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Insecticide susceptibility of the small brown planthopper, *Laodelphax striatellus* Fallén (Homoptera: Delphacidae), collected from East Asia

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Abstract

The insecticide susceptibility of the small brown planthopper, *Laodelphax striatellus* Fallén, collected from East Asia in 1992–1994, was examined by the topical application method. The LD_{50} values of organophosphorus insecticide for the northern Vietnam populations (HAI, HAN, VIN) and the JIN population (Yunnan Province, China) were smaller than those of the FU (Zhejiang Province, China), IB (central Japan) and KU (southwestern Japan) populations. The LD_{50} values of organophosphorus and carbamate insecticides for the northern Vietnam populations were almost the same as that for the Fukuoka (western Japan) population examined in 1967. The LD_{50} values of organophosphorus insecticides did not differ among the FU (Zhejiang Province, China), IB (central Japan), and KU (southwestern Japan) populations. The LD_{50} values of carbamates for the KU population were the largest, and those for the northern Vietnam population were the smallest. The carbamate susceptibility of acetylcholinesterase in the KU population was lower than that in the HAI population. Therefore, we considered that insensitivity of acetylcholinesterase for carbamate was one of the carbamate resistant factors in the KU population. The LD_{50} values of etofenprox, fenvalerate, and imidacloprid showed no differences among all the populations tested, respectively.

Key words: Acetylcholinesterase, aliesterase, East Asia, insecticide resistance, *Laodelphax striatellus*

INTRODUCTION

The small brown planthopper, *Laodelphax striatellus* Fallén (SBPH), is distributed from the Philippines to Siberia and Europe, mainly in the temperate zone (Esaki and Takeuchi, 1976; Kisimoto, 1989). This insect is able to overwinter in most parts of Japan (Kisimoto, 1989). A considerable number of SBPHs have been caught together with the brown planthopper and white-backed planthopper over the East China Sea (Kisimoto, 1972; Iijima, 1973; Hirao, 1974; Hirao and Ito, 1980; Ogawa et al., 1988). It is presumed that SBPH is able to migrate to Japan from overseas. It has been reported that the northeastern Japan SBPH populations indicated cytoplasmic incompatibility for SBPH populations collected from southwestern Japan and on the East China Sea (Noda, 1984, 1987).

SBPH is one of the most important insect pests infesting rice plants in Japan, transmitting the stripe virus disease. Therefore, SBPH has been controlled mainly by insecticides. Insecticide resistance of SBPH to malathion and lindane was first recognized by Kimura (1965, 1973) in the mid-1960's. Ozaki and Kassai (1971) also reported the resistance to some other organophosphorus insecticides and carbamates. There have also been several reports on the insecticide susceptibility of the SBPH populations collected in Japan (Takita, 1979; Hama, 1984; Nagata and Ohira, 1986; Ogawa, 1987; Sone et al., 1995). However, SBPH's insecticide susceptibility in East Asia is not well-known. Therefore, we surveyed the insecticide susceptibility of SBPH in this area.

MATERIALS AND METHODS

Insects. The collection sites and dates are shown in Table 1. The insects were reared on rice seedlings at 25°C under a 16 h photoperiod.

Chemicals. The following insecticides of technical grade were used: malathion (96%), diazinon (96%), fenitrothion (97%), dimethylvinphos (97%), monocrotophos (73%), acephate (97%), fenobucarb (98%), propoxur (99%), carbaryl (99%), carbofuran (99%), carbosulfan (92%), etofenprox (98%), fenvalerate (96%), and imida-

Table 1. Locality and collection time of tested populations of the small brown planthopper

