IRAC Public Health Team

August 25th , 2006

1st Meeting

August 25th, 2006

WHO Headquarters, Geneva, Switzerland

IRAC Public Health Team

August 25th, 2006

Members

- Ralf Nauen (Bayer CropScience) - Chairman
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Observer

- Pierre Guillet (WHO)
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Issues to be covered

- Disease vector resistance, e.g. mosquitoes
- Professional use
    - Resistance issues in
        - Cockroaches
        - Houseflies
        - Fleas
        - Bed bugs
- Resistance risk assessment in public health
- Resistance management issues non-ag pests

IRAC Public Health Team: 1st Meeting

WHO Geneva, 25/08/2006, Room C202

Agenda

08.30 - 9.00	Opening session (R. Nauen, P. Guillet, A. Kochi, Director, Global Malaria Programme), introduction of participants.
09.00 - 09.45	Finalization of the Vector Manual and next steps (All)
09.45 - 10.15	Insecticide mode of action in agriculture vs public health, IRAC´s classification scheme and mechanisms of resistance with special reference to vector control (R. Nauen)
10.15 -10.30	**Coffee break**

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WHO Geneva, 25/08/2006, Room C202

10.30 - 11.00	Vector control program of the Gates Foundation (K. Aultman)
11.00 - 11.30	Resistance as a field issue: consequences of vector ecology, behaviour, population dynamics and population genetics in development and spread of insecticide resistance. Current status of vector resistance and regional networks (P. Guillet).
11.30 - 12.00	Past and current problems faced in monitoring vector resistance (test kits, collection, validation and dissemination of information) (P. Guillet)
12.00 - 12.45	Discussions
12.45 - 14.00	**Lunch Break**

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14.00 - 15.00 Team discussion on selected points raised during concalls (all)

- Views on industry production of insecticide impreganted filter papers?
- Individual reports on industry supported monitoring programs
- How to arrange for a freeze storage stability test for impregnated filter papers?
- Regional vs global RM programs – Joint efforts between all parties on research initiatives (e.g. topics, supervision, funding etc.)
- Reference centers

15.00 -16.00 Short-term and long-term goals of the IRAC Public Health Team (all)

16.00 – 16.30 Joining forces in monitoring and management of vector resistance (all participants, tea served in the room)

16.30 – 17.00 AOB (next concall)

17.00 Departure to airport

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Check of completeness – Vector Manual

Avoiding the Development of Insecticide Resistance in Vectors and Pests of Public Health Importance

Pierre & Kate:	to review Ch 2 & 10
Ralf:	to review Ch 1,3,4 & 5 (done)
Mark/Phil:	to review Ch 6 (done)
Robin/John:	to review Ch 7 & 8 (done)
Janet/Ralf:	to review Ch 9 (done)

Next steps…

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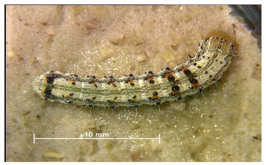
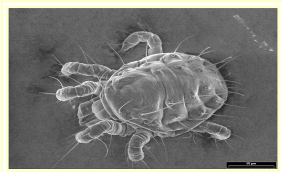
Insecticide mode of action and resistance

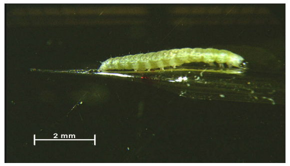
Ralf Nauen, PhD

Bayer CropScience AG, R-Insecticides, Monheim, Germany

Bayer CropScience







EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION (EPPO)

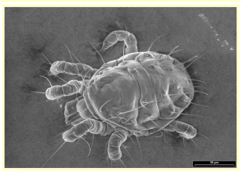
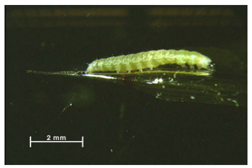
EPPO standard on Resistance Risk Analysis (PP 1/213)

Specific scope

This standard describes how the risk of resistance to plant protection products can be assessed and, if appropriate, systems for risk management can be proposed, in the context of official registration of plant protection products.

1. *Bemisia tabaci*
2. *Trialeurodes vaporariorum*
3. *Aphis gossypii*
4. *Myzus persicae*
5. *Leptinotarsa decemlineta*
6. *Spodoptera exigua*
7. *Frankliniella occidentalis*
8. *Tetranychus urticae*
9. *Panonychus ulmi*
10. *Cydia pomonella*
11. *Phorodon humuli*

Examples of species in the EPPO region which have developed resistance and for which sensitivity data should normally be provided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Key issues – resistance risk assessment

Monitoring

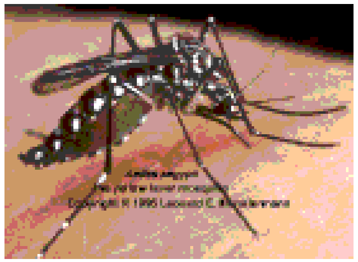
Mechanisms

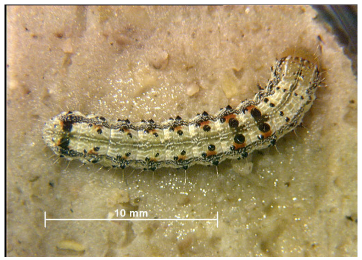
Management

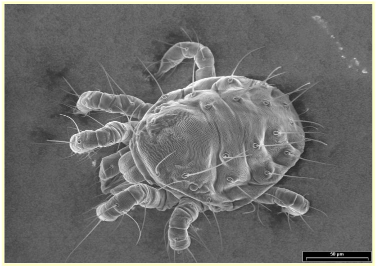
Baseline studies

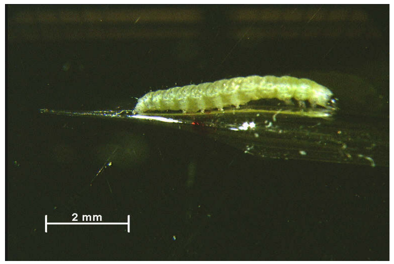
Bioassay methods are as diverse as the functional morphology of pest species and depend on the physicochemical a.i. properties

- Topical application
- Insect/leaf-dip tests
- Artificial diet tests
- Spray bioassays
- Filter paper assays
- Adult vial tests
- Systemic bioassays

