

Mechanisms of Resistance to Organophosphorus and Pyrethroid Insecticides in Asian Citrus Psyllid *Diaphorina citri*, Populations in Florida



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Abstract

Background: The Asian citrus psyllid (ACP) is the most devastating pest on citrus in Florida as it vectors causal pathogens of citrus greening, a disease also known as huanglongbing. Florida is the largest citrus producer in the US and the development of insecticide resistance in ACP populations is a serious threat to the citrus industry. Mechanisms of resistance in ACP are not fully understood. Thus, in order to develop a resistance management program, the primary type of resistance must be determined. We conducted several studies to investigate the mechanisms of resistance to two commonly used classes of insecticides in ACP populations.

Results: Enhanced metabolism by esterase (185.0-fold) and carboxyl esterase (30.33-fold) were major factors of resistance to organophosphorus insecticides. In addition, glutathione S-transferases (2.5-44 fold), mixed function oxidase (2.17-fold), and altered target site (3.67-fold) were also significant factors of resistance to pyrethroid insecticides.

Conclusion: Mechanisms of resistance in ACP populations to insecticides were both metabolic and altered target site; therefore synergist probes such as piperonyl butoxide, S, S, S-tri-n-butyl phosphorotrithionate, triphenyl phosphate and formamidin could be used successfully to design a resistance management strategy for the Asian citrus psyllid.

Keywords: Asian citrus psyllid; Citrus greening; Insecticide resistance; Mechanisms of resistance

Abbreviations: ACP: Asian Citrus Psyllid; PBO: Piperonyl Butoxide; SL: Susceptible Strain; CL: Confidence Limit; TEPP: Triphenyl Phosphate; SR: Synergism Ratio

Introduction

Florida is the largest citrus producer in the US, and the citrus industry contributes to about \$8.91 billion annually to the state [1]. The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, is the most devastating pest on citrus in Florida. First reported in Taiwan in 1907, it is spread throughout the citrus industries of Asia and was found in Florida in 1998 [2]. The ACP vectors

the pathogens that cause the disease known as citrus greening. Since 2006 the combination of pest and disease has resulted in an approximate loss of \$4.5 billion to Florida's citrus industry. The disease continues to spread and the entire U.S. citrus industry is threatened. Applications of insecticides have led to the development of insecticide resistance in ACP populations [3,4].

Therefore, in order to develop a resistance-monitoring program, the primary type of resistance must be determined. Mechanisms of ACP resistance are not fully understood. Insects have developed resistance to insecticides primarily as a result of three mechanisms: decreased penetration, reduced target site sensitivity, and enhanced metabolism [5,6]. There are three major enzymes associated with insecticide resistance: mixed-function oxidases, glutathione s-transferases, and esterases [7]. Mixed-function oxidases are known to have a wide range of substrates, thus has a role in the metabolism and detoxification of various compounds [8]. Esterase-mediated mechanism of resistance has been detected against all classes of insecticides containing ester moiety [9]. Glutathione s-transferases and Carboxyl esterase are enzymes that have been demonstrated to cause insect resistance to conventional insecticides [10,11]. Here we present results of several studies designed to determine the mechanisms of resistance to organophosphorus and pyrethroid insecticides in ACP populations from southern Florida. Such information is critical in the development of a successful resistance management strategy for the citrus industry

Materials and Methods

Insecticides and insect collections

All insecticides were technical grade samples (>98% purity). These include pyrethroids (cypermethrin) and organophosphates (malathion), which were purchased from Sigma-Aldrich (St. Louis, MO). The other chemical tested included the piperonyl butoxide (PBO). Diethyl maleate and formamidine were also purchased from Sigma-Aldrich (St. Louis, MO) and S, S, S, -tri-n-butyl phosphorotrithionate (DEF) was obtained from Chem Services (West Chester, PA).

