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High Temperature Effects on Yeast-like Endosymbiotes and Pesticide Resistance of the Small Brown Planthopper, *Laodelphax striatellus*

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Abstract: The newly-hatched nymphs of the small brown planthopper (SBPH), *Laodelphax striatellus*, including field and sensitive populations, were subjected to the high-temperature (35°C) treatment. The number of yeast-like endosymbiotes in SBPH reduced by 23.47%–34.23%, 57.86%–61.51% and 88.96%–90.71% after the high-temperature treatment for 1 d, 2 d, and 3 d, respectively. However, the size of yeast-like endosymbiotes was not obviously affected. Resistance of SBPH to three insecticides (imidacloprid, chlorpyrifos and fipronil) decreased with the increase of treatment time.
Key words: *Laodelphax striatellus*; yeast-like endosymbiote; high temperature; pesticide resistance; insect pest

The small brown planthopper (SBPH), *Laodelphax striatellus* Fallén (Homoptera: Delphacidae), is one of the major insect pests of food crops such as rice, corn, and wheat. Heavy infestation occurs in the middle and lower reaches of the Changjiang River and North China [1-2]. SBPH directly infests leaves and transmits some viral diseases [3-4], resulting in serious yield loss and grain quality decline. Currently, unreasonable use of insecticides promotes the resistance of SBPH to many kinds of pesticides [5]. That is a critical issue on controlling SBPH.

Recently, the insect endosymbiotes have attracted an increasing attention of researchers at home and abroad. Investigations show a large number of endosymbiotes in Homoptera insects, and there are tight links between endosymbiotes and their host insects on nutritional requirement and detoxification metabolism [6-8]. The resistance of aphids to organic pesticide is found to be related to the symbiotes in the aphid [9]. The symbiotes harbored in SBPH are yeast-like endosymbiotes (YLES), which are widely present in the abdominal fatty tissues of the host and obtained by larval progeny via transovarial transmission. The number of endosymbiotes increased with the age of its host insect [10].

Some environmental factors, such as high-temperature, antibiotics and pesticides, can inhibit the development of yeast-like endosymbiote in SBPH. SBPH without yeast-like endosymbiotes obviously differed from normal SBPH in their growth, development and fecundity [11-12]. However, the relationship between endosymbiotes and pesticide resistance of the host insect hasn't been reported yet.

In this experiment, the sensitivities of SBPH without YLES under the high temperature (35ºC) treatment to three commonly used insecticides (imidacloprid, chlorpyrifos and fipronil) were studied. The relationship between SBPH sensitivity to insecticides and YLES parasitized in SBPH was initially revealed.

MATERIALS AND METHODS

Experimental materials

Tested insects

The sensitive populations of SBPH were continuously reared on barley seedlings for 26–28 generations in the laboratory, and the field populations of SBPH were collected from rice fields in the Zhejiang Academy of Agricultural Sciences, Hangzhou, China.

Tested insecticides

The insecticides used in this study included 95% imidacloprid (Huzhou Rongshen Agrochemical Co, Ltd.,

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China), 97.7% chlorpyrifos (Zhejiang Xin'an Chemical Group Co, Ltd., China), and 87% fipronil (Bayer CropScience China Co., Ltd., China).

Methods

Quantity detection of yeast-like endosymbiotes in SBPH under high-temperature (35ºC) treatment

The newly-hatched nymphs of SBPH, including field and sensitive populations, were subjected to the high-temperature (35ºC) treatment for 1 d, 2 d and 3 d respectively in illumination cabinets, with a temperature of 35°C±1°C, a relative humidity of 80%–90% and a photoperiod of 16 h light and 8 h dark. After the high temperature treatment, they were raised in other cabinets to the 3rd instar under 25°C±1°C. Ten 3rd instar SBPHs from each treatment were collected, ground and homogenated with 500 μL 0.9% normal saline. Then 5 μL homogenate was loaded onto a blood cell counter and number of YLES was counted using a microscopy with 3–5 replications. The untreated SBPH was used as control (CK).

Size detection of yeast-like endosymbiotes in SBPH under high-temperature (35ºC) treatment

The abdomens of the 3rd instar SBPH from each treatment were frozen and sectioned with a cryostat microtome (Leica CM1900, Germany) and observed under a dissecting microscope (Leica S8APO, Germany). The thickness of cryostat section was 5–7 μm. The cryostat microtome was set to -30ºC for box and -40ºC for section joint. Sample preparation and crystal violet staining followed the methods from Chen et al [13]. The processed samples were placed on slides, observed and photographed (Leica DFC320; German) under a microscope (Leica DMLS2; German) after drying.

