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Status and preliminary mechanism of resistance to insecticides in a field strain of housefly (*Musca domestica*, L)

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ABSTRACT

Resistance profiles of houseflies (Gol-RR) collected from a field in Golmud city, Qinghai province, China, were determined for seven insecticides using topical bioassays. Resistance ratios of >1219.51, 153.17, >35.43, 6.12, 3.24, 1.73, and 0.86-fold were obtained for propoxur, cypermethrin, imidacloprid, indoxacarb, chlorpyrifos, fipronil, and chlorfenapyr, respectively, relative to a laboratory susceptible strain (SS). Synergism experiments showed that piperonyl butoxide (PBO), triphenylphosphate (TPP), and diethyl maleate (DEM) increased propoxur toxicity by >105.71, >7.88, and >5.15-fold in the Gol-RR strain, compared with 5.25, 2.00, and 1.39-fold in the SS strain, indicating the involvement of P450 monooxygenases, esterases, and glutathione-S-transferase in conferring resistance. Although cypermethrin resistance was significantly suppressed with PBO, TPP, and DEM in the Gol-RR strain, the synergistic potential of these agents to cypermethrin was similar in the SS strain, demonstrating that metabolism-mediated detoxification was not important for conferring resistance to cypermethrin in the Gol-RR strain. However, the three agents did not act synergistically with imidacloprid, indicating that other mechanisms may be responsible for the development of resistance to this insecticide. Acetylcholinesterase (AChE) activity was 13.70-fold higher in the Gol-RR than in the SS strain, suggesting the properties of the AChE enzyme were altered in the Gol-RR strain. Thus, rotation of chlorfenapyr insecticide with other agents acting through a different mode with minimal/no resistance could be an effective resistance management strategy for housefly.

Introduction

The housefly, *Musca domestica*, L., is a common insect that is widely distributed at waste sites such as livestock farms and garbage dumps. This species can spread various diseases, affecting the health of people and livestock, and carriage of >100 animal intestinal diseases has been reported (Pavela, 2008; Scott, 2016). Various measures to prevent and control diseases are practiced, among which chemical pesticide spraying is the most common. However, with elevated use of insecticides, the housefly is becoming increasingly resistant, exacerbated by its high fecundity and short lifespan. Many researchers have shown using laboratory experiments that the speed of resistance to many insecticides in housefly is surprisingly rapid. For example, a spinosad-selected strain developed a resistance ratio of 279-fold compared with a susceptible strain after selection on only 27 generations (G27) (Shi et al., 2010), 149.26-fold for emamectin (G5) (Khan et al., 2016), 4419.07-fold for beta-cypermethrin (G27) (Zhang et al., 2007), 211-fold for cyromazine (G7) (Khan and Akram, 2017), 430-fold for fipronil (G26) (Abbas et al., 2016), 106-fold for imidacloprid (G14) (Khan et al., 2014), 5945-fold for permethrin (G22) (Scott and Georghiou, 1985), and 176.34-fold for deltamethrin (G6) (Khan et al., 2015).

In China, housefly resistance to insecticides has also been demonstrated in the field (Huang et al., 2015), and is monitored annually by the department for Disease Control and Prevention (CDC). Wild populations often display multi-resistance, but the mechanisms remain poorly understood. Golmud city is located in the west of Qinghai province, China. Animal husbandry in this vast region generates animal faeces that provide an ideal place for housefly breeding. In the present study, we investigated insecticide resistance in houseflies at a refuse dump in the city, and explored

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the mechanisms of resistance in order to develop a strategy for controlling houseflies in this area.

Materials and methods

Housefly strains

Two housefly strains were used in the present study: a laboratory susceptible strain (SS), and a field strain (Gol-RR) collected from a rubbish dump in Golmud city, Qinghai province, China, in July 2017. Both strains were maintained in the laboratory as previous reported (Shi et al., 2002).

Compounds

Propoxur (94%), cypermethrin (95%), chlorpyrifos (98%), imidacloprid (95.2%), fipronil (95.7%), indoxacarb (93%), and chlorfenapyr (98.3%) were obtained from Shanghai Forever Chemical Co., Ltd. Reagent grade triphenylphosphate (TPP, 98%) was from Sinopharm Group Chemical Reagent, (Shanghai, China). Diethyl maleate (DEM, 98%) and piperonyl butoxide (PBO, 98%) were purchased from Chem. Service (West Chester, PA). Acetylthiocholine iodide, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), Triton X-100, and eserine were purchased from Sigma Chemical Company.

