EVIDENCE OF ANOPHELINE MOSQUITO RESISTANCE TO AGROCHEMICALS IN NORTHERN THAILAND

Hans J Overgaard[1,2,3], Simen R Sandve[1] and Wannapa Suwonkerd[4]

[1]Department of Ecology and Natural Resource Management, Agricultural University of Norway, Ås, Norway; [2]International Water Management Institute, Southeast Asia Regional Office, Bangkok, Thailand; [3]Department of Entomology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [4]Office of Vector-Borne Disease Control No. 10, Ministry of Public Health, Chiang Mai, Thailand

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. 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).

Correspondence: Hans J Overgaad, International Water Management Institute, Southeast Asia Reginonal Office, Bangkok, Thailand.
E-mail: hansovergaard@yahoo.se

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 evidence has yet been found in Southeast Asia.

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 anopheline 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 anopheline 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 insecticide 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 lowest χ^2-value was used to describe the data. If necessary, data were corrected using a heterogeneity factor. LT50 and LT90 for HIGH and LOW populations were compared by calculating lethal time ratios (LTR) with 95 % confidence intervals. If the LTR confidence limits do not include 1, there is a significant difference in response between the two populations at the 0.05 level. The Probit analysis and LTR calculations were done with PROBIT® and RELPOT®, respectively, written in Mathematica language (Wolfram, Champaign, IL), developed by Dr James Throne (Accessible from http://bru.gmprc.ksu.edu/sci/throne/).

RESULTS

The diagnostic doses established for a susceptible laboratory strain of *An. minimus* A 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* A collected 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 χ^2-values for both the HIGH (χ^2=6.99, df=2) and LOW (χ^2=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

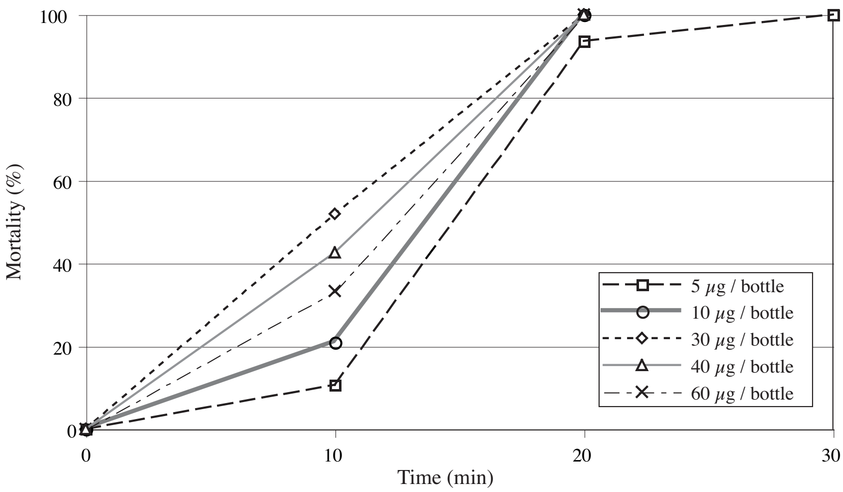

Fig 1- Establishment of diagnostic dose for susceptible *An. minimus* A lab-strain (Hang Dong F70) for methyl parathion (MeP). The diagnostic dose is 10 μg/bottle and the threshold of resistance 20 minutes.

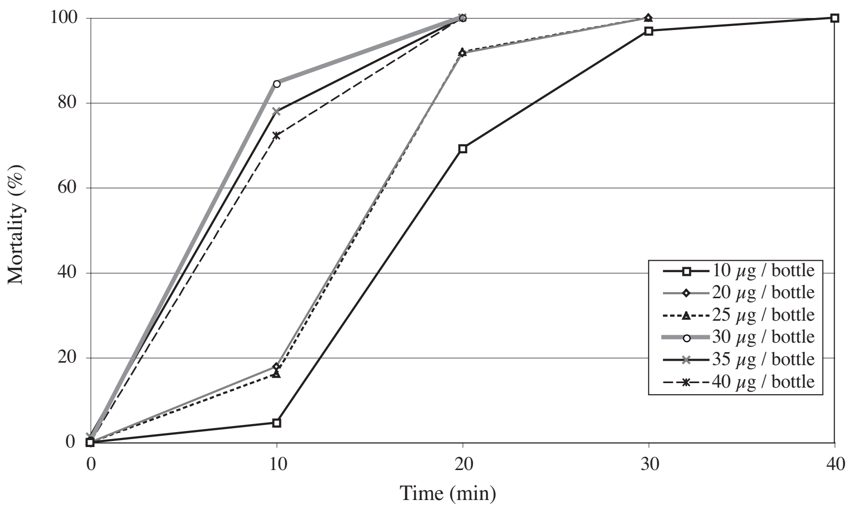

Fig 2- Establishment of diagnostic dose for susceptible *An. minimus* A lab-strain (Hang Dong F70) for cypermethrin (Cyp). The diagnostic dose is 30 µg/bottle and the threshold of resistance 20 minutes.

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).

Insecticide	Species	HIGH		LOW	
		Mortality	n	Mortality	n
MeP	*An. maculatus* s.s.	74 %	214	92 %	244
	An. sawadwongporni	94 %	237	99 %	244
	An. minimus A[a]	-	-	-	-
Cyp	*An. maculatus* s.s.	99 %	171	100 %	166
	An. sawadwongporni	100 %	166	100 %	154
	An. minimus A[a]	100 %	56	-	-

[a]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.

