Mechanism of Fipronil Resistance in *Laodelphax striatellus* (Hemiptera: Delphacidae)[1]

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Abstract The WX-F strain of *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae) was derived from a field-collected strain (WX strain) after 77 generations of continuous selection with fipronil. The selection resulted in 86.6-fold resistance to fipronil; WX-F showed no cross-resistance to dieldrin (1.8-fold) and low-level cross-resistance to endosulfan (3.7-fold) compared with the primary WX strain. Bioassay with synergists and analysis of detoxification enzyme activities indicated that metabolic detoxification is not significantly associated with fipronil resistance in the WX-F strain. Further molecular analysis indicated that the fipronil resistance was most likely attributed to target site insensitivity. Sequencing a cloned single cDNA fragment of the Rdl γ-aminobutyric acid (GABA) receptor gene showed a high frequency (80%) of the A2'N mutation in the fipronil-selected strain (WX-F). It carried a mutant LsRdl allele with the amino acid replacement of Ala (GCC) to Asn (AAC). This mutation was not detected in the 23 individuals in the WX strain. The high frequency of the LsRdl-Asn allele in the WX-F strain indicates that the A2'N mutation of the γ-aminobutyric acid (GABA) receptor gene may be associated with fipronil resistance in *L. striatellus*. In addition, a novel R305Q or R305W mutation was found in the cytoplasmic loop between M3 and M4 of the *L.striatellus* Rdl γ-aminobutyric acid (GABA) receptor subunit (Ls-Rdl). These were only found in cDNA clones carrying the A2'N mutation.

Key Words *Laodelphax striatellus*, fipronil, GABA receptor, insecticide resistance

The small brown planthopper, *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae), is widely distributed from Southeast Asia to Siberia and Europe. As a pest of rice, wheat, and corn, it causes serious damage to crops by the transmission of stripe virus, black streaked dwarf virus, and maize rough dwarf virus (Kisimoto 1967, Fang et al. 2001). In China, the small brown planthopper causes serious feeding damage or disease problems annually, and the density of the *L. striatellus* population has increased dramatically from 1999 - 2008 (Liu et al. 2006). To control the pest, neonicotinoid and phenyl-pyrazole insecticides, such as imidacloprid and fipronil, are used. Extensive applications of fipronil against rice planthopper have led to insecticide resistance problems in East and Southeast Asia (Matsumura and Otuka 2009, Matsumura et al. 2009, Zhao et al. 2011).

Fipronil is the first phenyl pyrazole insecticide introduced for pest control and is highly effective against both piercing-sucking and chewing insects (Moffat 1993). It is a potent blocker of the insect GABA-gated chloride channel or GABA receptor (Cole et al. 1993, Buckingham et al. 1994). Cyclodiene resistance is due to insensitivity of

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the cyclodiene/ picrotoxinin binding site on the gamma-aminobutyric acid subtype A ($GABA_A$) receptor-chloride ionophore complex (Ffrench-Constant et al. 1991). However, cyclodiene resistance is able to persist in pest populations even without further exposure to cyclodiene, representing a threat to novel insecticides interacting with the cyclodiene binding site, such as fipronil (Ffrench-Constant et al. 2000). This leads to concerns about whether there is cross-resistance between fipronil and cyclodiene insecticides.

The insect ionotropic γ-aminobutyric acid (GABA) receptor is a chloride channel and the important target for insecticides, such as cyclodienes and fipronil. Cyclodienes and fipronil are noncompetitive antagonists for the GABA receptor and inhibit the flux of chloride ions into the nerve cell, resulting in nervous system hyperexcitation (Ffrench-Constant et al. 1991). The insect GABA receptor subunit gene was first cloned from *Drosophila melanogaster* Meigen and noted as Rdl (resistant to dieldrin) (Ffrench-Constant et al. 1991). Dieldrin resistance in *D. melanogaster* was associated with a point mutation of Rdl gene that caused an amino acid substitution from alanine to serine at position 302 (Ffrench-Constant et al. 1993). To date, the homologous mutation also has been found in 11 species from 6 insect orders: *Tribolium castaneum* (Herbst) (Thompson et al. 1993, Masahiro et al. 1995), and *Hypothenemus hampei* (Ferrari) (Ffrench-Constant et al. 1994) in the order Coleoptera; *Aedes aegypti* (L.), *Drosophila simulans* (Sturtevant), *Musca demestica* (L.) (Thompson et al. 1993), and *Lucilia cuprina* (Wiedemann) (McKenzie 1996) in the order Diptera; *Blattella germanica* (L.) (Kaku and Matsumura 1994) in the order Dictyoptera; *Bemisia tabaci* (Gennadius) (Anthony et al. 1995) and *Myzus persicae* (Sulzer) (Anthony et al. 1998) in the order Hemiptera; *Ctenocephalides felis* (Bouché) (Daborn et al. 2004) in the order Siphonaptera, and; *Plutella xylostella* (L.) (Li et al. 2006) in the order Lepidoptera. However, the A302S mutation has not been reported in *L.striatellus*.