Because the type of YLES in SBPH was mainly oval, the length and the width of mature oval YLES were randomly measured by the software IPP (Arrowed in Fig. 1).

Susceptibility monitoring of SBPH to three insecticides

The susceptibility of SBPH to three insecticides (imidacloprid, chlorpyrifos and fipronil) was tested using the barley immersion test. The insecticide solutions were prepared with acetone and five serial dilutions were made. Then barley seedlings were immersed into the insecticide solutions for 30 s and dried. Three barley seedlings were placed into a test tube, and 20 third instars of SBPH were introduced into the test tube. The number of dead SBPHs was checked at 1 d, 2 d or 3 d after test, and the mortality was calculated. The experiment was replicated for three times.

Statistical analysis

The DPS processing system and double-factor variance analysis were used to compare the differences of length and width of YLES in differently treated SBPH. The LD-*P* lines and LC_{50} were obtained using the probit analysis method.

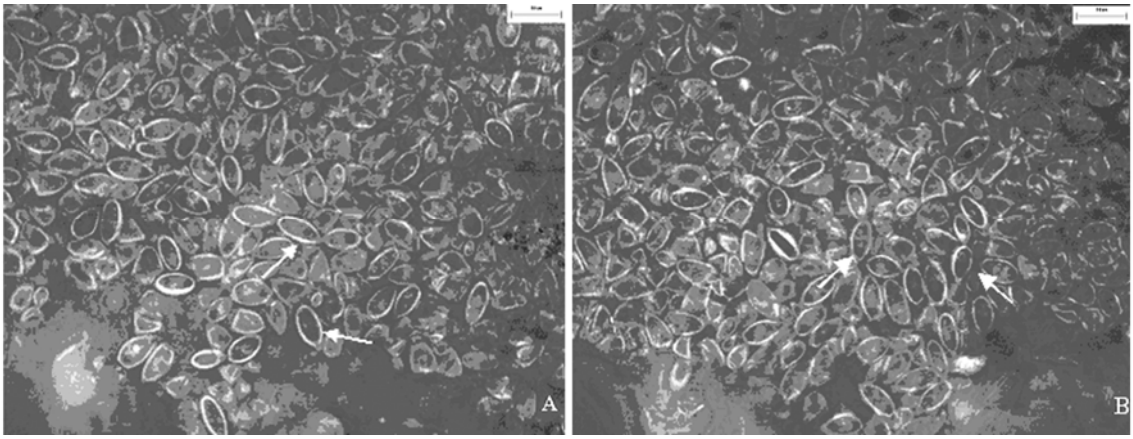

Fig. 1. Typical micrographs of the yeast-like endosymbiotes in the small brown planthopper (bars=10 μm).

A, Yeast-like endosymbiotes in the field population of the small brown planthopper; B, Yeast-like endosymbiotes in the sensitive population of the small brown planthopper.

RESULTS

Influence of high-temperature (35ºC) treatment on the quantity of YLES in SBPH

Compared with the control, the number of YLES in SBPH after the treatment for 1 d had no significant difference; however, the numbers of YLES in the field and sensitive populations were significantly decreased by 57.86% and 61.51% after the treatment for 2 d, respectively; and decreased by 90.71% and 88.96% after the treatment for 3 d, respectively (Table 1).

Influence of high-temperature (35ºC) treatment on the size of YLES in SBPH

In the control, the length of YLES in the field population was significantly longer than that in the sensitive population, and the width of YLES has no significant difference. After 2-day treatment, the width of YLES in the field population was less than that in the sensitive population (Fig. 2). However, the size of YLES was not obviously affected by the high-temperature (35ºC).

Alteration of the SBPH resistance to the three insecticides after high-temperature (35ºC) treatment

Toxicity of imidacloprid to SBPH

Table 2 shows that the toxicity of imidacloprid to the 3rd instars declined with the increase of treatment time, and declined rapidly after 3-day treatment. The toxicity indexes to SBPH were 0.78, 0.57 and 0.23 for the field population, and 0.87, 0.66 and 0.17 for the sensitive population after the treatments for 1 d, 2 d and 3 d, respectively, as compared with the control. This suggests that the resistance of SBPH to imidacloprid decreased with increasing treated time.