Population	Collection	
	Site	Time
HAI	Hai Fun Province, Vietnam	Apr. 1992
HAN	Hanoi, Vietnam	Mar. 1992
IN	Vinh Phu Province, Vietnam	Apr. 1993
JIN	Jinghong, Yunnan Province, China	May 1994
FU-A	Fuyang, Zhejiang Province, China	Jun. 1993
FU-B	Fuyang, Zhejiang Province, China	Jun. 1993
KU-A	Uto City, Kumamoto Prefecture, Japan	Jul. 1992
KU-B	Kikuchi district, Kumamoto Prefecture, Japan	May 1992
IB-A	Tateno, Tsukuba City, Ibaraki Prefecture, Japan	May 1993
IB-B	Nishimachi, Tsukuba City, Ibaraki Prefecture, Japan	Jun. 1993

cloprid (95%). Diazoxon (>96%) and fenitroxon (>96%) were synthesized using the method described by Shiotsuki and Eto (1987). Acetylthiocholine (>98%), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), and sodium lauryl sulfate (>98%) were purchased from Wako Chemical Industries, Ltd., α-naphthyl acetate and eserine from Sigma, and Fast Blue B salt from Tokyo Chemical Industry Co. Ltd.

Insecticidal tests. Female adults within 1 week after emergence were anaesthetized with carbon dioxide. A 0.05 μl droplet of acetone solution of insecticide was applied to the notum of the female adults using a microapplicator (Burkard®). The treated insects were kept at 25°C, 16L 8D, with rice seedlings in a plastic box (11×8×3.3 cm). Mortality was recorded 24 h after treatment, and LD_{50} was calculated using probit analysis (Bliss, 1935). More than 30 females were used for each concentration. Tests were carried out on 4–5 concentrations.

***In vitro* test.** Twenty females were homogenized in an ice-bath with 2 ml of 1/15 M phosphate buffer (pH 7.2) for measurement of aliesterase activity, and 200 males were homogenized with 5 ml of 1/15 M phosphate buffer (pH 7.4) for the inhibition test of acetylcholinesterase. The homogenate was filtered through nylon gauze, and then centrifuged at 800×g for 10 min to remove debris. The supernatant was used as the enzyme source.

Aliesterase activity was measured using the method described by Asperen (1962) employing 5 mM α-naphthyl acetate as the substrate. Inhibition of acetylcholinesterase (AChE) by insecticides was carried out using the method described by Ellman et al. (1961), employing 0.67 mM DTNB and 1 mM acetylthiocholine.

The protein contents of the homogenate were determined using the method described by Read and Northcote (1981), employing bovine serum albumin as a standard.

RESULTS

Insecticide susceptibility

As shown in Table 2, the LD_{50}s of organophosphorus insecticides and carbamates for the HAI, HAN and VIN populations were the smallest among the populations tested. The LD_{50}s of organophosphorus insecticides and most of the carbamates for the JIN population were also small. But the LD_{50} of carbaryl for the JIN population (95% confidence limit: 11–13 μg/g) was significantly larger than that for the HAI population (95% confidence limit: 2.0–3.2 μg/g). The LD_{50} values of malathion, fenitrothion, and diazinon for the FU populations were 72–88, 17–36 and 7.1–7.4 times as large as those for the HAI population, respectively. The LD_{50} values of carbamates for the FU populations were 4–19 times as large as those for the HAI population. LD_{50} values of the tested insecticides did not differ greatly between the FU and IB populations. The average LD_{50} values of carbamates for the KU populations were 3.9–13 times as large as those for the FU populations. However, the insecticide susceptibilities to pyrethroids and imidacloprid showed no differences among all the populations collected from

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Table 2. Insecticide susceptibility of the small brown planthopper