Lethal time	HIGH	LOW	LTR[a]
LT50	14.4 (11.2-18.1)	13.1 (12.5-13.8)	1.096 (1.007-1.193)
LT90	25.5 (20.0-39.1)	19.3 (18.2-20.7)	1.321 (1.180-1.478)

[a]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*, *ie* 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 (fenithrotion). 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* A.

ACKNOWLEDGEMENTS

We thank the staff of the Office of Vector-Borne Disease Control in Chiang Mai, particularly Raksakul Kantawong, Nantawan Suwannachote, Padaj Kaewkoun and Pongnarin Dee-in for mosquito collections and insect work; Dr James Throne, Biological Research Unit, GMPRC, Manhattan, Kansas and Magnus Dehli Vigeland for help with statistical analyses. We are grateful to Dr La-aied Prapanthadara at the Research Institute for Health Sciences, Chiang Mai University; Dr Andrew Noble at the International Water Management Institute – Southeast Asia Regional Office; and Dr Theeraphap Charoenviriyaphap, Department of Entomology, Kasetsart University, Bangkok for general support and suggestions to the manuscript. This study was financed by the Research Council of Norway, Swedish International Development Cooperation Agency (Sida), and the Agricultural University of Norway.

REFERENCES

Brogdon WG, Beach RF, Stewart JM, Castanza L. Microplate assay analysis of the distribution of organophosphate and carbamate resistance in Guatemalan *Anopheles albimanus*. *Bull World Health Organ* 1988;66:339-46.

Brogdon WG, Barber AM. Fenitrothion-deltamethrin cross-resistance conferred by esterases in Guatemalan *Anopheles albimanus*. *Pestic Biochem Physiol* 1990;3:130-9.

Brogdon WG, McAllister JC. Insecticide resistance and vector control. *Emerg Infect Dis* 1998a;4:1-11.

Brogdon WG, McAllister JC. Simplification of adult mosquito bioassays through use of time-mortality determinations in bottles. *J Am Mosq Control*

Assoc 1998b;14:159-64.

Brogdon WG, McAllister JC, Corwin AM, Cordon-Rosales C. Oxidase-based DDT-pyrethroid cross-resistance in Guatemalan *Anopheles albimanus. Pestic Biochem Physiol* 1999;64:101-11.

CDC. Evaluating mosquitoes for insecticide resistance - web-based instruction. Center for Infectious Diseases, Centers for Disease Control and Prevention, 2004. [Accessed October 2004]. Available at: URL: http://www.cdc.gov/ncidod/wbt/resistance/toc.htm

Chapin G, Wasserstrom R. Agricultural production and malaria resurgence in Central America and India. *Nature* 1981;293:181-5.

Charoenviriyahpap T, Aum-aung B, Ratanatham S. Current insecticide resistance patterns in mosquito vectors in Thailand. *Southeast Asian J Trop Med Public Health* 1999;30:184-94.

Diabate A, Baldet T, Chandre F, *et al*. The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. *Am J Trop Med Hyg* 2002;67:617-22.

Georghiou, GP. Overview of insecticide resistance. In: Green MB, LeBaron HM, Moberg WK, eds. Managing resistance to agrochemicals. Washington DC: American Chemical Society, 1990a:18-41.

Georghiou GP. The effect of agrochemicals on vector populations. In: Roush RT, Tabashnik BE, eds. Pesticide resistance in arthropods. New York: Chapman and Hall, 1990b:183-202.

Georghiou GP, Ariaratnam V, Breeland SG. *Anopheles albimanus*: development of carbamate and organophosphorus resistance in nature. *Bull World Health Org* 1971;46:551-4.

Hemingway J, Georghiou GP. Studies on the acetylcholinesterase of *Anopheles albimanus* resistant and susceptible to organophosphate and carbamate insecticides. *Pestic Biochem Physiol* 1983;19:167-71.

Jungbluth, F. Crop protection policy in Thailand. Economic and political factors influencing pesticide use. Hannover University, Germany; Pesticide Policy Project publication 1996:5.

Lines JD. Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. *Parasitol Today* 1988;4:S17-S20.

Malikul S. The current situation of the anti-malaria programme in Thailand. *Southeast Asian J Trop Med Public Health* 1988;19:355-9.

Overgaard HJ, Ekbom B, Suwonkerd W, Takagi M. Effect of landscape structure on anopheline mosquito density and diversity in northern Thailand: Implications for malaria transmission and control. *Lands Ecol* 2003;18:605-19.

Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Mol Biol* 2000;9:491-7.

Reid JA. Anopheline mosquitoes of Malaya and Borneo. *Stud Inst Med Res Malaysia* 1968;31:1-520.

Sharma, VP. Re-emergence of malaria in India. *Indian J Med Res* 1996;103:26-45.

Somboon P, Aramrattana A, Lines J, Webber R. Entomological and epidemiological investigations of malaria transmission in relation to population movements in forest areas of north-west Thailand. *Southeast Asian J Trop Med Public Health* 1998;29:3-9.

Somboon P, Prapanthadara L, Suwonkerd, W. Insecticide susceptibility tests of *Anopheles minimus* s.l., *Aedes aegypti, Aedes albopictus,* and *Culex quinquefasciatus* in northern Thailand. *Southeast Asian J Trop Med Public Health* 2003;34:87-93.

Throne JE, Weaver DK, Chew V, Baker JE. Probit analysis of correlated data: Multiple observations over time at one concentration. *J Econ Entomol* 1995;88:1510-2.

WHO. Resistance of vectors and reservoirs of disease to pesticides. *WHO Tech Rep Ser* 1986;737:1-87.