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ORIGINAL RESEARCH PAPER

Rapid development of resistance to diamide insecticides in the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae), in the tea fields of Shizuoka Prefecture, Japan

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Abstract We investigated the susceptibility of the smaller tea tortrix, *Adoxophyes honmai* Yasuda, to diamide insecticides in the Shimada-Yui tea fields in Shizuoka Prefecture, Japan, from 2006 to 2011. By 2011, the insects had developed significant resistance even to concentrations far above the registration concentrations of two diamides, flubendiamide and chlorantraniliprole. The lethal concentration 50 (LC_{50}) values of flubendiamide showed a rapid annual increase from 16.2 ppm in 2007 to 161 ppm in August 2011, exceeding the registration concentration of 100 ppm in 2010 and 2011. The LC_{50} values of chlorantraniliprole increased sharply from 25.3 ppm in 2010 to 98.8 ppm in August 2011, exceeding the registration concentration of 50 ppm. The LC_{50} values for flubendiamide and chlorantraniliprole at 10 days after treatment in insects collected in August 2011 were 105-fold and 77.2-fold higher, respectively, than those in a susceptible strain.

Keywords *Adoxophyes honmai* · Insecticide resistance · Diamide · Flubendiamide · Chlorantraniliprole

Introduction

The smaller tea tortrix, *Adoxophyes honmai* Yasuda, is one of the most destructive lepidopteran pests of tea, as is the oriental tea tortrix *Homona magnanima* Diakonoff. The damage caused by the larvae of these species delays the growth of new leaves and reduces yields if the insects experience an outbreak (Minamikawa and Osakabe 1979). To control *A. honmai* in Shizuoka Prefecture, the most important tea-producing district in Japan, insecticide is generally applied seasonally (4 times a year). Outbreaks of *A. honmai* have been a serious problem, particularly in the Makinohara area of Shizuoka Prefecture, for the past several years.

To date, no declines in the susceptibility of *A. honmai* to insecticides have been reported outside Japan. In the Makinohara area, some field populations of *A. honmai* have developed resistance to various classes of insecticides, including carbamates in 1986 (Ozaki and Takeshima 1984) and organophosphates and synthetic pyrethroids in 1997 (Kosugi 1999). In addition, Kosugi (1999) reported early indications of reduced susceptibility to benzoylurea and diacylhydrazine (DAH) analogs of insect growth regulators (IGRs) in 1997. Several new insecticides, including diamides, were registered recently and are already widely used in Shizuoka Prefecture for *A. honmai* control.

Diamides are a new class of insecticides (Lahm et al. 2007; Tohnishi et al. 2005). They are specific to lepidopteran pests and act on muscle contraction by binding to the ryanodine receptor and interfering with the receptor's role in calcium homeostasis (Cordova et al. 2006; Ebbinghaus-Kintscher et al. 2006). In Japan, flubendiamide and chlorantraniliprole were registered as diamide insecticides in 2007 and in 2009, respectively, for lepidopteran pests. Flubendiamide and chlorantraniliprole were first used in the tea fields of Shizuoka Prefecture in 2007 and 2010, respectively, to control *A. honmai* and *H. magnanima*.

High levels of resistance to diamides were reported only in the diamondback moth, *Plutella xylostella* L., in China (Wang and Wu 2012), the Philippines, and Thailand (Troczka et al. 2012). There have been no reports of resistance to diamides in other pests. In the present study,

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we show the results of a 6-year investigation (2006–2011) on the susceptibility of *A. honmai* to diamides in the tea fields of the Makinohara area, Shizuoka Prefecture. This is the first study to report a high level of resistance in *A. honmai* to diamides.