The A2'N mutation of the GABA receptor was first found in *Sogatella furcifera* Hovarth (Nakao et al. 2010). It was subsequently reported in *L. striatellus*. Both of the fipronil-resistant populations were collected directly from field in Japan, not from the laboratory-selected fipronil-resistant strain. The susceptible strain was not the primary one (Nakao et al. 2010, 2011), and they do not share the same genetic background. In this study, we investigated the mechanism involved in fipronil resistance in the hemipteran pest *L.striatellus* by using the primary and its fipronil-selected resistant strains to clarify the mechanism of fipronil resistance.

Materials and Methods

Insects. The primary strain (WX strain) of *L. striatellus* was collected from Jiangsu, China, and has been maintained in the laboratory more than 6 yrs without exposure to any insecticides. A fipronil-resistant strain (WX-F) was derived from the primary strain (WX strain) through 77 generations of continuous selection with fipronil by using the rice seedling dip method (Wang et al. 2008, 2010). The average mortality in the selection was between 40 and 60%. These 2 strains were reared on rice seedlings at 25 ± 1°C, 60 - 70% RH, and a photoperiod of 16:8 (L: D) h.

Toxicity bioassay. The rice seedling dipping method (Wang et al. 2008, 2010) was adopted for bioassay. Pesticide (fipronil, dieldrin, endosulfan) solutions were prepared in acetone and 10% Triton-100 (v/v) (emulsifier), and a series of concentrations were diluted with water. The experiment was conducted with 5 - 6 concentrations and repeated 3 times. Fifteen third-instar nymphs of *L. striatellus* were placed on the rice seedlings

and maintained under laboratory conditions of 25 ± 1°C, 60 - 70% RH, photoperiod of 16:8 (L: D) h. Numbers of dead nymphs were recorded 4 d after treatment.

Test for synergism. To investigate the synergistic effect of major metabolic enzyme inhibitors on the efficacy of fipronil, PBO, TPP, DEF, and DEM were added to each serial concentration of fipronil, respectively. Pretrial tests were conducted to determine the highest ineffectual concentration for each synergist (PBO 2.5 mg/L, TPP 10 mg/L, DEF 50 mg/L, DEM 50 mg/L), with no obvious detrimental effects on the third-instar nymphs of both strains of *L. striatellus*. Other experimental procedures were the same as the bioassay method described above. To assess the degree of synergism, the synergistic ratio (SR) was calculated by dividing the LC_{50} value of fipronil alone by the LC_{50} value of fipronil plus synergist.

Enzyme preparation. The third-instar nymphs were prepared and starved for 3 h to remove all digested rice seedling. All nymphs were homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.6, containing 1 mM EDTA, 1 mM DTT, 1 mM PTU, 1 mM PMSF and 20% glycerol). Insects were chilled on ice before homogenization. One hundred third-instar nymphs were homogenized in 0.6 mL buffer, the homogenate was centrifuged at 4°C, 10, 000 g for 15 min, and the solid debris and cellular material were discarded. The supernatant was transferred into a clean Eppendorf tube, placed on ice and, used immediately for mixed function oxidase (MFO), glutathione S-transferase (GST), esterase (EST), and total protein content assays.

Enzyme activity assays. The activity of mixed function oxidase (MFO) was assayed by the protocols of Rose et al. (1995). One hundred μL of 2 mM *p*-nitroanisole solution and 90 μL enzyme were added to each well of transparent 96-well Costar microplate (Corning Life Sciences, Lowell, MA). The microplate was incubated for 3 min at 27°C, and the reaction was initiated by the addition of 10 μL of 9.6 mM NADPH. The absorbance was read in a TECAN microplate reader (Infinite™ M200) at 405 nm and 27°C for 15 min. The activity of MFO was presented as nanomoles of *p*-nitrophenol per min per mg of protein.

