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INTERACTION BETWEEN SHORT-TERM HEAT PRETREATMENT AND FIPRONIL ON 2nd INSTAR LARVAE OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LINN)

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□ Based on the cooperative virulence index (*c.f.*) and LC_{50} of fipronil, the interaction effect between short-term heat pretreatment and fipronil on 2nd instar larvae of diamondback moth (DBM), *Plutella xylostella* (Linnaeus), was assessed. The results suggested that pretreatment of the tested insects at 30 °C for 2, 4 and 8h could somewhat decrease the toxicity of fipronil at all set concentrations. The LC_{50} values of fipronil increased after heat pretreatment and c.f. values in all these treatments were below zero. These results indicated that real mortalities were less than theoretical ones and antagonism was found in the treatments of fipronil at 0.39 and 0.78 mg/L after heat pretreatment at 30 °C at 2, 4 and 8 h. However, pretreatment at 30 °C for 12h could increase the toxicity of fipronil at all set concentrations, the LC_{50} of fipronil decreased after heat pretreatment and c.f. values in all these treatments were above zero, which indicated real mortalities were higher than theoretical ones. Pretreatment of the tested insects at 35 °C for 2, 4, 8 and 12h was found to increase the toxicity of fipronil at all set concentrations which resulted in the decrease of LC_{50} values of fipronil and c.f. above zero in all treatments with only one exception. Most interactions were assessed as synergism. The results indicated that cooperative virulence index (c.f.) may be adopted in hormetic effect assessment.

Keywords: short-term heat pretreatment, fipronil, diamondback moth (DBM), Plutella xylostella (Linn), hormetic, cooperative virulence index (c.f.), LC_{50}

1 INTRODUCTION

In the environment, organisms are often exposed to various stresses such as heat, cold, desiccation, CO_2, heavy metals, and different chemical poisons (Lindquist 1986; Hoffmann and Parsons 1991; Krishna *et al.* 1992; Ferrando *et al.* 1995). Among all these stresses, temperature is the most important factor that affects the abundance and distribution of organisms as well as their populations (Cossins and Bowler 1987; Clarke 2003; Hoffmann *et al.* 2003). Mild temperature hardening is one of the most frequent stresses that an organism might meet (Huang *et al.* 2007).

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Because of this, effects of short-term high temperature on different types of organisms have been extensively studied during the past decades. On the whole, the effect of short-term high temperature may be either beneficial or harmful depending on the level and duration of high temperature. Many studies have proven that brief exposure to a high temperature may increase the heat tolerance of an organism (heat hardening; see Hoffmann *et al.* 2003 and references therein). For example, exposed populations of *Drosophila melanogaster* to 29°C for up to several days helps them gain a higher thermotolerance. Twelve hours of exposure is most effective (Levins 1969). Treating 1-day-old adults of pea leafminer, *Liriomyza huidobrensis* (which are reared at 25-26 °C for population maintaiment) to 32 or 35°C for 4h significantly increases their later heat resistance (Huang *et al.* 2007). In response to some different low or modest level stresses, organisms have similar response mechanisms. The short-term high temperature treatment often helps organisms increase other stress tolerances known as cross protection, cross tolerance or cross resistance (Stebbing 1981; Calabrese and Baldwin 2003). For instance, after being treated with high but sublethal temperatures, 4th instar larvae of mosquitoes, *Anopheles stephensi* and *Aedes aegypti*, become more tolerant to propoxur (a carbamate insecticide) (Patil *et al.* 1996). Similarly, short-term high temperature treatment can increase cold tolerance of *D. melanogaster* (Bubliy and Loeschcke 2005) and flesh fly, *Sarcophaga crassipalpis* (Chen *et al.* 1987), and desiccation resistance of *D. melanogaster* (Hoffmann and Parsons 1989; Hoffmann, 1990). A short-term heat stress can even improve the tolerance of virus loads in mosquitoes (Watts *et al.* 1987). However, if the high temperature surpasses a threshold or duration, an extinction of a local population may occur. For instance, Leibee (1984) found that high temperature (35 °C) decreased pupal viability of *Liriomza trifolii.* At extreme temperatures, individual development of *Drosophila* will not proceed through the whole life cycle (Chakir *et al.* 2002). In *Drosophila* males, sterility is induced at temperatures above 30°C (David and Clavel 1969). Both the beneficial and harmful effects of different stresses of low or modest levels make up two continuous parts of a hormesis curve. Hormesis, synonymous with terms such as adaptative response, preconditioning, hardening, etc, in different research fields (Calabrese 2008), is an evolutionary natural selection process involving toxicological mechanisms as part of a strategy to enhance survival to low levels of stressor agents (Calabrese and Baldwin 2001; Calabrese 2008). It is defined as a dose-response phenomenon characterized by a low dose stimulation and a high dose inhibition, or vice versa depending on the endpoint measured (Chapman 2001). The hormesis phenomenon was first reported more than a century ago by Schulz, who used yeast as an experimental model (Calabrese 1999). Up to now, it has been proven that hormetic dose-response relationships occur in males and females of