Bioassays

Bioassays were performed using the topical method as described previously (Shi et al., 2002) with slight modifications. Droplets (l μl) containing six different concentrations (equal proportion) of insecticide (or l μl acetone as control) were applied to the dorsal thorax of 30 (3–4 day old) female houseflies using a mircoapplicator (Burkard, UK), which were then anesthetised for 45 s using ether. In order to evaluate the detoxification activity of P450 monooxygenases (P450), esterases (ESTs), and glutathione-S-transferase (GST), the maximum non-lethal concentration (0.1%) of PBO, TPP, and DEM were applied 1 h before the insecticide treatment. The temperature was maintained at 26 ± 1 °C throughout the bioassay period. All experiments were repeated in triplicate.

Acetylcholinesterase (AChE) enzyme activity assays

AChE activity was determined as described previously (Shi et al., 2002) with slight modifications. Head tissue from 20 adults was homogenised in 1 ml ice-cold 0.1 M sodium phosphate buffer, pH 7.0, containing 1% Triton X-100. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C, and the supernatant was collected and used as enzyme solution. A 10 μl sample of enzyme solution (or 10 μl buffer solution as control), 100 μl 75 mM ATCh, 200 μl 0.1 mM DTNB, and 2.19 ml buffer solution were incubated at 25 °C for 10 min. The reaction was stopped by the addition of 0.5 ml 0.01 mM eserine. The optical density (OD) at 412 nm was recorded with a Beckman DU-65 spectrophotometer.

Protein assays

Protein content was determined using the Bradford method (Bradford, 1976) with bovine serum albumin as a standard.

Data analysis

Analysis of 50% lethal dose (LD_{50}) values was performed using PoloPlus software. LD_{50} ratios were considered to be significantly different based on non-overlapping of 95% confidence intervals of LD_{50} and the 95% confidence intervals of RR or SR excluding 1.0 (Robertson et al., 2007). Significant differences of AChE enzyme activity between the SS and the Gol-RR strains were determined by Tukey's honestly significant difference tests following ANOVA ($p < 0.05$). Mortality was corrected using the Abbott's formula (Abbott, 1925).

Results

Toxicity of seven insecticides against SS and Gol-RR strains

Based on the LD_{50} values listed in Table 1, the SS strain showed the highest susceptibility to fipronil, followed by cypermethrin, chlorfenapyr, chlorpyrifos, indoxacarb, propoxur, and imidacloprid. However, the susceptibility of the Gol-RR strain to these insecticides was significantly reduced compared with the SS strain (non-overlapping of 95% CL of LD_{50} and 95% confidence intervals of RR excluding 1.0), except for chlorfenapyr. According to the reduction in the degree of sensitivity (from high to low), resistance ratios (RR) were >1219.51-fold for propoxur), followed by cypermethrin (153.17-fold), imidacloprid (>35.43-fold), indoxacarb (6.12-fold), chlorpyrifos (3.24-fold), fipronil (1.73-fold), and chlorfenapyr (0.86-fold).

Synergistic effects of detoxification enzyme inhibitors

The effects of PBO, TPP, and DEM on the toxicity of three insecticides (propoxur, cypermethrin, and imidacloprid) against the Gol-RR and SS strains are shown in Table 2. PBO, TPP, and DEM showed high synergy with propoxur in the Gol-RR strain (non-overlapping of 95% CL of LD_{50} and 95% confidence intervals of RR excluding 1.0). Synergism ratios were >105.71, >7.88, and >5.15 in the Gol-RR strain. Although cypermethrin resistance was significantly suppressed with PBO, TPP, and DEM in the Gol-RR strain, the synergistic potential of PBO, TPP, and DEM with cypermethrin in the Gol-RR strain was similar to that in the SS strain. However, PBO, TPP, and DEM did not increase the toxicity of imidacloprid against the Gol-RR strain, suggesting that other mechanisms may be responsible for the development of resistance to this insecticide.

AChE activity assays

The activity of AChE was tested in both strains, and the results are presented in Table 3. AChE activity in the Gol-RR strain was 13.70-fold ($p < 0.05$) higher than that in the SS strain, suggesting the properties of the AChE enzyme were altered in the Gol-RR strain.

Discussion

Consistent with a previous report (Khan et al., 2013), the results of the present study showed that the Gol-RR strain is very highly resistant to propoxur and cypermethrin, and highly resistant to imidacloprid, but has low resistance to indoxacarb, chlorpyrifos, and fipronil, and no resistance to chlorfenapyr, relative to the SS strain. The resistance status of the housefly strain collected from the field is consistent with the actual housefly control situation in China. Additionally, the China pesticide registration database (http://www.chinapesticide.gov.cn/hysj/index.jhtml) revealed that most insecticides currently used for the control of houseflies are typical traditional insecticides such as organophosphates, carbamates, and pyrethroids). Domestic reports of resistance in housefly are mainly focused on these three pesticide classes, and there are few reports of resistance to other types of insecticides. In the present study, we found that the Gol-RR strain has high resistance to imidacloprid, which may be related to the use of imidacloprid in China for the control of housefly. Wei et al. (2013) previously reported high resistance to pyrethroid insecticide

Table 1
Toxicity of various insecticides against SS and Gol-RR houseflies.

