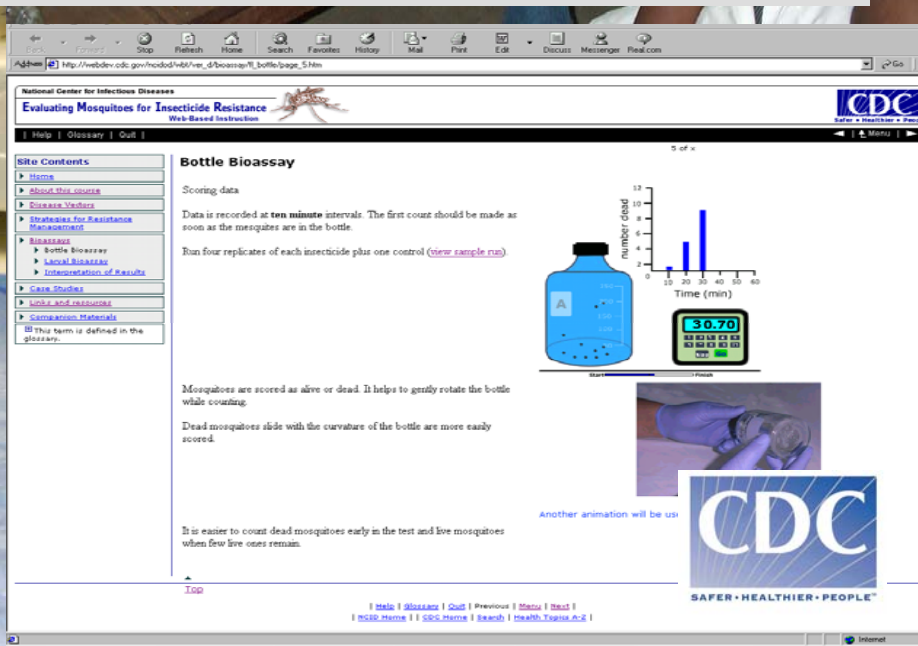
TACTICAL INSECTICIDE RESISTANCE SURVEILLANCE USING THE BOTTLE BIOASSAY

Santa Cruz, March 3rd, 2010

CDC Bottle Bioassay



Primary Tactical Question

WILL THIS FORMULATION OF THIS INSECTICIDE CONTROL THIS VECTOR AT THIS LOCATION AT THIS TIME?


IF NOT,
WHAT DO I DO NOW?

THE PRIMARY GOAL OF RESISTANCE SURVEILLANCE IS THE MEASUREMENT OF RESISTANCE:

AS IT EXISTS...

AT A PARTICULAR PLACE...

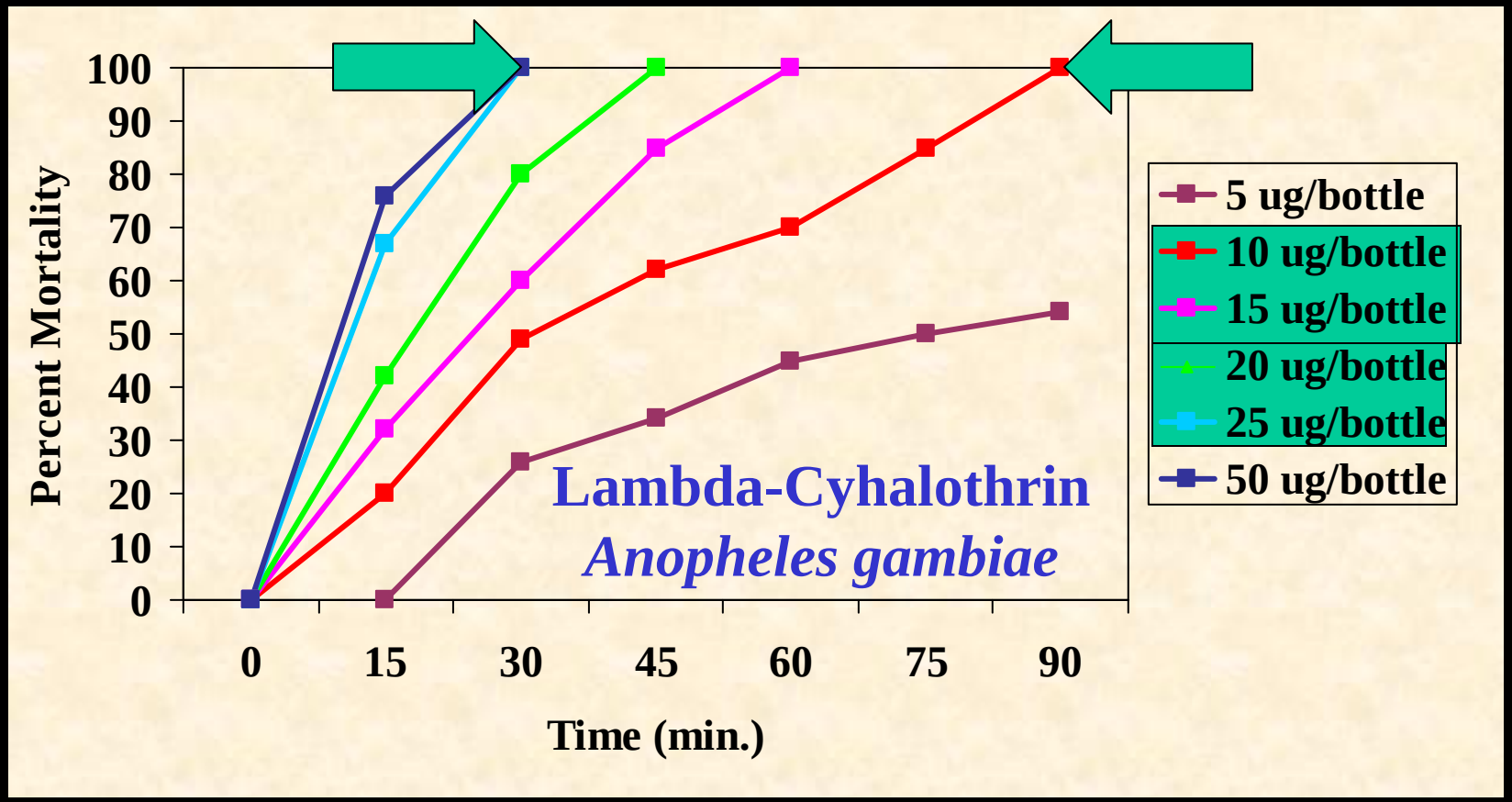
AT A PARTICULAR TIME.