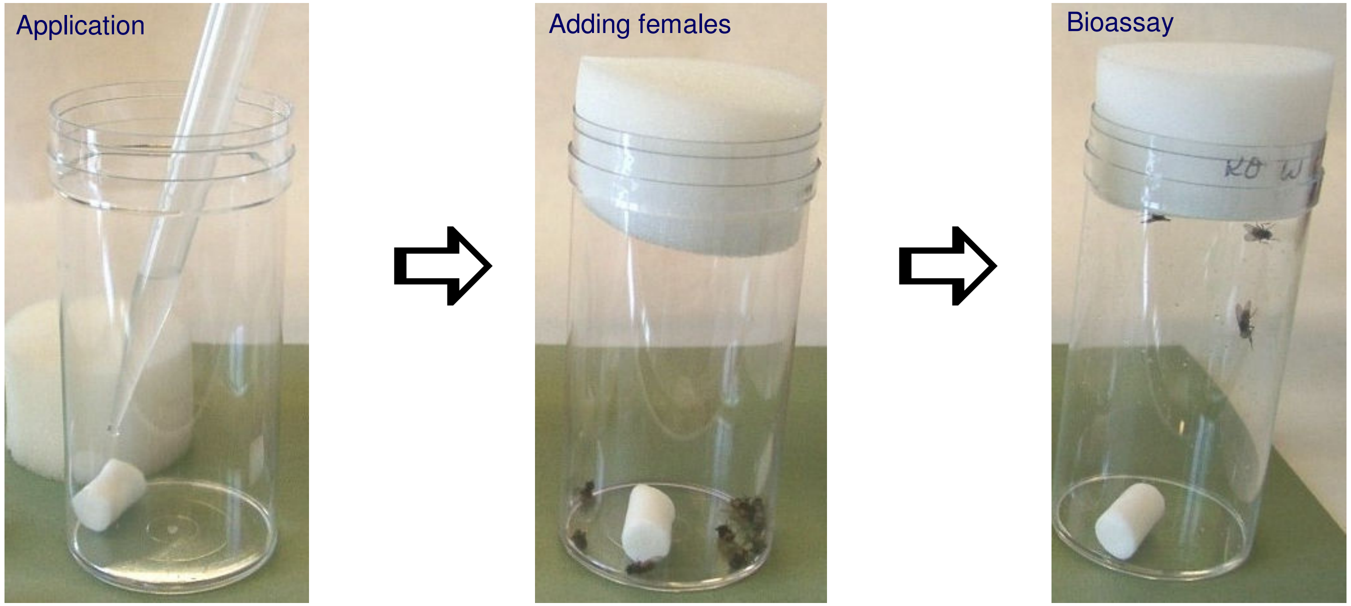




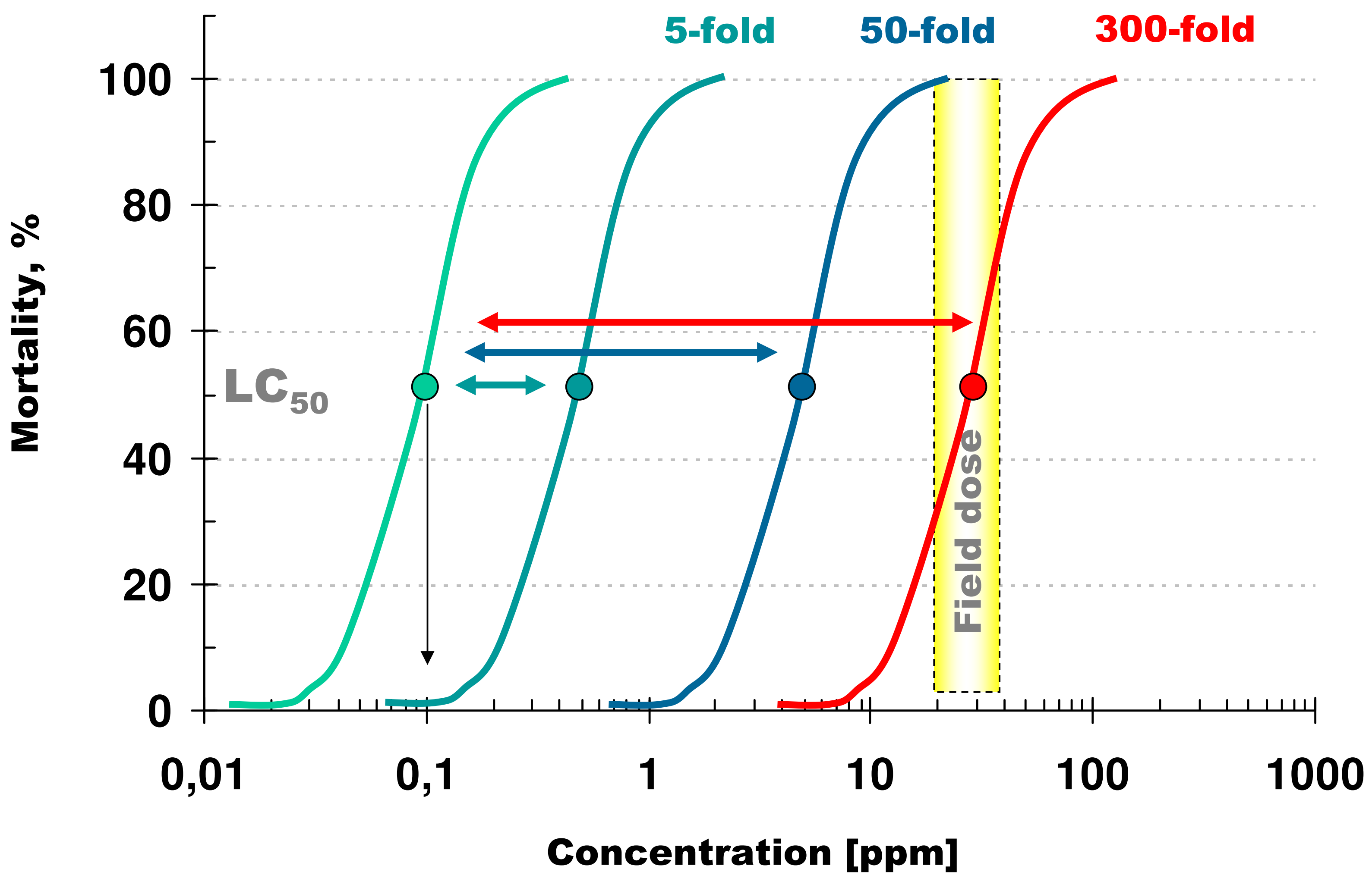




Feeding bioassay to monitor imidacloprid susceptibility in *Musca domestica*



Why to monitor for resistance?



Selection for imidacloprid resistance in BPH*

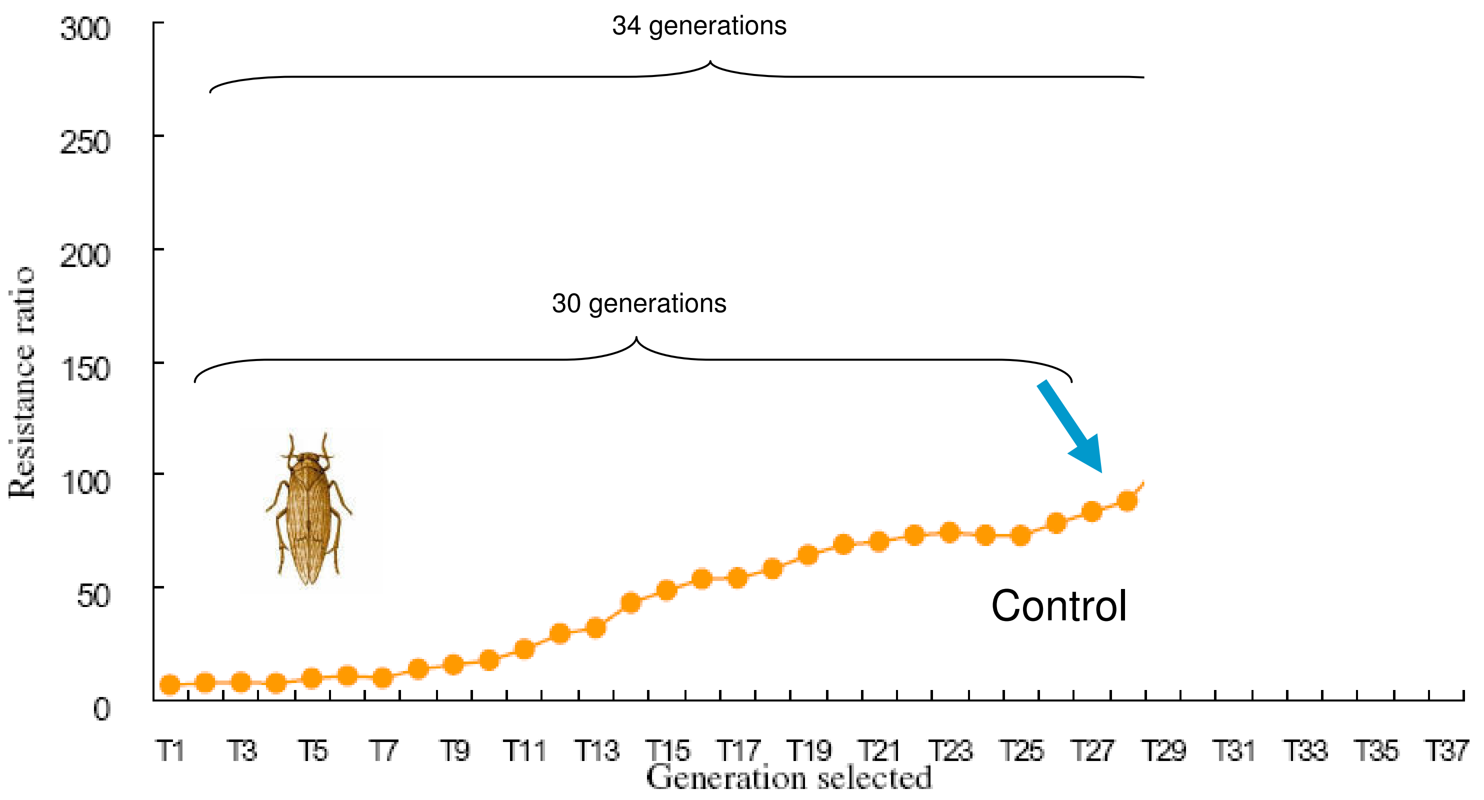


Fig 1 The dynamics of imidacloprid resistance in *Nilaparvata lugens* during resistance selection. Tn: generation selected.