numerous animal models in all principal age groups as well as across species displaying a broad range of differential susceptibilities to toxicants.

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the major cosmopolitan pest of *brassica* and other crucifer crops all over the world. It distributes in areas of different climatic types including tropical, subtropical and temperate zones and has the ability to migrate among different climatic zones (Chu 1986; Honda 1990; Honda *et al.* 1992; Chapman *et al.* 2002; Coulson *et al.* 2002). In tropical climates, DMB reproduces 20 generations or more a year. As a result, crop loss incurred by DBM may reach 90% (Verkerk and Wright 1996) and only few 4th instar larvae on a cabbage may make it unsalable (Shelton *et al.* 1983; Maltais *et al.* 1998). The total annual cost for DBM control throughout the world surpasses one billion US dollars (Talekar and Shelton 1993; Roux *et al.* 2007). For decades, insecticide use has been the most important control method targeting the 2nd and 3rd instar larvae and presently, fipronil and avermectin are two key insecticides used both in- and outside China (Scharf and Siegfried 1999; Ngim and Crosby 2001; Wilde *et al.* 2001; Sayyed *et al.* 2004; Zhou *et al.* 2004; Luo *et al.* 2008) .

The most favorable temperature for growth and development of DBM is 25°C and temperatures higher than 30°C or lower than 20°C are harmful (Ma and Chen 1993; Dan *et al.* 1995; Shirai 2000; Liu *et al.* 2002). Our prior studies have discovered the hormetic effect of short-term heat pretreatment on the toxicity of avermectin to 2nd instar DBM larvae and fipronil to 3rd instar DBM larvae (Gu *et al.* 2009a; b). In the present paper, the hormetic effect of short-term high temperature on fipronil tolerance of 3rd instar larvae of DBM is reported.

2 MATERIALS AND METHODS

2.1 Chemicals

Fipronil crude chemical (95.1% purity) was provided by Zhejiang Hisun Pharmaceutical Co., Ltd. Triton X-100 (a nonionic detergent) was bought at a local Fuzhou chemical market. Fipronil was serially diluted into 6.25, 3.13, 1.56, 0.78 and 0.39 mg/L with distilled water containing 0.1% (vol:vol) Triton X-100 for bioassay.

2.2 Insect stock culture

Pupae of DBM were originally collected from the vegetable fields in a suburb of Fuzhou City, Fujian Province, People's Republic of China. Twenty pairs of pupae were put into a plastic 1.5L softdrink bottle with lots of small round holes (1mm dia) in the lowerside and 4 bigger round holes (10mm dia) in the upperside of the wall. After pupation, the female and male adults mated and the eggs were laid on the inside wall. The

hatched 1st instar larvae dropped through the holes onto the leaves of cabbage plants, *Brassica oleracea* L. var.*capitata* L, grown in the pots 20 cm under the bottle. The insectary temperature was set at 25±1°C, 60%-70% RH with a photoperiod of 12:12 (L:D). Adults were fed with a 10% sugar solution saturated in cotton placed in the larger holes in the upper side of the bottle and the cotton was changed twice daily. The insects were reared for more than 30 generations without exposure to any insecticides. Same day aged 2nd instar larvae (body length about 2mm) were used for all experiments.