Materials and methods

Insects

From 2006 to 2011, *A. honmai* were collected from adjoining tea fields in Shimada-Yui (34.81°N, 138.18°E), located in the Makinohara area of Shizuoka Prefecture, Japan (Fig. 1). The *A. honmai* collected each June from 2006 to 2010 and in June and August in 2011, were treated as different strains. More than 20 adult females from each *A. honmai* strain were allowed to lay egg masses, following the rearing method of Noguchi (1991). The insects that hatched from the egg masses were reared in groups on an artificial diet (Insecta LFS, Nihon-Nosan Kogyo Co. Ltd., Yokohama, Japan) in an insectary at 25 °C under a 16-h L:8-h D photoperiod. The 2nd and 3rd instar larvae of the F_1 and F_2 generations were used for bioassays. A susceptible strain, called the Kanaya strain, was used as a control. The Kanaya strain was collected from the tea fields of Kanaya Tea Research Center, NARO Institute of Vegetable and Tea Science, in the 1960s. This strain was a gift from the Kanaya Tea Research Center and has been reared in our laboratory since the 1980s.

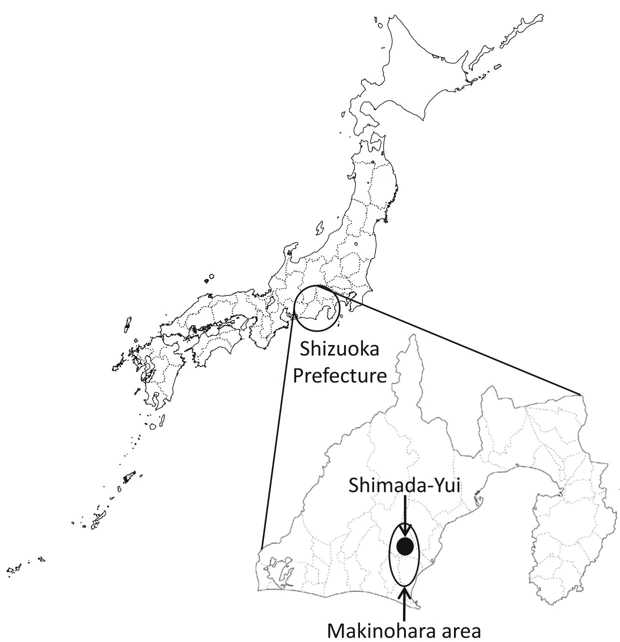

Fig. 1 Collection site of *Adoxophyes honmai* in the tea fields of Shimada-Yui, located in the Makinohara area of Shizuoka Prefecture, Japan

Leaf dipping bioassay

The leaf dipping bioassay method was used to test the susceptibility of *A. honmai* to the two diamides, flubendiamide and chlorantraniliprole. All bioassays were conducted using commercial formulations of flubendiamide (20 % water-dispersible granules from 2006 to 2010, and 18 % flowable in 2011 due to a formulation change, Nihon Nohyaku Co., Ltd., Japan) and chlorantraniliprole (10 % flowable, DuPont Co., Ltd., Japan). The insecticides were mixed with tap water to generate 4–6 serial dilutions.

Tea leaves were collected from the tea field at the Tea Research Center, Shizuoka Research Institute of Agriculture and Forestry, where pesticides are not used. The leaves were dipped in the insecticide solutions for 10 s and dried on paper towels (Kimtowel White, Nippon Paper Crecia Co., Ltd., Japan). Six or seven treated leaves were then transferred to a styrol cup (internal diameter 78 mm, depth 44 mm) containing a layer of filter paper. Ten 2nd and 3rd instar larvae of *A. honmai* were placed in each cup, which was then covered with a cap and maintained at 25 °C under a 16-h L:8-h D photoperiod. All treatments were repeated 3 times.

Larval mortality was scored at 7 days after treatment in 2006. It became apparent that 10 days are required to judge mortality accurately after treatment, because diamides have delayed mortal effects on *A. honmai*. Therefore, we scored mortality at 8 and 10 days after treatment in 2007 and subsequent years. We considered a larva that could not right itself after it was turned upside down to be dead. The dose–response data were calculated on the basis of percentage mortality, corrected with Abbott's (1925) formula. The lethal concentration 50 (LC_{50}) values were calculated by probit analysis (Bliss 1935). We started investigations of the susceptibility of *A. honmai* to flubendiamide and chlorantraniliprole in 2006 and 2010, respectively, according to the beginning of use of diamides in tea fields. The mortalities and the LC_{50} values of the Kanaya strain in response to flubendiamide and chlorantraniliprole were examined in 2007 and 2010, respectively.