Insects

The susceptible strain (SL) of adult ACP came from a population continuously reared at the Southwest Florida Research and Education Center, University of Florida. The susceptible strain was established in 2006 using field populations which had not been subjected to insecticides. The strain was maintained on orange jasmine, *Murraya paniculata* Jack (Sapindales: Rutaceae), without exposure to insecticides in an air conditioned glasshouse maintained at 27±4°C; 70±10% RH under natural light. The field populations (RM) of ACP were collected using an aspirator from commercial citrus groves in Immokalee, FL (26 41'04 N 81 26' 20W). They were fed on young seedlings of *Citrus macrophylla* Wester until used for bioassays.

Synergism bioassays

The mechanisms of resistance were determined by using known synergists as diagnostic probes. The glass vial bioassay technique developed by Kanga and Plapp [12] was used for the synergism bioassays. The synergists used in these experiments were piperonyl butoxide (PBO) a mixed-function microsomal oxidase inhibitor; S, S, S, -tri-n- butyl phosphorotrithionate (DEF)

a putative inhibitor of esterases; triphenyl phosphate (TEPP) an inhibitor of carboxylesterase; diethyl maleate (DEM) a glutathione s-transferase inhibitor [13] and formamidine as a target site synergist. The concentrations of synergists in the bioassay were 50µg/vial of PBO, 25µg/vial of DEF, 50µg/vial of DEM, 25µg/vial of TEPP, and 50µg/vial of formamidine. The amounts of synergists used in these experiments were the highest concentrations that are not toxic to the psyllids during the pre-experimental runs. Two sets of bioassays were conducted at each time. In one set, the psyllids were treated with a synergistic mixture of the selected insecticide, and second sets of psyllids were tested with the selected insecticide only. Each insecticide was tested using eight concentration levels (plus an ethanol control) with 10 replicates of 5 psyllids per vial. All treated vials were held at room temperature (27±1°C, 65% RH) and the psyllid mortality was recorded 24 hours after exposure. Adult ACP that were unable to walk for a short distance (>5mm) after gentle probing with a fine brush were considered dead.

Statistical analysis

Concentration-mortality data with or without synergists were subjected to Probit analysis using the POLO program [14]. Percentage mortality in the treatments was corrected for control mortality using Abbott's formula [15]. The effects of synergists were calculated by dividing the LC₅₀ for the insecticide alone by the LC₅₀ for the selected insecticide and synergist mixture. The responses to synergists were considered not significant if the 95% confidence limit (CL) of the synergist ratio at the LC₅₀ bracketed 1.0 [16]. A likelihood ratio test of equality was conducted to determine whether the regression lines of the two treatments were equal (the slopes and intercepts of the two lines were the same). A similar ratio test of parallelism was run to determine whether the regression lines were parallel (the slopes of the two lines were the same) [16].

Results and Discussion

Esterase-mediated resistance factor

The levels of toxicity (the LC₅₀) of malathion alone and malathion with DEF to ACP were significantly different as the 95% confidence limit of the synergist ratio at the LC₅₀ did not bracket 1.0 (Table 1). In addition, dose mortality regression lines for both treatments were not parallel ($\chi^2=93.32$; $df=1$; $P=0.0001$) and not equal ($\chi^2=221.81$; $df=2$; $P=0.0001$). The synergism ratio (SR) was 185.0-fold. These results suggested that esterase-mediated metabolism was a major resistance factor role in ACP populations.

Carboxylesterase resistance factor

The toxicity (LC₅₀) of insecticide malathion alone and malathion with TEPP to ACP were significantly different (Table 1). There was a significant increase of 30.33-fold in the toxicity to ACP of malathion with the synergist of TEPP compared to malathion alone. These results suggested that carboxylesterase was also a major factor of resistance in ACP populations to malathion.

Table 1: Toxicity of Malathion with and without DEF, PBO and TEPP to Asian citrus psyllid *Diaphorina citri*

Insecticides	na	Slope±SE	LC ₅₀ (95%CI)b	SRc	χ ²
Malathion	319	4.91±1.92	0.148 (0.01-1.47)	-	1.87
Malathion with DEF	166	0.50 ±0.08	0.0008 (0.0001-0.01)	185 (130.12- 226.01)	25.67
Malathion	319	3.20±0.35	0.18 (0.13-0.25)	-	82.82
Malathion with TEPP	206	1.04±0.17	0.006 (0.003-0.013)	30.33 (21.33- 37.05)	36.11

^aNumber of adult Asian citrus psyllids tested.

