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The effect of commercial herbicide exposure on the life history and insecticide resistance phenotypes of the major malaria vector *Anopheles arabiensis* (Diptera: culicidae)

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ABSTRACT

Herbicides, such as atrazine and glyphosate, are common agrochemicals known to pollute surface ground water. As such, aquatic invertebrates associated with agricultural activities can be exposed to varying doses of these xenobiotics. *Anopheles arabiensis*, a major malaria vector species in southern Africa, is often closely associated with agricultural activities. This study aimed to examine the effects of larval atrazine or glyphosate exposure on larval and adult life history traits on two laboratory strains of *An. arabiensis*; one insecticide susceptible (SENN), the other selected for resistance (SENN DDT). Atrazine delayed time to pupation in both strains, but markedly more so in SENN DDT. Glyphosate treatment reduced time to pupation in SENN DDT. Larval atrazine exposure decreased adult longevity in SENN, while both herbicide treatments significantly increased adult longevity in SENN DDT. Larval glyphosate exposure was the more potent enhancer of insecticide tolerance in adult mosquitoes. In SENN DDT, it reduced deltamethrin and malathion-induced mortality, and the LT50 s for these insecticides were increased in association with herbicide exposure. Glyphosate exposure also increased the LT50 s for malathion and deltamethrin in SENN. Exposure to both herbicides had contrasting effects on detoxification enzyme activities. Although both increased cytochrome P450 activity, they had opposite effects on those enzymes involved in reactive oxygen species detoxification. Glyphosate decreased glutathione S-transferase activity, but increased catalase activity with atrazine having the opposite effect. This study demonstrates that larval exposure to the herbicides atrazine and glyphosate can affect the insecticide susceptibilities and life history traits of epidemiological importance in *An. arabiensis*, with glyphosate being the more potent effector of insecticide resistance.

1. Introduction

A major contributor to Africa's malaria burden lies in the relative efficiency of its mosquito vectors (Sinka et al., 2010). One of these vectors is a member of the *Anopheles gambiae* species complex, *An. arabiensis*. Although not as efficient a vector as *An. gambiae s.s.*, *An. coluzzii* or *An. funestus* (Coetzee and Fontenille, 2004), it does present a particular challenge in areas where it is a major vector species, such as southern Africa.

Anopheles arabiensis is characterised by feeding and resting plasticity, as well as a tendency toward zoophily (Sinka et al., 2010). Therefore, it is not well controlled by traditional vector control methods, such as indoor residual spraying and insecticide treated nets (Kitau et al., 2012; Sharp et al., 1990). Its propensity for exophily and exophagy also means that it is capable of sustaining low levels of outdoor transmission known as residual malaria (Killeen, 2014). This makes it a major threat to malaria elimination efforts.

Anopheles arabiensis is also associated with agricultural activity, often being responsible for increased transmission in farmed regions (Abuelmaali et al., 2013; Jarju et al., 2009; Klinkenberg et al., 2005; Yadouleton et al., 2009). This can be ascribed to several reasons. For example, the aquatic stages of this species benefit from fertilizer applications and maturing plants (Mwangangi et al., 2006), and from maize pollen (Ye-Ebiyo et al., 2003, 2000). The increased fitness derived from improved larval nutrition may result in an increase in insecticide resistance in adult mosquitoes (Oliver and Brooke, 2013).

In regions under insecticide-based malaria vector control, the use of fertilizers in farming may inadvertently affect malaria transmission by altering the expression of insecticide resistance, where present, in adult mosquitoes. Although long suspected, with several examples suggesting

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that high levels of herbicide residues are associated with resistance in the field (Chouaibou et al., 2016), direct empirical evidence of agricultural pesticides driving resistance has only emerged relatively recently (Nkya et al., 2014a, b). Non-toxic agrochemicals have also been found to increase resistance in mosquito vectors (Darriet et al., 2012). Additionally, the effect of herbicides on insecticide tolerance has been demonstrated (Liang and Lichtenstein, 1974; Lichtenstein et al., 1973), making them agrochemicals of interest to vector control.

Herbicides are a complex and diverse group of agrochemicals that are used to supress the growth of weeds (Gianessi, 2005). Glyphosate and atrazine are the most commonly used herbicides in the world, with approximately 180–185 million pounds of glyphosate and 73–78 million pounds of atrazine used annually (Bara et al., 2014). Glyphosate is a non-selective post-emergent herbicide that inhibits the 5-enolpyruyl-shikimate-3-phosphate synthesis system (Duke and Powles, 2008). Atrazine is a triazine herbicide that inhibits photosystem II (Bara et al., 2014). Both herbicides are common contaminants of groundwater. Atrazine is a persistent, mobile molecule, with residues as high as 700–2300 µg detected in water (Pratt et al., 1988; Thurman et al., 1990. Glyphosate residues between 2 and 5200 µg/l have been detected in groundwater (Skark et al., 2004).

Herbicides are some of the key agrochemicals investigated in association with agriculture and resistance development. In *Aedes aegypti*, they have been shown to decrease larval sensitivity to *Bacillus thuringiensis* toxin (Boyer et al., 2006), and to imidacloprid, permethrin and propoxur - three insecticides with vastly different modes of action (Riaz et al., 2009). Apart from being a driver of insecticide resistance, sublethal herbicide concentrations can also affect mosquito life histories. These effects have been shown to vary by herbicide. Atrazine exposure caused longer aquatic stage development times in *Ae. aegypti* and *Ae. albopictus*, resulting in smaller adult females, and glyphosate exposure resulted in a male-biased sex ratio (Bara et al., 2014). Glyphosate was also found to prolong larval development time at high concentrations and to decrease proportional survivorship to adulthood in *Ae aegypti* (Morris et al., 2016).

