Bacterial *Rdl2* dsRNA increased the insecticidal activity of GABAR blockers and allosteric modulators against *Plutella xylostella*

Kunkun Wang, [a,b] Benjie Li, [a,b] Ye Yu, [a,b] Jiaqi Wei, [a,b] Jian Zhu, [a,b] Jiali Wang, [a,b] Fei Lin, [a,b*] Hanhong Xu, [a,b*]

[a]State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, South China Agricultural University, Guangzhou, Guangdong, People's Republic of China

[b]Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University, Guangzhou, Guangdong, People's Republic of China

[*]Corresponding Author: E-mail: hhxu@scau.edu.cn (Hanhong Xu) and resistanc@scau.edu.cn (Fei Lin).

ABSTRACT

BACKGROUND: The application of RNAi to control pests has attracted the attention of researchers. Our results indicated that knockout of *PxRdl2* can decrease resistance to fipronil in *Plutella xylostella*, providing a suitable target gene for RNAi-based pest control.

RESULTS: The differences in the sensitivity of two established homozygous knockout strains of *P. xylostella*. (*Rdl1*KO and *Rdl2*KO) and susceptible *P. xylostella* to a series of compounds were evaluated. Quinazolines and isoxazolines both showed stronger efficacy in the *Rdl2*KO strain. Therefore, we proposed a method based on the knockdown of the *P. xylostella Rdl2* gene as a tactic to enhance the toxicity of quinazolines and isoxazolines. To reduce costs and protect dsRNA against degradation, we applied a bacterial expression system using the L4440 vector to express *PxRdl2* dsRNA in HT115 *Escherichia coli*. Transformed bacteria (dsRNA-Bac) fed through leaves combined with quinazoline and isoxazolines proved to be more effective in both the susceptible and fipronil-resistant *P. xylostella*.

CONCLUSION: Our results provide a strategy for the development of novel insecticide spray formulations containing dsRNA-Bac, which synergize with insecticide toxins by suppressing *PxRdl2*, reducing the use of pesticides in the field.

Keywords: *Plutella xylostella*; RNAi; *PxRdl*; resistance management

1 INTRODUCTION

Since RNA interference (RNAi) was first discovered in the nematode *Caenorhabditis elegans*[1], it has been widely used as a powerful genetic tool for research on various taxa, including plants, insects, nematodes, bacteria, fungi, ect.[2,3]. RNAi has been considered a new generation of pest control technology because it has great advantages, such as safety for nontarget organisms and the ability to maintain pollution-free conditions. Although dsRNA injected directly into an organism is highly efficient, it is difficult to apply as pest control in the field because of the very high costs of and complexity in preparing dsRNA. Fortunately, ingestion of specific dsRNA through the oral cavity can trigger a silencing response in most body tissues in insects; this paves the way for the development of RNAi-based agricultural pest control if dsRNA can be protected from degradation in the environment and insect intestines by suitable delivery methods.

Currently, wrapping dsRNA in nanomaterials or expressing dsRNA in bacteria are the two main methods of dsRNA-insect delivery. It has been reported that nanomaterials can improve the efficiency of delivery allowing dsRNA to permeate cell membranes directly or by endocytosis[4]; thus, this has become a new type of delivery agent and has been used in pest RNAi control[5]. Employing bacteria as delivery vectors of dsRNA molecules is another approach that has been used in recent years and was first carried out in the bacteriophagous nematode *C. elegans*[6]. Considering the technology and economic-related issues of dsRNA synthesis *in vitro*, there have been many reports of bacteria expressing dsRNA to induce insect RNAi to control pests[7-9]. Other

limitations related to the application of dsRNA for pest control include the identification of the appropriate target genes and increasing the insect lethality. For this point, genes targeted by chemical insecticides and bio-insecticides might be ideal sites for dsRNA targeting. It has been reported that silencing *Sl102*, *MvIIS and OfCTP* through RNAi increases the toxicity of *Bacillus thuringiensis* (Bt)[10-12].

With the cultivation of cruciferous crops, the diamondback moth (DBM), *P. xylostella*, has developed into a major pest worldwide[13]. Following the misuse of insecticides in the field, *P. xylostella* has developed such severe resistance to most pesticides through multiple mechanisms that it is difficult to control[14,15]. Although RNAi was reported to be less efficient in lepidoptera[16], RNAi in *P. xylostella* through dsRNA injection or oral administration demonstrated that this approach is for this species[17,18]. Therefore, the control and resistance management of *P. xylostella* may be achieved by dsRNA ingestion and interference with specific target genes.

In insects, γ-aminobutyric acid receptors (GABARs) are the major inhibitory receptors widely distributed in the central nervous system[19] and are the target of natural compounds (picrotoxin) and synthetic pesticides (such as dieldrin, fipronil, fluxametamide and fluralaner)[20-22]. According to the classification of the Insecticide Resistance Action Committee (https://irac-online.org/modes-of-action/, IRAC), Compounds that act on GABAR are divided into two categories: blockers (Category 2, represented by fipronil and chlordane) and allosteric modulators (Category 30, represented by broflanilide and fluxametamide) (referred to as GABAR compounds in this paper). Resistance to dieldrin (*Rdl*) gene, which combines with other GABAR

subunits to constitute the target site of cyclodiene and phenylpyrazole pesticides, was cloned from dieldrin-resistant *Drosophila melanogaster* and named *Rdl*[23]. There are two *Rdls* in *P. xylostella*[24]. Our previous research showed that in *Xenopus oocytes*, the sensitivity of *PxRdl2* to fipronil was 40-fold lower than that in *P. xylostella Rdl1*. In addition, knockout of *PxRdl1* reduced the efficacy of fipronil, while knockout of *PxRdl2* increased its efficacy[25]. This may indicate that the *PxRdl2* gene plays an important role in resistance to GABAR compounds, which is beneficial for researching toxicology and resistance to insecticides. Based on previous results, we verified whether the knockout of *PxRdl2* or *PxRdl1* has effects on other GABAR compounds. Furthermore, we considered whether it is possible to develop *PxRdl2*-based RNAi to improve the efficacy of insecticides.

In this study, two homozygous knockout strains of *P. xylostella* (*Rdl1*KO and *Rdl2*KO) and susceptible strains were established to evaluate the differences in sensitivity of 5 compounds by bioassay. The bioassay results suggested that the newly synthesized quinazolines-4a and the two new isoxazolines (fluxametamide and fluralaner) displayed greater activity in the *Rdl2*KO strain than in the susceptible strain. Furthermore, *PxRdl2* interference by dsRNA, which induced expression by transformed HT115 *Escherichia coli* (named dsRNA-Bac in this paper), could improve the efficacy of quinazoline and isoxazolines (named RNAi compound in this paper) in both susceptible and fipronil-resistant *P. xylostella*. In addition, we also verified the effect of UV radiation on dsRNA-Bac to ensure that it could remain stable when exposed to sunlight in field applications. In summary, our study provides new

111 insights for pest control, improving safety for nontarget organisms. Thus, the results
112 may be beneficial for pesticide resistance management and reducing the usage of
113 pesticides in the field, which could be part of an integrated pest management (IPM)
114 approach.

115 **2 MATERIALS AND METHODS**

116 **2.1 Insect strain rearing and compounds**

117 Susceptible and fipronil-resistant *P. xylostella* were generously provided by Dr.
118 Minsheng You (Fujian Agriculture and Forestry University, China). Two homozygous
119 knockout strains (*Rdl1*KO and *Rdl2*KO) were previously established from susceptible
120 strains using CRISPR/Cas9 previously[25], and were reared on an artificial diet for more
121 than 30 generations in our laboratory. The four strains were kept at 25±1 °C and
122 65±5% relative humidity under 16:8 h (light: dark) conditions.
123 Compounds of fipronil (95%), fluxametamide (95%), chloraniliprole (95%),
124 fluralaner (95%) and abamectin (95%) were purchased from TargetMol (Shanghai,
125 China). Compounds 5a[26] and 4a[27] were synthesized in our laboratory.

