Proceedings of the Seventh International Conference on Urban Pests
William H Robinson and Ana Eugênia de Carvalho Campos (editors) 2011
Printed by Instituto Biológico, São Paulo, SP. Brazil.

SUSCEPTIBILITY LEVELS OF *PERIPLANETA AMERICANA* (DYCTIOPTERA: BLATTIDAE) TO TWO DIFFERENT INSECTICIDES IN POPULATIONS FROM SOUTHWEST COLOMBIA

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Abstract The levels of susceptibility to a pyrethroid (deltamethrin) and an organophosphate (malathion) and metabolic enzyme activities of adult *Periplaneta americana* females from populations collected in three cities of south western Colombia (Cali, Popayan, and Buenaventura), were characterized by bottle bioassays and biochemical tests. All populations were susceptible to both insecticides, with resistance ratios(RR) lower than 3. Although the control (Univalle) population, presented the lowest protein level, perhaps due to the smaller size and weight of the individuals kept in laboratory conditions for a long time, levels of esterases and oxidases did not show differences between the populations evaluated. Such baseline data will be valuable for reference and could help to control problems that arise in this pest in the future.
Key Words Biochemical assay, insecticide resistance, organophosphate, phyrethroid

INTRODUCTION

Periplaneta americana can be considered one of the most important urban pests worldwide (Robinson, 1996). It is a major vector in the mechanical transmission of many pathogens to humans as helminthes, viruses, protozoa and fungi (Peterson and Shurdut, 1999; Zarchi and Vatani, 2009).Their excreta and other wastes can contain a large number of allergens which induce severe respiratory allergies and hives (Bennett et al., 1996; Sookrung and Chaicumpa, 2010). This potential threat to human health is a major reason to maximize control efforts and elimination of this and other species of Blattodea (Valles et al., 1999). Despite the use of varied chemical control, relatively little is known of the resistance status of this pest.

The objective of this study was to determine the level of susceptibility to one organophosphate (malathion) and one pyrethroid (deltamethrin) in seven *P. americana* populations collected in three Colombian cities and relate the results to possible mechanisms of resistance.

MATERIALS AND METHODS

Collection Area

The samples were collected within the urban area of three cities located in the southwest of Colombia: Cali, Buenaventura and Popayan. In each of these cities the specimens of *P. americana* were collected in two or three different areas (Cali: Los Alamos, Ciudad Jardin, Departmental; Popayan: Santa Clara, Las Americas; and Buenaventura: Transformacion and Vista Hermosa). Each collection site was visited to obtain at least 40 adult males and females, sufficient to start a colony in the laboratory. Manual catches were made at night, between 21:00 to 24:00 hours, searching in peridomestic areas such as sewers, water and electricity meters and gardens.

The specimens were taken to the laboratories of entomology at the University del Valle (Cali) and established in plastic containers (0.17 m^3) following the conditions outlined by Bell (1981). A putative susceptible population (Univalle) kept ten years without any insecticide selection pressure was used as a control.

Bottle Bioassays

Glass bottles of 250 ml capacity were coated with 1 ml acetone containing a diagnostic concentration of either malathion 57 EC (15 mg) or deltamethrin 25 EC (1 mg). The bottles were rotated on a flat surface until all the acetone evaporated and left for 8-12 hours before use. Five adult females were put in each bottle and the number in knockdown counted every 10 minutes up to two hours. Each concentration was replicated six times. As a negative control, bottles were impregnated only with acetone.

Biochemical Assays

Enzyme activity and proteins determinations were carried out with coxa-III of 30 adult females from each population, this coxa is the biggest one and with more soft tissue that helps obtaining more material to do the assays. Each coxa was placed (separately) into a 1.5 ml Eppendorf tube and homogenised with 1 ml of potassium phosphate buffer (0.01 M - pH 7.2), centrifuged at 10,000 g for 20 sec. and the resulting supernatant used as the enzyme source.

To determine protein amount, esterase and oxidase activity, 20 µl of supernatant from each Eppendorf, in triplicate, were added to microplates. Protein determination was based on the method of Bradford (1976). To each well, 200 µl of 25% DYE solution (Bio-Rad Protein Assay Dye reagent concentrate) were added. After 10 min incubation, the absorbance was read at a wavelength of 630 nm.

To quantify the mixed function oxidases, 25 ml of a 3.3 ‘, 5.5’-Tetramethyl Benzidine and methanol (0.2%) solution was prepared and 75 ml of sodium acetate buffer (0.25 M - pH 5.0) were added. Aliquots (200 µl) of this were added to wells of a microplate and 25 µl H_2O_2 added. The absorbance was read at time 0 and after 10 minutes at a wavelength of 630 nm (Brogdon et al., 1988a, b).The substrate ß-naphthyl acetate was used to measure nonspecific esterase activity. A stock of 10% ß-naphthyl acetate in acetone was used to prepare 50 ml of potassium phosphate buffer (0.01 M - pH 7.2) containing 0.1% substrate. Aliquots (100 µl) of this solution was added to wells and incubated for 10 minutes at room temperature. After the incubation period,100 µl of a 0.1% solution of ultrapure water + o-Dianisidinetetrazotized were added. After another two minutes of incubation, the absorbance was read at a wavelength of 570 nm (Brogdon et al.,1988a, b).