SIMPLE

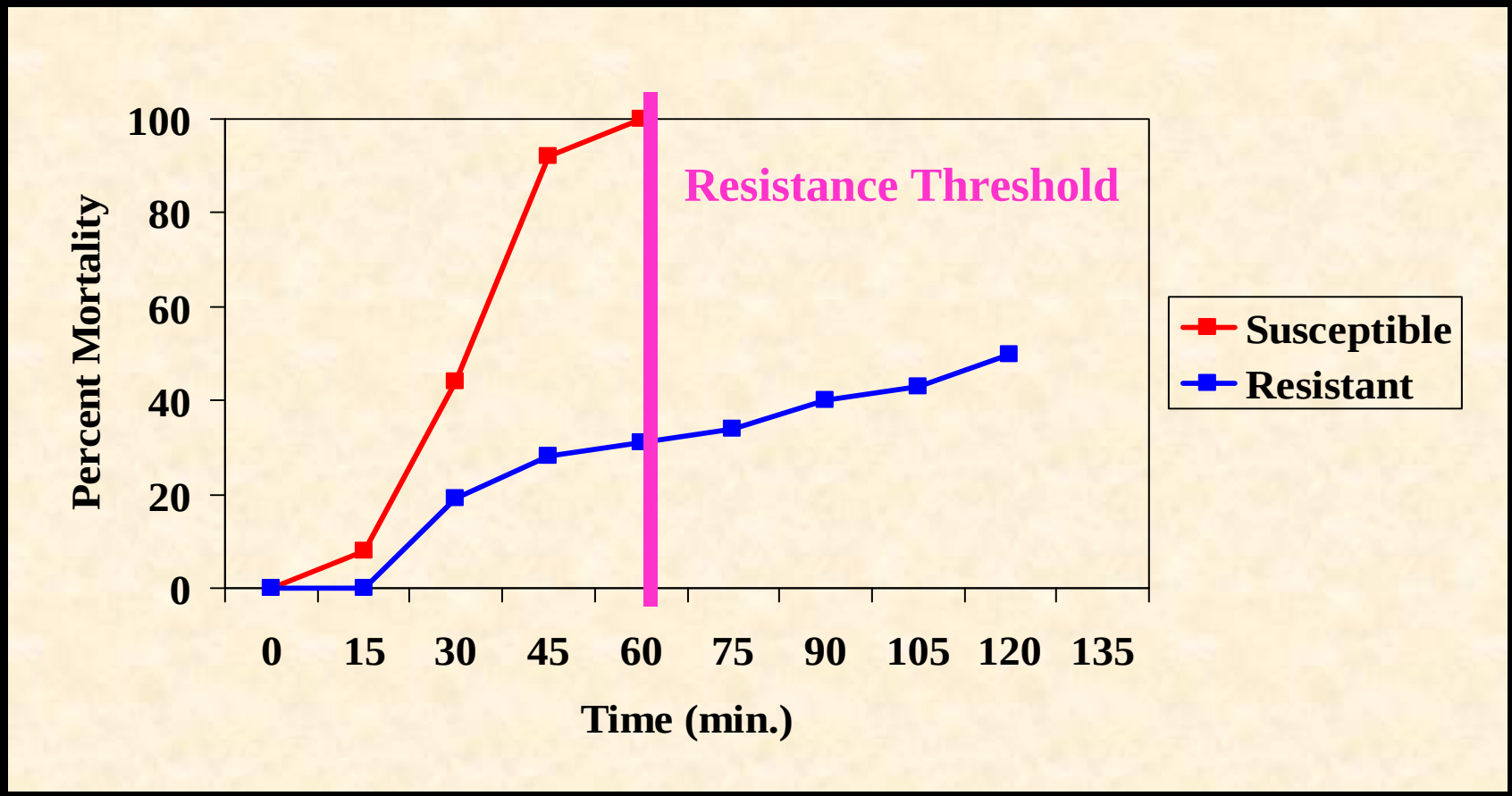
PRACTICAL

CHEAP

SEVERAL CONCENTRATIONS MAY FALL WITHIN USABLE RANGE...



UPPER RANGE LIMIT FOR SURVIVAL OF SUSCEPTIBLE POPULATION IS THE RESISTANCE THRESHOLD



Standardized diagnostic doses – Guayaquil 2008

Insecticida	**Concentración** (ug/botella)	**Tiempo** (minutos)
Deltametrina	12.5	30
Lambdacialotrina	12.5	30
Ciflutrina	12.5	30
Etofenprox	12.5	30
Cipermetrina	12.5	30
Permetrina	21.5	30
DDT	100	45
Malation	50	30
Fenitrotion	50	30
Propoxur	12.5	30
Bendiocarb	12.5	30

THESE DOSAGES WILL WORK QUITE WELL FOR:

Anopheles darlingi
Anopheles albimanus
Anopheles nuneztovari
Anopheles albitarsis
Anopheles aquasalis
Anopheles marajoara