2.3 Short-term heat pretreatment

For heat pretreatment, the temperature was set at 30°C and 35°C. Ten insects were released into an individual Petriplate (100mm dia) the bottom of which was covered with wet filter paper of the same size. The heat pretreatment was conducted in a digitized biochemistry incubator, produced by Hankang electronic Co, Ltd, Jintan City, Jiangsu Province, People's Republic of China for 0h, 2h, 4h, 8h and 12 h. Soon after heat pretreatment, the Petriplates were removed to 25±1°C, 60%-70% RH with a photoperiod of 12:12 (L:D) for fipronil bioassay.

2.4 Bioassay

The cabbage leaf disc dip method of bioassay as described by Tabashnik *et al.*(1987) was adopted in the present studies. Cabbage leaves were first washed with distilled water and dried for about 1h at room temperature. Cabbage leaf discs (10 mm dia) were then cut with a metal punch and dipped into a test solution prepared with distilled water containing 0.1% Triton X-100 for about 5s to facilitate uniform treatment with the active ingredient. For control, the leaf discs were dipped in distilled water containing 0.1% Triton X-100 without active ingredients for the same period. The leaf discs were placed slanting for about 2 minutes over a blotting paper in a tray to drain excess solution and then flattened to dry the test solution for about 2h at room temperature. Finally, 2 leaf discs were put into one petriplate to feed the insects. Each concentration had 3 replications and each replication contained 10 insects (1 petriplate). Larvae were allowed to feed on the treated leaf discs for 48h at 25 °C before being checked for mortality (Mohan and Gujar, 2003). An insect was regarded as dead if it had no response to a gentle touch of a tweezer.

2.5 Data analysis

Data obtained from the experiments were analyzed using analysis of variance ($P<0.05$) (Proc ANOVA; Tang and Feng 1997). Treatment means were compared by Tukey's F test, accepting significant differences

at P=0.05 (Tang and Feng 1997). The mortality data were transformed by the arcsine square root prior to significance analysis (Southwood and Henderson 2000) and were averaged within replications for each treatment (Sokal and Rohlf 1995; Yin *et al.* 2008).

Concentration-mortality data were analyzed by probit analysis using DPS (Tang and Feng 1997). Mortality rates were corrected using Abbott's formula (Abbott 1925) for each probit analysis.

The median lethal concentration (LC_{50}) in terms of mg active ingredient/L was estimated by subjecting mortality data to the maximum likelihood program of probit analysis (Tang and Feng, 1997). This program has a provision for control mortality. Tukey's F test was also used to compare the differences among the LC_{50}s.

Cooperative virulence index (c.f.) was calculated with the formulum proposed by Mansour *et al.* (1966), which was, c.f. = (real mortality- theoretical mortality)/theoretical mortalityX100, where theoretical mortality = the corrected mortality caused by heat treatment alone + the corrected mortality caused by fipronil alone - the product between them. The interaction result was assessed as, 'synergism' when c.f. was greater than or equal to 20; 'addition' when c.f. was greater than -20 and less than 20; and 'antagonism' when c.f. was less than or equal to -20.

3 RESULTS AND ANALYSIS

3.1 Interaction between short-term heat pretreatment at 30°C and fipronil on 2nd instar larvae of DBM, *Plutella xylostella* (Linn).

Heat treatment alone did not increase the mortality of the tested insects for no significant differences in mortality were found among the treatments (Tr1, Tr2, Tr3 and Tr4 in Table 1) and control (P>0.05). Mortality in the control was 6.67% but that in Tr4, where the tested insects were treated at 30 °C for 12 h, was just 10.00% and the difference was not significant (P>0.05).

Pretreatment of the insects at 30 °C for 12h seemed to modestly increase the toxicity of fipronil at all concentrations (Table 1). At fipronil levels of 1.56 mg/L, the mortality of the insects which were pretreated at 30 °C for 12h (Tr19) was 73.33%, but for those insects that experienced no prior heat treatment mortality was 60.00% (Tr15). However, heat pretreatment for a duration less than 12h seemed to decrease the toxicity of fipronil at all concentrations. In treatments of fipronil at 1.56 mg/L (Tr16, Tr17 and Tr18, where the durations of heat pretreatment were 2h, 4h and 8h, respectively), the mortalities were 56.70%, 51.11% and 57.41%, respectively and all were slightly lower than that in Tr15, which was 60.00%. But the differences were not significant (P>0.05).

