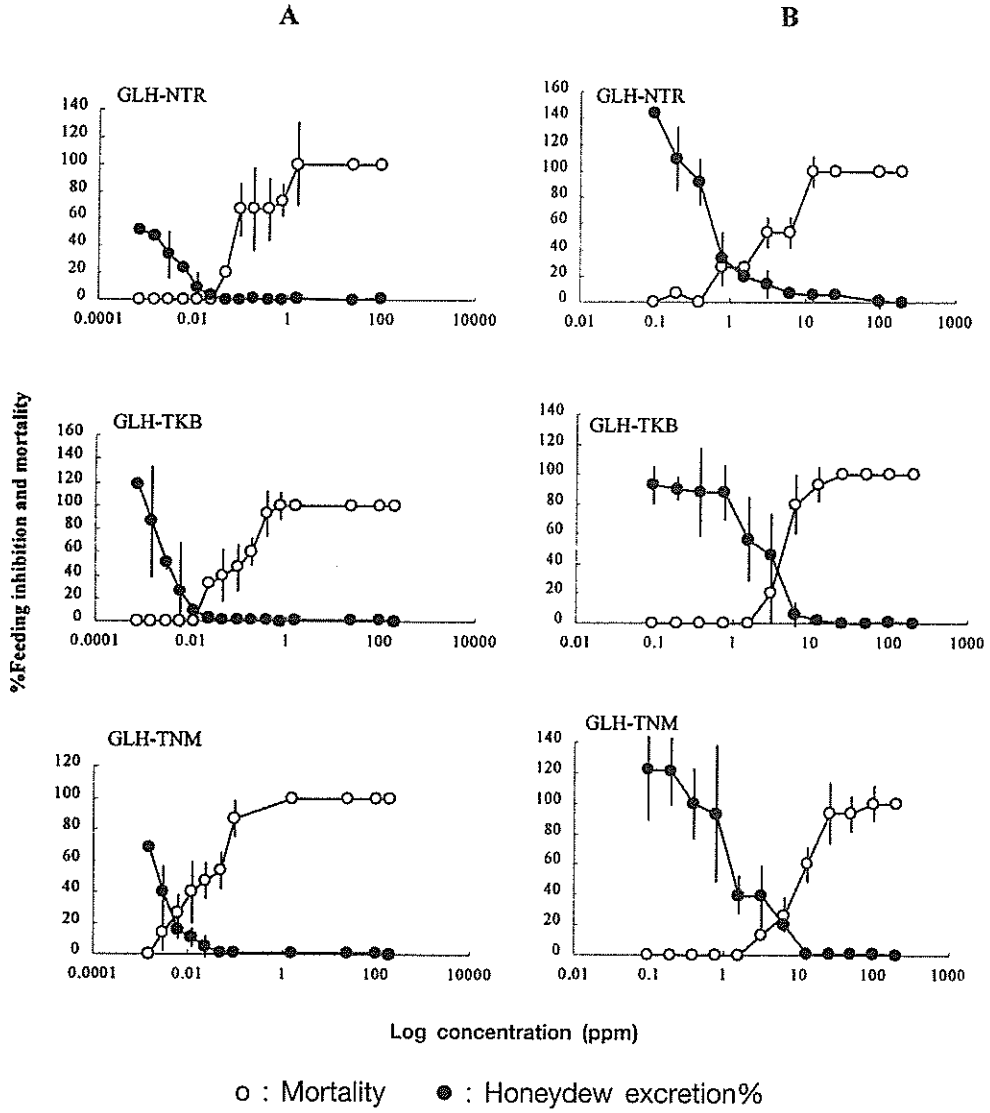

Figure 2. Relationship between feeding inhibition percentage and mortality of GLH treated with (A) imidacloplid and (B) cartap using the parafilm test. GLH-NTR, GLH-TKB and GLH-TNM refer to GLH-TKB, GLH-TNM and GLH-NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively. Vertical bars indicate standard deviation.