Anopheles gambiae

Anopheles stephensi

Anopheles dirus

THOUGH OPTIMUM DIAGNOSTIC TIMES CAN VARY

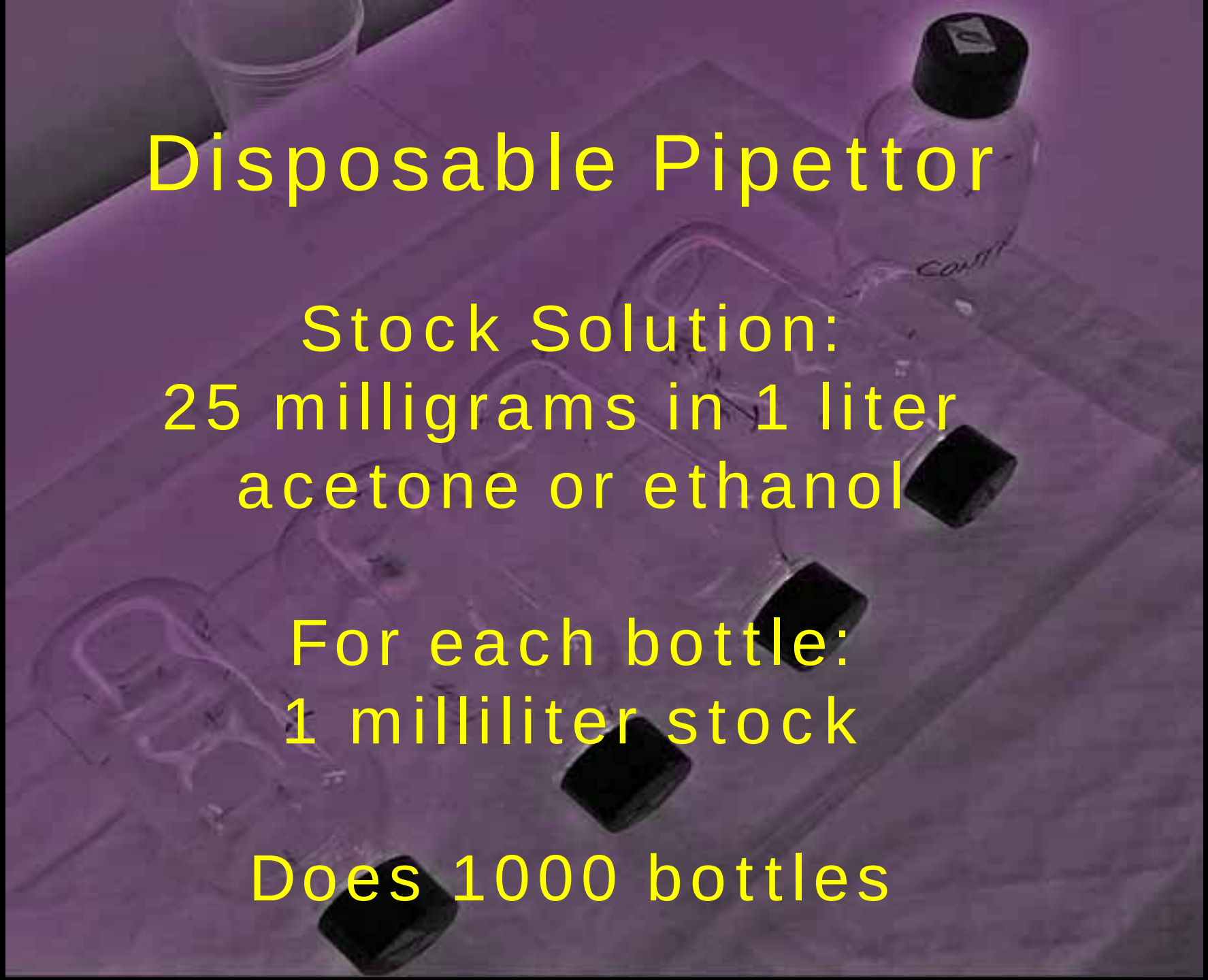

ETHYL
ALCOHOL


E
1
2
3
4


ETHYL
ALCOHOL
250
200
150
100
50


100
120
500
ETHYL
ALCOHOL


2
3
4



Disposable Pipettor

Stock Solution:
25 milligrams in 1 liter
acetone or ethanol

For each bottle:
1 milliliter stock

Does 1000 bottles

For formulations, calculate amount based upon active ingredient

For example:
Reagent grade (100 % active)
Use 25 milligrams

10% formulation:
Use 250 milligrams

Milligrams reagent grade divided by % in formulation

Colombia

Peru

Surinam

Brazil

Bolivia


UNIVERSIDAD
DE ANTIOQUIA
1803

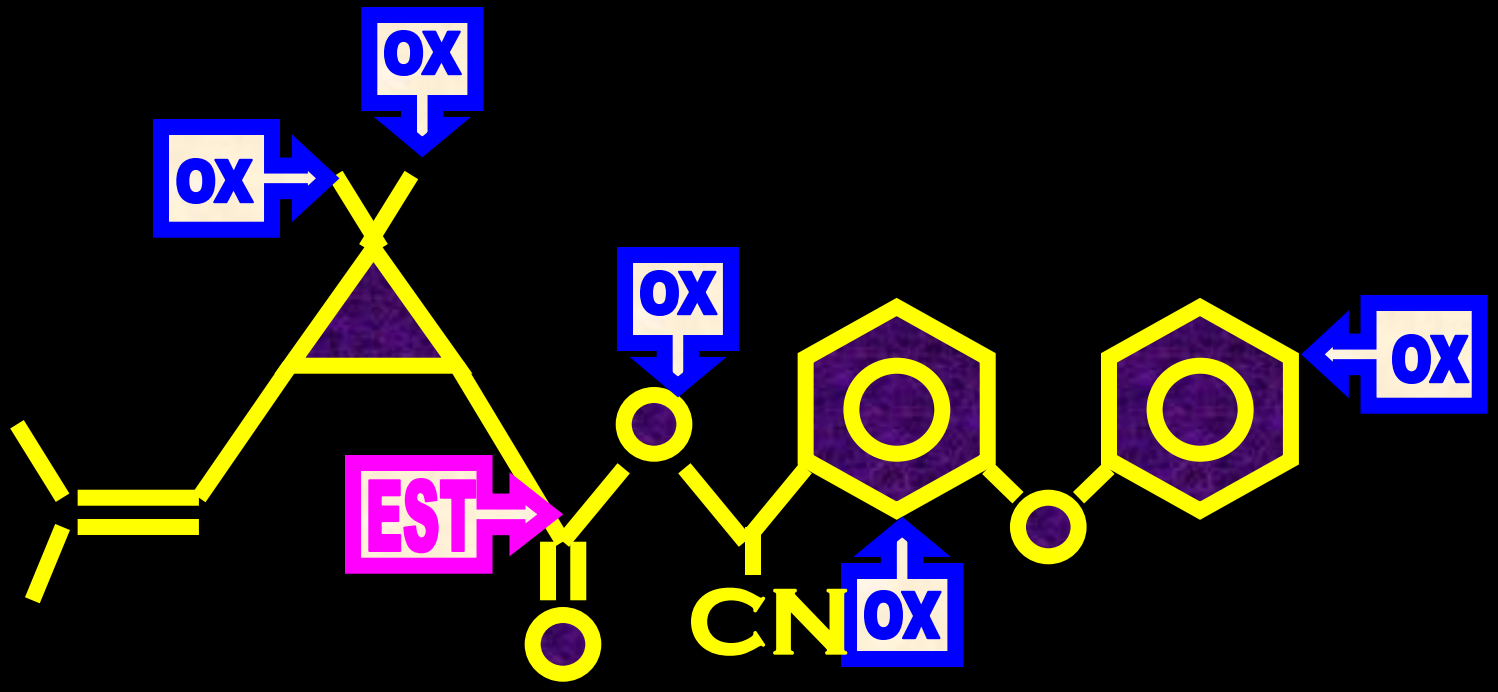
Gracias a todos !

It is not enough to know that resistance is present.

To decide what to do next, we must know why the mosquitoes are resistant.

For example, is there likely to be cross resistance to another insecticide or insecticide class?

Detoxification enzyme attack on a synthetic pyrethroid :



(1R, trans) (aR) (1)

(1R, trans) (aS) (2)

(1R, cis) (aR) (3)

(1R, cis) (aS) (4)

(1S, trans) (aR) (5)

(1S, trans) (aS) (6)

(1S, cis) (aR) (7)

(1S, cis) (aS) (8)

Configuration of the 8 isomers that constitute cypermethrin.

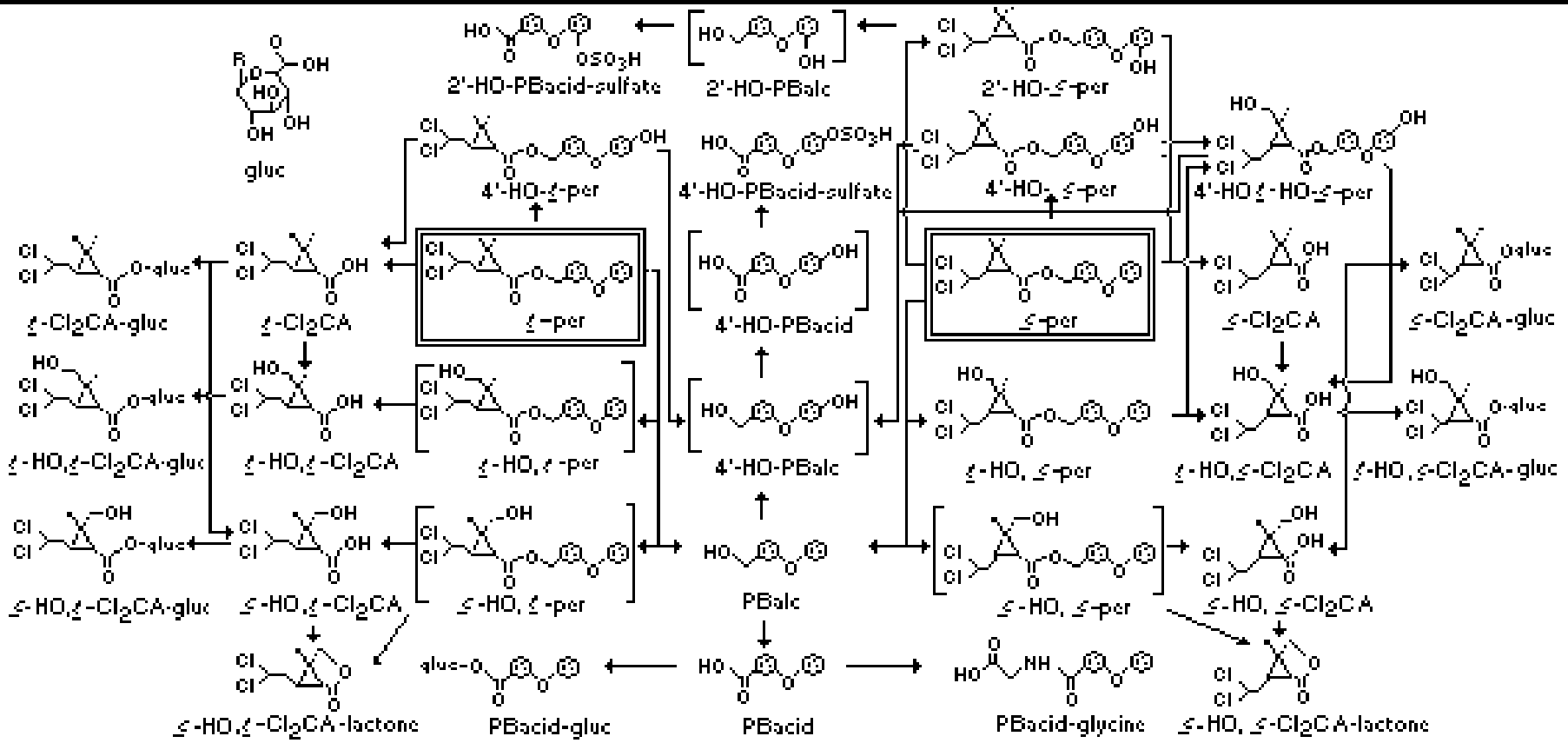
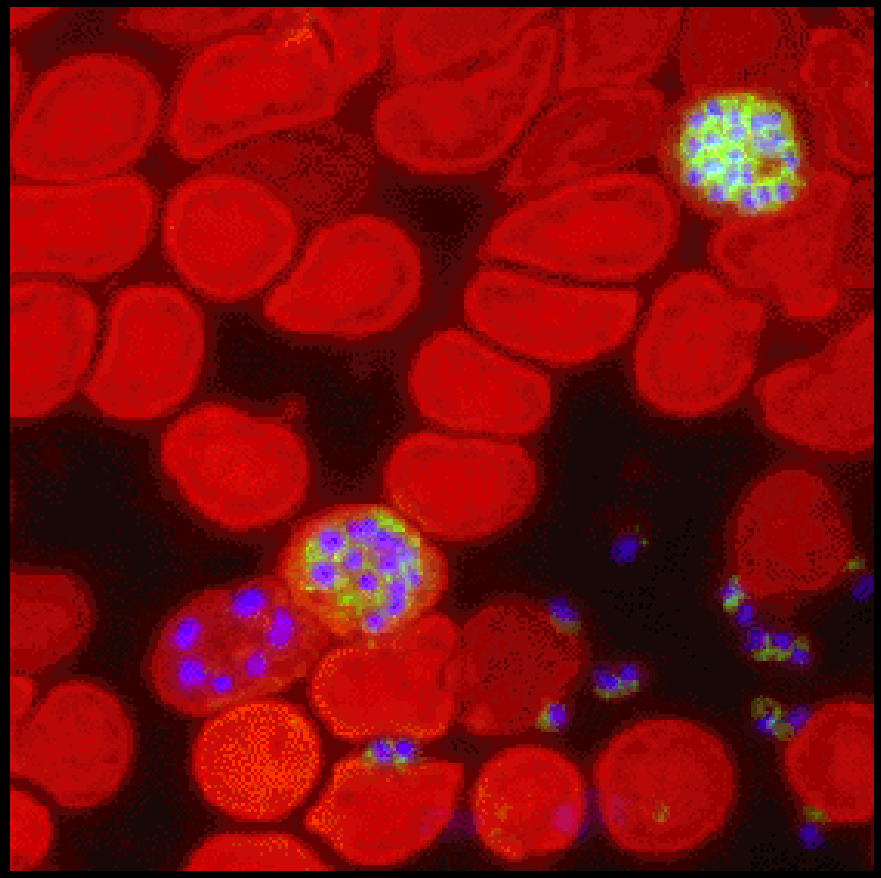


Figure 1. **Metabolic pathways for (1R, trans) - and (1R, cis)-permethrin indicating the abbreviations used for various metabolites. These pathways are also applicable to (1 RS, trans) - and (1RS, cis)-permethrin and to phenoxybenzyl alcohol and (1R, trans)-C1, CA. Additional pathways, which are not shown yield several minor metabolites, most of these being formed from cis-permethrin.**

BIOCHEMICAL ASSAYS RUN IN MICROPLATES

MOLECULAR ANALYSIS



The Impact of Extreme Genetic Diversity Upon Practical Diagnostics

“The standard PCR assay for detection of the kdr mutation in *An. gambiae* S form showed little association with pyrethroid resistance.