126 **2.2 Bioassays**

127 Two bioassays were conducted in this study. To evaluate the sensitivity of the
128 susceptible, *Rdl*KO and fipronil-resistant strains to the compounds, leaf-dip bioassays
129 were carried out as recommended by the IRAC
130 (https://irac-online.org/methods/plutella-xylostella-larvae/). The LCs of the different
131 compounds were determined through the leaf-dip bioassays. To better simulate the
132 outdoor spraying method applied in the field and to detect the impact of gene

133 silencing on the lethality of the compounds, RNAi compounds were subjected to
134 leaf-dip bioassays, in which dsRNA-Bac was evenly applied to the surface of the
135 leaves containing compounds.

136 For the *PxRdl2* RNAi compounds, a sublethal dose (LC_{20}) allowed the assessment of
137 any increase in the mortality rate caused by RNAi-induced gene silencing. Third
138 instar larvae were fed with leaves containing *PxRdl2* dsRNA-Bac (or *GFP*
139 dsRNA-Bac) and compounds. Each treatment used at least 10 larvae with 4
140 replications. Mortality was recorded every 12 h for 2 days. The theoretically reduced
141 dosage was calculated by the following formula: (LCs – LC_{20} of the *PxRdl2* RNAi
142 compounds)/LCs × 100%. The impact of *PxRdl1* dsRNA on the chemical toxicity of *P.*
143 *xylostella* was also assessed as described above, with the expectation that the LC_{50} of
144 each compound dosage was used.

145 **2.3 *In vitro* synthesis of *PxRdls* dsRNA**

146 Total RNA was extracted from 3rd instar larvae or bacteria using an RNAsimple Total
147 RNA Kit (TianGen, Beijing, China). The extracted RNA (1 μg) was used to reverse
148 transcribe cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect
149 Real Time) (TaKaRa Biotechnology, Dalian, China).

150 A 428-bp fragment of *PxRdl1* and a 441-bp fragment of *PxRdl2* were amplified from
151 cDNA extracted from larvae. The specific fragments were purified using an
152 E.Z.N.A.® Gel Extraction Kit (OMEGA Bio-tek, Guangzhou, China). The PCR
153 products were used as templates for the next PCR and primers containing the T7
154 polymerase promoter sequence at their 5’ ends were carried out. The PCR products

were used as templates to synthesize specific dsRNAs according to the manufacturer's instructions using the *in vitro* Transcription T7 Kit (TaKaRa Biotechnology, Dalian, China).

The 289-bp *GFP* fragment was amplified from the cloning vector pE*GFP*-N1-ro1*GFP* (Plasmid #82369, Addgene), and then, a similar method was applied to synthesize specific *GFP* dsRNA. All the primers in this paper were designed using Primer Premier 5 (Premier, Canada) and are shown in Table 1.

2.4 Construction of transformed HT115 *Escherichia coli* expressing dsRNA and overexpression of dsRNA

The 289-bp *GFP*, 428-bp *PxRdl1* and 441-bp *PxRdl2* fragments were cloned into L4440 vectors (Plasmid #1654, Addgene) via homologous recombination[28,29]. The recombinant L4440 vectors were transformed into HT115 *E. coli* competent cells. To produce dsRNA, the transformed bacteria were induced by isopropyl *β*-D-1-thiogalactopyranoside (IPTG). dsRNA was extracted from the bacterial cells for subsequent qRT-PCR absolute quantification.

2.5 qRT-PCR absolute quantification of dsRNA produced by the transformed bacteria

qRT-PCR was carried out using SYBR® Premix Ex Taq™ II (Takara Biotechnology) and a CFX96 Connect Real-Time system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Based on the absolute quantification method[30], the quantity of the dsRNA was confirmed by threshold (CT) values, which relate to an established standard curve. The standard curve for dsRNA was established by plotting the logarithm of a

177 10-10^5-fold dilution of the starting solution of template cDNA with inserts against the
178 corresponding CT values.

179 **2.6 Pretreatment of bacteria**

180 Bacteria were collected by centrifugation at 5000×g for 5 min at 4°C and suspended
181 in DEPC-H_2O. To inactivate the bacteria and promote the release of dsRNA,
182 sonication treatment was carried out by a low-temperature ultrahigh-pressure cell
183 crusher (Guangzhou Juneng Nano & Biotechnology Co., Ltd, Guangzhou, China) at
184 1800 bar. LB agar plates containing 100 μg mL^{-1} ampicillin were used to evaluate
185 whether the bacteria were totally inactivated.

186 **2.7 qRT-PCR relative quantification of *PxRdls* transcription**

187 dsRNA-Bac (100, 200 or 400 ng μL^{-1}) was evenly applied to the surface of the leaves,
188 and then naturally air-dried. Gene analysis was performed by qRT-PCR, which was
189 carried out by using gene-specific primers, after 2 days of feeding the
190 dsRNA-Bac-covered leaves to 3rd instar *P. xylostella* larvae. Gene-specific primers
191 were designed to test a segment of the mRNA external to the segment targeted by the
192 dsRNA. The qRT-PCR data were normalized to an internal control (*Actin* gene,
193 GenBank No. JN410820.1) and analysed by the $2^{-\Delta\Delta CT}$ method[31]. Each treatment used
194 5 larvae with 3 replications. The controls received *GFP* dsRNA-Bac.

195 **2.8 UV irradiation on dsRNA-Bac**

196 To better simulate the effects of field applications, a UV irradiation experiment was
197 carried out to determine the stability of dsRNA-Bac. *PxRdl2* dsRNA-Bac and *GFP*
198 dsRNA-Bac were placed on the surface of glassware. The glassware was placed on an

199 HD-650 desktop clean bench (Su Jing Purification Equipment, Zhejiang, China). A
200 UV lamp was used to irradiate the glassware for 0 h, 0.5 h, 1 h, 2 h or 4 h (254 nm, 8
201 W). Before UV irradiation, absolute quantification was carried out on the dsRNA-Bac.
202 The treated dsRNA-Bac was fed to *P. xylostella* through leaves for gene expression
203 analysis after two days.

204 **2.9 Statistical analysis**

205 For the leaf-dip bioassay data, SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used to
206 calculate the LCs. The LCs were analysed using regression-probit analysis, and "Log
207 base 10" was selected for transformation. The LC_{50} values were confirmed to be
208 significantly different if the 95% fiducial limits did not overlap. The other data were
209 analysed using GraphPad Prism, version 5.0 (GraphPad Software Inc., La Jolla, CA,
210 USA). Gene expression was analysed by one-way ANOVA and Tukey's test (alpha =
211 0.05). Survival curves of the *P. xylostella* 3rd instar larvae were compared using
212 log-rank analyses.

213 **3 RESULTS**

214 **3.1 Toxicological responses of the two *Rdl* knockout strains to five compounds**

215 To test roles of the two *Rdl* homologous genes in the interaction with the five tested
216 compounds, the responses of the two knockout strains and susceptible strain were
217 measured (Table 2 and Fig. 1: data for fipronil were adapted from our previous
218 publication[25]). The *Rdl*1KO strain showed greater resistance (2.85-fold) than the
219 susceptible strain to the newly synthesized compound quinazoline (4a). In contrast,
220 the toxicity of 4a to the *Rdl*2KO strain increased significantly as the resistance ratio

(RR) decreased to 0.45. These results revealed that while *PxRdl1* and *PxRdl2* are both vital determinants of the sensitivity of *P. xylostella* to 4a, they have adverse effects. Such a tendency was also observed in response to fipronil[25] and 5a treatment (unpublished data). The toxicity of fluxametamide to the *Rdl*1KO and *Rdl*2KO strains was significantly increased relative to that to the susceptible strain. Unlike fluxametamide, knockout of *PxRdl1* had no significant effect on fluralaner susceptibility, but knockout of *PxRdl2* increased fluralaner susceptibility (2.50-fold). No difference in toxicity was observed in the three strains for abamectin.

The negative control, chloraniliprole, exhibited toxicity similar to that of the two *Rdl* knockout strains compared with that of the susceptible strain. The increased susceptibility to knockout of *Rdl2* suggested that it could be an ideal target for RNAi in the development of synergists with GABAR compounds.