Liu & Han, *Pest Manag Sci, 2006*

*BPH = Brown planthopper, *Nilaparvata lugens*

Case study

Pollen beetle resistance to pyrethroid insecticides

Winter oilseed rape acreage Germany:	1,300,000 ha
Registered products for beetle control:	8
Mode of action classes:	1 (!)
Generations per season:	1

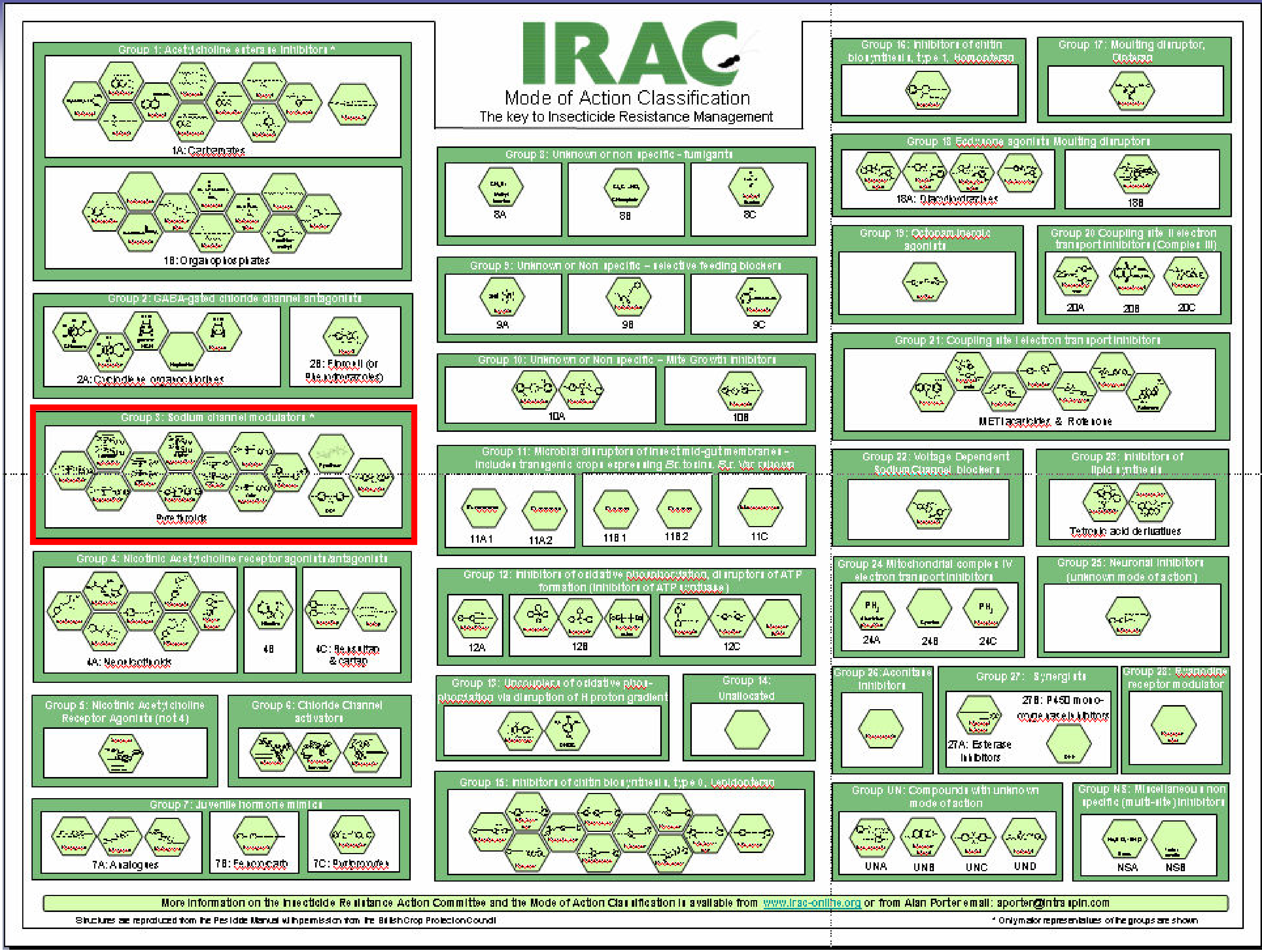
Meligethes aeneus

- **most important pest in oilseed rape**
- **damage of the flowering structures**
- **reduction in number of buds**

Insecticidal products in the German oilseed-rape market

➡ only pyrethroids!

	Active ingredient:
1. Fastac 100 SC	*Alpha-Cypermethrin*
2. Karate Zeon 100 CS	*Lambda-Cyhalothrin*
3. Trafo 50 WG	*Lambda-Cyhalothrin*
4. Decis flüssig 25 EC	*Deltamethrin*
5. Ripcord 100 EC	*Cypermethrin*
6. Sumicidin Alpha 50 EC	*Esfenvalerate*
7. Baythroid 50 EC	*Cyfluthrin*
8. Bulldock 25 EC	*Beta-Cyfluthrin*



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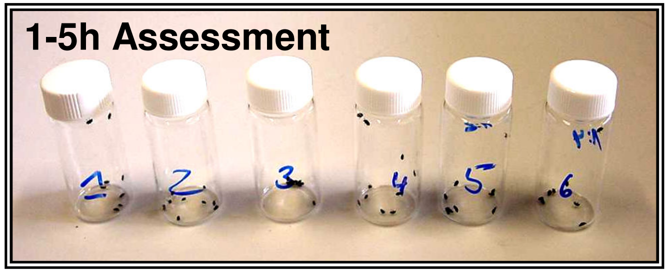
IRAC Mode of Action Classification v5.1, September 2005 [1]

Main Group and Primary Site of Action	Chemical Sub-group or exemplifying Active Ingredient	Active Ingredients
3 Sodium channel modulators	DDT Methoxychlor	DDT Methoxychlor
	Pyrethroids	Acrinathrin, Allethrin, d-cis-trans Allethrin, d-trans Allethrin, Bifenthrin, Bioallethrin, Bioallethrin S-cylclopentenyl , Bioresmethrin, Cycloprothrin, Cyfluthrin, beta-Cyfluthrin, Cyhalothrin, lambda-Cyhalothrin, gamma-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, beta-Cypermethrin, theta-cypermethrin, zeta-Cypermethrin, Cyphenothrin , (1*R*)-*trans*-isomers], Deltamethrin, Empenthrin , (*EZ*)- (1*R*)- isomers], Esfenvalerate, Etofenprox, Fenpropathrin, Fenvalerate, Flucythrinate, Flumethrin, tau-Fluvalinate, Halfenprox, Imiprothrin, Permethrin, Phenothrin [(1*R*)-trans- isomer], Prallethrin, Resmethrin, RU 15525, Silafluofen, Tefluthrin, Tetramethrin, Tetramethrin [(1*R*)-isomers], Tralomethrin, Transfluthrin, ZXI 8901

This document has been prepared using the most up-to-date information available to IRAC. It is provided to user groups, grower organisations, extension personnel, regulatory authorities such as the US EPA and all those involved in resistance management, as an agreed definitive statement by the agrochemical industry on the mode of action of insecticides currently in use.