Subsequent sequencing of the II56 domain containing the kdr mutation from nine surviving mosquitoes showed that eight were homozygous resistant and one heterozygous.

This correlated with the bioassay results and with previous studies on West African *An. gambiae*, but raised concerns about the reliability of the PCR assay for detection of the kdr mutation.”

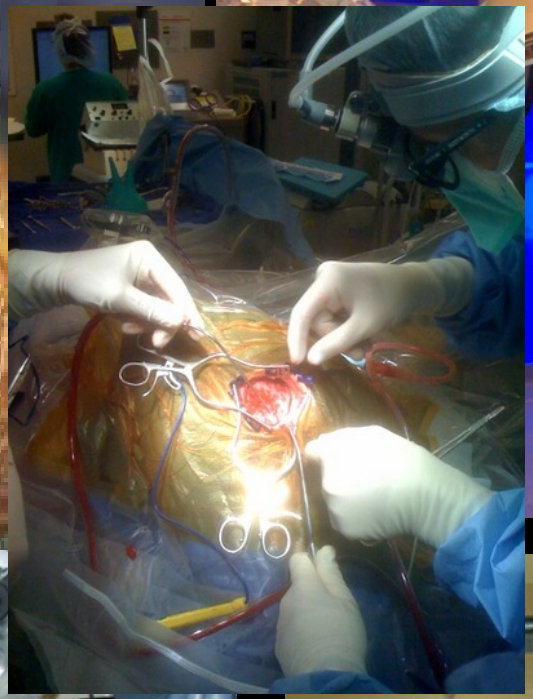
What is making the design of reliable molecular diagnostic assays so challenging?

There is gathering evidence that the sequence diversity within and between individual organisms and between small populations of organisms, be they parasites or vectors, is extreme.

This genetic diversity is causing severe problems in the design of broadly applicable molecular diagnostic methods.

THIS DIFFICULTY AND THE DIFFICULTY OF DOING BIOCHEMICAL AND MOLECULAR ASSAYS UNDER PRIMITIVE FIELD CONDITIONS CAUSED US TO INCORPORATE THE USE OF SYNERGISTS INTO THE BOTTLE BIOASSAY PROTOCOL.

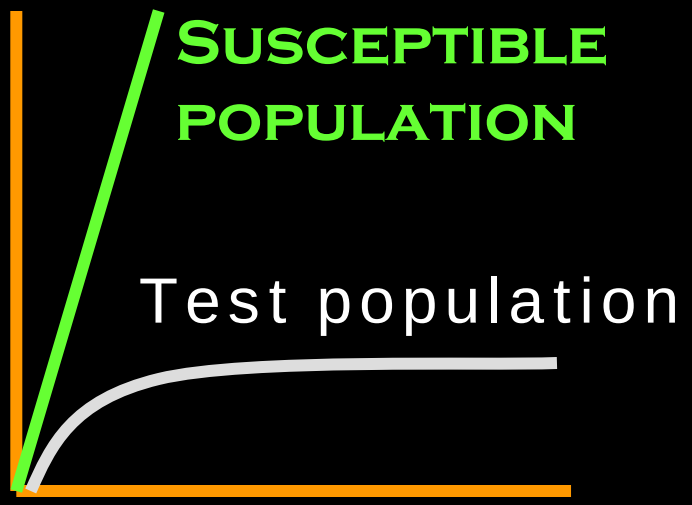





PSYCHO

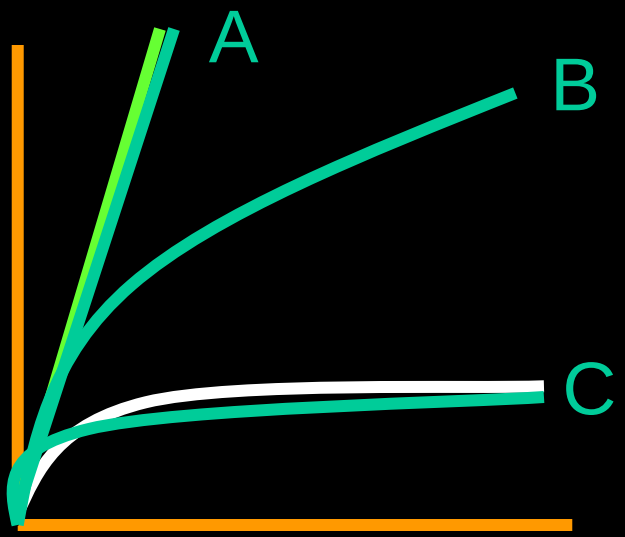
Pyrethroid Resistance Bottle Bioassay.

Bottle treated with: pyrethroid only



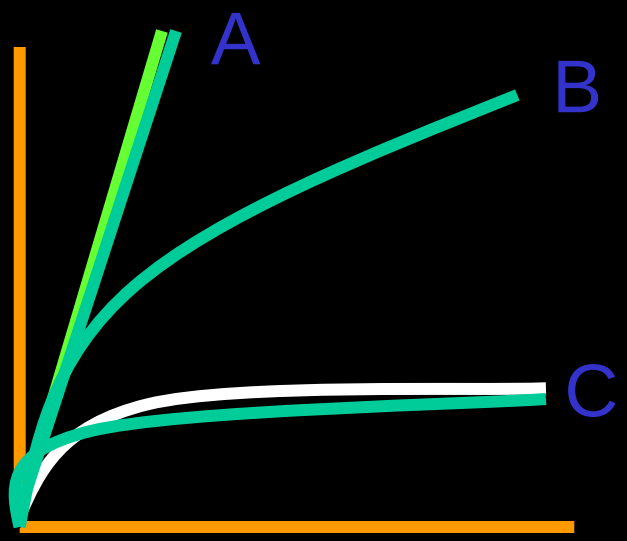
Esterase Resistance Bottle Bioassay.

Bottle treated with:
pyrethroid + DEF
(tributyl phosphoro trithioate)



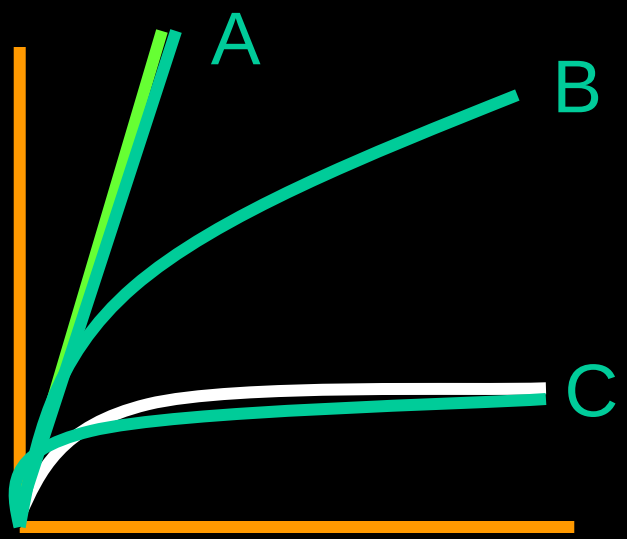
Oxidase Resistance Bioassay.

Bottle treated with: pyrethroid + piperonyl butoxide (PB)



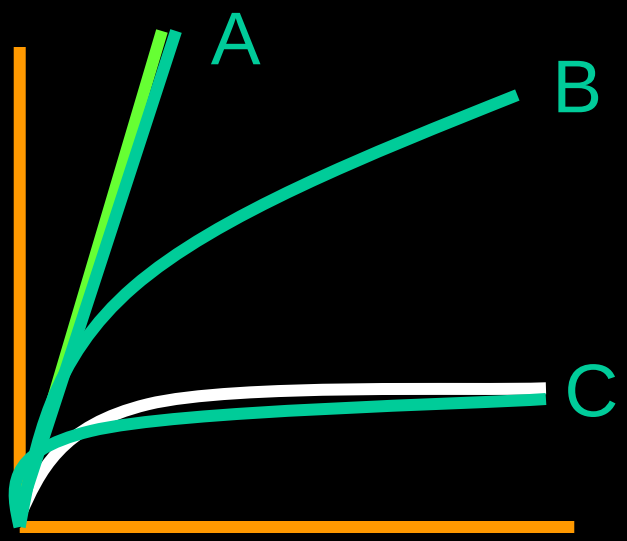
Glutathione s-Transferase Resistance Bioassay.