Insecticide	LD_{50}, μg/g										
	HAI	HAN	VIN	JIN	FU-A	FU-B	KU-A[a]	KU-B	IB-A[a]	IB-B	Fukuoka '67[b]
Malathion	2.5	3.5 [1.4][c]	4.6 [1.8][c]	2.9 [1.2][c]	180 [72][c]	220 [88][c]	380 [150][c]	330 [130][c]	130 [52][c]	95 [38][c]	1.6 [0.65][c]
Fenitrothion	1.0	2.1 [2.1]	1.9 [1.9]	1.6 [1.6]	17 [17]	36 [36]	38 [38]	31 [31]	27 [27]	18 [18]	0.75 [0.75]
Diazinon	3.8	2.5 [0.66]	4.8 [1.3]	2.5 [0.66]	28 [7.4]	27 [7.1]	74 [19]	45 [12]	28 [7.4]	21 [5.5]	1.9 [0.50]
Dimethylvinphos	—	—	2.4	—	—	—	—	—	—	—	—
Monocrotophos	—	—	0.48	—	—	—	—	—	—	—	—
Acephate	—	—	6.3	—	—	—	—	—	—	—	—
Fenobucarb	6.0	12 [2.0]	8.3 [1.4]	9.5 [1.6]	32 [5.3]	24 [4.0]	320 [53]	410 [69]	33 [5.5]	16 [2.6]	—
Propoxur	0.77	—	2.7 [3.5]	1.8 [2.3]	—	—	—	—	—	—	0.61 [0.79]
Carbaryl	2.6	3.6 [1.4]	3.5 [1.3]	12 [4.6]	42 [16]	49 [19]	450 [170]	—	14 [5.4]	12 [4.6]	1.6 [0.62]
Carbofuran	0.43	—	0.92 [2.1]	1.6 [3.7]	4.8 [11]	3.6 [8.4]	20 [47]	12 [28]	1.7 [3.9]	1.4 [3.2]	—
Carbosulfan	1.2	1.2 [1.0]	2.1 [1.8]	2.4 [2.0]	—	6.5 [5.4]	42 [35]	47 [39]	—	2.1 [1.8]	—
Etofenprox	1.3	3.0 [2.3]	1.9 [1.5]	3.0 [2.3]	1.0 [0.77]	3.3 [2.5]	2.7 [2.1]	2.1 [1.6]	1.7 [1.3]	1.5 [1.2]	—
Fenvalerate	5.6	2.9 [0.52]	3.4 [0.61]	3.6 [0.64]	2.4 [0.43]	6.7 [1.2]	—	4.9 [0.88]	1.9 [0.34]	2.9 [0.52]	—
Imidacloprid	0.22	—	0.11 [0.50]	0.20 [0.91]	0.32 [1.5]	—	0.61 [2.8]	—	0.26 [1.2]	—	—

[a] Data from Endo and Tsurumachi (2000).

[b] Data from Fukuda and Nagata (1969).

[c] Figures in parentheses indicate the ratio (LD_{50} for each population/LD_{50} for HAI population).

Table 3. Aliesterase activity of the small brown planthopper

	HAI	JIN	FU-A	KU-A[a]	IB-A[a]
Aliesterase activity nmol/10 min/μg protein	2.9±1.1	5.7±1.1 (2.0)[b]	32±4.8 (11)[b]	29±4.5 (10)[b]	27±3.6 (9.3)[b]

[a] Data from Endo and Tsurumachi (2000).

[b] Figures in parentheses indicate the ratio (Aliesterase activity of each population/Aliesterase activity of HAI population).

East Asia.

Aliesterase activity

The aliesterase activity of each population is shown in Table 3. The aliesterase activity of the JIN population was twice as large as that of the HAI population. Larger differences were seen in the FU-A, KU-A and IB-A populations in which the aliesterase activities were 9–11 times higher than those of the HAI population.

Insecticide susceptibility of AChE

The inhibition of AChE by carbamates and activated metabolites of organophosphorus insecti-

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Table 4. Inhibition of the small brown planthopper acetylcholinesterase

Insecticide	I_{50}±SE×10^{-7} M			
	HAI	FU-A	KU-A[a]	IB-A[a]
Diazoxon	2.0±0.6	1.2±0.6 (0.61)[b]	4.0±0.9 (2.0)[b]	1.9±0.4 (0.94)[b]
Fenitroxon	3.5±0.3	3.9±1.2 (1.1)	7.3±1.8 (2.1)	3.6±1.1 (1.0)
Fenobucarb	0.35±0.1	0.30±0.1 (0.85)	1.4±0.3 (4.0)	0.26±0.09 (0.74)
Carbaryl	0.46±0.2	1.1±0.3 (2.3)	3.1±0.6 (6.7)	0.48±0.09 (1.0)

[a] Data from Endo and Tsurumachi (2000).