Results

Corrected mortality

The effects of the two diamides against 2nd and 3rd instar larvae of *A. honmai* are shown in Table 1, using corrected mortality values. The mortalities of the Kanaya strain were 100 % for all treatments of flubendiamide and chlorantraniliprole. The mortalities of the Shimada-Yui strains at 10 days after treatment with flubendiamide for the 2,000

Table 1 Effect of two diamides on 2nd and 3rd instar larvae of *Adoxophyes honmai*

Insecticides (formulation, %AI)	Dilution[a]	Days after treatment	Corrected mortality[b] [%] (*N*[c])							
			Shimada-Yui population							Susceptible strain (Kanaya)
			2006	2007	2008	2009	2010	2011[d]		
								June	August	
Flubendiamide (W, 20)[e]	2,000	8	90.0[f] (30)	90.3 (30)	73.5 (26)	84.7 (30)	24.3 (31)	16.7 (30)	21.5 (30)	100 (30)
		10	–[g]	96.8	85.2	92.7	52.5	34.1	32.0	100
	8,000	8	86.7[f] (30)	45.2 (30)	14.8 (28)	38.8 (30)	0 (28)	3.70 (26)	0 (29)	100 (29)
		10	–	51.6	26.2	46.0	15.3	7.41	0	100
Chlorantraniliprole (F, 10)	2,000	8	–	–	–	–	72.0 (29)	36.7 (30)	20.9 (27)	100 (30)
		10	–	–	–	–	72.0	45.6	21.9	100
	8,000	8	–	–	–	–	22.6 (30)	3.33 (30)	0 (29)	100 (30)
		10	–	–	–	–	27.1	10.4	0	100

W Water-dispersible granules, *F* flowable

[a] 2,000 dilution is ordinary concentration of each insecticide

[b] Mortality corrected using the method of Abbott (1925)

[c] Number of larvae tested

[d] Shimada-Yui population was collected twice in June and August 2011

[e] Formulation of flubendiamide in June and August 2011 is 18 % flowable because the formulation was changed. 18 % flowable represents an equivalent dose of insecticide

[f] These data are from 7 days after treatment

[g] No data

and 8,000 dilutions were 96.8 and 51.6 %, respectively, in 2007, but by August 2011 these values had dropped to 32.0 and 0 %, respectively. The mortalities of the Shimada-Yui strains at 10 days after treatment with chlorantraniliprole for the same two dilutions were 72.0 and 27.1 %, respectively, in 2010, but they fell in August 2011, to 21.9 and 0 %, respectively.

LC_{50} values

Table 2 shows the susceptibility of 2nd and 3rd instar larvae of *A. honmai* to two diamides, flubendiamide and chlorantraniliprole, from 2006 to 2011. The LC_{50} values of flubendiamide at 8 and 10 days after treatment in the Kanaya strain were 1.75 and 1.54 ppm, respectively. The LC_{50} values of chlorantraniliprole in Kanaya were 1.57 and 1.28 ppm, respectively. The LC_{50} values of flubendiamide at 10 days after treatment in the Shimada-Yui strains increased annually from 16.2 ppm in 2007 to 161 ppm in August 2011, exceeding 100 ppm, which was the concentration of flubendiamide registered for use on the tea crop in Japan in 2010 and 2011. The LC_{50} values of chlorantraniliprole also rose from 25.3 ppm in 2010 to 98.8 ppm in August 2011, exceeding 50 ppm, which was the concentration of chlorantraniliprole registered for use on the tea crop in Japan in 2011. The LC_{50} value at 10 days after treatment with flubendiamide in August 2011 was 105-fold higher in the Shimada-Yui strains than in the Kanaya strain. The LC_{50} value for chlorantraniliprole in the Shimada-Yui strains was 77.2-fold higher than that in the Kanaya strain in 2011.