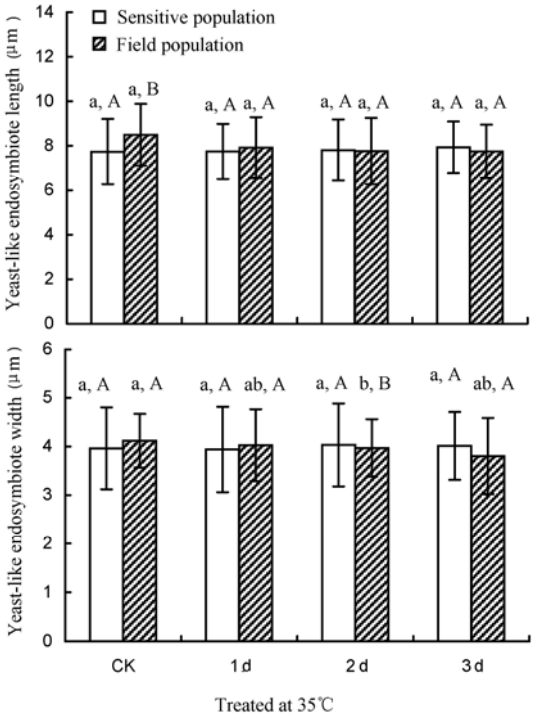

Fig. 2. Length and width of yeast-like endosymbiotes in the small brown planthopper under high temperature treatment.

The same lowercase letters for the same population stand for no significant difference between different treatments and the same uppercase letters for the same treatment stand for no significant difference between populations (Duncan's test, $P>0.05$).

Toxicity of chlorpyrifos to SBPH

After high-temperature treatments for 1 d, 2 d and 3 d, the resistance of SBPH to chlorpyrifos decreased obviously. The toxicity indexes to SBPH of field and sensitive populations were 0.77 and 0.82 after 1-day treatment; and 0.27 and 0.30 after 3-day treatment, corresponding to the control respectively (Table 3).

Toxicity of fipronil to SBPH

The toxicity of fipronil to the 3rd instars of SBPH showed a declining trend under the high-temperature (35ºC) treatment. The toxicity indexes of fipronil to SBPH

Table 1. Number of yeast-like endosymbiotes in the 3rd-instar nymph of the small brown planthopper after the high temperature (35ºC) treatment.

Treatment	Field population		Sensitive population	
	Number of YLES ($\times10^4$)	Reduction rate (%)	Number of YLES ($\times10^4$)	Reduction rate (%)
CK	9.8±1.30 a	-	6.34±1.14 a	-
1 d	7.5±1.20 ab	23.47	4.17±0.92 ab	34.23
2 d	4.13±1.13 bc	57.86	2.44±0.74 bc	61.51
3 d	0.91±0.11 c	90.71	0.70±0.05 c	88.96

Data followed by the same lowercase letters within a column indicate no significant difference by the Duncan's test at $P>0.05$.

Table 2. Toxicity of imidacloprid to the small brown planthopper.

Insect	Treated time at high temperature	Toxicity regression equation	LC_{50} (95 % confidence interval)[a] (mg/L)	*P*	Toxicity index [b]
Field population	CK	y=1.3758x+1.7261	239.57 (174.43–342.43)	0.0000	1.00
	1 d	y=1.*6142*x+1.3479	187.82 (98.56–437.00)	0.0002	0.78
	2 d	y=1.4593x+1.8768	137.74 (103.87–189.10)	0.0000	0.57
	3 d	y=1.4566x+2.4601	54.77 (33.61–103.07)	0.0002	0.23
Sensitive population	CK	y=1.2883x+2.8818	45.87 (12.17–223.8)	0.0065	1.00
	1 d	y=1.7023x+2.2844	39.94 (21.56–98.17)	0.0004	0.87
	2 d	y=1.2013x+3.5826	30.49 (18.74–58.86)	0.0002	0.66
	3 d	y=1.2425x+3.9106	7.67 (4.99–14.43)	0.0006	0.17

[a] Toxicities of an insecticide in treatments are considered to be significantly different if their respective 95% confidence intervals of the LC_{50} values don't overlap.

[b] Toxicity index= LC_{50} of the treated insects / LC_{50} of CK.

Table 3. Toxicity of chlorpyrifos to the small brown planthopper.