Bottle treated with: pyrethroid + Ethacrynic Acid



kdr Resistance Bioassay.

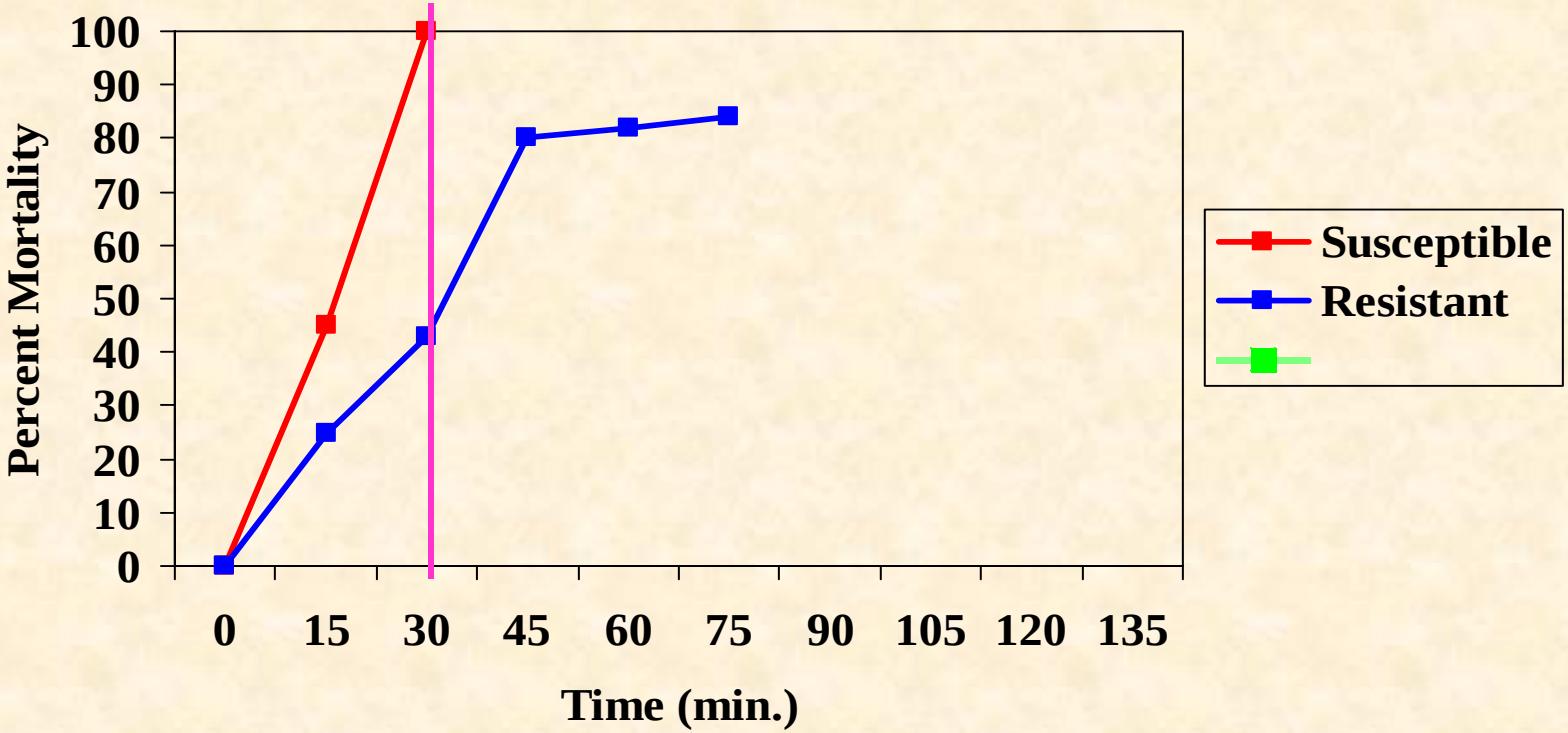
Bottle treated with:
pyrethroid + all 3 inhibitors



