EVIDENCE OF ANOPHELINE MOSQUITO RESISTANCE TO AGROCHEMICALS IN NORTHERN THAILAND

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Abstract. The objective of this study was to assess insecticide resistance in anopheline mosquito populations in agroecosystems with high and low insecticide use in a malaria endemic area in Chiang Mai province in northern Thailand. Anopheline mosquitoes were collected in May and June 2004 from two locations with different agricultural insecticide intensity (HIGH and LOW), but similar in vector control strategies. The F1-generation of Anopheles maculatus s.s. and An. sawadwongporni were subjected to diagnostic doses of methyl parathion (MeP) and cypermethrin (Cyp), both commonly used insecticides in fruit orchards in Thailand. An. minimus A from the HIGH location was subjected to diagnostic doses to Cyp. CDC bottle bioassays were used to determine insecticide susceptibility. Time-mortality data were subjected to Probit analyses to estimate lethal time values (LT50 and LT90). Lethal time ratios (LTR) were computed to determine differences in lethal time response between populations from HIGH and LOW locations. The mortality of An. maculatus to MeP was 74% and 92% in the HIGH and LOW locations, respectively. There was no indication of resistance to Cyp for all species tested in either location. The corresponding figures for An. sawadwongporni were 94% and 99%. There was no indication of resistance to Cyp for all species tested in either location. The LT90 and LT50 values of An. maculatus s.s. subjected to diagnostic doses of MeP were significantly different between locations (p<0.05). Reduced susceptibility to MeP in mosquito populations in the HIGH location is caused by intensive agricultural pest control and not by vector control activities, because organophosphates have never been used for vector control in the area. Our results indicate that there are still susceptible anopheline populations to pyrethroids, which is consistent with other research from the region. Therefore, there is presently no direct threat to vector control. However increased use of pyrethroids in agriculture may cause problems for future vector control.

INTRODUCTION

Insecticide resistance in disease vectors is a serious threat to the control of vector-borne diseases, because often the only approaches left to control such diseases are insecticide-based strategies, such as insecticide treated nets, indoor residual spraying, and insecticide treatment of breeding habitats. The Database of Arthropods Resistant to Pesticides of Michigan State University – Center for Integrated Plant Studies lists 63 species of malaria mosquitoes resistant to one or more of the main groups of insecticides, ie organochlorines, organophosphates, pyrethroids, and carbamates (http://www.cips.msu.edu/resistance). Cross-resistance in mosquito vectors is commonly found, eg between DDT and pyrethroids (Brogdon et al, 1999; Ranson et al, 2000), between organophosphates and pyrethroids (Brogdon and Barber, 1990), and between organophosphates and carbamates (Hemingway and Georghiou, 1983). Multiple resistance is also known, because insecticides from different chemical groups have been used sequentially to control mosquitoes (Georghiou, 1990a; Brogdon and McAllister, 1998a).

Research on insecticide resistance in disease vectors has mainly focused on public health insecticides. However, the facts that approximately 90% of all insecticides worldwide are used for agricultural purposes (WHO, 1986) and that agriculture has become increasingly resource-intensive deserve attention as to what role agriculture plays in resistance development in disease vectors. Agricultural activities have often been blamed for disease vector insecticide resistance, but few attempts have been made to determine and confirm the direct impact of agrochemicals. Lines (1988) and Georghiou (1990b) reviewed the relationship between agrochemicals and insecticide resistance in mosquito vectors. Insecticide resistance in disease vectors due to selection pressure from agrochemicals has been reported from Central America (eg Georghiou et al, 1971; Chapin and Wasserstrom, 1981; Brogdon et al, 1988), Africa (Diabate et al, 2002), and South Asia (Sharma, 1996), but no clear
In 1951, the nation-wide Malaria Control Program in Thailand adopted the insecticide control strategy using DDT indoor residual spraying (Malikul, 1988). However, following environmental and public health concerns, DDT was banned for agricultural use in 1983, but could still be used in vector control. From 1992, synthetic pyrethroids became the insecticides of choice in malaria vector control and were used to impregnate bed nets and for indoor residual spraying. DDT was phased out in malaria vector control during 1995 and 1999. Recent research from northern Thailand has shown that An. minimus, the most common malaria vector in Thailand, is still susceptible to DDT and pyrethroids (Somboon et al., 2003).

In Thailand, diversification of the agricultural sector has led to an increase in more pesticide intensive crop systems, such as fruit cultivation (Jungbluth, 1996). Recent research from rural areas in northern Thailand has shown a decrease in anophele density with an increase in fruit orchard area (Overgaard et al., 2003). This relationship may be caused by intense use of insecticides in fruit orchards and might lead to selection of insecticide resistance in mosquito populations (Lines, 1988; Georghiou, 1990b).

We conducted a study to assess insecticide resistance in anophele mosquito populations in agroecosystems with high and low insecticide use. We tested mosquito susceptibility to an organophosphate, methyl parathion, and a pyrethroid, cypermethrin, which are common agricultural insecticides used in fruit orchards in Thailand (Overgaard, unpublished data). Here, we present the results of susceptibility tests undertaken on An. maculatus s.s., An. sawadwongporni, and An. minimus A from a malaria endemic district in Chiang Mai Province, northern Thailand.