3.2 Production of bacteria expressing *PxRdls* dsRNA

To construct a vector expressing dsRNA, the *GFP* and *PxRdls* PCR products were cloned into the L4440 vector, which was induced by the addition of IPTG to overexpress dsRNA under the double T7 promoters and used to transform HT115 *E. coli* cells. The quantity of dsRNA produced by the HT115 *E. coli* (Fig. 2a) was quantified by qRT-PCR (Fig. 2b, Fig. 2c, Fig. 2d). For *GFP*, the PCR efficiency (E = 95.516%) was calculated according to the slope and the coefficient of correlation (R^2) of the standard curve (slope = -3.358, y intercept = 18.835, R^2 = 0.996). For *PxRdl1*, the PCR efficiency (E = 108.930%) was calculated according to the slope and the coefficient of correlation (R^2) of the standard curve (slope = -3.125, y intercept =

12.116, R^2 = 0.998). For *PxRdl2*, the PCR efficiency (E = 92.780%) was calculated according to the slope and the coefficient of correlation (R^2) of the standard curve (slope = -3.508, y intercept = 5.898, R^2 = 0.998).

3.3 Gene silencing in *P. xylostella* by feeding individuals leaves containing dsRNA-Bac

Quantitative analysis of the expression of the two *PxRdl* genes in 3rd instar larvae was performed by qRT-PCR two days after the larvae were fed leaves containing dsRNA produced by HT115 *E. coli*. The results demonstrated that the transcription levels of the targeted *PxRdl1* and *PxRdl2* genes were significantly affected by the dsRNA treatment and were positively correlated with the experimental dose used ($P < 0.05$) (Fig. 3a, Fig. 3b).

3.4 The effect of UV irradiation on dsRNA-Bac

After dsRNA-Bac was irradiated with UV for different times, the treated dsRNA-Bac was fed to 3rd instar larvae through leaves. The gene expression analysis data showed that dsRNA-Bac had strong stability and could maintain activity under strong UV irradiation for 1 h. The activity of dsRNA-Bac decreased when exposed to UV for more than 2 h ($P < 0.05$) (Fig. 3c).

3.5 *PxRdl2* dsRNA-Bac enhances the toxicity of quinazolines and isoxazolines in susceptible *Plutella xylostella*

RNAi compounds were used to test the efficacy of the simultaneous administration of *PxRdl2* dsRNA-Bac and compounds (fipronil, quinazolines and isoxazolines). This experiment aimed to more closely reproduce the possible effects of the two active

ingredients (dsRNA and compounds) applied in the field. The results showed that simultaneous administration of *PxRdl2* dsRNA-Bac and compounds led to obviously higher mortality in *PxRdl2*-silenced larvae than in controls (Fig. 4) (log-rank test for 3rd instar larvae exposed to different compounds: fipronil: chi-square = 4.501, $df = 3$, $P = 0.0339$; fluxametamide: chi-square = 14.80, $df = 3$, $P = 0.0001$; fluralaner: chi-square = 12.33, $df = 3$, $P = 0.0004$; 5a: chi-square = 14.95, $df = 3$, $P = 0.0001$; 4a: chi-square = 34.26, $df = 3$, $P < 0.0001$).

In addition, to further evaluate the effect of RNAi compound-mediated toxicity enhancement, the theoretical reduction in compound usage was calculated. When the compounds were used alone, the LCs^b in Table 3 shows the dose corresponding to mortality, indicating the final mortality of *PxRdl2* RNAi compounds after 48 h. According to the calculation results, the enhanced toxicity of the compounds mediated by RNAi could reduce the doses needed of different compounds (fipronil: 45.00%; fluxametamide: 56.82%; fluralaner: 45.45%; 5a: 59.31%; and 4a: 72.36%) (Table 3).

3.6 *PxRdl1* dsRNA-Bac-mediated gene silencing led to reduced toxicity of quinazolines and isoxazolines in susceptible *Plutella xylostella*

The LC_{50} values of fipronil and the quinazolines and isoxazolines were used for a *PxRdl1* RNAi compound bioassay similar to that for *PxRdl2*. The data showed that *PxRdl1* silencing reduced the toxicity of fipronil and the quinazolines and fluxametamide, except for fluralaner (Fig. 5) (log-rank test of the 3rd instar larvae for different compounds, fipronil: chi-square = 11.11, $df = 3$, $P = 0.0009$; fluxametamide: chi-square = 4.672, $df = 3$, $P = 0.0307$; fluralaner: chi-square = 0.5698, $df = 3$, $P =$

287 0.4503; 5a: chi-square = 4.159, *df* = 3, *P* = 0.0414; 4a: chi-square = 8.844, *df* = 3, *P* =
288 0.0029).

289 **3.7 *PxRdl*2 dsRNA-Bac enhances the toxicity of quinazoline and isoxazolines in**
290 **fipronil-resistant *Plutella xylostella***

291 Fipronil-resistant *P. xylostella* are extremely resistant to fipronil, and its resistance has
292 increased 1881-fold. For 4a, the resistance also increased 14-fold. Compound 5a and
293 two isoxazolines had good effects against fipronil-resistant *P. xylostella*. These 3
294 compounds were used to the *PxRdl2* RNAi compounds. The data showed that these
295 three compounds could increase the toxicity to fipronil-resistant *P. xylostella* of
296 *PxRdl2* RNAi compounds (Fig. 6) (log-rank test of 3rd instar larvae for different
297 compounds, fluxametamide: chi-square = 11.47, *df* = 3, *P* = 0.0007; fluralaner:
298 chi-square = 4.964, *df* = 3, *P* = 0.0259; 5a: chi-square = 14.65, *df* = 3, *P* = 0.0001). In
299 addition, the enhanced toxicity mediated by RNAi could reduce the dose required of
300 different compounds, such as fluxametamide (58.06%), fluralaner (43.14%), and 5a
301 (45.61%) (Table 4).

302 **4 DISCUSSION**

303 Recent studies have shown that the existence of multiple copies of homologous *Rdl*
304 further enriches the functional library of GABARs in insects[24,32,33]. These different
305 *Rdl* subunits may combine to produce GABARs with a series of physiological and
306 pharmacological properties[32,33]. It has been reported that knockout of *Rdl1* or *Rdl2*
307 alone does not affect the survival of *H. armigera* or *P. xylostella*[25,34]. These results
308 indicated the functional redundancy of multiple homologous *Rdl* genes in *H. armigera*

and *P. xylostella*. We also tried to knockout both *Rdl* genes in *P. xylostella*, but unfortunately, no viable homozygous knockouts were obtained (the associated data and process were not shown in this paper). This may further illustrate the functional redundancy of the two *Rdls*, which cannot be deleted at the same time.

Three strains of the *Rdl* genotype in *P. xylostella* were used in bioassays of different compounds to clarify the role of the two *Rdls* as anti-compounds. Data from our previous bioassays showed that the *Rdl1*KO strain obtained 10.4-fold resistance to fipronil and that the *Rdl2*KO strains obtained 4.4-fold sensitivity to fipronil compared to the sensitivity of susceptible strains[25]. For 4a, knockout of *PxRdl1* significantly increased resistance, by 2.85-fold, whereas knockout of *PxRdl2* significantly increased susceptibility, by 2.21-fold. 5a is a possible antagonist of GABAR[26], our unpublished results also proved that 5a has a similar trend to 4a and fipronil. This suggested that both *PxRdl1* and *PxRdl2* were involved in mediating fipronil and newly synthesized quinazolines toxicity. Furthermore, *PxRdl2* played an important role in anti-quinazolines activities, and *PxRdl2* was more tolerant than *PxRdl1* to quinazolines. In addition, different pharmacological properties between the two *PxRdls* may resolve their counter contribution to the sensitivities of *P. xylostella* to these compounds.