Insecticide	Strain	LD_{50} (95% CI) (10 μg/fly)	Slope (SE)	χ^2 (df)	RR (95% CI)
Propoxur	SS	3.28 (2.63–4.10)	3.84 (0.36)	3.48(4)	
	Gol-RR	>4000[b]			>1219.51[a]
Cypermethrin	SS	0.13 (0.07–0.17)	3.37 (0.29)	1.36(4)	
	Gol-RR	19.65 (15.86–24.10)	2.90 (0.20)	2.44(4)	153.17[a] (101.78–230.52)
Imidacloprid	SS	11.29 (5.76–17.15)	1.52 (0.13)	1.33(4)	
	Gol-RR	>400[c]			>35.43[a]
Indoxacarb	SS	1.13 (0.86–1.46)	2.75 (0.22)	4.48(4)	
	Gol-RR	6.91 (2.93–10.66)	1.79 (0.14)	1.13(4)	6.12[a] (2.45–8.89)
Chlorpyrifos	SS	0.82 (0.53–1.36)	2.91 (0.30)	7.79(4)	
	Gol-RR	2.65 (2.18–3.23)	3.16 (0.29)	0.94(4)	3.24[a] (2.44–4.30)
Fipronil	SS	0.07 (0.05–0.09)	2.93 (0.27)	1.75(4)	
	Gol-RR	0.12 (0.08–0.18)	2.72 (0.28)	4.57(4)	1.73[a] (1.21–2.48)
Chlorfenapyr	SS	0.14(0.07–0.20)	2.29 (0.24)	1.31(4)	
	Gol-RR	0.12 (0.04–0.20)	1.60 (0.14)	1.31(4)	0.86 (0.38–1.94)

Resistance ratio, RR = LD_{50} of Gol-RR strain/LD_{50} of SS strain.
[a] Significant differences based on non-overlapping of 95% confidence intervals of LD_{50} and the 95% confidence intervals of RR excluding 1.0.
[b] Mortality is only 10%.
[c] Mortality is only 20%.

Table 2
Synergism of PBO, TPP, and DEM with other insecticides in SS and Gol-RR houseflies.

Strain	Insecticide	LD_{50} (95% CI) (10 μg/fly)	Slope (SE)	χ^2	SR (95% CI)
SS	Propoxur	3.28 (2.63–4.10)	3.84 (0.36)	3.48(4)	
	+PBO	0.63 (0.49–0.82)	3.08 (0.28)	3.53(4)	5.25[a] (3.81–7.25)
	+TPP	1.64 (1.26–2.09)	3.20 (0.27)	1.56(4)	2.00[a] (1.45–2.77)
	+DEM	2.36 (1.82–3.04)	2.92 (0.26)	2.71(4)	1.39 (0.99–1.93)
	Cypermethrin	0.13 (0.07–0.17)	3.37 (0.33)	1.36(4)	
	+PBO	0.01 (0.01–0.01)	4.23 (0.41)	3.55 (4)	11.24[a] (7.60–16.62)
	+TPP	0.02 (0.02–0.03)	2.43 (0.19)	1.51(4)	5.75[a] (3.69–8.98)
	+DEM	0.09 (0.06–0.14)	2.52 (0.26)	4.61(4)	1.39 (0.89–2.16)
	Imidacloprid	11.29 (5.76–17.15)	1.52 (0.14)	1.33(4)	
	+PBO	2.58 (1.64–3.58)	2.00 (0.21)	0.61(4)	4.38[a] (2.36–8.13)
	+TPP	8.46 (5.61–12.68)	1.50 (0.16)	1.46(4)	1.34 (0.71–2.51)
	+DEM	4.08 (2.97–5.36)	2.57 (0.23)	1.51(4)	2.78[a] (1.56–4.91)
Gol-RR	Propoxur	>4000			
	+PBO	37.84 (27.01–57.94)	1.83 (0.17)	2.89(4)	>105.71[a]
	+TPP	507.93 (331.46–1000.72)	1.46 (0.11)	1.54(4)	>7.88[a]
	+DEM	776.99 (490.27–1842.58)	1.54 (0.14)	0.75(4)	>5.15[a]
	Cypermethrin	19.65 (15.86–24.10)	2.90 (0.30)	2.44(4)	
	+PBO	1.02 (0.73–1.47)	1.80 (0.17)	2.22(4)	19.20[a] (12.91–28.57)
	+TPP	3.35 (2.18–4.69)	1.84 (0.16)	2.01(4)	5.87[a] (3.86–8.92)
	+DEM	9.85 (7.80–12.54)	3.36 (0.34)	1.42(4)	1.99[a] (1.46–2.72)
	Imidacloprid	>400			
	+PBO	>400			
	+TPP	>400			
	+DEM	>400			

Synergistic ratio, SR = LD_{50} without synergist/LD_{50} with synergist.
[a] Significant differences based on non-overlapping of 95% confidence intervals of LD_{50} and the 95% confidence intervals of SR excluding 1.0.