Glutathione-S-transferase (GST) activity was measured using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate on transparent 96-well Costar microplates according to the methods of Yang et al. (2004). One μL of the enzyme solution (diluted 10-fold in 0.1 M pH 7.6 sodium phosphate buffer) was mixed with 100 μL 1.2 mM CDNB and 100 μL 6 mM glutathione (GSH) in each well. Enzyme activity was recorded in a TECAN microplate reader at 340 nm and 27°C using the kinetic mode for 10 min. The activity of GST was determined using the extinction coefficient of 9.6 mM/cm for CDNB. The results were expressed as nanomoles glutathione conjugate per min per mg of protein.

Total esterase (EST) activity was determined using α-naphthyl acetate (α-NA) as a substrate on transparent 96-well Costar microplates according to the methods of Yang et al. (2004). One μL of enzyme solution (diluted 10-fold in 0.1 M pH 7.6 sodium phosphate buffer) was mixed with 200 μL of substrate solution (5 mL 0.2 M pH 6.0 phosphate buffer, 10 mg Coomassie Brilliant Blue G-250 and 0.1 mL 100 mM α-NA) in each well. Enzyme activity was measured at 450 nm and 27°C using a TECAN microplate reader in the kinetic mode for 10 min. The activity of the esterase was expressed as nanomoles of α-naphthol per minute per milligram of protein.

Total protein content was determined by the methods described by Bradford (1976) using bovine albumin as a standard.

cDNA fragments of GABA gene clone. Total cellular RNA was isolated from individual adult of *L. striatellus* by using Trizol reagent (Invitrogen) following the manufacturers instructions. cDNA was obtained by RT-PCR. A cDNA fragment of 1204 bp

encoding *L.striatellus* Rdl GABA gene was amplified using a pair of specificity primers: [LsRdl-F: 5-ATGTTAGGAGACGTTAACA-3, and LsRdl-R: 5-TC AGTTCCCGCTC-GGAGC-3]. Polymerase chain reaction (PCR) (50 μL) contained 2 ul of cDNA, 5 μL of 10X standard PCR buffer, 4 μL of 25mM Mg^{2+}, 10 pmol of each primer, 1 μL of 10mM dNTP, and 1U Taq DNA polymerase. The initial step of the amplification reaction denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 30s, 50°C for 30s, 72°C for 90s, and a final extension at 72°C for 10 min. PCR products of the expected size were excised and purified by using the DNA agarose gel purification kit and cloned by using the pUCm-T vector system.

Detection of the mutation in the GABA gene fragment by using sequencing of PCR reagents. To compare the mutation of the Rdl GABA gene from the WX-F strain and WX strain, a 1204 bp fragment including 4 membrane-spanning regions of Rdl γ-amino butyric acid (GABA) receptor gene from individual adult *L. striatellus* was cloned by using the Trizol reagent (Invitrogen), following the manufacturer instructions. Each strain was cloned using at least 20 individuals.

Data analysis. Median concentration values (LC_{50}) and their 95% fiducial limits (FL) were estimated using POLO-Plus program (LeOra 2002). Significant differences of LC_{50} were determined by nonoverlapping 95% fiducial limits (FL). Resistance Ratio (RR) was calculated by dividing the LC_{50} value of the resistant strain by the LC_{50} value of the primary strain. Resistance levels were classiðed on the basis of the standard described by Shen and Wu (1995) as susceptible (RR < 3-fold), minor resistance (RR=3 - 5-fold), low resistance level (RR=5 - 10-fold), medium resistance level (RR=10 - 40-fold), high resistance level (RR=40 - 160-fold), and extremely high resistance level (RR > 160-fold). The activity of MFO, GST and EST was subjected to a DPS analysis followed by *t*-test ($\alpha < 0.05$). Significance was accepted at $\alpha = 0.05$ in all statistical tests used in this study. LsRdl-like cDNA was confirmed by similarity search of GenBank using Blastx NR (http: //www.ncbi.nlm. nih.gov/blast/) and multiple sequences alignment of the cDNA by using bioedit and clustalx software (Thompson et al. 1997). Transmembrane helix was predicted using TMpred on-line tools (http://www.cbs.dtu.dk/services/ TMHMM-2.0/).

Results

Cross-resistance between fipronil and cyclodiene insecticides (dieldrin and endosulfan). No evidence of cross-resistance between fipronil and the cyclodiene insecticides (dieldrin and endosulfan) was seen in comparing the WX-F strain with the WX strain. When compared with the WX strain, the WX-F strain had no cross-resistance to dieldrin (1.8-fold) and only very low level cross-resistance to endosulfan (3.7-fold) (Table 1).