The novel isoxazoline insecticides fluxametamide and fluralaner were also used in the sensitivity tests of the three strains. The toxicity of the two isoxazolines to the three strains showed different trends, which may verify the novel mode of action compared to that of fipronil and two quinazolines. The key mode of action of avermectin in

invertebrates is to activate glutamate-gated chloride channels (GluCls), although it has also been proposed that avermectin binds to and activates GABAR[35]. However, in our study, knockout of *PxRdl1* or *PxRdl2* did not affect sensitivity to abamectin, confirming that abamectin may not be active on *PxRdl1* or *PxRdl2* individually. Susceptibility to the negative control, chloraniliprole, was not significantly affected by knockout of either *PxRdl1* or *PxRdl2*. In summary, in pharmacological reactions, our results indicated that *PxRdl2* plays an important role in resisting quinazolines and isoxazolines.

Pest control based on RNAi, due to its specificity and minimal or nonexistent impact on nontarget species, provides new opportunities for the development of sustainable IPM plans. Baum et al[36] demonstrated that genetically engineered dsRNA expression in maize (*Zea mays*) could cause larval mortality in the western corn rootworm (WCR), *Diabrotica virgifera*. This aroused great interest from researchers related to the use of dsRNA produced by transgenic plants as a novel pest control agent. This new type of pest control tool has been widely applied in coleoptera because of its high RNAi efficiency[37]. Therefore, it should be further studied and expanded to control other important agricultural pests, such as lepidopteran. The reaction efficiency of lepidopteran insects to RNAi is uneven, and the dsRNA delivery mode will affect its interference efficiency. It has been reported that *H. armigera* and *S. littoralis* were sensitive to orally delivered dsRNA[10,38]. Moreover, there were also reports of RNAi in *P. xylostella* using orally delivered dsRNA[39], which could provide us with references to control *P. xylostella* using RNAi.

353 In our previous research, *PxRdl2* was confirmed to play an important role in the fight
354 against fipronil and the function of *PxRdl2* in fighting quinazolines and isoxazolines
355 was verified in this article. This discovery allows the extension of a new pest control
356 method aimed at enhancing the toxicity of insecticides through RNAi-mediated
357 *PxRdl2* gene silencing in *P. xylostella*. However, the development of efficient, safe
358 and economically sustainable RNAi delivery strategies is vital to achieve this goal. In
359 this paper, we probed the utilization of bacteria as potential carriers of dsRNA
360 targeting the *PxRdl2* gene and evaluated their effects on quinazolines and isoxazolines
361 for the control of *P. xylostella*. The recombinant vector L4440 was transformed into
362 HT115 *E. coli* to induce the expression of a specific dsRNA fragment, which may be
363 the preferred method for the mass production of dsRNA. Expression of dsRNA by
364 bacteria was effective in silencing the target gene *PxRdls* and showed a quantitative
365 dependence when administering *P. xylostella* dsRNA-Bac via leaves (Fig. 3a, Fig. 3b).
366 It was previously reported that the dsRNA molecule was protected by the bacterial
367 envelope and was not easily degraded (in both the environment and the insect gut),
368 which could allow dsRNA to exist/release for a longer time[38,40]. This provides support
369 for the application of RNAi technology in the field, where light (UV) is an important
370 factor affecting the effect of dsRNA. In the laboratory, UV light is an important means
371 for sterilization and digestion of nucleic acids. To better simulate field light factors,
372 UV irradiation was carried out to test the stability of dsRNA-Bac. Compared with *in*
373 *vitro* synthesized dsRNA[41], our data showed that dsRNA-Bac was resistant to UV
374 exposure and needed to be continuously irradiated with strong UV for more than 2 h

obviously to lose part of its activity (Fig. 3c). It was speculated that dsRNA-Bac may be more stable under the protection of the bacterial envelope than dsRNA directly exposed to the external environment. This experiment proved the stability of dsRNA-Bac under light exposure, because UV radiation in the laboratory is several times stronger than the intensity of solar UV radiation in the field, which laid the foundation for the use of dsRNA-Bac for RNAi in the field.

Feeding *P. xylostella* dsRNA-Bac confirmed that the target gene (*PxRdls*) was successfully silenced, which prompted us to evaluate the effect of *PxRdls* gene silencing on insecticidal activity. The data suggested that *PxRdl2* gene silencing induced by dsRNA-Bac has a strong synergistic effect with quinazolines and isoxazolines. Significantly, the mortality of larvae increased in the case of simultaneous feeding of compounds and dsRNA-Bac (Fig. 4). In addition, the theoretically reduced doses of the compounds were calculated and ranged from 45.00% to 72.36%. To further verify these results, *PxRdl1* dsRNA-Bac was used for RNAi compound treatment. For fipronil and the quinazolines and fluxametamide, the results showed that the influence of *PxRdl1* was the opposite of the influence of *PxRdl2* (Fig. 5), which further verifies that *PxRdl2* is a key gene in the response to the compounds. However, fluralaner did not show the exact opposite results (Fig. 5c), and more research may be required to explore the mechanism driving this response. There was high resistance to fipronil and 4a in the fipronil-resistant *P. xylostella* (Table 2). Fortunately, 5a and two isoxazolines were very effective against the fipronil-resistant strain. In the subsequent *PxRdl2* RNAi compounds, silencing the *PxRdl2* gene also

increased the toxicity of the three compounds to the fipronil-resistant strain (Fig. 6). The calculated theoretical reduction in the doses of the three compounds was from 43.14% to 58.06% (Table 4).

In summary, oral *PxRdl2* dsRNA-Bac combined with GABAR compounds could increase the activity of the compounds against *P. xylostella*, and these combinations are suitable for both susceptible and resistant strains. Stable dsRNA-Bac can be prepared by bacteria in large quantities, which greatly reduces the associated costs, supporting the development of pesticide sprays. Therefore, a spray may be developed that includes dsRNA-Bac as a synergist of insecticides, which is beneficial for reducing pesticide doses, delaying pest resistance and protecting nontarget organisms.

ACKNOWLEDGEMENTS

We would like to thank Professor Xiaorong Wang for her support in insect rearing. This work was supported by the National key R&D program of China (2018YFD0200300), the Project of Science and Technology in Guangdong Province (2018A030313188), the Natural Science Foundation of Guangdong Province (2017A030310490) and the Research and Innovation Team of Key Technologies in Modern Agricultural Industry in Guangdong Province (2019KJ130).

Competing financial interests

The authors declare no competing financial interests.

REFERENCES

1 Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC, Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**: 806-811(1998).

2 Koch A and Kogel K, New wind in the sails: Improving the agronomic value of crop plants through RNAi-mediated gene silencing. *Plant Biotechnol J* **12**: 821-831(2014).

3 Zotti M, Dos Santos EA, Cagliari D, Christiaens O, Taning CNT and Smagghe G, RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. *Pest Manag Sci* **74**: 1239-1250(2018).

4 Al-Jamal KT, Nerl H, Müller KH, Ali-Boucetta H, Li S, Haynes PD, Jinschek JR, Prato M, Bianco A, Kostarelos K and Porter AE, Cellular uptake mechanisms of functionalised multi-walled carbon nanotubes by 3D electron tomography imaging. *Nanoscale* DOI: **3**: 2627-2635(2011).

5 Sun Y, Wang P, Abouzaid M, Zhou H, Liu H, Yang P, Lin Y, Hull JJ and Ma W, Nanomaterial□wrapped ds*CYP15C1*, a potential RNAi□based strategy for pest control against *Chilo suppressalis*. *Pest Manag Sci* **76**: 2483-2489(2020).

6 Timmons L, Court DL and Fire A, Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263**: 103-112(2001).

7 Chikami Y, Kawaguchi H, Suzuki T, Yoshioka H, Sato Y, Yaginuma T and Niimi T, Oral RNAi of *diap1* results in rapid reduction of damage to potatoes in

442 *Henosepilachna vigintioctopunctata*. *J Pest Sci* **94**: 505–515(2021).

443 8 Dhandapani RK, Gurusamy D, Duan JJ and Palli SR, RNAi for management of
444 Asian long-horned beetle, *Anoplophora glabripennis*: Identification of target
445 genes. *J Pest Sci* **93**: 823-832(2020).

446 9 Bento FM, Marques RN, Campana FB, Demétrio CG, Leandro RA, Parra JRP and
447 Figueira A, Gene silencing by RNAi via oral delivery of dsRNA by bacteria in
448 the South American tomato pinworm, *Tuta absoluta*. *Pest Manag Sci* 7**6**:
449 287-295(2020).