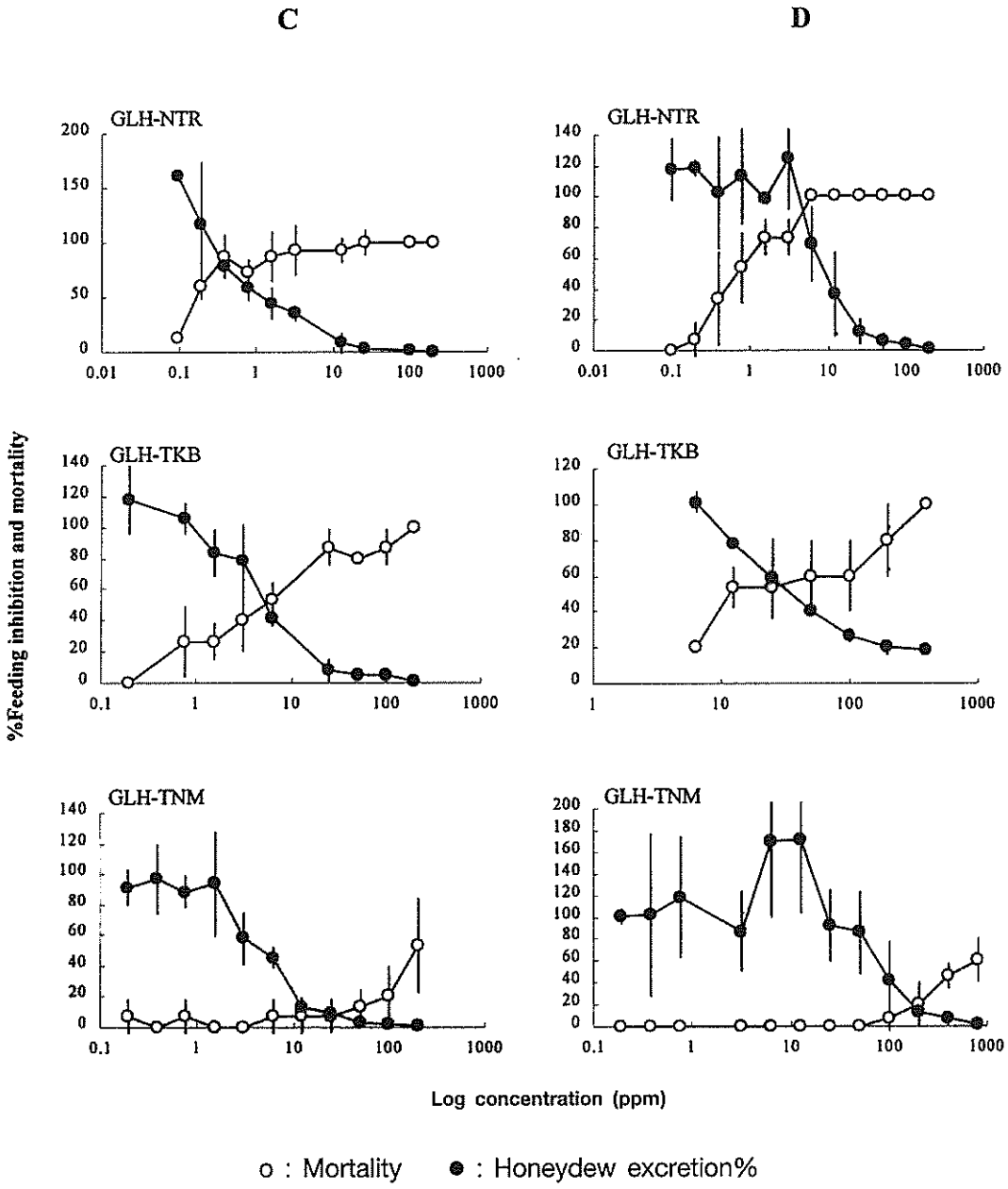

Figure 3. Relationship between feeding inhibition and mortality of GLH treated with (C) propaphos and (D) disulfoton using the parafilm test. GLH-NTR, GLH-TKB and GLH-TNM refer to GLH-TKB, GLH-TNM and GLH-NTR represent GLH populations collected from Tsukuba, Taniyama, and Natori respectively. Vertical bars indicate standard deviation.

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 acetylcholinesterase acetylcholinesterase sensitivity method are widely used to evaluate the toxicity of insecticides to GLH, but there have been few studies on how to evaluate both the feeding rate and the toxicity of systemic insecticides. A preliminary assay of antifeeding chemicals and insecticides for GLH applied through a stretched parafilm membrane has been reported (Kono *et al.*, 1975; Sogowa, 1971). Previous studies have revealed that the parafilm test is a simple and rapid monitoring method for evaluating the efficiency of systemic insecticide as a feeding inhibition for GLH. The insecticide susceptibility of the two GLH strains, TNM and NTR, to cartap and propaphos has been reported by this method (Yedaly, 2000).