Effect of synergists on fipronil resistance. Synergistic ratios of DEF = 0.93, TPP = 0.89, PBO = 0.91, and DEM = 0.77-fold in the WX strain and 1.00, 1.18, 1.18, and 1.27-fold in the WX-F strain (Table 2). These results indicate that esterases, P450-monooyxgenases, and glutathione-S-transferases might be not associated fipronil resistance in the WX-F strain.

Metabolic enzyme activities. To test whether the metabolic enzymes are involved in fipronil resistance, metabolic enzyme activities, including P450 monooxygenases (against the substrates p-NA), esterases (against the substrate α-NA), and GSTs (against the substrates CDNB) were tested between the WX strain and WX-F strain. The results showed no significant difference in detoxification enzyme activities

Table 1. Cross-resistance of the fipronil-selected WX-F strain of *L. striatellus*.

Pesticide	Strain	N*	LC_{50} (mg/liter) (95%FL)	Slope(±SE)	χ^2 (d f)	RR^{88}
Fipronil	WX	315	0.51(0.36 - 0.68)	1.76(±0.27)	0.76(4)	-
	WX-F	315	44.15(27.89 - 66.99)	1.29(±0.22)	1.27(4)	86.6
Dieldrin	WX	270	17.78(12.66 - 24.51)	1.58(±0.26)	2.92(3)	-
	WX-F	270	32.18(20.28 - 48.67)	1.39(±0.27)	2.10(3)	1.8
Endosulfan	WX	270	19.68(12.34 - 28.36)	1.34(±0.24)	0.21(3)	-
	WX-F	315	72.88(40.26 - 107.59)	1.56(±0.30)	3.12(4)	3.7

* Number of nymph tested.
** $RR=LC_{50}$ (WX-F)/LC_{50} (WX).

between these 2 strains (Table 3) indicating that metabolic mechanisms are not important in the high fipronil-resistant strain.

Cloning and sequencing of LsRdl gene. A single cDNA fragment was cloned (1204bp) from the WX-F strain and WX strain. The sequence encoded about 401 amino acid residues and shared 99% amino acid sequence identity with the *L. striatellus* Rdl gene. This gene was designated as LsRdl. In this study, 23 individuals of the WX strain and 20 individuals of WX-F strain were cloned. Alignment of the sequences of the GABA receptor gene yielded 89 sites where the replacement of amino acids from the WX strain and WX-F strain occur. It contained 11 loci from part of the WX strain and WX-F strain simultaneously. Thirty-nine loci only were found in the part of the WX-F strain. Other thirty-nine loci only were found in the part of the WX strain

Table 2. Synergism of DEM TPP PBO and DEF to ðpronil in the WX strain and WX-F strain of *L. striatellus*.

Strain	Pesticide	n*	LC_{50} (mg/liter) (95%FL)	Slope(±SE)	χ^2 (d f)	SR**
WX	Fipronil	315	0.51(0.36 - 0.68)	1.76(±0.27)	0.76(4)	-
	Fipronil+DEM	315	0.66(0.43 - 1.08)	1.34(±0.21)	0.59(4)	0.77
	Fipronil+TPP	315	0.57(0.36 - 0.91)	1.11(±0.20)	1.64(4)	0.89
	Fipronil+PBO	315	0.56(0.36 - 0.88)	1.14(±0.19)	1.61(4)	0.91
	Fipronil+DEF	315	0.55(0.37 - 0.78)	1.45(±0.23)	0.18(4)	0.93
WX-F	Fipronil	315	44.15(27.89 - 66.99)	1.29(±0.22)	1.27(4)	-
	Fipronil+DEM	315	34.87(17.92 - 65.48)	0.82(±1.08)	1.12(4)	1.27
	Fipronil+TPP	315	37.47(21.81 - 61.06)	1.07(±0.20)	0.54(4)	1.18
	Fipronil+PBO	315	37.42(21.77 - 61.46)	1.05(±0.20)	1.90(4)	1.18
	Fipronil+DEF	315	44.04(28.60 - 65.60)	1.39(±0.27)	0.65(4)	1.00

* Number of nymph tested.
** $SR=LC_{50}$(fipronil alone)/LC_{50}(fipronil+synergist)

Table 3. Metabolic enzyme activities of third-instar nymph from the primary WX strain and the fipronil-resistant WX-F strain of *L. striatellus*.