450 10 Caccia S, Astarita F, Barra E, Di Lelio I, Varricchio P and Pennacchio F,
451 Enhancement of *Bacillus thuringiensis* toxicity by feeding *Spodoptera littoralis*
452 larvae with bacteria expressing immune suppressive dsRNA. *J Pest Sci* **93**:
453 303-314(2020).

454 11 Al BA, Jung JK and Kim Y, Alteration of insulin signaling to control insect pest by
455 using transformed bacteria expressing dsRNA. *Pest Manag Sci* **76**:
456 1020-1030(2019).

457 12 Guan R, Li H and Miao X, RNAi pest control and enhanced BT insecticidal
458 efficiency achieved by dsRNA of chymotrypsin-like genes in *Ostrinia furnacalis*.
459 *J Pest Sci* **90**: 745-757(2017).

460 13 Grzywacz D, Rossbach A, Rauf A, Russell DA, Srinivasan R and Shelton AM,
461 Current control methods for diamondback moth and other brassica insect pests
462 and the prospects for improved management with lepidopteran-resistant Bt
463 vegetable brassicas in Asia and Africa. *Crop Prot* **29**: 68-79(2010).

14 Troczka B, Zimmer CT, Elias J, Schorn C, Bass C, Davies TGE, Field LM, Williamson MS, Slater R and Nauen R, Resistance to diamide insecticides in diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is associated with a mutation in the membrane-spanning domain of the ryanodine receptor. *Insect Biochem Molec* **42**: 873-880(2012).

15 Endersby NM, Viduka K, Baxter SW, Saw J, Heckel DG and McKechnie SW, Widespread pyrethroid resistance in Australian diamondback moth, *Plutella xylostella* (L.), is related to multiple mutations in the para sodium channel gene. *B Entomol Res* **101**: 393-405(2011).

16 Shukla JN, Kalsi M, Sethi A, Narva KE, Fishilevich E, Singh S, Mogilicherla K and Palli SR, Reduced stability and intracellular transport of dsRNA contribute to poor RNAi response in lepidopteran insects. *Rna Biol* **13**: 656-669(2016).

17 Zhang Z, Kong J, De Mandal S, Li S, Zhihua, Zheng, Jin F and Xu X, An immune-responsive PGRP-S1 regulates the expression of antibacterial peptide genes in diamondback moth, *Plutella xylostella* (L.). *Int J Biol Macromol* **142**: 114-124(2019).

18 Pana Z, Xub L, Liua B, Zhang J, Chen Z, Chen Q and Zhua Y, PxAPN5 serves as a functional receptor of Cry2Ab in *Plutella xylostella* (L.) and its binding domain analysis. *Int J Biol Macromol* **105**: 516-521(2017).

19 Sattelle DB, GABA receptors of insects, in: Advances in Insect Physiology, ed. by Evans PD and Wigglesworth VB, Academic Press, pp. 1-113 (1990).

20 Buckingham SD and Sattelle DB, GABA receptors of insects, in: Comprehensive

Molecular Insect Science, ed. by Gilbert LKand Gill SS, pp. 107-142 (2005).

21 Gassel M, Wolf C, Noack S, Williams H and Ilg T, The novel isoxazoline ectoparasiticide fluralaner: Selective inhibition of arthropod γ-aminobutyric acid- and l-glutamate-gated chloride channels and insecticidal/acaricidal activity. *Insect Biochem Molec* **45**: 111-124(2014).

22 Asahi M, Kobayashi M, Kagami T, Nakahira K, Furukawa Y and Ozoe Y, Fluxametamide: A novel isoxazoline insecticide that acts via distinctive antagonism of insect ligand-gated chloride channels. *Pestic Biochem Phys* **151**: 67-72(2018).

23 Ffrench-Constant RH, Mortlock DP, Shaffer CD, MacIntyre RJ and Roush RT, Molecular cloning and transformation of cyclodiene resistance in *Drosophila*: An invertebrate gamma-aminobutyric acid subtype a receptor locus. *Proc Natl Acad Sci U S A* **88**: 7209-7213(1991).

24 Yuan G, Gao W, Yang Y and Wu Y, Molecular cloning, genomic structure, and genetic mapping of two Rdl-orthologous genes of GABA receptors in the diamondback moth, *Plutella xylostella*. *Arch Insect Biochem* **74**: 81-90(2010).

25 Li BJ, Wang KK, Chen DP, Yan Y, Cai XL, Chen HM, Dong K, Lin F and Xu HH, Distinct roles of two RDL GABA receptors in fipronil action in the diamondback moth (*Plutella xylostella*). *Insect Sci* **0***:* 1-13 (2021).

26 Jiang X, Yang S, Yan Y, Lin F, Zhang L, Zhao W, Zhao C and Xu H, Design, Synthesis, and Insecticidal Activity of 5,5-Disubstituted 4,5-Dihydropyrazolo[1,5-a]quinazolines as Novel Antagonists of GABA

508 Receptors. *J Agr Food Chem* **68**: 15005-15014(2020).

509 27 Yang S, Lai Q, Lai F, Jiang X, Zhao C and Xu H, Design, synthesis, and
510 insecticidal activities of novel 5-substituted
511 4,5-dihydropyrazolo[1,5-a]quinazoline derivatives. *Pest Manag Sci* **77**:
512 1013-1022(2021).

513 28 Kostylev M, Otwell AE, Richardson RE and Suzuki Y, Cloning should be simple:
514 *Escherichia coli* DH5α-Mediated assembly of multiple DNA fragments with
515 short end homologies. *Plos One* **10**: e0137466(2015).

516 29 Motohashi K, Evaluation of the efficiency and utility of recombinant enzyme-free
517 seamless DNA cloning methods. *Biochemistry and Biophysics Reports* **9**:
518 310-315(2017).

519 30 Rutledge RG, Mathematics of quantitative kinetic PCR and the application of
520 standard curves. *Nucleic Acids Res* **31**: e93(2003).

521 31 Livak KJ and Schmittgen TD, Analysis of relative gene expression data using
522 Real-Time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. **25**: 402-408(2001).

523 32 Del Villar Sand Jones A, Cloning and functional characterisation of the duplicated
524 RDL subunits from the pea Aphid, *Acyrthosiphon pisum*. *Int J Mol* **19**:
525 2235(2018).

526 33 Ménard C, Folacci M, Brunello L, Charreton M, Collet C, Mary R, Rousset M,
527 Thibaud J, Vignes M, Charnet P and Cens T, Multiple combinations of RDL
528 subunits diversify the repertoire of GABA receptors in the honey bee parasite
529 *Varroa destructor*. *J Biol Chem* **293**: 19012-19024(2018).

530 34 Wang J, Zhao X, Yan R, Wu S, Wu Y and Yang Y, Reverse genetics reveals
531 contrary effects of two Rdl-homologous GABA receptors of *Helicoverpa*
532 *armigera* on the toxicity of cyclodiene insecticides. *Pestic Biochem Phys* **170**:
533 104699(2020).
534 35 Wolstenholme AJ, Glutamate-gated chloride channels. *J Biol Chem* **287**:
535 40232-40238(2012).
536 36 Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S,
537 Plaetinck G, Munyikwa T, Pleau M, Vaughn T and Roberts J, Control of
538 coleopteran insect pests through RNA interference. *Nat Biotechnol* **25**:
539 1322-1326(2007).
540 37 Zhu KY and Palli SR, Mechanisms, applications, and challenges of insect RNA
541 interference. *Annu Rev Entomol* **65**: 293-311(2020).
542 38 Lim ZX, Robinson KE, Jain RG, Sharath Chandra G, Asokan R, Asgari S and
543 Mitter N, Diet-delivered RNAi in *Helicoverpa armigera* – Progresses and
544 challenges. *J Insect Physiol* **85**: 86-93(2016).
545 39 Guo Z, Kang S, Zhu X, Xia J, Wu Q, Wang S, Xie W and Zhang Y, The novel ABC
546 transporter ABCH1 is a potential target for RNAi-based insect pest control and
547 resistance management. *Sci Rep-Uk* **5**: 13728(2015).
548 40 Vatanparast Mand Kim Y, Optimization of recombinant bacteria expressing dsRNA
549 to enhance insecticidal activity against a lepidopteran insect, *Spodoptera exigua*.
550 *Plos One* **12**: e183054(2017).
551 41 San Miguel K and Scott JG, The next generation of insecticides: dsRNA is stable

as a foliar-applied insecticide. *Pest Manag Sci* **72**: 801-809(2016).

