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Contrasting patterns of tolerance between chemical and biological insecticides in mosquitoes exposed to UV-A

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ABSTRACT

Mosquitoes are vectors of major human diseases, such as malaria, dengue or yellow fever. Because no efficient treatments or vaccines are available for most of these diseases, control measures rely mainly on reducing mosquito populations by the use of insecticides. Numerous biotic and abiotic factors are known to modulate the efficacy of insecticides used in mosquito control. Mosquito breeding sites vary from opened to high vegetation covered areas leading to a large ultraviolet gradient exposure. This ecological feature may affect the general physiology of the insect, including the resistance status against insecticides. In the context of their contrasted breeding sites, we assessed the impact of low-energetic ultraviolet exposure on mosquito sensitivity to biological and chemical insecticides.

We show that several mosquito detoxification enzyme activities (cytochrome P450, glutathione S-transferases, esterases) were increased upon low-energy UV-A exposure. Additionally, five specific genes encoding detoxification enzymes (*CYP6BB2*, *CYP6Z7*, *CYP6Z8*, *GSTD4*, and *GSTE2*) previously shown to be involved in resistance to chemical insecticides were found over-transcribed in UV-A exposed mosquitoes, revealed by RT-qPCR experiments. More importantly, toxicological bioassays revealed that UV-exposed mosquitoes were more tolerant to four main chemical insecticide classes (DDT, imidacloprid, permethrin, temephos), whereas the bioinsecticide *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) appeared more toxic.

The present article provides the first experimental evidence of the capacity of low-energy UV-A to increase mosquito tolerance to major chemical insecticides. This is also the first time that a metabolic resistance to chemical insecticides is linked to a higher susceptibility to a bioinsecticide. These results support the use of *Bti* as an efficient alternative to chemical insecticides when a metabolic resistance to chemicals has been developed by mosquitoes.

1. Introduction

Mosquitoes are considered as the most dangerous animals worldwide due to their capacity to transmit major human diseases, such as malaria, dengue or yellow fever (Gubler, 1998; Ross, 1901; Tomori, 2004). They represent a heavy sanitary and economic burden, especially in highly-impacted developing countries. Because of the lack of cost-effective and long-lasting drug treatments or vaccines for most mosquito-borne diseases, control measures historically and currently target vector populations through the use of highly efficient chemical insecticides (Hemingway et al., 2006; Killeen et al., 2002). Nevertheless, chemical usage has detrimental side effects, such as high environmental persistence and low mosquito specificity by their toxicity to non-target insect species (Diabate et al., 2002). Moreover, mosquito populations show full resistance to all chemical insecticide classes used in vector control, threatening the long-term efficacy of chemical-based control interventions (Enayati and Hemingway, 2010; Hemingway and Ranson, 2000). The increasing awareness for the need of a more integrated strategy to control mosquito populations has led to the development of alternative strategies, including the use of genetically engineered mosquitoes (de Valdez et al., 2011; Scott et al., 2002) or entomopathogenic microorganisms, such as the insect reproductive parasite *Wolbachia* (Iturbe-Ormaetxe et al., 2011), toxic fungal species (Blanford et al., 2005; Scholte et al., 2005), resident mosquito microbial symbionts (Cirimotich et al., 2011) or the bioinsecticide *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) (Goldberg

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and Margalit, 1977). Technical drawbacks and high costs combined with lower efficacy compared to chemicals have strongly delayed the widespread use of bioinsecticides as a long-term alternative to chemicals. Biological insecticides intrinsically target distinct aspects of the mosquito physiology when compared to chemical insecticides; for instance, chemicals used in current control programs are neurotropic agents blocking synaptic transmission in the insect central nervous system (Soderlund, 2008), whereas *Bti*, the major bioinsecticide used worldwide, directly disrupts the intestinal gut (Vachon et al., 2012). Therefore, *Bti* may represent a promising alternative to chemicals by sustaining its toxicity toward mosquito populations highly resistant to chemicals. Although this hypothesis has been largely accepted and relayed by the scientific community (Bravo et al., 2011; Lacey et al., 2001; Roh et al., 2007; Schnepf et al., 1998), to our knowledge it has never been experimentally validated.

Numerous biotic and abiotic factors were shown to affect the efficacy of insecticides on mosquito populations, such as temperature (Christiansen et al., 2004; Sibanda et al., 2011), water pH (Mittal et al., 1995), altered nycthemeral rhythm (Yang et al., 2010), presence of organic matter (Tetreau et al., 2012b) or water pollution (Poupardin et al., 2008; Riaz et al., 2009). A large part of the mosquito life cycle consists of four aquatic larval stages, which live in highly contrasted natural or man-made breeding sites, such as agricultural fields or highly urbanized sites. Suitable mosquito breeding sites could vary widely in term of light exposure from large opened areas with maximal exposure to shaded water pools with high vegetation coverage in forestry settings. Major disease vectors, such as the malaria vector *Anopheles gambiae*, the dengue vector tiger mosquito *Aedes albopictus*, the yellow fever mosquito *Aedes aegypti* or *Culex quinquefasciatus* transmitting West Nile virus and filarial worms, show distinct preferences in their breeding sites; *An. gambiae* breeding sites are often described as clean shallow water pounds, whereas *Aedes* and *Culex* mosquitoes are more opportunistic, underlying the invasive features of the tiger mosquito for instance through European mainland (Benedict et al., 2007). With such contrasted breeding sites, mosquito larvae can be exposed to an important UV gradient, from woodland ponds and sewers, to large open areas of stagnant water or uncovered containers. To date, there is no report on the effect of UV light on the insecticide susceptibility of mosquitoes, despite a huge literature on the impact of UV on insect oxidative stress responses, general physiology and populations (Ballare et al., 2001; Ingram, 1992; Mazza et al., 2002; Wang et al., 2001). Using the yellow fever mosquito vector *Ae. aegypti* as a versatile model species for breeding site type, the present article reports an opposite effect of low-energy UV-A light on the mosquito susceptibility to five major chemical insecticide classes (namely the organochlorine DDT, the organophosphate temephos, the pyrethroid permethrin, the carbamate propoxur and the neonicotinoid imidacloprid) and to the bioinsecticide *Bti*. This contrasting pattern of tolerance was linked to an increase of all detoxification enzyme activities and a mRNA over-expression of several candidate genes previously associated with insecticide resistance. All the results are discussed with regard to mosquito control strategies and resistance management.