The effect of heat pretreatment on fipronil tolerance of the tested insects could also be seen in the changes of LC_{50} values of fipronil

TABLE 1. Combined toxicity between short-term heat pretrement at 30°C and fipronil on 2nd instar larvae of DBM, *Plutella xylostella* (Linn.)

Treatment	Duration of heat-pretreatment (h)	fipronil concentration (mg/L)	Mortality (Average±SE)
CK	0	0	6.67± 6.67c
Tr1	2	0	7.04±3.53c
Tr2	4	0	6.67±6.67c
Tr3	8	0	10.00±5.77bc
Tr4	12	0	10.00±5.77bc
Tr5	0	0.39	49.63±8.25a
Tr6	2	0.39	40.00±5.77ab
Tr7	4	0.39	40.74±9.26ab
Tr8	8	0.39	39.26±3.23ab
Tr9	12	0.39	53.33±4.63a
Tr10	0	0.78	57.04±1.48a
Tr11	2	0.78	43.33±8.82a
Tr12	4	0.78	41.48±1.48ab
Tr13	8	0.78	43.60±3.96a
Tr14	12	0.78	60.00±5.77a
Tr15	0	1.56	60.00±5.77a
Tr16	2	1.56	56.70±1.68a
Tr17	4	1.56	51.11±8.89a
Tr18	8	1.56	57.41±4.90a
Tr19	12	1.56	73.33±3.33a
Tr20	0	3.13	60.74±3.23a
Tr21	2	3.13	57.41±4.90a
Tr22	4	3.13	55.56±8.01a
Tr23	8	3.13	58.52±1.48a
Tr24	12	3.13	72.59±2.59a
Tr25	0	6.25	70.00±0.00a
Tr26	2	6.25	66.67±6.67a
Tr27	4	6.25	74.44±10.08a
Tr28	8	6.25	69.26±5.15a
Tr29	12	6.25	75.56±7.29a

Note: Data followed with the same lower case letter are not significantly different (P=0.05, Tukey's F test). Same as below.

(Table 2). Since heat pretreatment at 30°C for 12h could increase the toxicity of fipronil at all concentrations, the LC_{50} of fipronil to those insects pretreated at 30°C was 0.31mg/L, lower than those that experienced no prior treatment which was 0.60 mg/L, This difference was not significant ($P>0.05$). Because heat pretreatment at 30 °C for less than 12h could decrease the toxicity of fipronil at all concentrations, the LC_{50} values of fipronil in those treatments all increased and were significantly higher in the treatments which experienced 2h or 4h heat treatment ($P<0.05$).

The interaction results between short-term heat pretreatment at 30°C and fipronil varied with the duration of the heat pretreatment (Table 3). When heat pretreatment lasted for 12h, all the cooperative virulence index (c.f.) values were above zero indicating that the real mortalities

TABLE 2. Effect of short-term heat pretreatment at 30°C on the LC_{50} of fipronil to 2nd instar larvae of DBM, *Plutella xylostella* (Linn.) (48h)

Duration of heat-pretreatment (h)	Toxicity equation	Coefficient (r)	LC_{50} (mg/L)	95%confidence interval (mg/L)
0	y=5.09+0.41x	0.96	0.60^{bc}	0.37-0.97
2	y=4.87+0.61x	0.97	1.65^{a}	1.25-2.17
4	y=4.84+0.76x	0.94	1.63^{a}	1.07-2.48
8	y=4.88+0.68x	0.98	1.52^{ab}	1.19-1.95
12	y=5.28+0.54x	0.93	0.31^{c}	0.13-0.73

TABLE 3. Assessment of interaction between short-term heat pretreatment at 30°C and fipronil on 2nd instar larvae of diamondback moth, *Plutella xylostella* (Linn)