Figure Legends

Fig. 1 Resistance levels of the *Rdl1*KO (*PxRdl1* knockout strain) and the *Rdl2*KO (*PxRdl2* knockout strain) to chemical insecticides compared with the susceptible strain of *Plutella xylostella*. Resistance ratio = LC_{50} of *Rdl1*KO or *Rdl2*KO divided by LC_{50} of susceptible. * mean their LC_{50}s are significantly different from susceptible.

Fig. 2 Production of HT115 *Escherichia coli* cells expressing dsRNA. **a** Expression of dsRNA by transformed HT115 *E. coli*; the total RNA were carried out to RT-PCR, and the production of amplification were resolved on 1% agarose gel. The specific primers used for *GFP*, *PxRdl1* or *PxRdl2* genes produced amplicons of the expected fragments in transformed HT115 *E. coli* (lines 1, 2 and 3), whereas the same primers did not produced any fragment from non-transformed HT115 *E. coli* (WT) (lines 4, 5 and 6). **b c** and **d** Standard curves used on qRT-PCR absolute quantification of *GFP*, *PxRdl1* and *PxRdl2* dsRNA produced by *E. coli* suspensions.

Fig. 3 mRNA expression of *PxRdls* gene in *Plutella xylostella* 3rd instar larvae orally treated for 2 days with dsRNA-Bac. **a** and **b** The *PxRdl1* and *PxRdl2* gene are down-regulated upon ingestion of dsRNA-Bac administered by leaves, which was dose-dependent; **c** The *PxRdl2* dsRNA-Bac treated different UV irradiation times (0h, 1h, 2h, 4h) was fed to *P. xylostella* 3rd instar larvae for 2 days. UV irradiation for less than 1h had no obvious effect on the activity of dsRNA-Bac. *GFP* dsRNA-Bac were used as control. The values are the mean ± standard errors (* $P < 0.05$, Tukey' s test).

574 **Fig. 4** *PxRdl2* RNAi-compounds in susceptible *Plutella xylostella* 3rd instar larvae

575 Susceptible 3rd instar larvae were treated for 2 days with leaves containing with

576 *PxRdl2* dsRNA-Bac (corresponding to 400 ng μL^{-1} of dsRNA) and with compounds

577 (LC_{20}). **a** for fipronil, * $P = 0.0339$; **b** for fluxametamide, *** $P = 0.0001$; **c** for

578 fluralaner *** $P = 0.0004$; **d** for 5a *** $P = 0.0001$; **e** for 4a *** $P < 0.0001$ (based on

579 log-rank test).

580 **Fig. 5** *PxRdl1* RNAi-compounds in susceptible *Plutella xylostella* 3rd instar larvae

581 Susceptible 3rd instar larvae were treated for 2 days with leaves containing with

582 *PxRdl1* dsRNA-Bac (corresponding to 400 ng μL^{-1} of dsRNA) and with compounds

583 (LC_{50}). **a** for fipronil, *** $P = 0.0009$; **b** for fluxametamide, * $P = 0.0307$; **c** for

584 fluralaner $P = 0.4503$; **d** for 5a * $P = 0.0414$; **e** for 4a ** $P = 0.0029$ (based on

585 log-rank test).

586 **Fig. 6** *PxRdl2* RNAi-compounds in fipronil-resistant *Plutella xylostella* 3rd instar

587 larvae

588 Fipronil-resistant 3rd instar larvae were treated for 2 days with leaves containing with

589 *PxRdl2* dsRNA-Bac (corresponding to 400 ng μL^{-1} of dsRNA) and with compounds

590 (LC_{20}). **a** for fluxametamide, *** $P = 0.0007$; **b** for fluralaner, * $P = 0.0259$; **c** for 5a,

591 *** $P = 0.0001$ (based on log-rank test).

592 Table 1 Detailed information of primers used in this study.

Primer name	Nucleotide sequence (5'-3')	Function
GFP-F	CAGTGCTTCAGCCGCTACCC	Amplification of *GFP*
GFP-R	AGTTCACCTTGATGCCGTTCTT	
Rdl1-F	AGCAATACTGGATTCATT	Amplification of *PxRdl1*
Rdl1-R	TCTCTATATGGCACAGTTGT	
Rdl2-F	TGGTCTTCGCTAGTCTTT	Amplification of *PxRdl2*
Rdl2-R	AATTTGTTGATTCCCTTC	
T7-*GFP*-F[a]	**TAATACGACTCACTATA**GGGC AGTGCTTCAGCCGCTACCC	*In vitro* synthesis of *GFP* dsRNA
T7-*GFP*-R[a]	**TAATACGACTCACTATA**GGG AGTTCACCTTGATGCCGTTCTT	
T7-*Rdl1*-F[a]	**TAATACGACTCACTATA**GGG AGCAATACTGGATTC	*In vitro* synthesis of *PxRdl1* dsRNA
T7-*Rdl1*-R[a]	**TAATACGACTCACTATA**GGGT CTCTATATGGCACA	
T7-*Rdl2*-F[a]	**TAATACGACTCACTATA**GGGT GGTCTTCGCTAGTCT	*In vitro* synthesis of *PxRdl2* dsRNA
T7-*Rdl2*-R[a]	**TAATACGACTCACTATA**GGGA TTTGTTGATTCCCTTCG	
qPCR-AQ-*GFP*-F	GCCGCTACCCCGACCACAT	Absolute quantification of dsRNA produced by the transformed bacteria
qPCR-AQ-*GFP*-R	CGCCCTCGAACTTCACCTC	
qPCR-AQ-*Rdl1*-F	GCGTAGAGACATTATCAGTT	
qPCR-AQ-*Rdl1*-R	AATTCGTTGCTCGTAGTAG	
qPCR-AQ-*Rdl2*-F	GGCTGCTGAGAAGAAA	
qPCR-AQ-*Rdl2*-R	GGGAGTGTGGGACCTA	
qPCR-*Actin*-F	TGGCACCACACCTTCTAC	Relative quantification of *PxRdls* transcription
qPCR-*Actin*-R	CATGATCTGGGTCATCTTCT	
qPCR-*Rdl1*-F	GAAGTTGCCTCCAGACTGC	
qPCR-*Rdl1*-R	CCACCCTTTGAATGTGCC	
qPCR-*Rdl2*-F	GTCAGTCAGCTACGACAAACG	
qPCR-*Rdl2*-R	AAATCCAGGGTAAAATCCAT	

[a]The bold nucleotide sequences are the T7 promoter.