MATERIALS AND METHODS

Location

The study was carried out close to the Myanmar border in Chiang Dao district, Chiang Mai Province, northern Thailand (Lat N 19° 44′, Long E 98° 54′). Two villages were selected based on their general agricultural plant protection practices, ie the intensity of insecticide use. Thus, Mueang Na village (HIGH) is characterized by a high agrochemical input as a consequence of widespread insecticide-intensive commercial fruit cultivation in the area. The other village, Huay Mae Kiang village (LOW), is a smaller village characterized by less intensive, smaller scale subsistence agriculture, with low agricultural pesticide input. This village is situated within a Royal Development Project area, where sustainable agricultural resource management is promoted. The distance between the two villages is approximately 7 km. The surrounding areas consist of rugged forest-covered mountains.

Mosquito collections

Human bait collections of adult mosquitoes were undertaken at both locations during two field trips; one in May and the other in June 2004, each lasting for five consecutive days. In the HIGH location there were 3 collection sites and in the LOW location there were 4 collection sites. Two persons collected mosquitoes landing on their exposed legs at each site between the hours of 18:00 and 24:00. Mosquitoes were also collected 2-3 times per night on a long open net surrounding a buffalo shelter situated in each location. The day after collection, mosquitoes were taken to the field laboratory and identified by species using morphological characteristics. Mosquitoes were then transported back to the laboratory in Chiang Mai city for subsequent bioassays. Time-mortality data from HIGH and LOW locations were compared within each species of the most abundant malaria mosquitoes collected, ie An. maculatus s.s., An. sawadwongporni, and An. minimus A.

Insecticide susceptibility bioassay and statistical analysis

The insecticide susceptibility bioassay followed the bottle bioassay tests developed by the Centers for Disease Control and Prevention (Brogdon and McAllister, 1998b; CDC, 2004). Diagnostic doses and thresholds for resistance for methyl parathion (MeP) and cypermethrin (Cyp) were established for a susceptible An. minimus A laboratory strain (Hang Dong F70). The diagnostic dose is the lowest concentration that kills 100% of susceptible mosquitoes in the shortest time and the threshold for resistance is the time interval where 100% mortality occurs in a susceptible population. Wild-caught An. maculatus s.s., An. sawadwongporni, and An. minimus A from the two villages were reared to the F1-generation and 2-7 day old, sugar-fed females were tested with the diagnostic doses of MeP and Cyp.

Lethal time values, LT50 and LT90, with 95% confidence intervals for MeP bioassay data were estimated for An. maculatus s.s. populations from HIGH and LOW locations using a Probit analysis of correlated data (Throne et al., 1995). No other LT values with confidence limits could be calculated because of too few observations. The chi-square goodness-of-fit test was used to determine how well regression lines fitted the observed data. The data transformation with
RESULTS

The diagnostic doses established for a susceptible laboratory strain of *An. minimus* were 10 μg/bottle and 30 μg/bottle for methyl parathion (MeP) and cypermethrin (Cyp), respectively, and the threshold for resistance was 20 minutes for both insecticides (Figs 1 and 2).

Reduced mortality to MeP was found in *An. maculatus* s.s. and *An. sawadwongporni* originating from the HIGH location (Table 1). *An. maculatus* s.s. exposed to the diagnostic dose for MeP showed a 74% and 92% mortality from the HIGH and LOW locations, respectively. The corresponding figures for *An. sawadwongporni* were 94% and 99%, showing a similar but reduced pattern of mortality to MeP. There were not enough *An. minimus* to undertake bioassays with MeP.

The results of bioassays with Cyp showed full susceptibility to this insecticide in both locations for all species (Table 1). The log-Probit transformation gave the lowest χ²-values for both the HIGH (χ²=6.99, df=2) and LOW (χ²=3.60, df=1) regression lines. Lethal time ratios with 95% confidence intervals for both LT50 and LT90 showed significant differences between *An. maculatus* s.s. HIGH and LOW populations (Table 2).

DISCUSSION

We have shown strong indications of reduced insecticide susceptibility in anopheline populations collected in areas with high agricultural insecticide use as opposed to mosquitoes from areas with low agricultural insecticide use in a malaria endemic district in northern Thailand. Lower methyl parathion (MeP) susceptibility of *An. maculatus* s.s. from the HIGH location compared to the LOW location has not occurred due to vector control activities, because organophosphates have never been used in mosquito control in the area and the mosquito control strategies are identical in the two locations. During the 1990s DDT was replaced with pyrethroids for malaria control in Thailand. Thus, the current vector control methods in the study areas are indoor residual spraying using deltamethrin and insecticide treated nets using lambdacyhalothrin and permethrin (Charoenviriyaphap et al., 1999). We claim that intensive agrochemical pest control activities – most likely by the use of organophosphates in fruit orchards – caused the
Fig 2- Establishment of diagnostic dose for susceptible An. minimus A lab-strain (Hang Dong F70) for cypermethrin (Cyp). The diagnostic dose is 30 μg/bottle and the threshold of resistance 20 minutes.