Less information on the effect of herbicide exposure is available for anophelines. Kibuthu et al., (2016) showed that *An. arabiensis* tend to lay fewer eggs in glyphosate contaminated water, and that larval development is delayed following oviposition. No effects were observed on adult longevity.

Based on the information available to date, the aim of this study was to examine variation in the life histories and insecticide susceptibilities of insecticide resistant and susceptible *An. arabiensis* laboratory strains following exposure to two common commercial herbicides.

2. Materials and methods

2.1. Materials

Two laboratory strains of *An. arabiensis* were used. The SENN strain was colonised from Sennar, Sudan, in 1980. From this strain, SENN DDT was selected for resistance to insecticides. SENN DDT is resistant to DDT, permethrin, deltamethrin, λ-cyhalothrin and malathion (Oliver and Brooke, 2014, 2017). The strains are housed in the Botha de Meillon insectary of the National Institute for Communicable Diseases in Johannesburg, and are reared as described in Hunt et al., (2005).

2.2. Methods

2.2.1. The effect of herbicide exposure on development

Fifty SENN and SENN DDT 1st instar larvae (less than 24 h after hatching) were placed in 1000 ml water (214 × 155 x 80 mm), supplemented with a final concentration of 0.01 M glyphosate or 0.01 M atrazine. Larvae in untreated water served as a control. The larvae were fed as per the protocol described in (Oliver and Brooke, 2013). Larval development was monitored until pupation. The experiment was replicated 3 times from 3 cohorts originating from 3 different egg batches.

2.2.2. The effect of larval herbicide exposure on adult longevity

Fifty SENN and SENN DDT 1st instar larvae were treated as described in the larval development experiment. Adults were collected from the treatments (atrazine treated females n = 25, atrazine treated males n = 24, glyphosate treated females, n = 26, glyphosate treated males, n = 25 for each strain) and monitored until death. Thirty males and 30 females emerging from the untreated water were used as controls. The experiment was replicated 3 times from 3 cohorts originating from 3 different egg batches. Adults were allowed *ad libitum* access to 10% sucrose, which was changed twice weekly. Longevity was assessed using the Kaplan-Meier estimator, with the log-rank test used as a test for significance.

2.2.3. Insecticide tolerance as determined by WHO bioassay and LT50 estimation (time to 50% mortality)

Two hundred and fifty SENN DDT 1st instar larvae (less than 24 h old) were placed in 2000 ml (335 × 270 x 140 mm) of water supplemented with 0.01 M atrazine or glyphosate. An identical number of 1st instar larvae reared in untreated water served as a control. All treatments were fed an equal amount of food. Adults were collected upon emergence and supplied with 10% sucrose *ad libitum*. Females were not allowed blood during their lifetime. At the age of 3 days, male and female adults were exposed to either 5% malathion (organophosphate OP) or 0.05% deltamethrin (pyrethroid PYR) according to the standard WHO bioassay method (WHO, 2016). An unexposed treatment served as an environmental control, while WHO-supplied OP and PYR controls were also used in each replicate as per WHO guidelines (WHO, 2016). This experiment was replicated 3 times from 3 different cohorts originating from 3 separate egg batches. Changes in insecticide tolerance were between the treatment and control cohorts were assessed using two sample *t*-test with a 95% confidence interval.

As insecticide tolerance in the insecticide susceptible SENN strain cannot be determined using WHO bioassays, changes in insecticide tolerance was determined by assessing changes in lethal time. Emergent SENN and SENN DDT male and female adults were also used to determine the amount of exposure time required to induce 50% mortality (LT50) in this strain using either 10 µg/ml malathion or deltamethrin for the resistant SENN DDT strain or 1 µg/ml malathion or deltamethrin for the susceptible SENN strain according to the CDC bottle bioassay method (Brogdon and McAllister, 1998). Similarly, emergent SENN adults following larval exposure to either atrazine or glyphosate were used to determine this strain's LT50 against malathion or deltamethrin, (see (Oliver and Brooke, 2018). Adults emerging from untreated water served as a control. A range of exposure times were used to determine the respective LT50s: 2, 4, 8, 16 and 32 min for SENN and 10, 20, 40 and 80 min for SENN DDT. The differing exposure times were chosen to compensate for the differences in insecticide susceptibility between strains. Exposure time required to induce 50% mortality by replicate was determined using probit analysis (Finney, 1952).The experiment was replicated in triplicate from 3 different cohorts, originating from 3 different egg batches. Differences in the means were determined by 1-way ANOVA and 2-sample t-tests, with a 95% confidence interval.

2.2.4. The effect of larval herbicide exposure on adult detoxification and oxidative stress enzyme activity

SENN and SENN DDT larvae were treated as described for the WHO bioassays. Adults that emerged were supplied with 10% sucrose, and females were not allowed a blood meal during their lifetime. At the age of 3 days, the adults were cold-killed and stored at −70 °C. Cytochrome P450, Glutathione S-transferase, General esterase and catalase activities were determined as described in (Oliver and Brooke, 2018).