Statistical Analysis

Bottle bioassays. To calculate the KT_{50} (time taken for 50% of the population to be in knockdown) of each population studied, a PROBIT analysis was performed with the program Polo Plus 1.0 (2002). Resistance ratios (RR) of the different populations were determined by comparing the KT_{50} of the populations collected in the three cities with the KT_{50} of the susceptible population:

Biochemical assays. The average values of the negative control were subtracted from the absorbance of the esterases and oxidases. Each of the absorbance values was standardised as activity per unit (µg) of protein dividing these data by the results obtained in total protein assays to adjust for differences in body mass of individuals that might require correction factors for the enzyme assays. To compare the enzyme activity of the populations studied, the final values were subjected to an analysis of variance (ANOVA) using SPSS 15.0 (2006).

RESULTS

Bottle Bioassays

Malathion. The KT_{50}’s for each population is shown in Table 1. Although some slight differences occurred between the populations, with the susceptible control being most susceptible and Cali (Ciudad Jardin) having the highest KT50, no significant resistance to malathion was considered to be present (RR<3).

Deltamethrin. Quicker knockdown was observed with the pyrethroid. TheKT_{50}’s for each population is shown in Table1. Although some slight differences occurred between the populations, with the susceptible control being most susceptible and Cali (Los Alamos) having the highest KT50, no significant resistance to deltamethrin was considered to be present (RR<3).

$$RR = \frac{KT_{50}\ field\ population}{KT_{50}\ suscpetible\ population\ (Univalle)}$$

Biochemical Assays

The control population (Univalle) had an average protein level lower than the rest of the populations (Figure 1a). This was not surprising since the females of this population, were on average, smaller in size and weight (Length = 3.17 ± 0.25 cm, weight = 0.93 ± 0.15 g) in relation to females from other populations evaluated (Buenaventura: length.= 3.39 ± 0.25 cm., weight = 1.18 ± 0.18 gr., Cali: length .= 3.24 ± 0.19 cm., weight = 1.11 ± 0.15 gr.). The females from Popayan-Santa Claraalso had less protein than other field strains, but not as low as the control.

There were no significant differences in esterase activity between the populations (Figure 2b). Indeed, the population with the highest esterase levels was Univalle (absorbance value: 1.39). Similarly, there were no significant differences in haem content between the populations ($p>0.001$) (Figure 2c).

Table 1. Knock down times (min) KT_{50} of seven *P. americana* populations from three cities of the southwest part of Colombia. Thirty adult females were evaluated at a 15 µg/µl concentration of malathion and 1 µg/ml concentration of deltamethrin using bottle bioassays (CDC).

Population	Malathion		Deltamethrin	
	KT_{50} (min) (CI 95%)	**RR***	**KT_{50} (min) (CI 95%)**	**RR***
Control (Univalle)	42.96 (40.38-45.39)	-	25.74 (21.36-30.02)	-
Cali (Ciudad Jardin)	61.98 (59.44-64.42)	1.44	37.78 (33.27-41.85)	1.29
Cali (Los Alamos)	57.06 (54.21-59.79)	1.33	40.64 (35.94-46.25)	1.58
Cali (Departamental)	49.21 (46.53-51.78)	1.14	22.62 (18.51-26.25)	0.88
Buenaventura (La Transformacion)	56.52 (53.39-59.59)	1.31	35.90 (33.20-38.49)	1.39
Buenaventura (Vista Hermosa)	49.35 (46.78-51.83)	1.15	37.95 (34.59-41.48)	1.47
Popayan (Las Americas)	53.54 (51.02-55.97)	1.25	27.81 (24.73-30.64)	1.08
Popayan (Santa Clara)	43.11 (40.48-45.59)	1.00	29.40 (25.22-33.38)	1.14

*RR = KT_{50} field population / KT_{50} control population (Univalle)

DISCUSSION

Although all populations evaluated were deemed susceptible to malathion and deltamethrin, bottle bioassays may underestimate the magnitude of the resistance and could fail to detect low levels of resistance (Milio et al., 1987). However, these results agree with the few works that have been performed with *P. americana* worldwide (Vythilingam and Sutivigit,1994; Ho et al.,1994).

The absence of resistance is not a guarantee that control problems will not occur in the near future, even within these populations. Factors influencing the development of resistance to insecticides include habitats, ecological requirements and behavioral traits such as gregariousness, night habits and omnivorous diets; but the over-riding determinants are selection pressure from applied chemicals and the relatively long life-cycle. *P. americana* takes between 4-12 months to complete its life cycle and the number of generations per year are not as many as in other species (i.e. *Blattella germanica*) therefore the expression of resistance genes could take more time.