593 Table 2 Toxicity of different compounds to susceptible, fipronil-resistant and *Rdl*KO
594 *Plutella xylostella*.
595

Compound[a]	Strain[b]	N[c]	Slope ± SE	LC_{50}(mg L^{-1}) (95% FL[d])	RR[e]
fipronil[25]	susceptible	238	2.66 ± 0.02	0.22 (0.19-0.26)	-
	*Rdl1*KO	242	2.27 ± 0.22	2.29 (1.90-2.76)	10.41
	*Rdl2*KO	239	2.58 ± 0.01	0.05 (0.04-0.06)	0.23
	resistant	243	4.43 ± 0.48	413.83 (366.49-459.29)	1881.05
fluxametamide	susceptible	246	2.65 ± 0.43	0.079 (0.051-0.59)	-
	*Rdl1*KO	252	4.06 ± 0.44	0.042 (0.037-0.047)	0.53
	*Rdl2*KO	253	3.21 ± 0.37	0.043 (0.038-0.050)	0.54
	resistant	247	2.19 ± 0.21	0.31 (0.18-0.51)	3.92
fluralaner	susceptible	243	4.40 ± 0.85	0.010 (0.0090-0.012)	-
	*Rdl1*KO	242	5.53 ± 0.73	0.0090 (0.0060-0.015)	0.90
	*Rdl2*KO	252	4.67 ± 0.48	0.0040 (0.0040-0.0050)	0.40
	resistant	246	2.44 ± 0.31	0.065 (0.033-0.333)	6.50
5a	susceptible	244	3.16 ± 0.35	1.74 (0.86-3.18)	-
	resistant	244	3.19 ± 0.41	5.24 (4.58-5.94)	3.01
4a	susceptible	241	3.01 ± 0.34	3.93 (3.41-4.60)	-
	*Rdl1*KO	242	5.32 ± 0.58	11.19 (10.32-12.17)	2.85
	*Rdl2*KO	251	2.50 ± 0.29	1.78 (1.51-2.11)	0.45
	resistant	250	4.41 ± 0.50	57.95 (40.35-79.44)	14.75
abamectin	susceptible	252	1.69 ± 0.24	0.012 (0.0080-0.015)	-
	*Rdl1*KO	236	2.30 ± 0.27	0.015 (0.012-0.018)	1.25
	*Rdl2*KO	254	2.33 ± 0.25	0.016 (0.014-0.020)	1.33
chloraniliprole	susceptible	236	1.30 ± 0.19	1.32 (0.88-1.81)	-
	*Rdl1*KO	233	1.41 ± 0.19	1.70 (1.23-2.27)	1.29
	*Rdl2*KO	238	1.11 ± 0.18	1.58 (1.03-2.25)	1.20

[a]4a is a compound synthesized by Shuai Yang et al., 2020; 5a is a compound synthesized by Xunyuan Jiang et al., 2020 (same below) (Only susceptible and resistant data is showed).
[b]Susceptible: susceptible strain; *Rdl*1KO: *PxRdl1* knockout strain; *Rdl2*KO: *PxRdl2* knockout strain; resistant: fipronil-resistant strain.
[c]Number of larvae tested.
[d]95% Fiducial limits.
[e]Resistance ratio was calculated by LC_{50} of *Rdl1*KO, *Rdl2*KO or resistant divided by LC_{50} of susceptible.

596 Table 3 The LCs of 5 compounds in susceptible *Plutella xylostella*

597

compound	LC_{20}[a]	LC_{45}[b] (45%)[c]	LC_{55}[b] (55%)[c]	LC_{60}[b] (60%)[c]	LC_{65}[b] (65%)[c]	LC_{80}[b] (80%)[c]	Reduce dosage(%)[d]	LC_{50}[e]
fipronil	0.11	0.20					45.00%	0.22
fluxametamide	0.038		0.088				56.82%	0.079
fluralaner	0.0060			0.011			45.45%	0.010
5a	0.94				2.31		59.31%	1.74
4a	2.07					7.49	72.36%	3.93

[a]LC_{20} for *PxRdl2* RNAi-compounds.

[b]LCs: The dose corresponding to the mortality of the compounds used alone.

[c]The final mortality of *PxRdl2* RNAi-compounds after 48 h.

[d]Theoretically reduce dosage = (LCs – LC_{20} of the *PxRdl2* RNAi compounds)/LCs × 100%.

[e]LC_{50} for *PxRdl1* RNAi-compounds.

598

599

600

601 Table 4 The LCs of 5 compounds in fipronil-resistant *Plutella xylostella*

602

compound[a]	LC_{20}[b]	LC_{40}[c] (40%)[d]	LC_{50}[c] (50%)[d]	Reduce dosage(%)[e]
fipronil	267.24	-		
fluxametamide	0.13		0.31	58.06%
fluralaner	0.029	0.051		43.14%
5a	2.85		5.24	45.61%
4a	37.34	-		

[a]High resistance to fipronil and 4a, no RNAi-compounds.

[b]LC_{20} for *PxRdl2* RNAi-compounds.

[c]LCs: The dose corresponding to the mortality of the compound used alone.

[d]The final mortality of *PxRdl2* RNAi-compounds after 48 h.

[e]Theoretically reduce dosage = (LCs – LC_{20} of the *PxRdl2* RNAi compounds)/LCs × 100%.