Resistance monitoring bioassay

- Baseline-susceptibility
- Monitoring

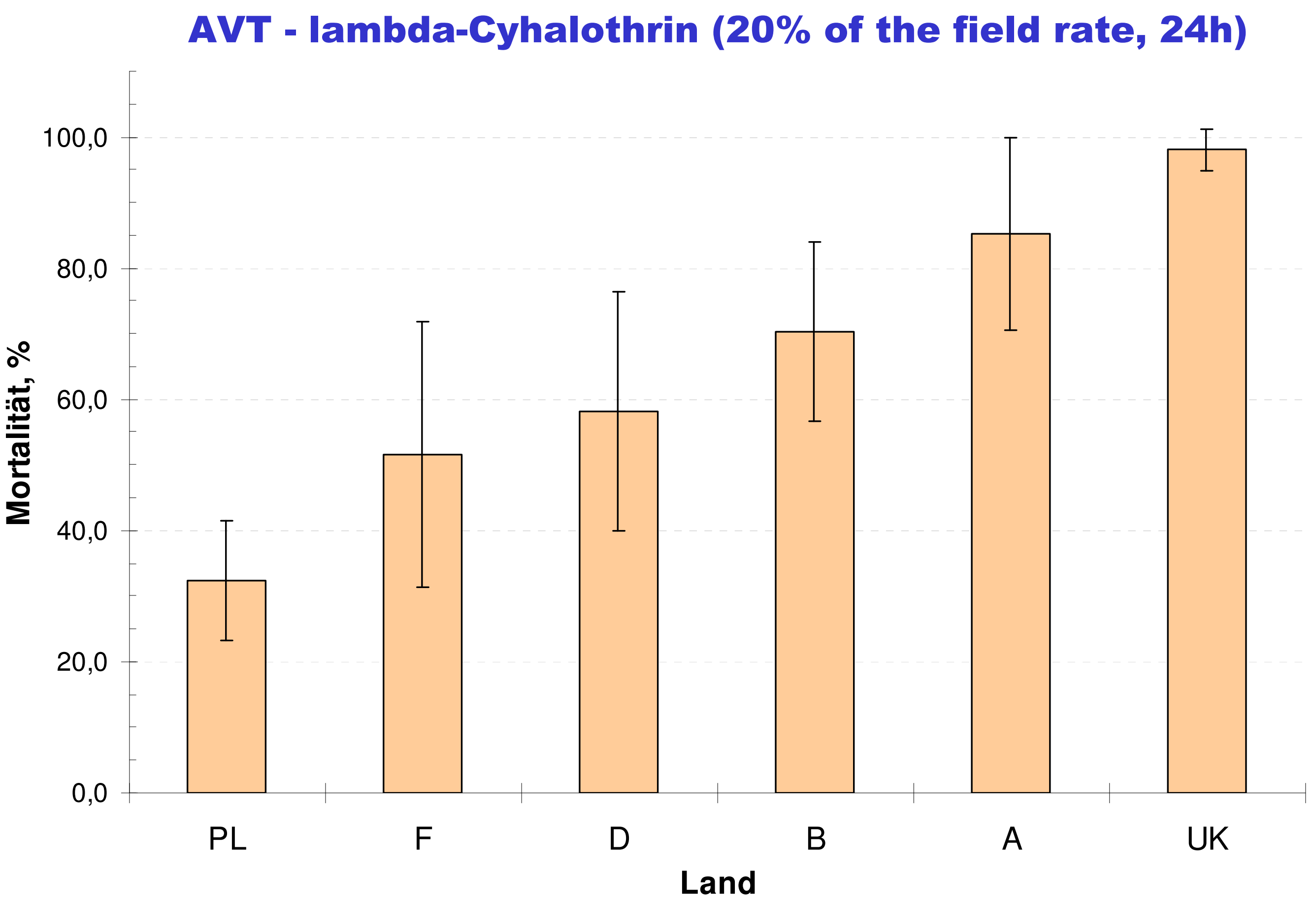


AVT
(Adult vial test)

Pyrethroids

Organophosphates

Pollen beetle pyrethroid resistance monitoring in different European countries – mean values ± SD of all populations checked for each country in 2006



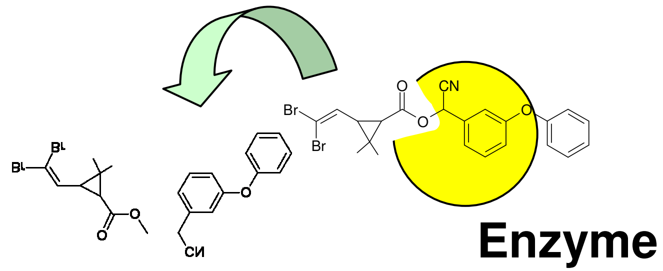
Resistance mechanisms

Some important examples leading to field failure of insecticides under practical conditions in agriculture

Resistance mechanisms

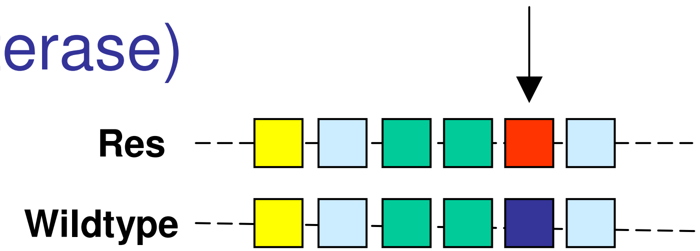
Metabolic Resistance (enzymatic cleavage)

- Esterases
- Monooxygenases
- Glutathione S-transferases



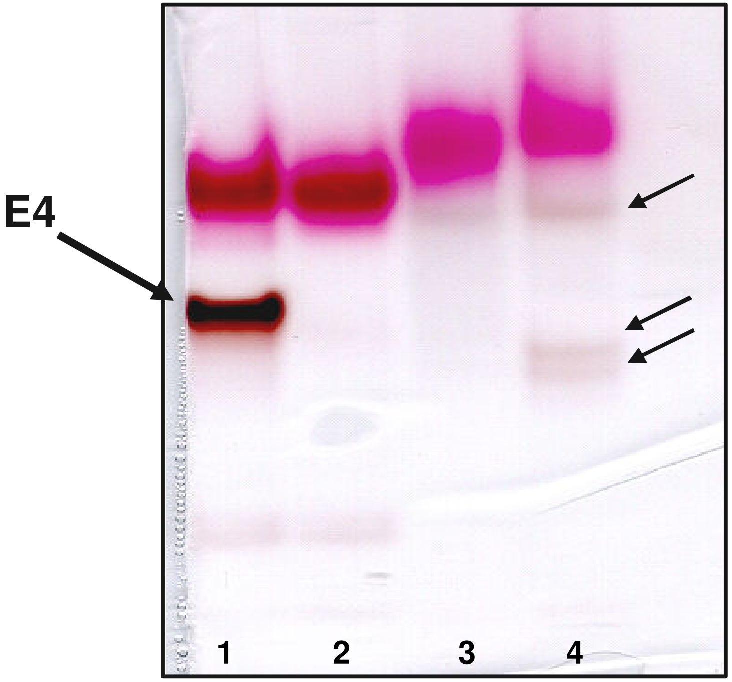
Target site resistance

- MACE (Modified acetylcholinesterase)
- Kdr („Knock-down-resistance“)
- Rdl („Resistance to dieldrin“)



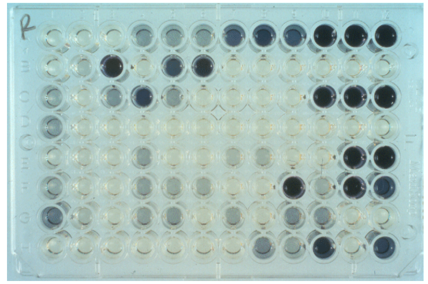
Reduced penetration

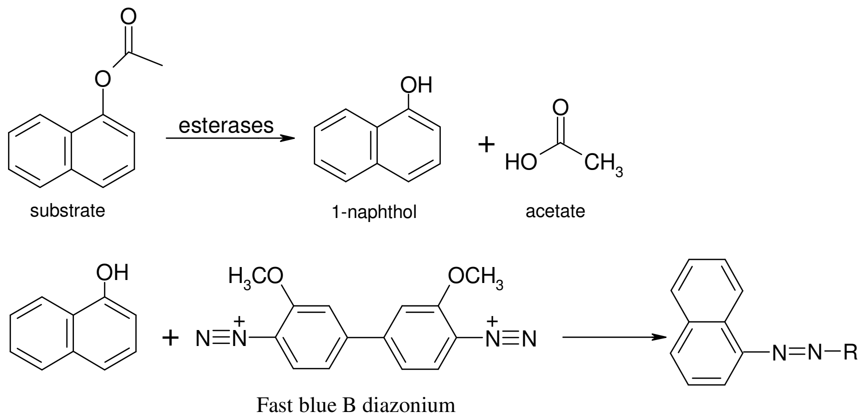
Esterases confer OP, carbamate and pyrethroid resistance in aphids such as *Myzus persicae*



Native PAGE of aphid homogenates

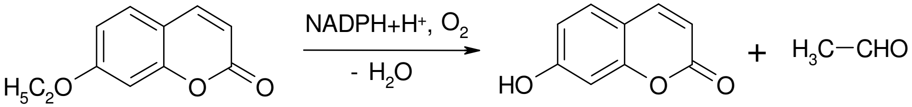
1 *M.persicae* JR
2 NS
3 *P.humuli* H2-99
4 H08-03