			Mortality (%)			Interaction	
Treatment	Duration of heat-pretreatment (h)	Fipronil concentration (mg/L)	Real value	Corrected value	Theoretical value	Cooperative virulence index (c.f.)	Interaction assessment
CK	0	0	6.67				
Tr5	0	0.39	49.63	46.03			
Tr10	0	0.78	57.04	53.97			
Tr15	0	1.56	60.00	57.14			
Tr20	0	3.13	60.74	57.94			
Tr25	0	6.25	70.00	67.86			
Tr1	2	0	7.04	0.39			
Tr6	2	0.39	40.00	35.71	46.24	–22.77	antagonism
Tr11	2	0.78	43.33	39.28	54.15	–27.45	antagonism
Tr16	2	1.56	56.70	53.61	57.31	–6.46	addition
Tr21	2	3.13	57.41	54.36	58.10	–6.43	addition
Tr26	2	6.25	66.67	64.28	67.98	–5.44	addition
Tr2	4	0	6.67	0.00			
Tr7	4	0.39	40.74	36.51	46.03	–20.69	antagonism
Tr12	4	0.78	41.48	37.30	53.97	–30.88	antagonism
Tr17	4	1.56	51.11	47.62	57.14	–16.67	addition
Tr22	4	3.13	55.56	52.38	57.94	–9.59	addition
Tr27	4	6.25	74.44	72.62	67.86	7.02	addition
Tr3	8	0	10.00	3.57			
Tr8	8	0.39	39.26	34.92	47.96	–27.19	antagonism
Tr13	8	0.78	43.60	39.57	55.61	–28.84	antagonism
Tr18	8	1.56	57.41	54.36	58.67	–7.34	addition
Tr21	8	3.13	58.52	55.55	59.44	–6.53	addition
Tr26	8	6.25	69.26	67.06	69.00	–2.81	addition
Tr4	12	0	10.00	3.57			
Tr9	12	0.39	53.33	50.00	47.96	4.26	addition
Tr14	12	0.78	60.00	57.14	55.61	2.75	addition
Tr19	12	1.56	73.33	71.43	58.67	21.74	synergism
Tr24	12	3.13	72.59	70.63	59.44	18.84	addition
Tr29	12	6.25	75.56	73.81	69.00	6.96	addition

TABLE 4. Combined toxicity between short-term heat pretrement at 35°C and fipronil on 2nd instar larvae of DBM, *Plutella xylostella* (Linn.)

Treatment	Duration of heat-pretreatment (h)	fipronil concentration (mg/L)	Mortality (Average±SE)
CK	0	0	13.33±3.33^{jk}
Tr1	2	0	3.33±3.33^{k}
Tr2	4	0	13.33±3.33^{jk}
Tr3	8	0	16.67±3.33^{j}
Tr4	12	0	16.67±3.33^{j}
Tr5	0	0.39	26.67±3.33^{hij}
Tr6	2	0.39	20.00±5.77^{ij}
Tr7	4	0.39	43.33±3.33^{fghi}
Tr8	8	0.39	43.33±3.33^{fghi}
Tr9	12	0.39	53.33±3.33^{defgh}
Tr10	0	0.78	36.67±3.33^{ghij}
Tr11	2	0.78	46.67± 3.33^{efgh}
Tr12	4	0.78	46.67± 3.33^{efgh}
Tr13	8	0.78	66.67±3.33^{abcdef}
Tr14	12	0.78	60.00±5.77^{bcdefg}
Tr15	0	1.56	56.67±3.33^{cdefg}
Tr16	2	1.56	50.00±5.77^{defgh}
Tr17	4	1.56	56.67±6.67^{cdefg}
Tr18	8	1.56	73.33±6.67^{abcde}
Tr19	12	1.56	63.33±3.33^{abcdefg}
Tr20	0	3.13	60.00±5.77^{bcdefg}
Tr21	2	3.13	76.67±3.33^{abcd}
Tr22	4	3.13	60.00±5.77^{bcdefg}
Tr23	8	3.13	80.00±5.77^{abc}
Tr24	12	3.13	73.33±3.33^{abcde}
Tr25	0	6.25	76.67±3.33^{abcd}
Tr26	2	6.25	83.33±3.33^{ab}
Tr27	4	6.25	80.00±5.77^{abc}
Tr28	8	6.25	86.67±3.33^{a}
Tr29	12	6.25	83.33±3.33^{ab}