ABIDJAN, IVORY COAST

Anopheles gambiae

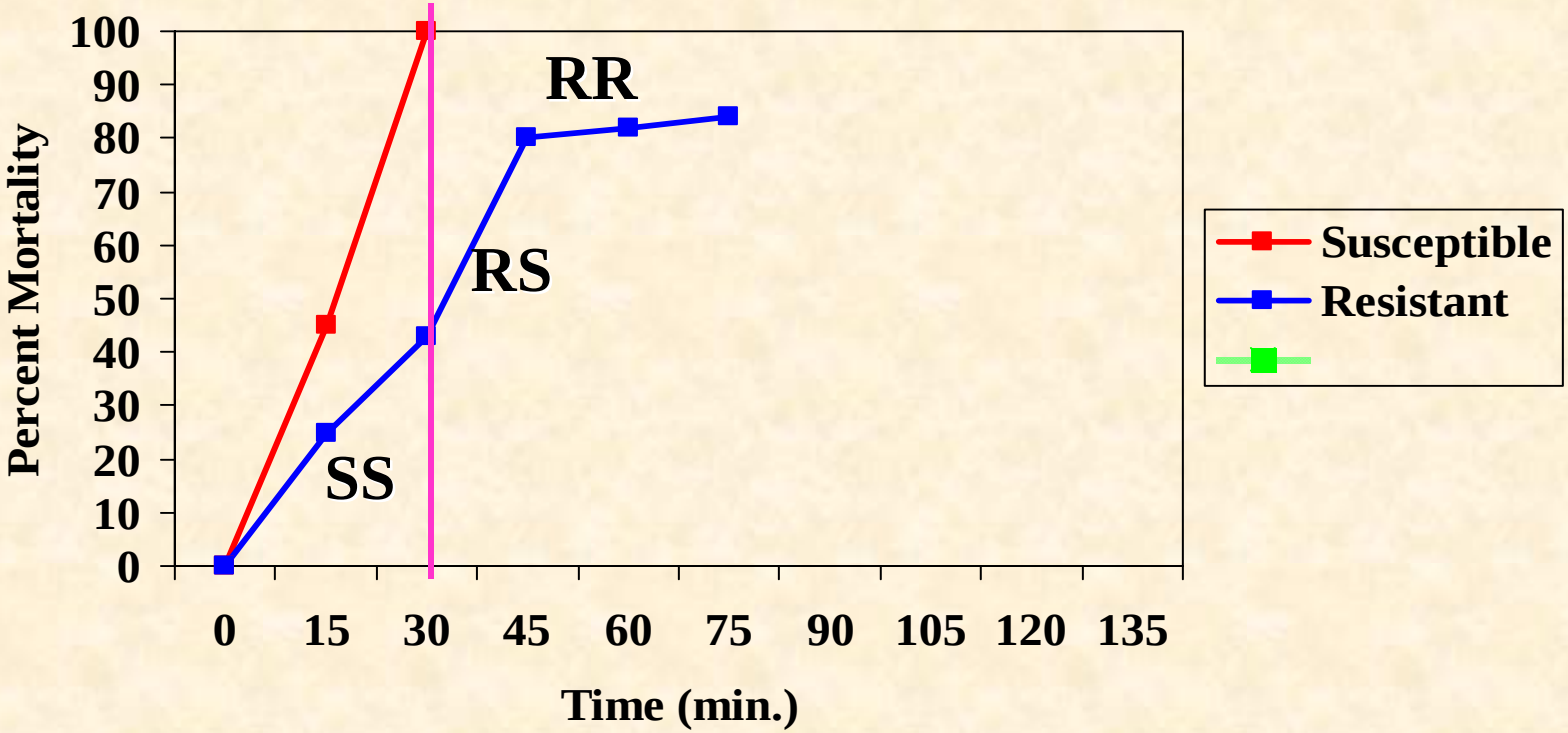
Permethrin



ABIDJAN, IVORY COAST

Anopheles gambiae

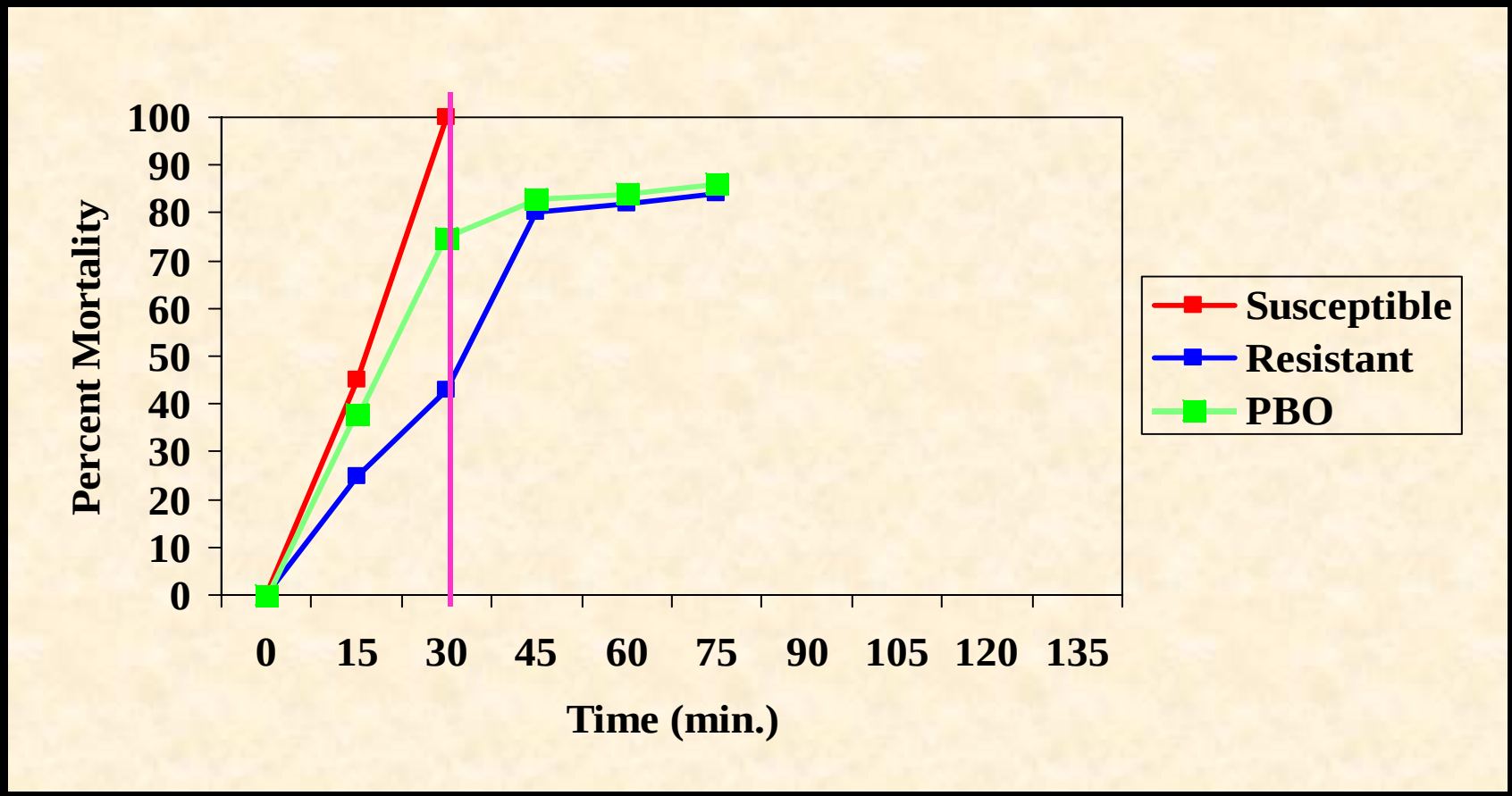
Permethrin



ABIDJAN, IVORY COAST

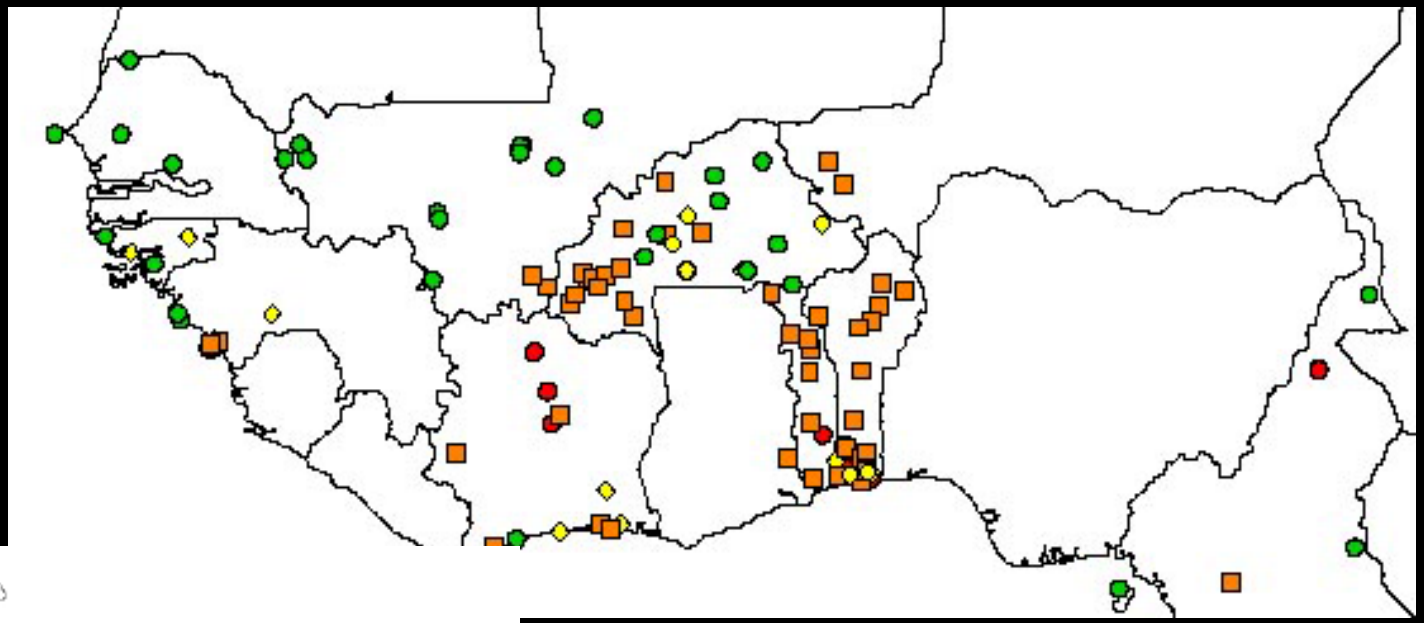
Anopheles gambiae

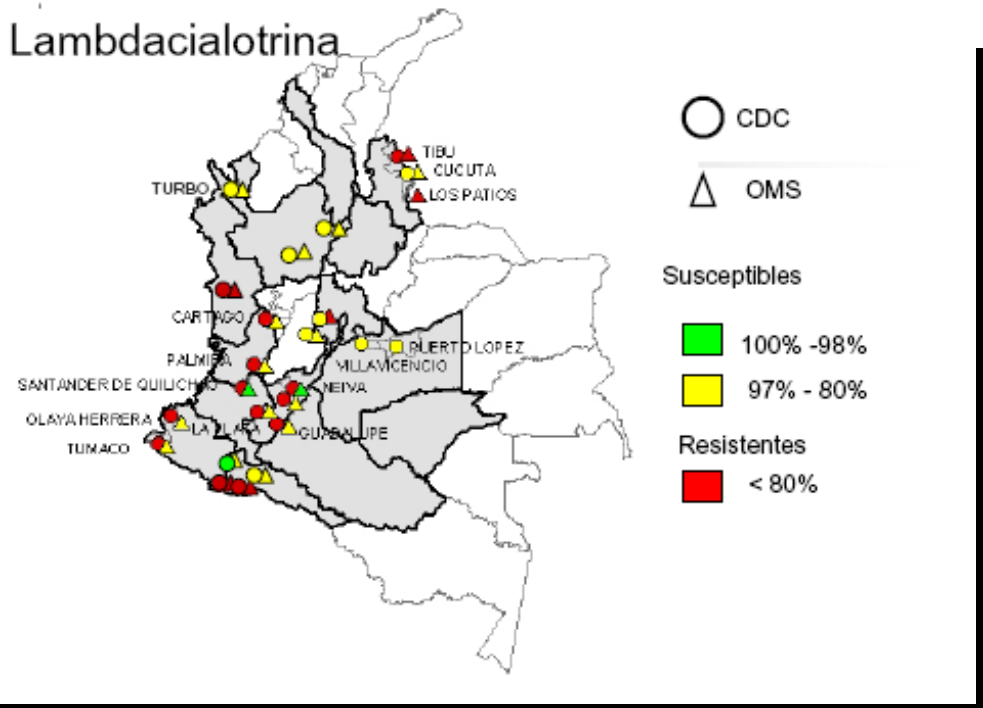
Permethrin



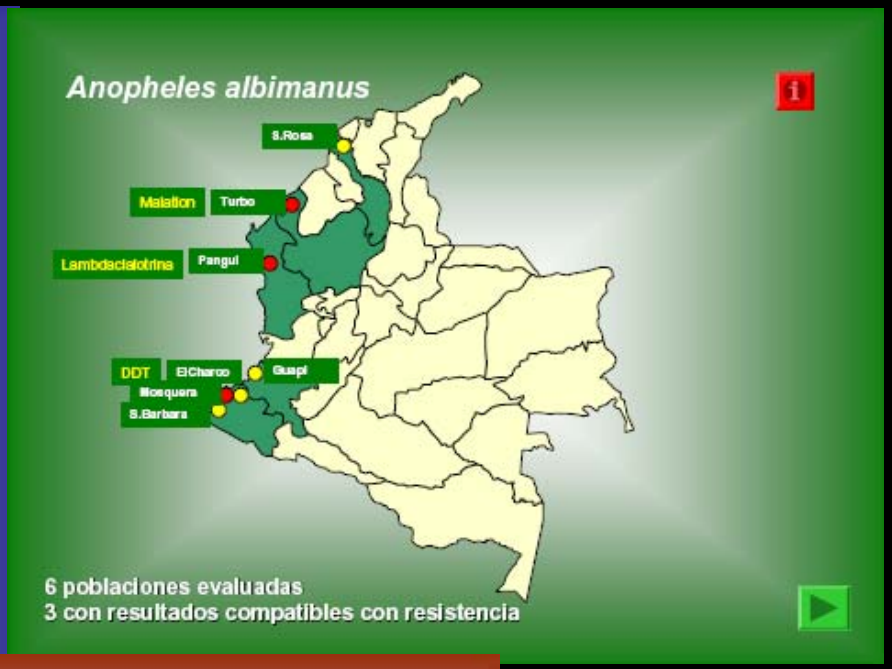
Practical Decision-Making Based Upon Insecticide Resistance Surveillance Data

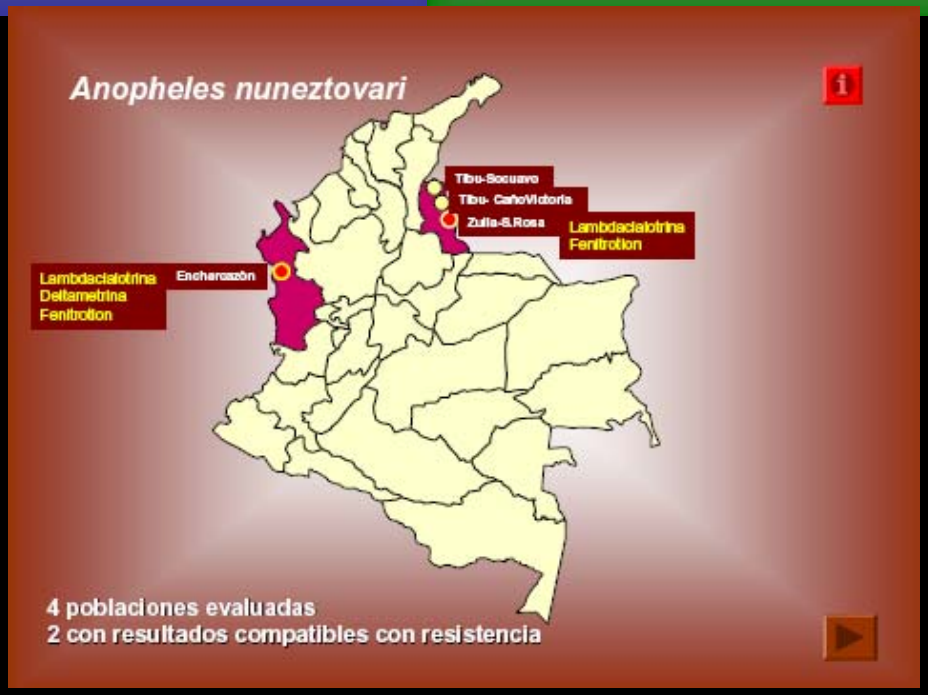
Each resistance problem is potentially unique.




Lambdacialotrina
TIBU
CUCUTA
LOS PATIOS
TURBO
CARTAGO
PALMIRA
PUERTO LOPEZ
VILLAVICENCIO
SANTANDER DE QUILICHAO
NEIVA
OLAYA HERRERA
LA PLATA
GUADALUPE
TUMACO
CDC
OMS
Susceptibles
100% -98%
97% - 80%
Resistentes
< 80%