Discussion

This is the first report of a high level of diamide resistance in *A. honmai*. This is also the first reported case of resistance to diamides in any pest in Japan. High levels of resistance to diamides have been reported only in *P. xylostella* in cruciferous vegetable production areas of China (Wang and Wu 2012), the Philippines, and Thailand (Troczka et al. 2012). Wang and Wu (2012) reported that the LC_{50} value for a *P. xylostella* strain in southern China against chlorantraniliprole was 265 ppm, which was 2,000-fold higher than that for a susceptible strain, ROTH (0.132 ppm). Troczka et al. (2012) reported that the LC_{50} values of *P. xylostella* strains in the Philippines and Thailand against chlorantraniliprole were, respectively, >4,100-fold (>200 ppm) and >200-fold (>60 ppm) higher than that of the susceptible strain, HS (0.048 ppm). The LC_{50} values of *P. xylostella* strains in the Philippines and Thailand against flubendiamide were, respectively,

Table 2 Susceptibility of 2nd and 3rd instar larvae of *Adoxophyes honmai* to two diamides from 2006 to 2011

Insecticides (formulation, %AI, registration concentration [ppm])	Days after treatment	Shimada-Yui strain							
		2006				2007			
		N[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]	*N*[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]
Flubendiamide (W, 20, 100)[d]	8	120	6.92[e] (0.693 to 13.1)	1.41	3.95[f]	120	13.6 (10.6 to 17.2)	2.70	7.77
	10	–[g]	–	–	–	120	16.2 (12.9 to 20.6)	2.78	10.5
Chlorantraniliprole (F, 10, 50)	8	–[g]	–	–	–	–[g]	–	–	–
	10	–[g]	–	–	–	–[g]	–	–	–

Insecticides (formulation, %AI, registration concentration [ppm])	Days after treatment	Shimada-Yui strain							
		2008				2009			
		N[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]	*N*[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]
Flubendiamide (W, 20, 100)[d]	8	120	55.5 (49.1 to 63.7)	2.79	31.7	180	35.2 (30.1 to 42.0)	2.24	20.1
	10	120	41.8 (37.1 to 47.2)	2.81	27.1	180	24.4 (21.4 to 28.0)	2.16	15.8
Chlorantraniliprole (F, 10, 50)	8	–[g]	–	–	–	–[g]	–	–	–
	10	–[g]	–	–	–	–[g]	–	–	–

Insecticides (formulation, %AI, registration concentration [ppm])	Days after treatment	Shimada-Yui strain											
		2010				2011[h]							
						June				August			
		N[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]	*N*[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]	*N*[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope	RR[c]
Flubendiamide (W, 20, 100)[d]	8	180	>100[i] (–)	–	–	150	1,174 (454 to >10,000)	0.927	671	150	196 (175 to 221)	3.03	112
	10	180	110 (80.8 to 173)	1.30	71.4	150	141 (119 to 176)	2.04	91.5	150	161 (144 to 181)	3.11	105
Chlorantraniliprole (F, 10, 50)	8	180	26.3 (21.2 to 33.8)	1.80	16.8	150	64.6 (55.4 to 78.0)	2.24	41.1	150	114 (101 to 132)	2.79	72.6
	10	180	25.3 (20.7 to 31.9)	1.96	19.8	150	50.0 (43.2 to 59.0)	2.16	39.1	150	98.8 (86.7 to 114)	2.48	77.2

Insecticides (formulation, %AI, registration concentration [ppm])	Days after treatment	Susceptible strain (Kanaya)		
		N[a]	LC$_{50}$[b] [ppm] (95 % CI)	Slope
Flubendiamide (W, 20, 100)[d]	8	145	1.75 (1.36 to 2.23)	2.76
	10	150	1.54 (1.03 to 1.97)	2.92
Chlorantraniliprole (F, 10, 50)	8	180	1.57 (1.36 to 1.79)	2.48
	10	180	1.28 (1.10 to 1.47)	2.55

W Water-dispersible granules, *F* flowable, *CI* Confidence interval

[a] Number of larvae tested

[b] LC$_{50}$ values were calculated by probit analysis

[c] Resistance ratio: LC$_{50}$ of each data point/LC$_{50}$ of susceptible strain

[d] Formulation of flubendiamide in June and August 2011 is 18 % flowable because the formulation was changed. 18 % flowable represents an equivalent dose of insecticide

[e] These data are from 7 days after treatment

[f] RR is calculated by LC$_{50}$ of susceptible strain at 8 days

[g] No data

[h] Shimada-Yui strain was collected twice in June and August 2011

[i] Could not be calculated

>1,300-fold (>200 ppm) and >750-fold (>60 ppm) higher than that of the HS strain (0.15 ppm) (Troczka et al. 2012).