were higher than theoretical. In Tr19 (fipronil concentration of 1.56mg/L) , c.f. was 21.74 which suggested that synergism occurred. However, when the duration of heat pretreatment was less than 12h, all c.f. values were below zero, which implied that the real mortalities were less than theoretical ones. Antagonsim was found in fipronil treatments of 0.39 mg/L or 0.78mg/L (Tr6, Tr7, Tr8, Tr11, Tr12 and Tr13), for all c.f. values in these treatments were below -20.

3.2 Interaction between short-term heat pretreatment at 35°C and fipronil on the 2nd instar larvae of DBM, *Plutella xylostella* (Linn).

Similar to that at 30°C, heat treatment at 35°C alone did not significantly increase the mortality of the tested insects (P>0.05, Table 4). The mortality was 13.33% in the control and 16.67% both in Tr3 and Tr4 in

TABLE 5. Effect of short-term heat pretreatment at 35°C on the LC_{50} of fipronil on the 2nd instar larvae of DBM, *Plutella xylostella* (Linn.) (48h)

Duration of heat-pretreatment (h)	Toxicity equation	Coefficient (r)	LC_{50} (mg/L)	95%confidence interval (mg/L)
0	y=4.56+1.32x	0.98	2.15[a]	1.75-2.65
2	y=4.56+183x	0.96	1.74[a]	1.28-2.37
4	y=4.86+0.88x	0.94	1.45[ab]	0.98-2.13
8	y=5.22+1.09x	0.97	0.63[b]	0.42-0.94
12	y=5.16+0.78x	0.98	0.62[b]	0.44-0.87

which the insects were treated at 35°C for 8 and 12h. No significant differences were found ($P>0.05$).

Compared with the treatments that experienced no prior heat stress, short-term heat pretreatment at 35°C increased the toxicity of fipronil at all set concentrations. The only two exceptions were found in Tr6 and Tr16 where the duration of heat pretreatment was 2h, and the fipronil concentations were 0.39 mg/L and 1.56mg/L (Table 4). When fipronil concentration was 0.78mg/L, in Tr10 (where the insects were only treated with fipronil), the mortality was 36.67%. Mortality increased with duration of heat pretreatment and in Tr14 (where the insects were treated at 35°C for 12h prior to fipronil), it reached 60.00%.

Since short-term heat pretreatment at 35°C increased the toxicity of fipronil in nearly all treatments, the LC_{50} values of fipronil decreased after heat pretreatment (Table 5). The LC_{50} of fipronil to insects with no prior heat treatment was 2.15mg/L and decreased to 0.62mg/L when the duration of heat pretreatment was 12h. This difference was significant ($P<0.05$).

In accordance with the effect of heat pretreatment on the toxicity of fipronil shown in Table 4, c.f. values between short-term heat pretreatment at 35°C and fipronil were above zero in almost all treatments with the exceptions of that in Tr6 and Tr16 (Table 6). In many treatments, c.f. was even higher than 20, which suggested synergism occurred.

4 DISCUSSION

Organisms typically overcome an unpredictable environment via fast-inducible and reversible responses. These mechanisms are more effective and less costly compared with fixed changes in basal resistance (Jørgensen *et al.* 2006) and cross protection is adopted while faced with different stresses (Stebbing 1981; Calabrese and Baldwin 2003). This has been proven in many studies (Chen *et al.* 1987; Watts *et al.*, 1987; Hoffmann and Parsons 1989; Hoffmann, 1990; Patil *et al.* 1996; Bubliy and Loeschcke 2005). Our previous work has shown the hormetic effect

TABLE 6. Assessment of interaction between short-term heat pretreatment at 35°C and fipronil on 2nd instar larvae of diamondback moth, *Plutella xylostella* (Linn)