Anopheles darlingi
Nechi
Zaragoza
Lambdacialotrina
DDT
Bata
SanVicente
Bella
Suiza
Solano
Porvenir
C.Cocha
Quebradita
9 poblaciones evaluadas
1 con resultados compatibles con resistencia


Anopheles albimanus
S.Rosa
Malation
Turbo
Lambdacialotrina
Pangui
DDT
El Charco
Guapi
Mosquera
S.Barbara
6 poblaciones evaluadas
3 con resultados compatibles con resistencia

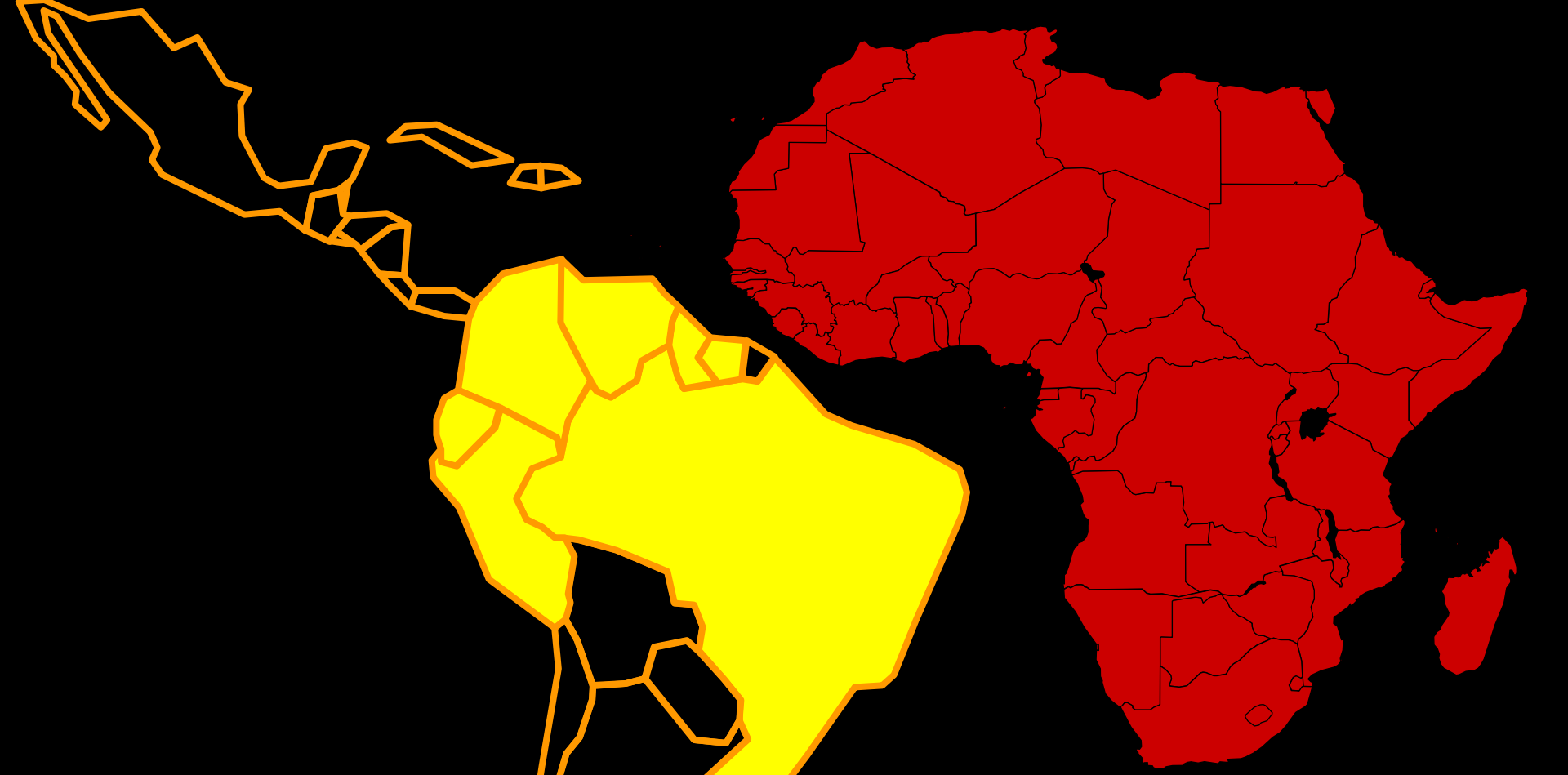

Anopheles nuneztovari
Tibu-Socuavo
Tibu- CañoVictoria
Zulia-S.Rosa
Lambdacialotrina
Fenitrotion
Lambdacialotrina
Deltametrina
Fenitrotion
Encharazón
4 poblaciones evaluadas
2 con resultados compatibles con resistencia

Because resistance is focal, resistance surveillance must be conducted at high resolution to optimize application of the appropriate control strategy.