Detoxification enzyme	Specific activity*		
	WX strain	WX-F strain	Rate**
Oxidases (p-NA)	630.4(±60.0)a	567.5(±68.8)a	0.90
Esterases (a-NA)	9035.6(±72.4)a	8989.0(±18.0)a	0.99
GSTs (CDNB)	431.4(±9.47)a	427.3(±7.40)a	0.99

* Means (±SE) followed by different lowercase letters in the same row are significantly different ($\alpha < 0.05$). Enzyme activity was showed as means ± SE (n mol/min/mg protein).
** Rate= the enzyme activity in WX-F strain / the activity in WX strain.

(data not shown). And, there was only the wild-type allele at the second position in the second transmembrane domain (M2) of LsRdl gene (encoding allele LsRdl-Ala) in the WX strain. The WX-F strain, in addition to the wild-type allele LsRdl-Ala, carried a mutant LsRdl allele with the amino acid replacement of Ala (GCC) to Asn (AAC). Besides the A2'N mutation, a novel R305Q or R305W mutation was found in the cytoplasmic loop between M3 and M4. Moreover, the R305Q or R305W was only found in cDNA clones carrying the A2'N mutation (Fig. 1).

Association of the A2'N mutation of the LsRdl with fipronil resistance. At least 20 individuals from each strain were cloned in the current study. As expected, we found that all individuals tested from the WX strain were genotypes for the LsRdl-Ala allele. However, the mutant LsRdl-Asn allele frequency in the WX-F strain was 80% (Table 4).

Discussion

Cyclodiene-resistant insects generally have low levels of cross-resistance to fipronil. For example, a strain of *B. germanica* that was highly resistant to dieldrin (LD_{50} resistance ratio > 17 000) was only 6.7- to 7.7-fold cross-resistant to fipronil (Thompson et al. 1997). And, the selection strain of *P. xylostella* resulted in 300-fold resistance to fipronil, only 3.5- and 6.5-fold cross-resistance to dieldrin and endosulfan, respectively (Li et al. 2006). In our study, a selection resulted in 86.6-fold increase resistance to fipronil, and it showed no cross-resistance to dieldrin (1.8-fold) and low level cross-resistance to endosulfan (3.7-fold), respectively, between in the WX-F strain and WX strain. Therefore, these results further confirm that the binding site of fipronil and cyclodiene insecticides in the Rdl GABA receptor are not identical.

Resistance to insecticides also can arise from increased detoxification. Several types of enzymes such as cytochrome P450 monooxygenases, esterases, and glutathione S-transferases (GSTs) are involved in the detoxification of various insecticides. Our results showed no significant difference in detoxification enzyme activities between these 2 strains, and the same results also were reported with *P. xylostella* (Li et al. 2006). This indicates that metabolic mechanisms are not involved in the resistance of *L. striatellus* to fipronil. However, metabolic mechanisms might be involved in resistance to fipronil when the level of resistance is low or moderate.

The A302S mutation in the Rdl gene confers high levels of dieldrin resistance in many insects, but it has not been found in *L. striatellus*. Apart from the conserved

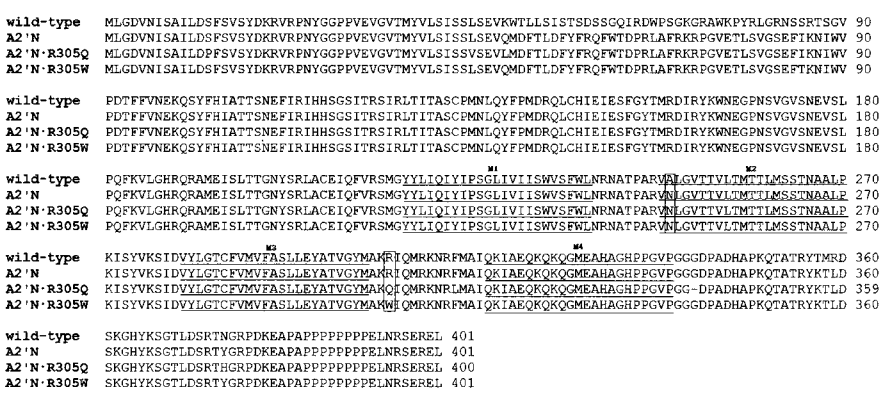

Fig. 1. Amino acid sequences of the wild-type and the mutant type GABA gene (Ls-Rdl) from *L.striatellus* (GenBank ID: JQ389120 for wild-type, JQ389119 for A2'N, JX051000 for A2'N·R305Q, JX051001 for A2'N·R305W). Four membrane- spanning regions (M1–M4) are underlined. The positions of mutations A2′ and R305 were shown by a rectangular box.