[b] Figures in parentheses indicate the ratio (I_{50} for each population/I_{50} for HAI population).

cides (diazoxon and fenitroxon) is shown in Table 4. The I_{50} values of diazoxon, fenitroxon, fenobucarb and carbaryl for FU-A and IB-A AChE were almost the same as those for HAI AChE, respectively, except for the I_{50} of carbaryl for FU-A AChE, which showed a slight insensitivity. The I_{50} values of the examined compounds for KU-A AChE were 2 and 4–7 times as large as those for HAI AChE.

DISCUSSION

In Japan and Korea, several reports have described a local difference in the insecticide susceptibility of SBPH as determined by topical application (Choi et al., 1975; Song et al., 1976; Takita, 1979; Hama, 1984; Nagata and Ohira, 1986; Lee et al., 1987; Ogawa, 1987; Sone et al., 1995). These results showed that the LD_{50} values of carbamate for the SBPH populations from northern Japan and Korea were generally smaller than those from southern Japan. As compared with the earliest data available in 1967 (Fukuda and Nagata, 1969, see Table 2), the 1985 Hokkaido population (northern Japan) gave a 34-fold larger LD_{50} for fenitrothion (Ogawa, 1987), and the 1992 Nagano populations (middle Japan) gave a 31-fold larger LD_{50} for propoxur (Sone et al., 1995), respectively. Therefore, it has been concluded that SBPH was developing insecticide resistance to organophosphorus insecticides and carbamates in various districts in Japan during those periods. However, the resistance ratios of the northern Vietnam populations (HAI, HAN, and VIN) to organophosphorus insecticides and carbamates based on the 1967 baseline data (Fukuda and Nagata, 1969) ranged from 1.3–2.8-fold and 1.3–4.4-fold, respectively. This may suggest that the northern Vietnam populations have not thus far been exposed to intensive selection pressure by insecticides. The JIN population from Yunnan was also susceptible to insecticides, as well as the northern Vietnam populations, except for a slight increase in LD_{50} for carbaryl.

Nagata and Ohira (1986) reported that insecticide susceptibility of SBPH collected on the East China Sea was almost equal to that of SBPH collected from various locations in Kyushu in their 1980 monitoring. However, our results showed a large difference in LD_{50} for the carbamates tested (fenobucarb and carbaryl etc.) between the FU populations collected from China and the Kyushu populations (KU-A and KU-B), though the LD_{50} values for the organophosphorus insecticides were almost the same for the two countries. Kisimoto (1989) described that SBPH is able to overwinter in most parts of Japan. Therefore, this suggests there exists a resident population developing carbamate resistance that overwinters on inland Kyushu, Kumamoto Prefecture, which is not affected by migrant populations from abroad, presumably from China or northern Vietnam.

The LD_{50} values of the 1992 KU populations collected in Kyushu for carbaryl were 6-times larger than those determined in 1980, while those for the organophosphorus insecticides were almost the same as those determined in Kyushu in 1980 (Nagata and Ohira, 1986) and in 1986 (Ogawa, 1987). Therefore, it can be concluded that SBPH was still developing carbaryl resistance during 1980–1992 in Kyushu, although organophosphorus insecticide resistance remained unchanged during this period. Thus, a definite local difference in insecticide susceptibility was detected for SBPH in East Asia; however, local differences in susceptibility to newly developed insecticides such as imidacloprid and etofenprox, being registered in 1992 and 1987 in Japan, respectively, have not been observed thus far.

It is well-known that aliesterase affects the organophosphorus insecticide resistance of SBPH (Ozaki and Kassai, 1970; Hama, 1984). We recognized that the aliesterase activity of the FU-A population (organophosphorus insecticide resistant) was 11 times higher than that of the HAI population (organophosphorus insecticide susceptible). Miyata et al. (1976) reported that one of the aliesterase isozymes (E7) of the malathion resistant strain decomposed malathion more than the susceptible strain did. We considered that the E7 of the FU-A population might decompose malathion to a greater extent than the HAI population.

Endo and Tsurumachi (2000) reported that insensitive AChE was one of the factors of carbamate and organophosphorus insecticide resistance in SBPH. Insensitivity of KU-A AChE to fenobucarb and carbaryl was 4.0 and 6.7 times larger than that of HAI, respectively. Therefore, it was presumed that one of the factors in the resistance of KU-A to carbamates might be associated with the insensitivity of AChE to carbamates.

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