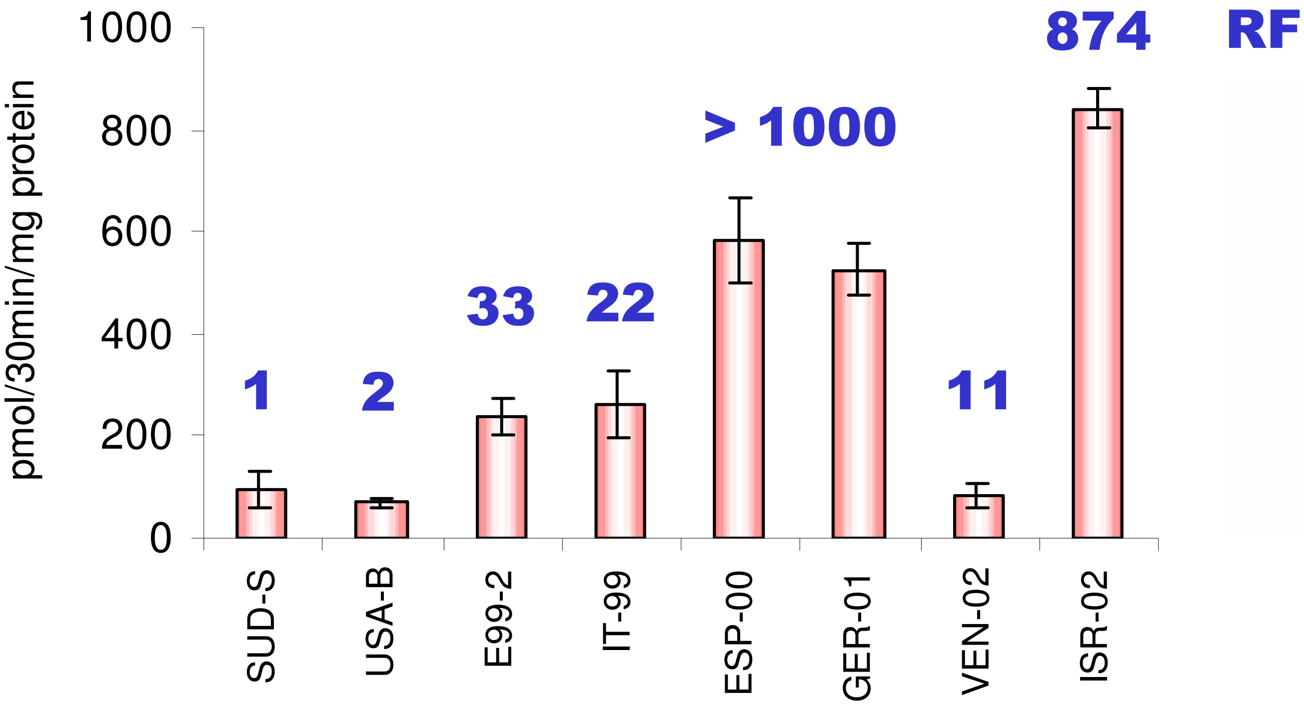


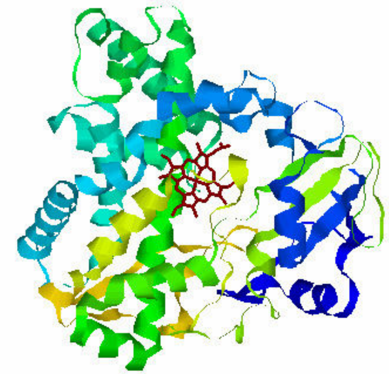


Nauen et al. (1996) Bull, Entomol. Res. 86

Monooxygenases confer neonicotinoid resistance in cotton whiteflies, *Bemisia tabaci*



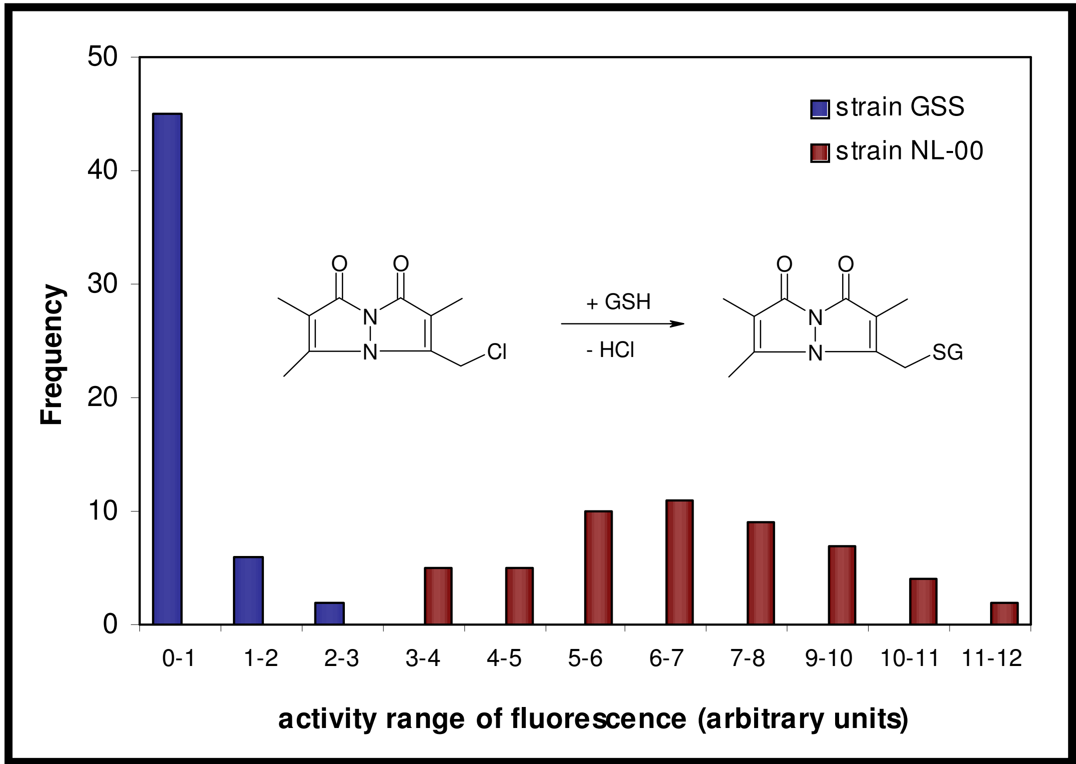




Rauch & Nauen (2003) Arch. Insect Biochem. Physiol. 54

7-Ethoxycoumarin-O-deethylase activity is a biochemical marker linked to neonicotinoid resistance in *Bemisia tabaci*

Elevated glutathione S-transferase levels in abamectin resistant two-spotted spider mites, *T. urticae*

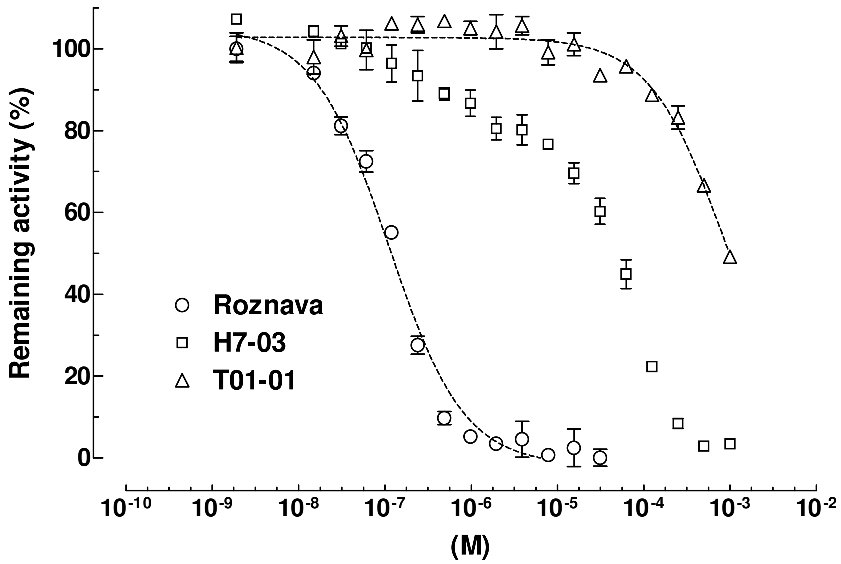


Strain NL-00 exhibited a >50-fold resistance to abamectin

	Strain	K_m (SD)	V_{max} (SD)
		µM	mOD/min/equivalent
	WI	79 (3,0)	46 (0,6)
	GSS	75 (5,8)	25 (0,7)
	NL-00	90 (5,5)	57 (1,2)
)	WI	591 (45,8)	49 (1,1)
	GSS	648 (32,4)	35 (0,5)
	NL-00	669 (45,9)	96 (2,0)
		µM	Fluorescence/equivalent
MCB	WI	64 (9,3)	1243 (69,2)
	GSS	60 (13,1)	1016 (83,4)
	NL-00	86 (9,7)	5114 (245,3)