(deltamethrin) and low levels of cross-resistance to Imidacloprid in housefly. Surprisingly, our results showed that the Gol-RR strain was very highly resistant to propoxur, however, the resistance ratio to chlorpyrifos was low (only 3.24-fold). These results suggest there might be differences in the major resistance mechanism(s) to propoxur and chlorpyrifos, even though they share the same target (AChE) for insecticide action. Low resistance to fipronil was also observed in the Gol-RR strain, possibly reflecting its low usage due to the short period from registration and relatively high cost compared with carbamates, organophosphates, and pyrethroids.

Low resistance to indoxacarb is probably due to cross-resistance, since this agent has not been registered for control of houseflies. The Gol-RR strain exhibited no cross-resistance to chlorfenapyr (also not registered for controlling houseflies), with a resistance ratio of only 0.86-fold. Similar results were reported for *Culex pipiens pallens* regarding resistance to cypermethrin (Yuan et al., 2015), and some researchers have even reported negative cross-resistance in some insects including housefly (Scott et al., 2004), horn fly (Sheppard and Joyce, 1998), and tobacco budworm (Pimprale et al., 1997). This insecticide has been recommended by the WHO for use in public health due to only slight toxicity to humans (WHO toxicological classification III). However, it has not been registered for the control of houseflies in china. Chlorfenapyr is a pyrrole insecticide with a unique mechanism of action that does not confer cross-resistance to neurotoxic insecticides (Raghavendra et al., 2011), hence it is a candidate for controlling houseflies.

The biochemical mechanism of resistance was investigated, and PBO, TPP, and DEM had a more significant effect on the toxicity of propoxur in the Gol-RR strain than the SS strain, suggesting that metabolic detoxification is involved in resistance to propoxur. The biochemical mechanism of propoxur resistance has been investigated previously in German cockroach, and propoxur resistance was suppressed with PBO and DEF (TPP and DEM are inhibitors of esterase) (Chai and Lee, 2010; Sanchez-Arroyo et al., 2001). In

Table 3
Activity of AChE in SS and Gol-RR strains of houseflies.

Target enzymes	Strains	Activity[a] (SE)	AR
AChE	SS	0.20 (0.02)	
	Gol-RR	2.74 (0.15)	13.70[b]

[a] Activity = OD/min/mg protein.
Activity ratio, AR = activity of the Gol-RR strain/the SS strain.
[b] Indicates the value is significantly different from that of the SS strain ($p < 0.05$).

the present study, synergism tests on cypermethrin showed that cytochrome P450, GST and EST enzymes were involved in the metabolic resistance of cypermethrin in the Gol-RR strain. Upon comparing with the SS strain, we found that these enzymes did not appear to be responsible for the main resistance mechanisms, and there might be other resistance mechanisms involved. However, Zhang et al. (2007) reported that EST is involved in resistance in a beta-cypermethrin-resistant housefly strain. In addition, we also found that P450, GST, and EST enzymes were not involved in imidacloprid resistance in the Gol-RR strain. Kavi et al. (2014) previously reported that PBO is not involved in resistance in an imidacloprid-resistant housefly strain.

Comparison of the target enzyme AChE in the two strains revealed that the activity of AChE was different, suggesting the insecticide sensitivity of AChE was altered in the Gol-RR strain. Similarly, Yuan et al. (2009) found that the biochemical properties of AChE in a propoxur-resistant housefly (>100-fold) were different from those of the enzyme in the SS strain. Tao et al. (2006) identified point mutations in the AChE gene in the propoxur-resistant strain. Therefore, we inferred that the AChE gene in the Gol-RR strain may also be mutated, resulting in high resistance to propoxur. In addition, the Gol-RR strain also displayed high resistance to cypermethrin. Scott (2016) reported that target-site insensitivity to pyrethroids is due to three mutations [*kdr*(L1014F), *kdr-his*(L1014H), and *super-kdr*(M918T + L1014F)] in the voltage-sensitive sodium channel. However, the mutations associated with propoxur resistance in the Gol-RR strain remains unknown and requires further investigation, as does the mechanism of resistance at the molecular level.

In the present study, we found that propoxur and imidacloprid are not effective against the Gol-RR strain. New and effective insecticides are urgently needed to replace agents that are no longer effective, and more effective methods of prevention and control are also required. Fortunately, chlorfenapyr is still very effective for controlling houseflies with high resistance to other insecticides, suggesting this agent could be used more widely.

Conflicts of interest

The authors declare that they have no conflict of interest.

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