WE MUST NOW FOCUS ON PROVIDING ANSWERS TO THE "BIG QUESTIONS" ...

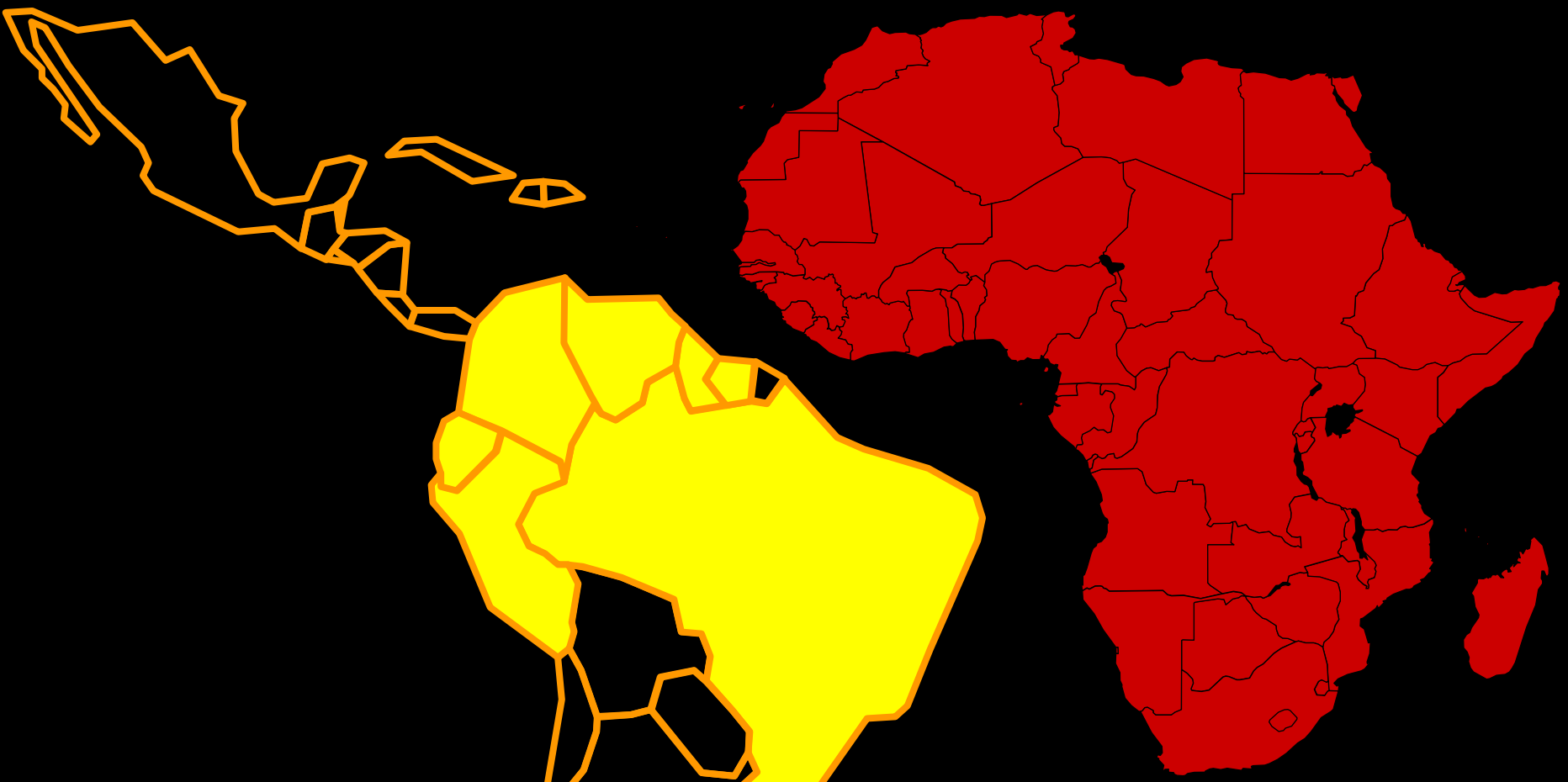
What is the relative resistance Selection efficiency of IRS vs LLINs ?

How Will Resistance Affect the AMI, PMI and MMI And Dengue Control?



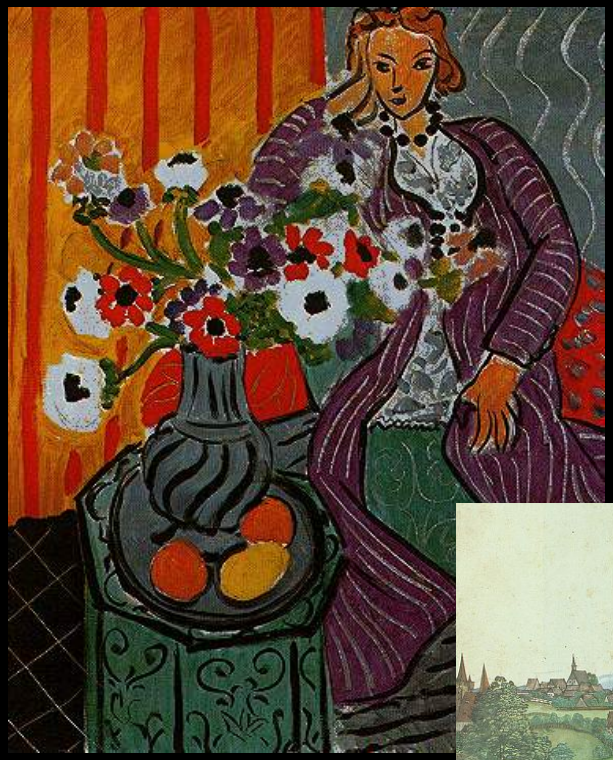
Where will Pyrethroids work And where will they not?

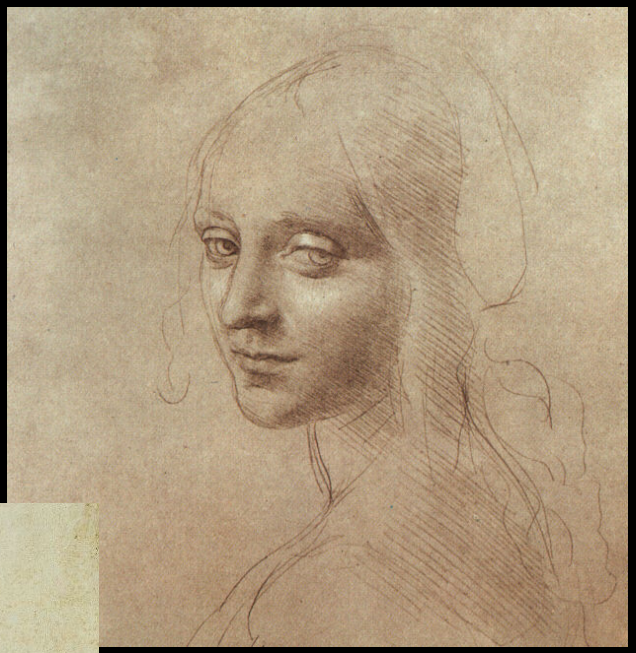

What are the mechanisms
Responsible for resistance ?



When and Where are LLINs and IRS Appropriate?

Every solution may be unique.





Tactical Versus Strategic Insecticide Resistance Management Strategies for Disease Vectors

Strategy

Tactics

Primary Strategic Question

How should control interventions be used to minimize development of resistance?

IPM Strategy

- Use rotations of chemicals
- Time applications of chemicals
- Use mixtures
- Provide refugia (mosaic application of insecticides)

Challenges

- Requires deeper understanding of vector.
- Budget and authority to broadly implement.

Primary Tactical Question

Will this formulation of this insecticide control this vector at this location at this time?

If not,
what do I do next?

Surveillance-Response Tactics

- Establish baselines
- Periodic testing of vector populations
- Correlate changes with control efficacy
- Change control strategy when data indicate

Response Choices

- Switch chemicals
- Apply chemicals focally
- Use source reduction where chemicals are ineffective
- Concentrate on personal protection where chemicals are ineffective

The insecticide chosen:

- Least expensive
- Safest
- Most readily available
- Most effective

If you are not assessing the efficacy of control, it serves no purpose to conduct resistance monitoring. Even if you see resistance, you will not know if it is important or not.

Together, we have learned many things.

However, we need to know much more...

QUESTIONS ?