2. Methods

2.1. Mosquito strain and experimental design

A laboratory *Ae. aegypti* strain Bora–Bora susceptible to all insecticides has been used. After egg hatching, larvae were reared in tap water and fed with standardized amount of larval food (hay pellets) at a temperature of 27 °C with 80% of relative humidity and a 15/9 h light/dark period. Three different conditions of UV exposure were performed; larvae were not exposed to UV ("UV 0") or exposed 2 h ("UV 2") or 15 h ("UV 15") every day during 4 days (from egg hatching to 3rd instar larval stage) to UV-A (λ = 365 nm) at 70 W/m^2 (Vilbert-Lourmat 115.L 15 W lamp), which represents a very low UV-index of 1.14 (calculated according to the standardized ISO/CIE procedure; correction factor for 365 nm: 0.000407). In our settings, a 2 h daily exposure may represent a daily noon maximal exposure in an otherwise shaded breeding site, whereas 15 h daily exposure represents open areas with long-lasting exposure with no coverage on the mosquito breeding site.

2.2. Effect of UV on cell and tissue mortality.

To monitor the effect of UV on mosquito cell mortality, living larvae from the three conditions were incubated for 3 h in a solution of 40 μg ml^{-1} of propidium iodure (PI). Thereafter larvae were put in water for 1 h. A positive control of cell mortality test was performed by incubating larvae with 70% ethanol for 5 min. Larvae were then observed using a stereo zoom microscope (SZX16, Olympus) under normal light ("Visible") and epifluorescence (excitation: 450–490 nm and emission $\geq$520 nm ("PI") or both ("Merge").

2.3. Enzyme activities and ROS measurement

800 fresh larvae per condition (2 h-, 15 h-exposure and control) were homogenized by a glass-teflon homogenizer on ice (10 strokes) in 0.05 M phosphate buffer (pH 7.2) containing 0.5 mM DTT, 2 mM EDTA and 0.8 mM PMSF. The homogenate was then centrifuged at 10,000 $\times$ *g* for 20 min at 4 °C and the resulting supernatant was ultracentrifuged at 100,000 $\times$ *g* for 1 h at 4 °C. The pellet (microsomal fraction) was resuspended into 500 μL 0.05 M phosphate buffer and used for cytochrome P450 monooxygenases activities measurement. The supernatant (cytosolic fraction) was used for all other enzyme activity measurements. Three replicates were performed for each condition.

P450 activities were measured by using 7-ethoxyresorufin (EROD, Sigma) as substrate as described in Reynaud et al. (2002) with modifications for micro-plate measurements. The activity was assayed at 30 °C in a medium containing 20 μg microsomal proteins, Glucose-6-Phosphate (G6P) (5 mM), NADPH (0.1 mM), G6P dehydrogenase (1 U mL^{-1}), 7-ethoxyresorufin (5 μM), and phosphate buffer (50 mM Na_2PO_4; pH 7.4) in a final volume of 200 μL. Fluorescence (excitation 537 nm; emission 583 nm) was recorded every 30 s during 40 min in a Varioskan Flash Multimode Reader (multiplate reader; Thermo Scientific). Glutathione-S-transferase (GST) activities were measured by using 1-chloro-2,4-dinitrobenzene (CDNB, Sigma) as substrate as described in Riaz et al. (2009) with slight modifications. The reaction mixture contained 20 μg protein from the cytosolic fraction, 1.3 mM reduced glutathione (Sigma) and 1 mM CDNB in 0.1 M phosphate buffer; pH 6.9. The absorbance of the reaction solution at 340 nm was measured after 1 min. Esterase activities were measured by using α-naphthyl acetate and β-naphthyl acetate as substrates (Sigma) (Van Asperen, 1962) as described in (Poupardin et al., 2008). Catalases activities were measured spectrophotometrically at 240 nm during 60 s, using 200 μL proteins from the cytosolic fraction in 1.6 mL buffer and 10 mM H_2O_2, as described in (Dubovskii *et al.*, 2010). The activity of catalase (H_2O_2 decomposition) was expressed in μmol min^{-1} using the extinction coefficient of 0.0394 mM^{-1} cm^{-1} for hydrogen peroxide.

The amount of reactive oxygen species (ROS) was measured by reduction of the redox dye nitroblue tetrazolium (NBT) (Sigma, France) as described in Reynaud et al. (2001) with modifications. Twenty larvae were added to 1 mL of a 1 mg L^{-1} NBT solution for 2 h in the dark at room temperature. Larvae were washed twice in ethanol, air dried and the formazan produced was then dissolved