The LC_{50} values of propaphos and disulfoton for the TNM strain collected from an intensively treated area in the 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 the northern Japan. However, the feeding inhibitions of propaphos and disulfoton were fond to be strong with the organophosphate 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 diseases 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, mortality and feeding inhibition, based on those in control (Figures 2 and 3). The intersection point indicated the property of a systemic insecticide as an antifeedant when tested by parafilm test. 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 (Nagata and Hayakawa, 1998; Nagata *et al.*, 2000). This study supports previous work by the parafilm

test demonstrating feeding inhibitory action of cartap and propaphos against GLH (Yedaly, 2000).

When antifeedant activity of some insecticides as a strategy were considered for preventing virus diseases, carefulness should be made for 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 (Reissig *et al.*, 1982; Wu *et al.*, 2001). With this consideration in mind, we have to be concerned about with the effect of the insecticide residue on the rice plant and on the insects after treatment.

ACKNOWLEDGEMENTS

Sincere appreciation to Dr. K. Katsuya, Dr. T. Yamashita and Dr. M. Hara for their useful comments and suggestions. This work was supported by the Tsukuba International Centre and the Japan International Cooperation Agency.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.

Bliss, C.I. 1935. The calculation of the dosage-mortality curve. *Ann. Appl. Biol.* 22: 919-924.

Ghorpade, S.A. 1990. Esterase activity and associated resistance of the rice brown planthopper, *Nilaparvata lugens* (Stål) to insecticides. *J. Pesticide Sci.* 3: 152-157.

Ghorpade, S.A. 1993. Study on oxidase activity in strains of rice brown planthopper, *Nilaparvata lugens* (Stål) resistant to insecticides. *J. Insect Sci.* 6: 85-88.

Hirai, K. 1994. Current status of insecticide susceptibility in the *Nilaparvata lugens* Stål, in Japan. *J. Pesticide Sci.* 19: 225-227.

Kato, Y., Nomura, M., and Miyata, T. 1999. Negatively correlated cross-resistance between N-Methyl carbamate and monocrotophos in green rice leafhopper, Nephotettix cincticeps Uhler. *J. Pesticide Sci.* 24: 369-368.

Kono, Y., D. Nagaarashi and M. Sakai. 1975. Effects of cartap, chlordimeform and diazinon on the probing frequency of green leafhopper (Hemiptera: Deltocephalidae). *Appl. Entomol. Zool.* 10: 58-60.

Nagata, T., M.O. Yedaly, and S. Kojima. 2000. Insecticide resistance of the green rice leafhopper, *Nephotettix cincticeps* Uhler, to the systemic insecticides

administered through parafilm membrane. Laboratory of Applied Entomology and Zoology, Ibaraki University (unpublished).

Nagata, T. and T. Hayakawa. 1998. Antifeeding activity of aconitic acid and oxalic acid on brown planthopper, *Nilaparvata lugens* (Stål) and green rice leafhopper, *Nephotettix cincticeps* (Uhler). Japan. *J. Appl. Entomol. Zool.* 42: 115-121.

Nagata, T., T. Masuda, and S. Moriya. 1979. Development of insecticide resistance in the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Appl. Entomol. Zool.* 14: 264-269.

Nomura, M., Y. Kato and T. Miyata. 1999. Monitoring of organophosphate and carbamate resistance and identifying a combination of insecticides showing negatively correlated cross resistance in the green rice leafhopper *(Nephotettix cincticeps* Uhler) (Hemiptera: Deltocephalidae). *Appl. Entomol. Zool.* 34: 525-530.

Reissig, W.H., E.A. Heinrichs and S.L. Valencia. 1982. Insecticide-induced resurgence of the brown planthopper, *Nilaparvata lugens,* on rice varieties with different levels of resistance. *Environ. Entomol.*, 11: 165-168.

Sogawa, K. 1971. Preliminary assay of antifeeding chemicals for brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). *Appl. Entomol. Zool.* 6: 215-218.

Widiarta, I.N., W. Hermawan, S. Oya, S. Nakajima and F. Nakasuji. 1997. Antifeedant activity of Andrographis paniculata (Acanthaceae) against the green rice leafhopper, *Nephotettix cincticeps* (Hemiptera: Cicadellidae). *Appl. Entomol. Zool.* 32: 561-566.

Wu, J., J., Xu, S., Yuan, J., Liu, Y., Jiang, and J., Xu. 2001. Pesticide-induced susceptibility of rice to brown planthopper *Nilaparvata lugens.* Entomol. Exp. Appl. 100: 119-126.

Yedaly, M.O. 2000. The insecticide resistance of the green rice leafhopper, *Nephotettix cincticeps* Uhler, to the systemic insecticides administered through parafilm membrane. Pages 61-71. *In* Reports of Experiment in Rice Research Techniques Course, Tsukuba International Centre.