Nauen & Stumpf (2002) Anal, Biochem. 303
Rauch & Nauen (2002) Pestic. Biochem. Physiol. 72

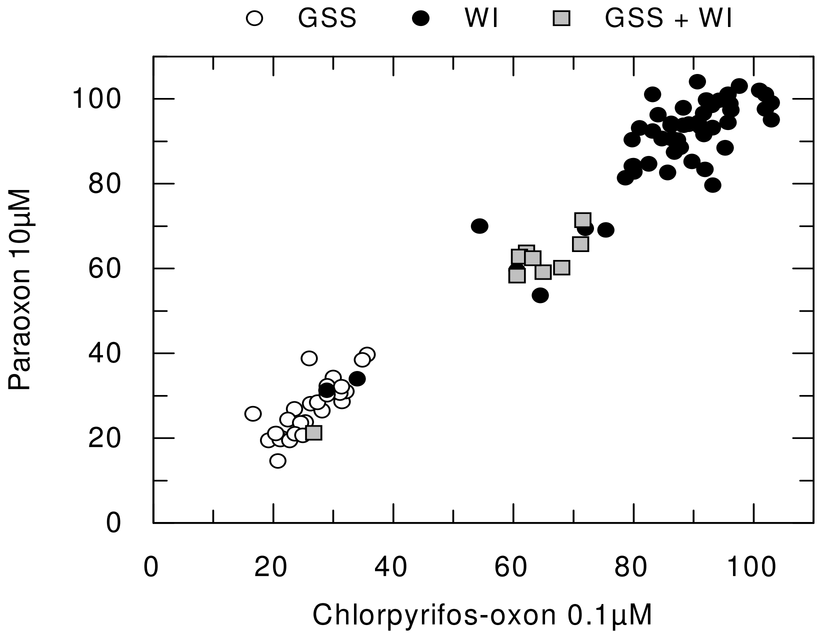
Insensitive acetylcholinesterase confers OP and carbamate resistance in several pest species



Pirimicarb resistance in *Phorodon humuli* due to MACE

Benting & Nauen (2004) Pest Manag Sci
Nauen et al. (2006) Pestic Biochem Physiol, submitted

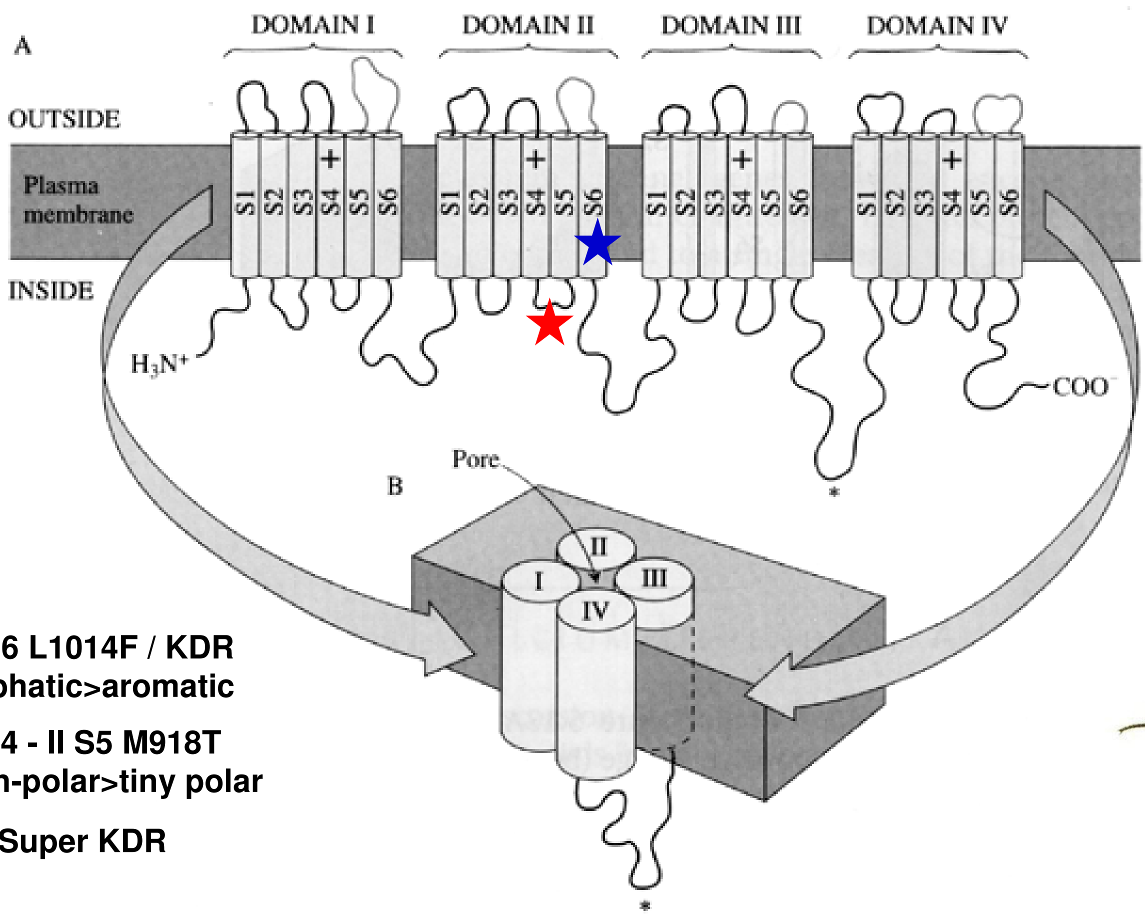
OP resistance in *T. urticae* due to insensitive AChE



