Insecticide resistance management in a multi-resistant malaria vector scenario

A Mexican trial shows sustainability

A large-scale field trial in Mexico evaluated resistance management strategies for dealing with, delaying or even stopping insecticide resistance selection. Biological and biochemical assays showed that high level resistance development was reduced and kept at low levels by using rotation or mosaic schemes rather than single insecticide regimes.

Insecticide resistance is an important issue in malaria control, with some vectors already multi-resistant. Agricultural usage of insecticides has also increased the selection pressure on disease vectors that rest and breed on the crops. The use of insecticides is currently the major method of prevention and control of many vector-borne diseases. Dengue control, for example, relies on the use of larvicides, such as organophosphates, or space sprays. Although alternatives such as bio-insecticides and insect growth regulators (IGR) are available, their higher costs often prevent their use in developing countries. Since only a few new molecules for vector control are being developed, new approaches to retain efficacy of currently available public health insecticides are

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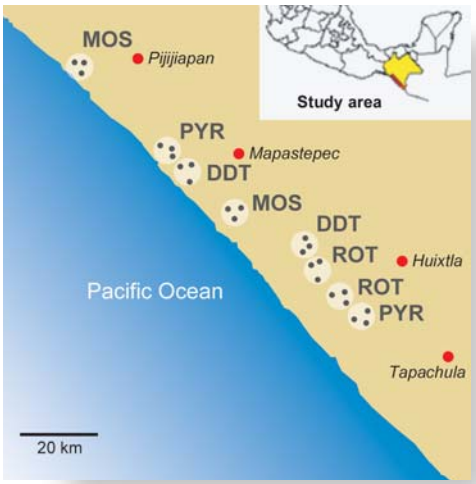
Photo: Adalberto Rios Szalay / Sexto Sol / gettyimages



MAP OF THE STUDY AREA indicating the groups of villages and the treatments.

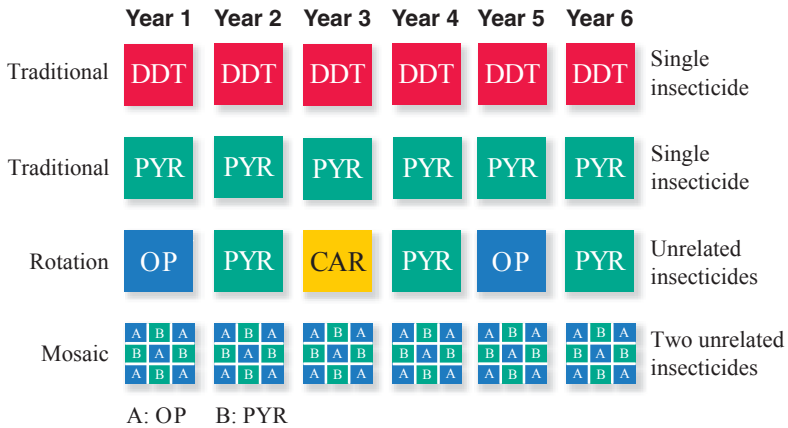
MOS = mosaic application,
PYR = single use of a pyrethroid,
DDT = single use of DDT,
ROT = annual rotation of insecticides.

clearly needed. Resistance management strategies are an option that could delay or in the most favorable scenario, stop resistance development while maintaining disease control.

To establish whether predicted methods of resistance management would work under operational conditions, the IRAC public health group sponsored an ambitious resistant management program against *Anopheles albimanus,* the multi-resistant New World malaria vector *(see box page 29: IRAC*).

Designing operational conditions

In the coastal plain of Chiapas, Mexico, a large-scale field trial was undertaken from 1996-2002 to evaluate rotations and mosaics of insecticides (*see map above*). The site was chosen because of the history of insecticide use in Mexico. Extensive agricultural and public health insecticide use during the 1960's and 1970's selected multiple insecticide resistance mechanisms in *An. albimanus*, the main coastal malaria vector. Subsequent changes in land use, the reduction in cotton farming and the success of malaria control activities consequently decreased insecticide use. This resulted in a well-documented regression towards insecticide susceptibility in *An. albimanus* to all insecticides except DDT – as measured by diagnostic WHO mortality tests (*see table page 26: Experimental design*). DDT resistance was maintained by continued use of this insecticide for malaria control activities in Mexico.



EXPERIMENTAL DESIGN
Four treatment regimes were assigned to the selected villages:

PYR = pyrethroid (deltamethrin)
OP = organophosphate (pirimiphos-methyl)
CAR = carbamate (bendiocarb)

Twenty-four villages were selected and grouped into sets of three villages, which were randomly assigned to one of four treatment regimes (*see map page 25*).

All insecticides involved in the study were applied as part of normal anti-malarial activities three times per year, with the exception of DDT, which was sprayed twice per year. Insecticides were sprayed with a Hudson X-Pert® sprayer with nozzle No. 8002. Wall bioassays to monitor residual efficacy of insecticides were conducted one day and then every month, after spraying. Good killing effect of mosquitoes was achieved with all products at the applied dosages (pirimiphos-methyl) at 2 g a.i./m^2, deltamethrin at 0.025 g a.i./m^2, bendiocarb at 0.4 g a.i./m^2 and DDT at 2 g a.i./m^2), with mosquito mortalities averaging around 75% four months after insecticide application.