Due to the mobility of individuals to and from human dwellings, the impact of the gene flow between populations from inside and outside (with different selection pressures) could be delaying the evolution rate of

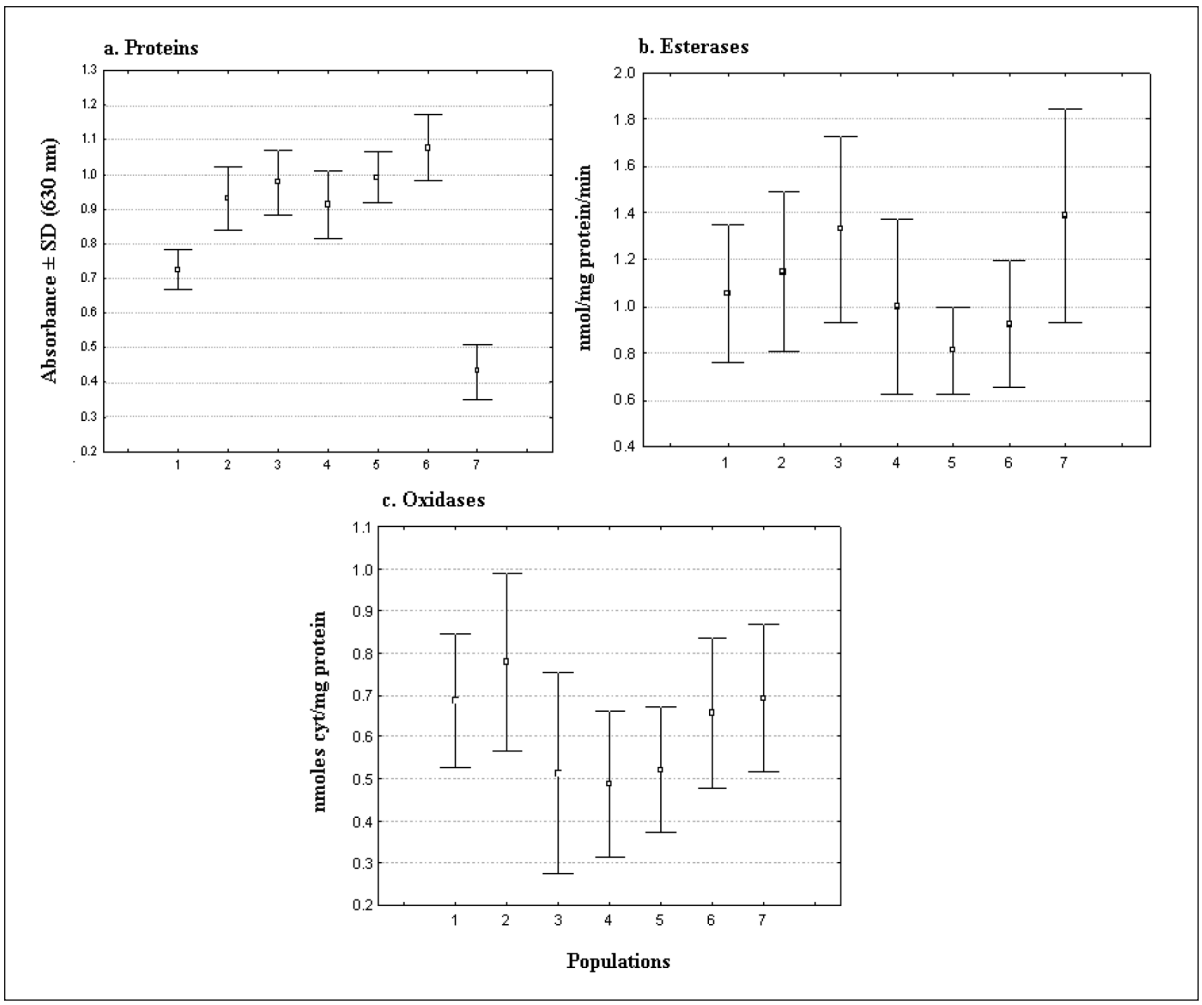

Figure 1. Average ± standard deviation of the absorbance (OD) of total proteins (a.), esterases (b.), oxidases (c.) of seven *P. americana* populations from three Colombian cities. 1. Popayan-Santa Clara, 2. Popayan-Americas, 3. Buenaventura-Transformacion, 4. Cali-Ciudad Jardin, 5. Cali-Departamental, 6. Cali-Alamos, 7. Control-Univalle

resistance (McKenzie, 1996). Transportation of goods would further spread resistance genes should they arise, but equally this would serve to 'dilute' resistance genes in areas of high selection by the influx of susceptible genes from areas of refugia (i.e. areas not sprayed).

The exact history of direct selection pressure in outside locations is difficult to ascertain, as there are no official records of applications. However, is possible that this species receives some selection pressure outside the homes in Cali and Buenaventura due to insecticide applications provided by the Health Departments to control the primary vectors of diseases like malaria and dengue fever. And this could be affecting the lost of susceptibility in some of the populations in these two cities.

The insecticides used locally are still efficient to control this pest, but is a priority maintained surveillance over these populations in order to detect changes in the susceptibility levels to the most widely used insecticides. This study will serve to provide base-line measures for future bio- and biochemical- assays. These data could be used as reference for further studies for monitoring the susceptibility status of these populations.

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