^bConcentrations are expressed in µg insecticide per vial.

^cSynergist ratio (SR) calculated by dividing the LC₅₀ for insecticide alone by the LC₅₀ for insecticide with the synergist.

Glutathion-S-transferase resistance factor

The pyrethroid cypermethrin alone was significantly less toxic to ACP compared to cypermethrin with the synergist DEM (Table 2). In addition, the dose mortality regression lines for both

treatments were parallel ($\chi^2=0.023$; $df=1$; $P=0.88$) but not equal ($\chi^2=8.52$; $df=2$; $P=0.014$). Data indicated a significant increase of 2.5-fold in synergism ratio with DEM and suggested that glutathione- s-transferases were involved in insecticide resistance in ACP populations.

Mixed-function oxidase

Table 2: Toxicity of cypermethrin with and without DEM, PBO and Formamidine to Asian citrus psyllid *Diaphorina citri*.

Insecticides	na	Slope±SE	LC ₅₀ (95%CI)b	SRc	χ ²
Cypermethrin	257	1.42±0.22	0.02 (0.013-0.032)	-	0.9
Cypermethrin with DEM	212	1.40±0.20	0.008 (0.005-0.012)	2.5 (1.75-3.75)	4.61
Cypermethrin	257	1.174±0.118	0.013 (0.004-0.039)	-	2.77
Cypermethrin with PBO	157	6.091±181.347	0.006 (0.001-0.031)	2.17 (1.53-3.89)	20
Cypermethrin	257	1.43±0.22	0.022 (0.007-0.049)	-	0.89
Cypermethrin with Formamidine	156	4.20±6.37	0.006 (0.002- 0.0154)	3.67 (2.18-5.38)	18.5

^aNumber of adult Asian citrus psyllids tested.

^bConcentrations are expressed in µg insecticide per vial.

^cSynergist ratio (SR) calculated by dividing the LC₅₀ for insecticide alone by the LC₅₀ for insecticide with the synergist.

Data indicated a significant increase of 2.17-fold in toxicity of cypermethrin with the synergist PBO compared to cypermethrin alone to ACP (Table 2). In addition, the dose mortality regression

lines for cypermethrin alone and cypermethrin with PBO were parallel ($\chi^2=14.24$; $df=2$; $P=0.001$) and not equal ($\chi^2=10.56$; $df=1$; $P=0.001$). The synergism ratio (SR) was 2.17- fold indicating that

mixed-function microsomal oxidases were one of the major factors of resistance in ACP populations to cypermethrin.

Target site resistance factor

The levels of toxicity (LC_{50}) of the pyrethroid cypermethrin alone and cypermethrin with the synergist formamidine to ACP were significantly different (Table 2). The dose mortality regression lines for both treatments were not parallel ($\chi^2=17.81$; $df=2$; $P=0.0001$) and not equal ($\chi^2=3.91$; $df=1$; $P=0.0048$). Data indicated a significant increase of 3.67-fold in synergism ratio with formamidine. The results suggested that altered target site was a major factor of resistance in ACP populations. From the overall data, DEF and TEPP significantly synergized the toxicity of malathion and suggested that enhanced metabolism by esterase and carboxylesterase was a major mechanism of resistance to organophosphorus insecticides in ACP populations. Similarly, significant increase in synergism activities with DEM, PBO and formamidine indicated that mixed-function oxidases, glutathione s-transferases, and altered target site insensitivity are also major mechanisms of resistance to pyrethroid insecticides in populations of Asian citrus psyllid. Mechanisms of resistance in ACP populations to these classes of insecticides appear to be both metabolic and altered target site, therefore synergist probes such as piperonyl butoxide, S, S, S-tri-n-butyl phosphorotrithionate, triphenyl phosphate and formamidine could be used successfully to design a resistance management strategy for the ACP population.

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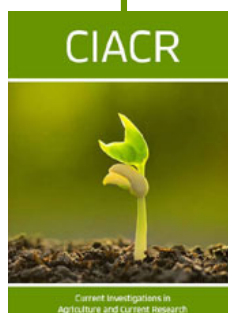


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