Mortality of *Anopheles albimanus*

Insecticide	Concentration (%)	Mortality (%) 1982	1983	1990	1997
DDT	4	38	39	47	40
Malathion	5	84	93	99	100
Fenitrothion	1	44	57	99	100
Fenthion	2.5	97	100		99
Chlorphoxim	4	98	99	100	100
Propoxur	0.1	89	95		100
Deltamethrin	0.025	64	57	86	99
Cypermethrin	0.1		82		100
Bendiocarb	0.1		87		100
Pirimiphos-methyl	4		99		100

MORTALITY of *Anopheles albimanus* from the Chiapas coastal plain to WHO diagnostic adult doses of different insecticides during the early 1980's and late 1990's.

Photo: Cuauhtemoc Villarreal

A NUMBER OF VILLAGES were selected for a large-scale field trial.

Field-caught mosquitoes in the lab

The frequency of all resistance mechanisms was monitored before and during the intervention period by biochemical assays, along with WHO diagnostic bioassays using insecticide impregnated papers. Field samples of mosquitoes were collected on a regular basis approximately three months after each spray round and the F1 generation reared from the field-caught mosquitoes were used for all assays. When few mosquitoes were available, priority was given to biochemical assays since this method was the most sensitive for detection of small changes in resistance. Biochemical assay results were compared with the susceptible *An. albimanus* Panama strain. Logistic regression analyses were used to determine the effect of the different treatment regimes on the frequency of different resistance mechanisms. Pyrethroid treatment and pre-spray were set as reference variables in the analysis. Since no changes were observed in DDT resistance levels under any treatment scheme during the whole study period, data from DDT treated villages were excluded from the analyses.

It had been anticipated that DDT resistance should have declined over time when the DDT selection pressure was relaxed. It did not. There are two possible reasons for this. Either the DDT still on the walls (given the longevity of the active agent) was sufficient to maintain positive selection – or perhaps more likely the resistance had been selected so long ago and then maintained that any negative selection associated with DDT resistance genes had been counterbalanced by other genetic changes, thus removing the negative fitness costs of the resistance genes.

Rotation or mosaic schemes more effective?

Bioassays showed that continuous use of a pyrethroid gradually increased pyrethroid

Photo: Cuauhtemoc Villarreal

resistance in the mosquito field population over the first four years: resistance then remained stable for the next two years. In the rotation and mosaic schemes, pyrethroid and organophosphate resistance were selected at low levels and remained stable. No carbamate resistance was observed in the rotation scheme.

The biochemical assays *(see box below)* showed that although enzyme activity patterns varied, the chances of high level resistance development using a rotation or a mosaic regime were significantly lower than the rate at which resistance was selected using a pyrethroid alone.

Delaying resistance selection

Both the rotation and mosaic strategies performed well operationally and were accepted by the local population. Hence, rotations or mosaics should be implemented as part of normal malaria control operations, to reduce the likelihood of resistance development. Even in areas where resistance is already present these strategies may still work well and delay high level resistance selection.

Resistance biochemistry

Organophosphate and carbamate resistance in *Anopheles* is often due to a change in the insecticide's target site, acetylcholinesterase (AChE). Odds ratios for individuals with altered AChE above the normal insecticide susceptible range were significantly higher for the rotation and mosaic treatments compared to the single pyrethroid treatment during most of the study period, and after the application of both organophosphate and carbamate in the rotation system.

Altered AChE was the main mechanism conferring resistance against organophosphates and carbamates in Mexico and resistance increased slightly due to this mechanism with both the rotation and mosaic regime.

Esterase-based organophosphate and pyrethroid resistance is also common in mosquitoes. Odds ratio for individuals with esterase levels (measured with the substrate ρNPA) above the normal susceptible range indicated that the rotational regime kept that mechanism at or below "acceptable" levels, as compared to the single use of a pyrethroid. This suggests that esterases play an important role in conferring pyrethroid resistance in *An. albimanus*.

Odds ratios for individuals with esterase levels using α-naphthyl acetate as a substrate were above the normal susceptible range for both the rotation and mosaic regimes, hence they selected for individuals with this type of resistance mechanism.

The odds ratios for individuals with cytochrome P^{450}s above the normal susceptible range also indicated that by the fourth year of using the pyrethroid or the rotation, a significantly higher frequency of individuals with this resistance mechanism were selected. There was no evidence of selection of a glutathione transferase-based mechanism by any of the four treatments.

WAITING FOR the house to be sprayed after moving goods and furniture outside.

The format of the rotation scheme should take into account the previous history of insecticide resistance or insecticide use.

Where resistance management is undertaken resistance levels should be monitored regularly. In the course of this trial the biochemical assays, although variable, were more reliable and practical than the bioassays. More insects could be processed compared to the WHO method and a greater amount of information was generated per mosquito when sample numbers were low.

CONCLUSION

New public health insecticides have been brought to market at a slower rate than insecticide resistance has developed, and regulatory issues have further reduced the available insecticide choice. Better resistance management of current and new public health insecticides, to delay or even stop resistance selection, is needed if vector control is to be sustainable in the long-term.

Article (with plots of odds ratios) on the enclosed Public Health CD-ROM

IRAC

Insecticide Resistance Action Committee

IRAC is an inter-company group formed in 1984 to provide insecticide and acaricide resistance management strategies to help reduce the development of resistance in insect and mite pests.

The key to managing resistance is to reduce selection pressure caused by the over-use or misuse of an insecticide, because this could result in the selection of resistant forms of the pest and the consequent evolution of populations that are resistant to that insecticide.

IRAC believes that Resistance Management should be an integral part of Integrated Pest Management and provides for sustainable agriculture and improved public health.

Photo: IRAC

IRAC is acting as a Specialist Technical Group of CropLife.

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