In our study, the LC$_{50}$ values at 10 days after treatment of the Shimada-Yui strains in August 2011 were 105-fold (161 ppm) higher for flubendiamide and 77.2-fold (98.8 ppm) higher for chlorantraniliprole than those in the susceptible strain, Kanaya (1.54 and 1.28 ppm for flubendiamide and chlorantraniliprole, respectively) (Table 2). The resistance ratio of *A. honmai* was lower than that of *P. xylostella* strains in China, the Philippines, and Thailand. The lower values of *A. honmai* are probably due to a difference in baseline susceptibility or in the bioassay method between *A. honmai* and *P. xylostella*.

The mortalities of the Shimada-Yui strains at 10 days after treatment with the two diamides at the 2,000 dilution (the ordinary concentration used in the field) indicated that the practical efficacy of this concentration was limited by 2011. Each year, a maximum of four to five applications of insecticide are used in the tea fields in

Shizuoka Prefecture. The pest control calendar is compiled by the agricultural cooperative association in Shizuoka Prefecture to rotate insecticides that have different modes of action; therefore, it is unlikely that rapid resistance developed due to overuse of the diamides. In contrast, Wang and Wu (2012) and Troczka et al. (2012) reported that the high level of resistance of *P. xylostella* to diamides in China, the Philippines, and Thailand was probably due to intensive use.

There are three possibilities to explain the rapid development of resistance to diamides in *A. honmai*. The first is that the initial frequency of the diamide-resistance gene in the Shimada-Yui strains was already high. Georghiou and Taylor (1977) point out that the speed of development is related to the initial frequency of the gene for resistance. It is likely that this frequency was high in the Shimada-Yui strains, as mortality for the registration concentration did not reach 100 % in the first year of its application in the tea fields (96.8 and 72.0 % for flubendiamide in 2007 and chlorantraniliprole in 2010, respectively) (Table 1). Additional data supporting this explanation are that there was not a sufficient margin between the registration concentration of the diamides (100 and 50 ppm for flubendiamide and chlorantraniliprole, respectively) and the LC_{50} values of the Shimada-Yui strains for the diamides in the first year of application (Table 2).

The second possibility is that cross-resistance to the diamides and other insecticides developed in *A. honmai*. Previous work showed that both field-derived strains and laboratory-selected strains of *P. xylostella* that are resistant to currently used insecticides do not have cross-resistance to chlorantraniliprole (Wang et al. 2010). Furthermore, Wang and Wu (2012) suggest that resistance to chlorantraniliprole in *P. xylostella* resulted from field selection itself, rather than from cross-resistance to other insecticides. Circumstantial evidence confirms that susceptibility to the DAH analogs of IGRs tends to decline in a tea-producing area, and the diamides show the same trend in the same tea-producing area (Uchiyama and Ozawa unpublished data). Therefore, it is possible that *A. honmai* has developed resistance rapidly as a result of cross-resistance between diamides and other insecticides. We are currently conducting research regarding cross-resistance between the diamides and other insecticides in *A. honmai*.

The third possibility is that resistance to diamides might have developed earlier than resistance to other classes of insecticides because the diamides have a long residual activity period in *A. honmai* (Uchiyama 2012). If an insecticide has a long residual activity period in fields, the insect pests, including *A. honmai*, are exposed to the insecticide for a long time. As a result of this long-term exposure in *A. honmai*, resistance to the diamides may develop earlier than resistance to other classes of insecticides. In our previous study, we reported the rapid development of resistance in *A. honmai* to the DAH analogs of IGRs (Uchiyama et al. 2013). Because the IGRs have a long period of residual activity in *A. honmai*, like the diamides, it is possible that resistance to the IGRs developed rapidly in *A. honmai*.

It is necessary to immediately monitor the susceptibility of *A. honmai* to the diamides in other tea-producing areas in Japan, including other parts of Shizuoka Prefecture. Furthermore, it will be necessary to examine why resistance to diamides in *A. honmai* developed so quickly by investigating resistance mechanisms, such as cross-resistance and inheritance of resistance.

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