Stumpf et al. (2001) Pestic Biochem Physiol 69

Knock down resistance / pyrethroids and DDT

Target: voltage-gated Sodium Channel, transduction of nerve activity



★ **II S6 L1014F / KDR aliphatic>aromatic**

★ **II S4 - II S5 M918T non-polar>tiny polar**

★ ★ **Super KDR**

Musca domestica

IRAC Public Health Team CropLife

Mutations of the voltage-gated sodium channel in pest insects

CTT
CTC
CTA
CTG
TTA
TTG
} Leucin = susceptible

CAT
CAC
} Histidin = KDR
H. virescens

TTT
TTC
} Phenylalanin = KDR

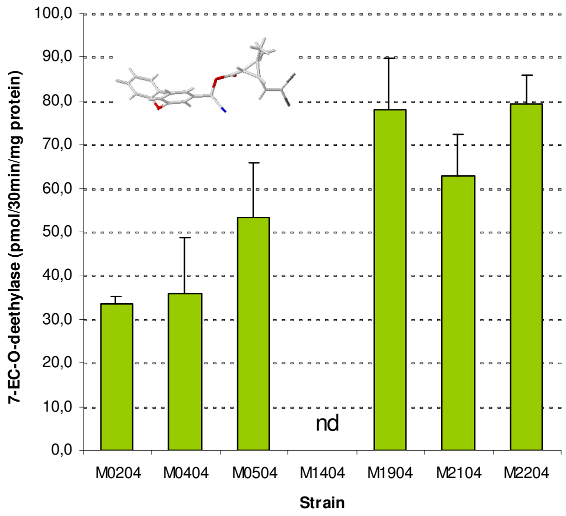
TCA
TCG
} Serin = KDR
Mosquitos

```
M. domestica (2987)     TGGCGATGTCAGCTGTATACCCTTCTTCTTGGCCACGGTCGTGATAGGCAATCTTGTGGTTCTTAATCTTTTCTTAGCTT
B. germanica (2924)     TTGGAGACTGGTCCTGCATCCCGTTCTTCTTGGCCACTGTCGTCATTGGAAACTTGGTTGTGTTGAACCTCTTCTTGGCCT
D. melanogaster (3301)  TGGCGATGTCTCGTGCATTCCCTTCTTCTTGGCCACCGTTGTCATCGGCAATCTTGTGGTACTTAACCTTTTCTTAGCCT
P. humuli (259)         TCGGAGAACCCACGTGTATACCATTCTTCTTGGCTACTGTTGTCATTGGTAACCTCGTGGTTCTCAATCTTTTCTTGGCGT
P. xylostella (269)     TGGGGACGTCTCCTGTATCCCCTTCTTCTTGGCCACCGTCGTCATTGGCAACTTTGTGGTCCTCAACTTGTTC-------
M. persicae (269)       TCGGAGAACCAACGTGTATACCATTCTTCTTGGCTACTGTTGTCATTGGTAACTTCGTGGTCCTCAACCTCTTCTT-----
L. decemlineata (2217)  TTGGAGATGTATCCTGTATCCCATTTTTCCTAGCCACAGTTGTTATTGGCAATCTTGTTGTTTTGAATCTCTTCTTGGCCT
A. gossypii (275)       TCGGAGAACCAACGTGTATACCATTCTTCTTGGCATCTGTTGTCATCGGTAACCTTGTGGTACTTAATCTTTTCTTGGCGT
H. virescens (2354)     TCGGAGATGTCTCTTGTATACCCTTCTTCTTGGCTACCGTCGTCATTGGCAATCTTGTGGTACTTAACCTTTTCTTGGCCC
B. microplus (404)      TCTCAGGCTGGCCCTGCATCCCCTTCTTTCTCGCTACTGTAGTCATCGGGAACCTTGTGGTGCTCAACCTTTTCCTCGCCT
B. tabaci (275)         TTGGTGATGTGTCCTGTATTCCTTTTTTTTAGCCACTGTCGTTATCGGTTACCTTGTAGTTTTAAATCTTTTCTTAGCGT
A. gambiae (257)        TCGGTGATGTATCCTGCATACCATTTTTCTTGGCCACTGTAGTGATAGGAAATTTAGTCGTGCTTAACCTTTTCTTAGCCT
Consensus (3330)        TCGGAGATGT TCCTGTATACC TTCTTCTTGGCCACTGT GTCATTGG AACCTTGTGGT CT AACCTTTTCTT GCCT
```

So what confers pyrethroid resistance in European populations of pollen beetles?

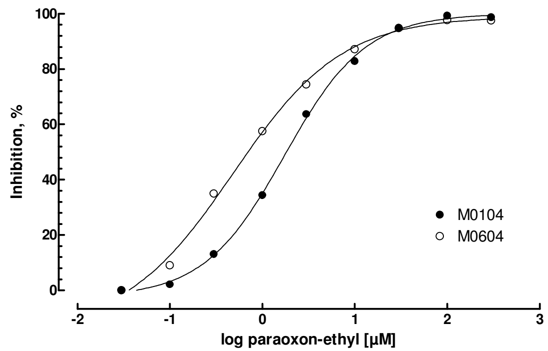
Pyrethroid-resistance pollen beetles is conferred by elevated levels of monooxygenases, but not esterases and kdr

1)

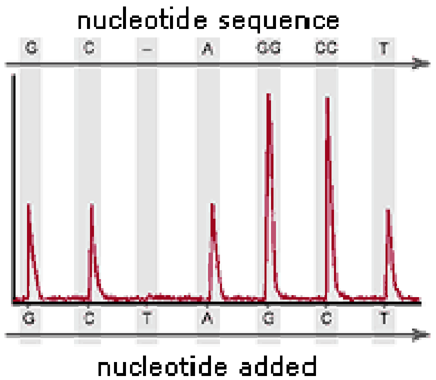


1)	P450	+
2)	Kdr	-
3)	MACE	-
4)	Esterases	-

3)



2)



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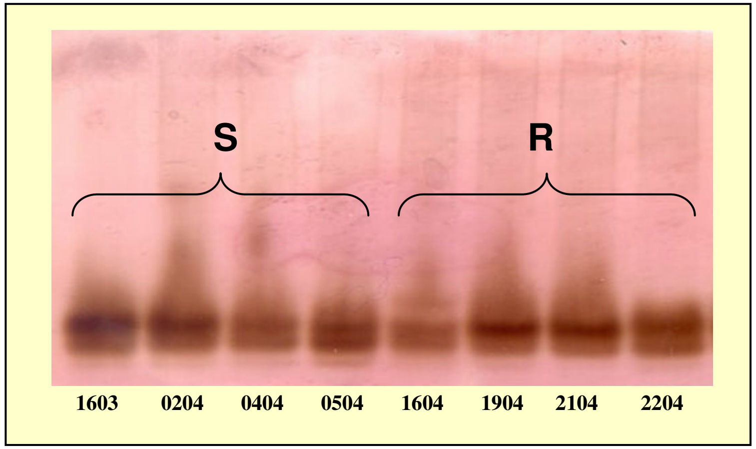
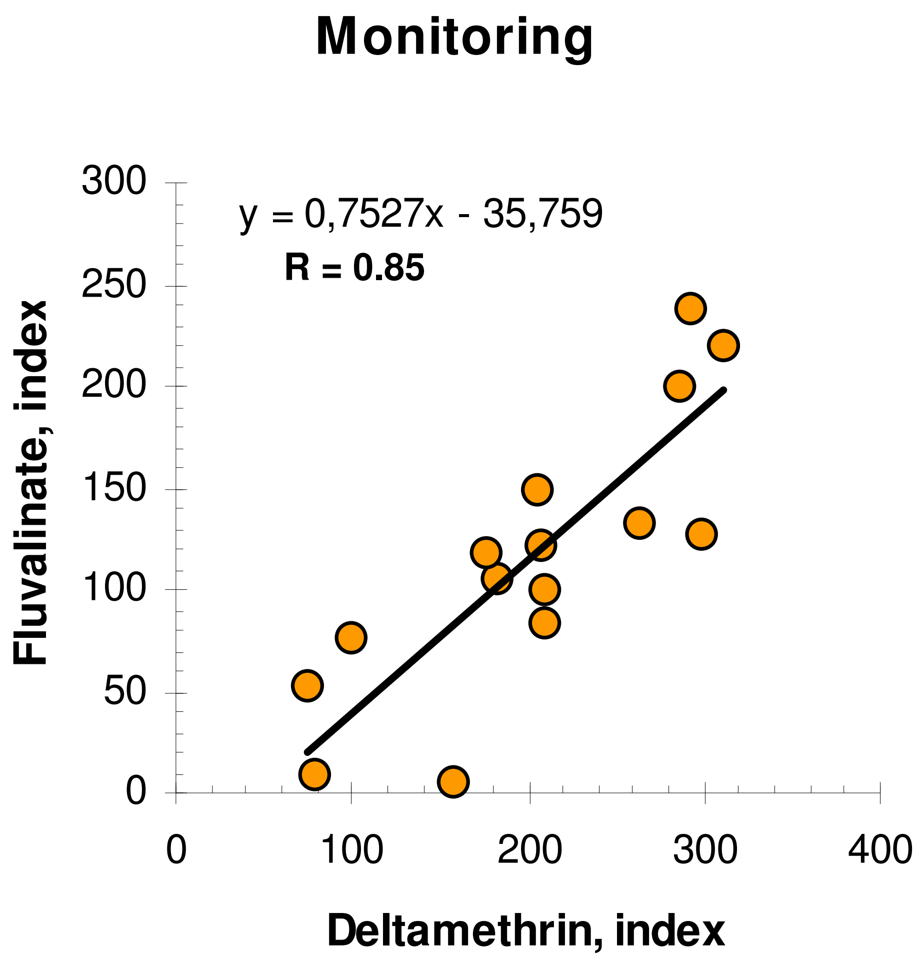