A301G mutation (responding to A302S in *D. melanogaster*), an additional mutation (T350M) in the third transmembrane domain has been confirmed to contribute to fipronil resistance in *D. simulans* (Goff et al. 2005, Gao et al. 2007). However, in our study, those 2 mutations were not found. In a recent study, the A2'N mutation of Rdl GABA receptor was first found in *S. furcifera* and confirmed that it conferred fipronil resistance in *S. furcifera* and *L. striatellus* populations (Nakao et al. 2010, 2011). Our methods, however, differed from those of Nakao et al. (2010, 2011) whose fipronil-resistant populations of *S. furcifera* and *L. striatellus* were collected directly from field and not from the laboratory-selected fipronil-resistant strain. Furthermore, the susceptible populations were not the primary ones of the resistant populations, so they may not be derived from the same gene pool. In our study, the resistant strain was selected with fipronil for 77 generations, and the susceptible strain was the first generation of the resistant strain, both of them sharing the same gene pool. We identified the A2'N mutation of the LsRdl gene in the fipronil selection resistant strain (WX-F strain). Sixteen of 20 individuals of fipronil-resistant *L.striatellus* showed the genotype for Asn (AAC) at the second position in the second transmembrane domain (M2) of LsRdl gene, but there was only one genotype (Ala) of the LsRdl gene at the second position in M2 domain of LsRdl gene in 23 individuals of the primary strain (WX strain).

Table 4. Distribution of A2'N mutation in different strains of *L. striatellus*.

		LsRdl genotypes	
strain	No Individual tested	Ala/Ala	Asn/Ala
WX	23	23	0
WX-F	20	4	16

Alanine belongs to the nonpolar hydrophobic amino acids, but asparagine is a polar neutral amino acids. The mutation may lead to a change the affinity between fipronil and Rdl GABA receptor. In addition to the A2'N mutation, a novel R305Q or R305W mutation was found in the cytoplasmic loop between M3 and M4. However, the R305Q or R305W was only found in cDNA clones carrying the A2'N mutation, and they appeared only once in our study. The same mutation (A2'N·R340Q) also has been confirmed that it conferred fipronil resistance in *S. furcifera* (Nakao et al. 2012). These results further confirm that the A2'N mutation of LsRdl gene may be associated with fipronil resistance in *L.striatellus*. The A2'N·R305Q or A2'N·R305W double mutation may also confer fipronil resistance in *L.striatellus*. However, it could not exclude some other mutation(s) (date not shown), or the same mutation at low frequency in the WX strain. There may be some other mutation(s) in other target receptors that contribute to fipronil resistance (Zhao et al. 2004, Kacimi et al. 2009).

Glutamate-gated chloride channels (GluCls) also are targets of fipronil in some insects (Zhao et al. 2004, Kacimi et al. 2009). GluCl subunits have been suggested to coassemble with GABA receptor subunits (Ludmerer et al. 2002, Zhao et al. 2010), although the GluCl subunits in *Drosophila* and *Musca* can function as homomeric receptors when they were expressed in the oocytes of the African clawed frog, *Xenopus laevis* (Daudin) (Cully et al. 1996, Eguchi et al. 2006). It is not reported whether the A2'N mutant Rdl GABA receptor subunit coassembles with GluCl subunits and affects sensitivity to fipronil, but effects of fipronil on GluCls can be considered an important factor affecting fipronil resistance.

In conclusion, we confirmed that fipronil-resistant insects have no or low levels of cross-resistance to cyclodiene and that metabolic mechanisms are not obviously related to the high levels of fipronil resistance. The high level of resistance is most likely attributed to target site insensitivity. We found a novel R305Q or R305W mutation in *L. striatellus* GABA receptor. The mutations were located in the cytoplasmic loop between M3 and M4 and found only in clones carrying the A2'N mutation. The A2'N mutation of the Rdl GABA receptor will be useful in the development of new monitoring methods and the discovery of new insecticides. Many questions remain to be answered through further studies, such as whether A2'N mutation of the Rdl GABA receptor can be found in other insect species, does its spread in various locations, and/or other mutation(s) in the LsRdl gene or other Rdl-like subunit(s) contribute to fipronil resistance.

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