603

Figure 1

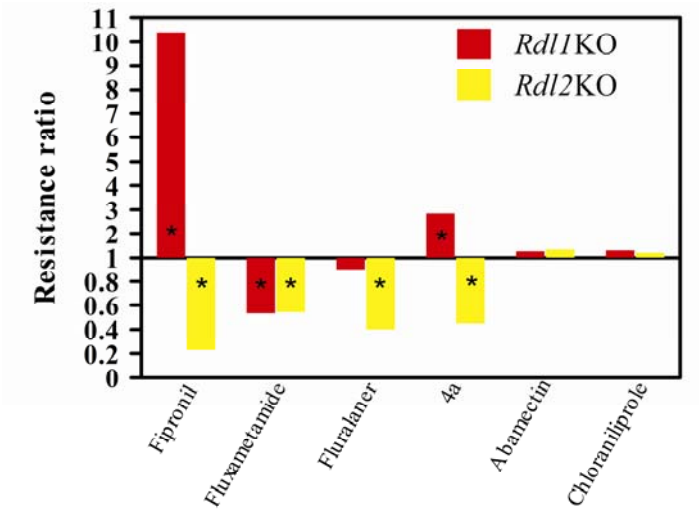

Figure 2

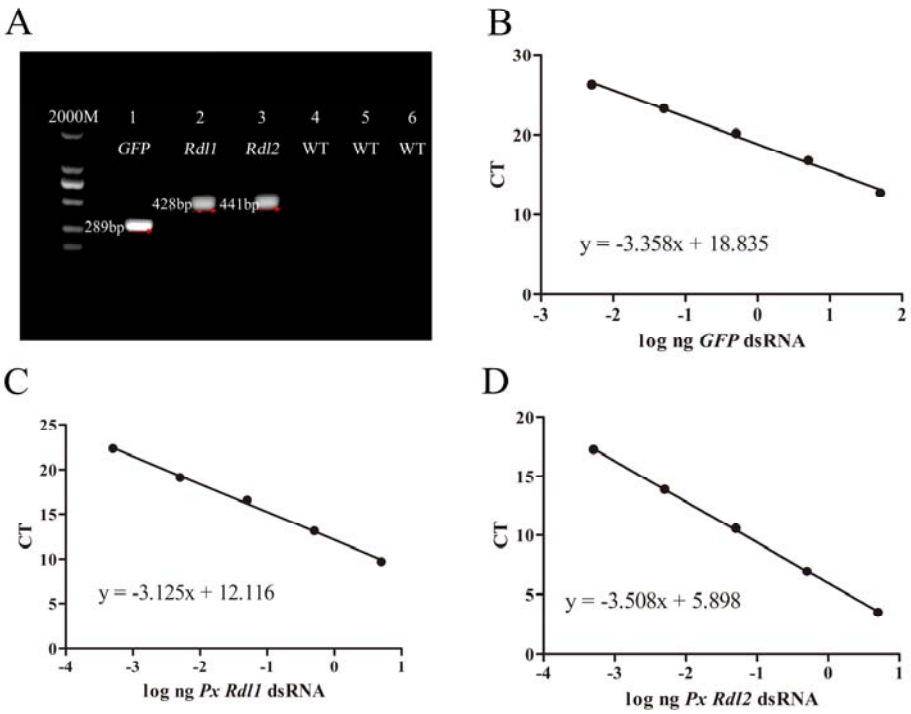

Figure 3

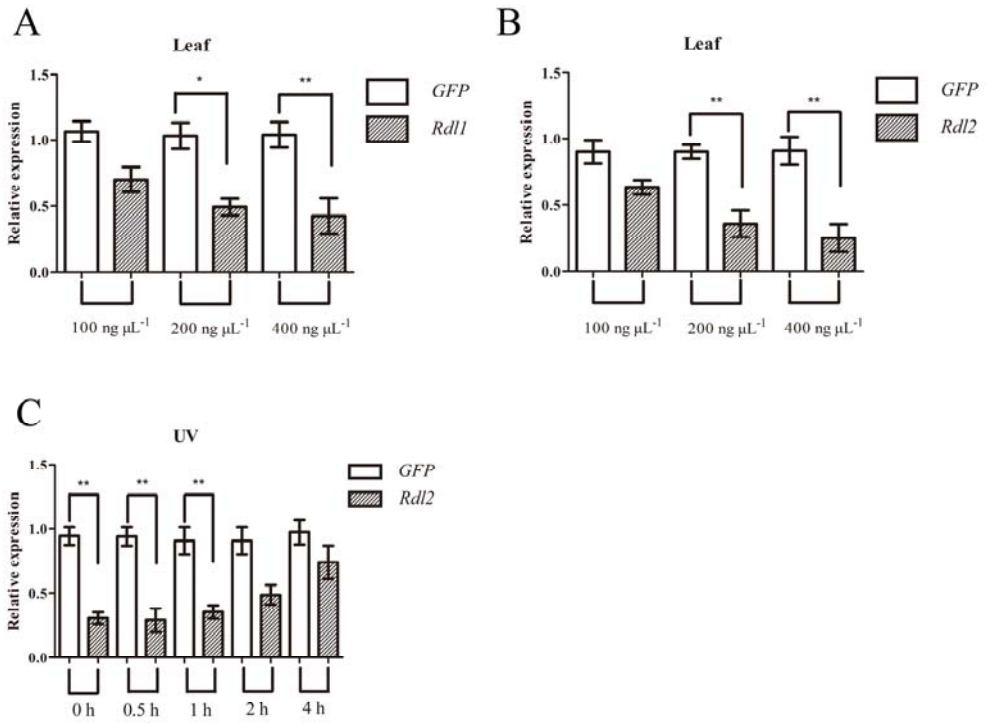

Figure 4

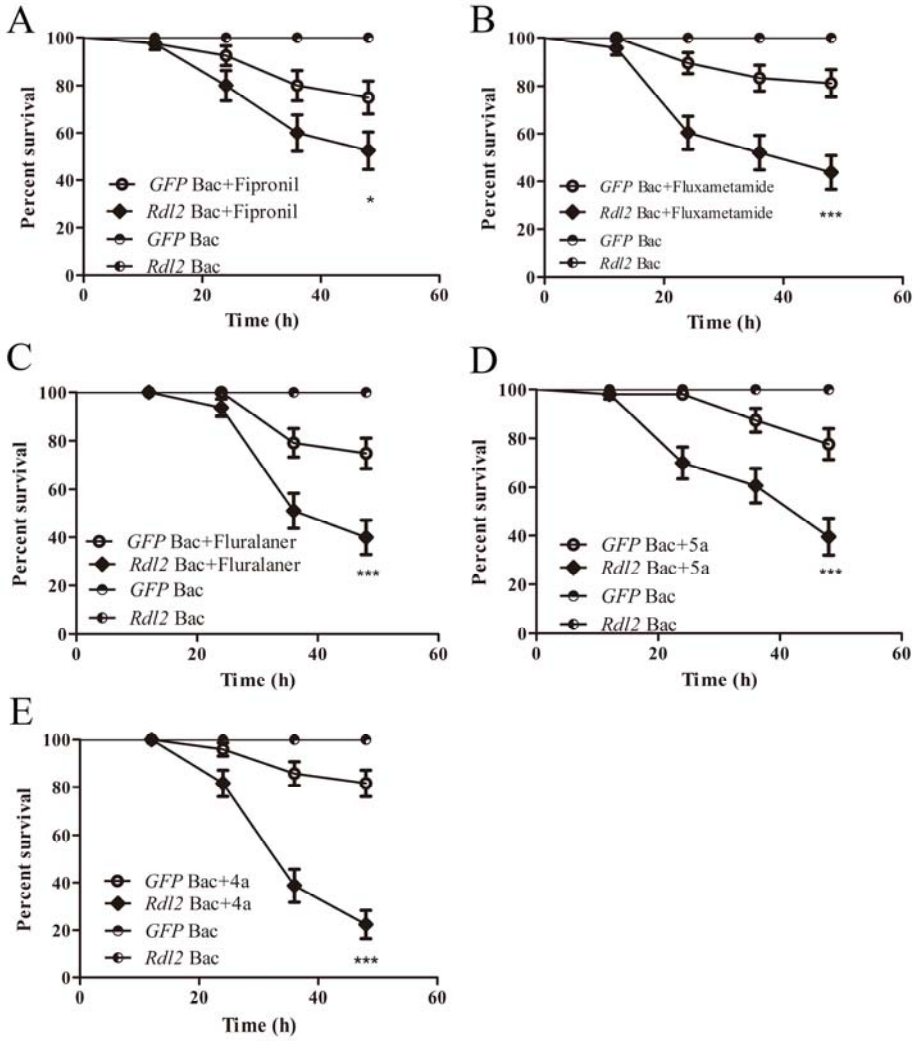

Figure 5

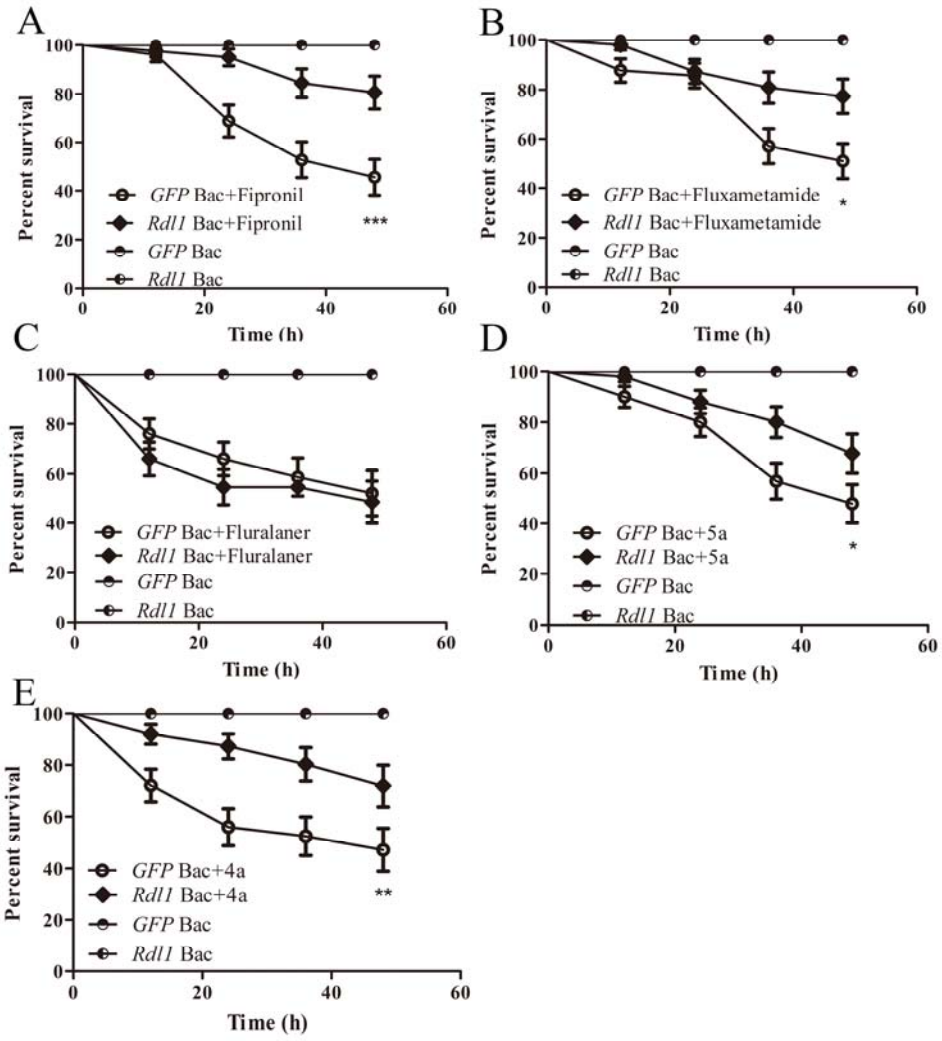

Figure 6