Table 1
Mortality of F1 progeny anopheline females from an area with high agricultural insecticide use, Muang Na village (HIGH) and an area with low agricultural insecticide use, Huay Mae Kiang village (LOW) in Chiang Mai province, northern Thailand subjected to diagnostic doses of methyl parathion (MeP) and cypermethrin (Cyp).

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Species</th>
<th>HIGH</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mortality</td>
<td>n</td>
<td>Mortality</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeP</td>
<td>An. maculatus s.s.</td>
<td>74 %</td>
<td>214</td>
<td>92 %</td>
<td>244</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An. sawadwongporni</td>
<td>94 %</td>
<td>237</td>
<td>99 %</td>
<td>244</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An. minimus A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyp</td>
<td>An. maculatus s.s.</td>
<td>99 %</td>
<td>171</td>
<td>100 %</td>
<td>166</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An. sawadwongporni</td>
<td>100 %</td>
<td>166</td>
<td>100 %</td>
<td>154</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An. minimus A</td>
<td>100 %</td>
<td>56</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

*Insufficient numbers of wild An. minimus A were collected to undertake complete bioassays.

Table 2
Lethal time values (LT50 and LT90, in minutes) and lethal time ratios (LTR) with 95% confidence intervals (in parenthesis) for An. maculatus s.s. subjected to diagnostic doses of methyl parathion (MeP). Mosquitoes were collected from an area with high agricultural insecticide use, Muang Na village (HIGH) and an area with low agricultural insecticide use, Huay Mae Kiang village (LOW) in Chiang Mai Province, northern Thailand.

<table>
<thead>
<tr>
<th>Lethal time</th>
<th>HIGH</th>
<th>LOW</th>
<th>LTR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT50</td>
<td>14.4 (11.2-18.1)</td>
<td>13.1 (12.5-13.8)</td>
<td>1.096 (1.007-1.193)</td>
</tr>
<tr>
<td>LT90</td>
<td>25.5 (20.0-39.1)</td>
<td>19.3 (18.2-20.7)</td>
<td>1.321 (1.180-1.478)</td>
</tr>
</tbody>
</table>

*If LTR confidence limits do not include 1, there is a significant difference in response between the two populations at the 0.05 level.
apparent pattern of resistance in *An. maculatus* s.s. The results for *An. sawadwongporni* showed a similar, but weaker, pattern as *An. maculatus*, which further supports the concept that fruit orchard insecticides affect resistance development in anophelines. Unfortunately, we were not able to collect enough *An. minimus* specimens to test for methyl parathion susceptibility.

*An. maculatus* s.s. is common in forested foothill areas and breeds in similar habitats as *An. minimus*, i.e., along the edges of slow-moving streams, but also in ponds, marshes, and paddy fields (Reid, 1968). *An. maculatus* s.s. is commonly associated with malaria transmission in the south of Thailand (Reid, 1968), but has also been incriminated in northern Thailand (Somboon et al., 1998). Considerable agrochemical-insect contact may occur during mosquito flight between breeding habitats and blood sources and resting places, potentially increasing insecticide selection pressure. Insecticide residuals in breeding habitats originating from run-off or drift may also confer increased selection pressure for resistance at the larval stage. In the Mueang Na area (HIGH), there is an abundance of small streams and other potential breeding habitats among the pesticide-intensive fruit orchards.

Aerial photographs from 1995 of the Mueang Na area showed that fruit orchards had not yet been established. If insecticides from fruit orchards confer resistance (which seems very likely), our results indicate that resistance in malaria mosquitoes in this area has developed in a time-span of less than 10 years.

One aspect that may compromise future control efforts is the potential for cross-resistance between organophosphates and pyrethroids. Resistance to pyrethroids has commonly been associated with cross-resistance to DDT (Brogdon et al., 1999; Ranson et al., 2000), however Brogdon and Barber (1990) found that an esterase-based resistance mechanism in *An. albimanus* conferred cross-resistance between pyrethroids (deltamethrin) and organophosphates (fenitrothion). In areas of predominantly organophosphate agricultural pest control, such cross-resistance may pose a potential threat to future vector control.

Our results also show that the three anopheline species studied here were still susceptible to pyrethroids. This confirms results from another study undertaken in the area, in which *An. minimus* was still found to be susceptible to both pyrethroids and DDT (Somboon et al., 2003). Based on these facts, malaria mosquito control as undertaken today is still likely to be effective in this area. However, increased use of pyrethroids in agriculture may cause problems for future vector control, because of the apparent mosquito-insecticide contact in this environment, increasing the chances of insecticide resistance development. Thus, it is important to continue regular monitoring of pyrethroid resistance in mosquito vectors.

The results presented here will be followed-up by biochemical microplate assays to confirm if reduced methyl parathion susceptibility in *An. maculatus* s.s. and *An. sawadwongporni* is caused by physiological resistance. We will also complete susceptibility tests on *An. minimus*.

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