Insect	Treated time at high temperature	Toxicity regression equation	LC_{50} (95 % confidence interval)[a] (mg/L)	*P*	Toxicity index [b]
Field population	CK	y=3.9197x-0.9420	33.14 (21.67–57.93)	0.0002	1.00
	1 d	y=2.1226x+2.0556	25.38 (8.15–861.94)	0.0075	0.77
	2 d	y=1.7087x+3.1616	12.18 (5.58–72.47)	0.0034	0.38
	3 d	y=1.6579x+3.4302	8.99 (5.64–18.48)	0.0007	0.27
Sensitive population	CK	y=1.8310x+2.9233	14.94 (5.26–523.68)	0.0091	1.00
	1 d	y=1.7399x+3.1267	12.22 (6.87–32.71)	0.0044	0.82
	2 d	y=2.2050x+3.0896	7.41 (5.39–11.39)	0.0003	0.50
	3 d	y=2.7475x+3.3246	4.53 (2.77–12.24)	0.0011	0.30

[a] Toxicities of an insecticide in treatments are considered to be significantly different if their respective 95% confidence intervals of the LC_{50} values don't overlap.

[b] Toxicity index= LC_{50} of the treated insects / LC_{50} of CK.

Table 4. Toxicity of fipronil to the small brown planthopper.

Insect	Treated time at high temperature	Toxicity regression equation	LC_{50} (95 % confidence interval)[a] (mg/L)	*P*	Toxicity index [b]
Field population	CK	y=1.8610x+1.4142	0.93 (0.39–1.89)	0.0187	1.00
	1 d	y=2.1885x+0.9444	0.74 (0.44–1.02)	0.0070	0.80
	2 d	y=1.*7383*x+2.4310	0.31 (0.07–395.46)	0.0141	0.33
	3 d	y=2.0940x+2.7651	0.12 (0.05–1.18)	0.0057	0.13
Sensitive population	CK	y=1.5622x+2.5096	0.38 (0.11–17.33)	0.0061	1.00
	1 d	y=1.7582x+2.4004	0.32 (0.21–0.43)	0.0004	0.84
	2 d	y=1.2728x+3.6125	0.13 (0.05–0.22)	0.0016	0.34
	3 d	y=1.*0425*x+3.9733	0.10 (0.06–0.17)	0.0008	0.26

[a] Toxicities of an insecticide in treatments are considered to be significantly different if their respective 95% confidence intervals of the LC_{50} values don't overlap.

[b] Toxicity index= LC_{50} of the treated insects / LC_{50} of CK.

decreased, being 0.80 and 0.84 for the field and sensitive populations, respectively, corresponding to the control after 1-day treatment, and decreased rapidly after 2-day and 3-day treatments (Table 4).

DISCUSSION

It has been reported that the endosymbiotes in insects usually evolve with the host, and play a very important role in the host insect growth and reproduction. Recently, the endosymbiotes have attracted the increasing attention of researchers at home and abroad, particularly the relationship between yeast-like symbiotes and their host insect nutritional requirements. Noda et al [14] reported that the endosymbiotes in SBPH could provide lipid nutrition for the host, and Sasaki et al [15] found that the endosymbiotes of the brown planthopper is probably related to the reuse of uric acid. However, the research on the effects of the endosymbiotes on their host's

detoxification metabolism and insecticide resistance is inadequate currently.

The previous research suggested that reducing the number of YLES would lead to many harmful effects on its host insects, such as the delay of host insect growth, and the decrease of host insect survival and emergence rate, adult weight and fecundity [7, 16-17]. The number of YLES in rice planthopper would be reduced by physical and chemical methods artificially. Xu et al [11] found that the number of YLES in the brown planthopper was reduced obviously when the host was treated with methamidophos and omethoate. In this study, the high-temperature (35ºC) treatment on the newly-hatched nymphs of SBPH resulted in the decreased number of YLES, which is consistent with those reported previously. High-temperature treatment has been confirmed to be an effective method for reducing the number of YLES in SBPH [18]. However, the mechanism involved in internal change of YLES under high-temperature treatment needs to be investigated, such as the influence of high temperature on the YLES individuals on physiological and molecular levels.

The endosymbiotes in the host insects were also reported to function in detoxification to insecticide, mycotoxin, and phytotoxin. For example, YLES in the cigarette beetle could produce hydrolase to metabolize the toxic substance in food [19]. In this study, the toxicity indexes of imidacloprid, chlorpyrifos and fipronil to SBPH were decreasing when SBPH were subjected to the high-temperature (35ºC) for 1 d, 2 d and 3 d. The tendency was the same with the change of the number of YLES. However, the size of YLES was not obviously changed after the treatments. Therefore, we suggest that the resistance of SBPH might correlate with the number of symbiotic YLES. The detoxification mechanism of symbiotic YLES in its host and its influence on the host insecticide resistance need to be further investigated.

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