			Mortality (%)			Interaction	
Treatment	Duration of heat-pretreatment (h)	Fipronil concentration (mg/L)	Real value	Corrected value	Theoretical value	Cooperative virulence index (c.f.)	Interaction assessment
CK	0	0	13.33				
Tr5	0	0.39	26.67	15.39			
Tr10	0	0.78	36.67	26.93			
Tr15	0	1.56	56.67	50.00			
Tr20	0	3.13	60.00	53.85			
Tr25	0	6.25	76.67	73.08			
Tr1	2	0	3.33	0.00			
Tr6	2	0.39	20.00	7.70	15.39	–49.99	antagonism
Tr11	2	0.78	46.67	38.46	26.93	42.85	synergism
Tr16	2	1.56	50.00	42.31	50.00	–15.38	addition
Tr21	2	3.13	76.67	73.08	53.85	35.71	synergism
Tr26	2	6.25	83.33	80.77	73.08	10.53	addition
Tr2	4	0	13.33	0.00			
Tr7	4	0.39	43.33	34.62	15.39	124.97	synergism
Tr12	4	0.78	46.67	38.46	26.93	42.85	synergism
Tr17	4	1.56	56.67	50.00	50.00	0.00	addition
Tr22	4	3.13	60.00	53.85	53.85	0.00	addition
Tr27	4	6.25	80.00	76.92	73.08	5.26	addition
Tr3	8	0	16.67	3.85			
Tr8	8	0.39	43.33	34.62	18.65	85.66	synergism
Tr13	8	0.78	66.67	61.54	29.74	106.93	synergism
Tr18	8	1.56	73.33	69.23	51.93	33.33	synergism
Tr21	8	3.13	80.00	76.92	55.62	38.29	synergism
Tr26	8	6.25	86.67	84.62	74.11	14.17	addition
Tr4	12	0	16.67	3.85			
Tr9	12	0.39	53.33	46.16	18.65	147.55	synergism
Tr14	12	0.78	60.00	53.85	29.74	81.07	synergism
Tr19	12	1.56	63.33	57.69	51.93	11.11	addition
Tr24	12	3.13	73.33	69.23	55.62	24.46	synergism
Tr29	12	6.25	83.33	80.77	74.11	8.98	addition

of short-term heat pretreatment on avermectin and fipronil tolerance of DBM larvae (Gu *et al.* 2009a, b). Pretreating 2nd instar larvae of DBM at 35°C for 2 or 4h may antagonize the toxicity of avermectin at lower concentrations but not at 30°C (Gu *et al.* 2009a). Similarly, pretreating 3rd instar larvae of DBM at 30°C and 35°C for 2h or 4h can increase their fipronil tolerance (Gu *et al.* 2009b). Here, the hormetic effect of short-term heat pretreatment at 30°Con fipronil tolerance of 2nd instar larvae of DBM was seen (Table 1 to Table 3).

The interaction results between short-term heat pretreatment at 30°C and fipronil is shown in Table 3. When the duration of heat pretreatment was less than 12h, the hormetic effect was comparatively high, and even

could antagonize the toxicity of fipronil at 0.39mg/L and 0.78 mg/L (Tr6, Tr7, Tr8, Tr11, Tr12 and Tr13). In other treatments, although interaction did not result in antagonism, heat pretreatment still decreased the toxicity of fipronil for c.f values were below zero. Furthermore, it demonstrated again that c.f could be used for the hormetic effect assessment (Gu *et al.* 2009a; 2009b)

A comparison of the interaction between short-term heat pretreatment and avermectin (Gu *et al.* 2009a) and fipronil, revealed several different responses. The hormetic effect of short-term heat pretreatment on avermectin tolerance of tested insects was found both at 30°C and 35°C when the heat duration was no longer than 8h. However, the hormetic effect of short-term heat pretreatment on fipronil tolerance was only found at 30°C and at a duration of less than 12 h. Usually, the underlying mechansims of cross-tolerance between heat shock and other stresses are thought to be the upregulation of heat shock proteins (Hsps) (Feder and Hofmann 1999; Sørensen *et al.* 2003) and that has also been considered as the underlying mechanism of cross protection between short-term high but sublethal temperatures and propoxur in 4th instar larvae of mosquitoes, *Anopheles stephensi* and *Aedes aegypti* (Patil *et al.* 1996). Moreover, several studies have confirmed that upregulation of Hsps brought by short-term thermal stress may affect ion channels, receptors, and the sodium pump in the central nervous system (CNS) (Wu and Fisher 2000; Robertson 2004; Trotta *et al.* 2004). The target of both avermectin and fipronil are chloride ions in the CNS. So whether short-term heat pretreatment can also affect chloride ion and whether that plays a role in the mechanism of the horemetic effect of short-term heat pretreatment on avermectin and fipronil tolerance of DBM larvae should be investigated. The difference in toxicity mechanism between avermectin and fipronil lies in that fipronil blocks the chloride ion channel (Ikeda *et al.* 2003), but the effect of avermectin is the opposite (Pong *et al.* 1982). Whether this can partly account for the differences found in the hormetic effect of short-term heat pretreatment on the tolerance of DBM larvae needs to be studied.

The hormetic effect of short-term heat pretreatment on the tolerance of fipronil also exists in 3rd instar larvae of DBM (Gu *et al.* 2009b). But in the 3rd instar larvae, the hormetic effect has been found at both 30°C and 35°C, which differs from that in 2nd instar larvae where it was only found at 30°C. Pretreatment at 35 °C mainly resulted in a harmful effect (Table 3, Table 6). For up-regulation of heat shock proteins, three indices are very important and species-specific: the minimum heat-shock temperature required to induce the heat shock response (HSR) (Ton), the temperature of maximal response (Tmax), and the shut-off temperature (Toff) (Dietz and Somero 1993; Tomanek and Somero 1999; Barua and Heckathornl 2004). Further studies show that induction temperatures of

the HSR vary with developmental stage, growth temperature, and season (Dietz and Somero 1992; Hofmann and Somero 1995; Roberts *et al.* 1997; Chapple *et al.* 1998; Tomanek and Somero 1999; Currie *et al.* 2000; Buckley and Hofmann 2002; Barua and Heckathornl 2004). Whether these three indices are different between the 2nd and 3rd instar larvae of DBM and whether this has caused the different hormetic effect of short-term heat pretreatment on fipronil tolerance in the two DBM instars are still unknown.

Survival and fertility are two important indices of a population. For survival, the hormetic effect of short-term heat shock has been repeatedly reported. For fertility, Jørgensen *et al.* (2006) have found that mild heat stress may increase the male fertility of *Drosophila buzzatii* and that also occurs in *D. melanogaster* (Krebs and Loeschcke 1994). Research results in these two areas are often contradictory because of “trade-offs”. “Trade-offs” give advantage in survival at the cost of a disadvantage in fertility or vice versa. “Trade-offs” always coexists with hormesis and that is why disputes on whether the hormetic dose-response can be used as a default model are not resolved. For example, temperature hardening does improve thermotolerance of *L. huidobrensis*, but their egg production is remarkably decreased after 4 h exposure to 10, 32, or 35°C, and the 35°C exposure almost completely halted egg deposition (Huang *et al.* 2007). The reason is because heat shock protein synthesis is a process of energy consumption (Koehn and Bayne 1989; Hoffmann 1995), usually results in a concomitant reduction in the synthesis of other proteins (Parsell and Lindquist 1994), and finally brings about harmful effects on some other biological characteristics. So the interaction results often vary with the indices that are chosen and what to select for study becomes very important. In our opinion, at least in pest control, that is not an irresolvable issue. The DBM always needs to be controlled within a comparatively short time mainy through the use of insecticides to decrease its survival. But for the health of humans and the environment, overuse of insecticides is to be avoided. Then the interaction between environmental conditions and low dose insecticides on survival should be of concern. However, for some forest pests such as *Dendrolimus punctatus* Walker, which usually reproduces 2-5 generations a year in China, it is acceptable to inhibit its population by decreasing their fertility. Then the interaction between environmental conditions and low dose insecticide on their fertility should be closely watched. But in both of these two situations the possible hormetic effect should always be taken into consideration.

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