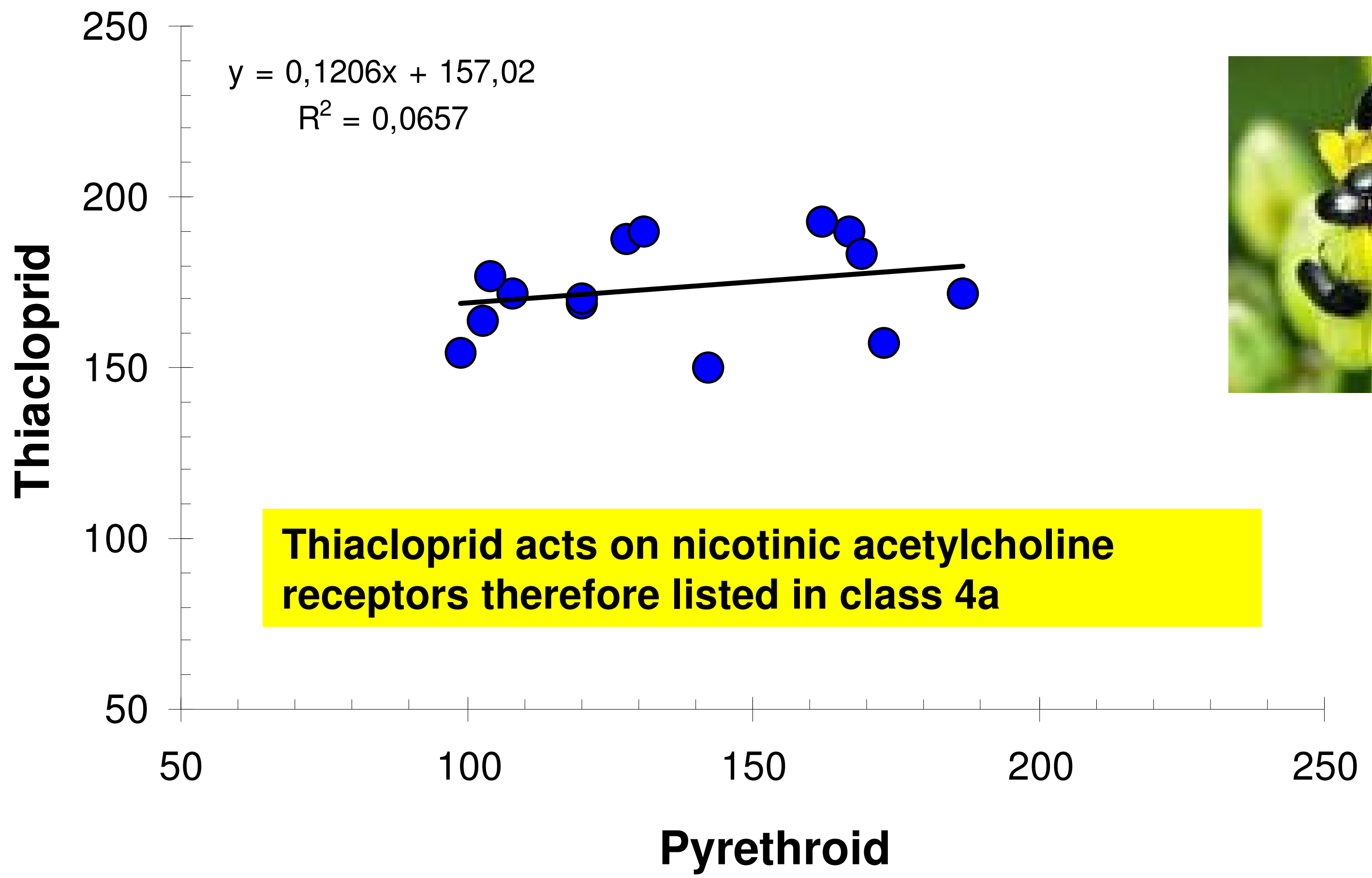
Substrate: alpha-naphthylacetate (gradient PAGE)

Pyrethroid cross resistance in pollen beetles



Correlation between deltamethrin and tau-fluvalinate efficacy points to pyrethroid cross-resistance in pollen beetles

No correlation between thiacloprid (IRAC class 4A insecticide) and pyrethroid (class 3 insecticides) efficacy on pollen beetles



Remember the brown planthopper example?

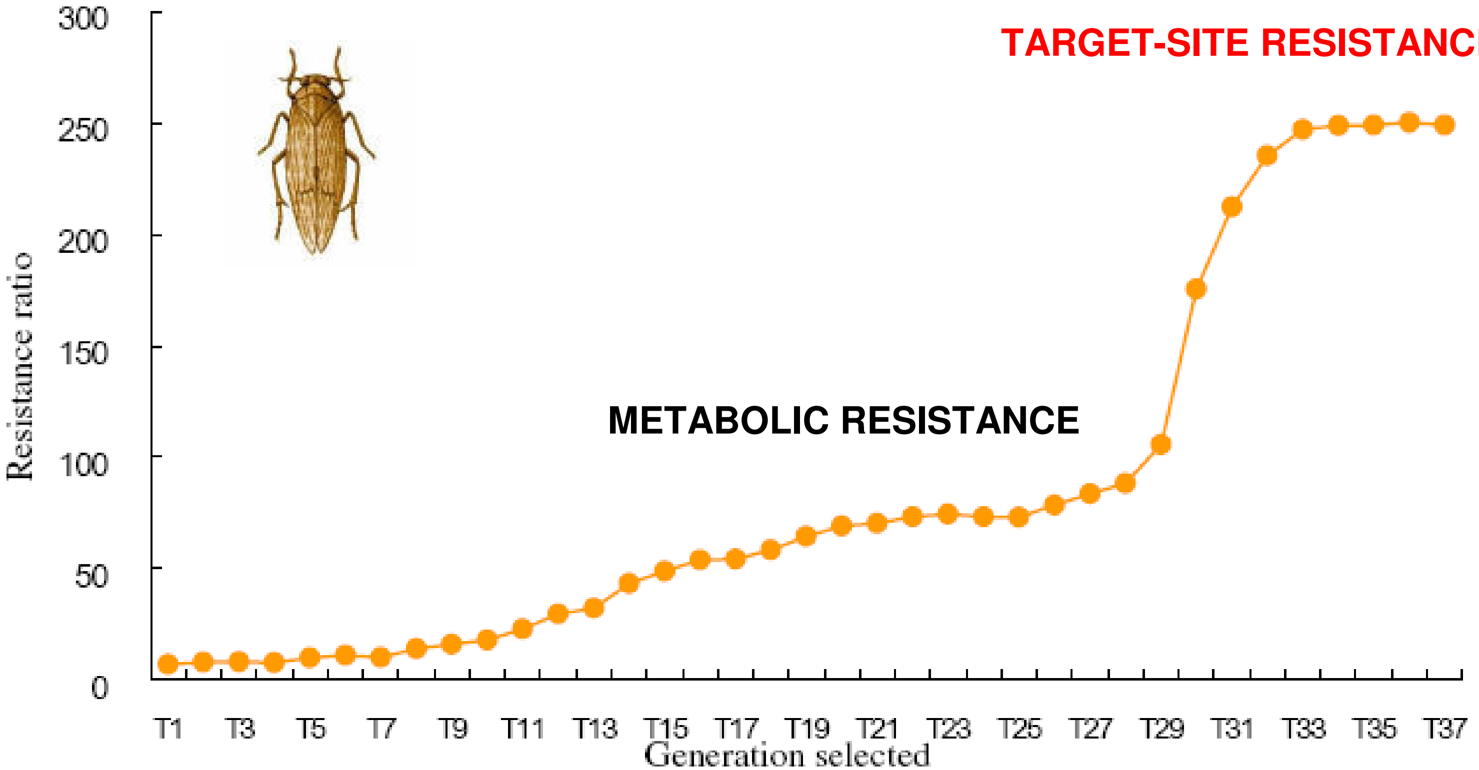


Fig 1 The dynamics of imidacloprid resistance in *Nilaparvata lugens* during resistance selection. Tn: generation selected.

Liu & Han, *Pest Manag Sci, 2006*

PNAS PNAS PNAS PNAS

A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper)

Zewen Liu*, Martin S. Williamson†, Stuart J. Lansdell‡, Ian Denholm†, Zhaojun Han*§, and Neil S. Millar‡

*Key Laboratory of Monitoring and Management of Plant Diseases and Insects, Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, China; †Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom; and ‡Department of Pharmacology, University College London,

review September 24, 2004)

Nl 1S	KFGSWT Y DGNHVDL	
Nl 1R	KFGSWT S DGNHVDL	
Nl 3S	KFGSWT Y NGAQVDL	
Nl 3R	KFGSWT S NGAQVDL	

Y151S

een slow to emerge but now involves a number
sect pests (10). In the best-studied example of
esistance, involving the whitefly *Bemisia tabaci*,
ributable to enhanced oxidative detoxification of
by overexpressed monooxygenases, rather than
anges in nAChRs (11). For other major insec-
oth target-site modifications and enhanced de-
e been identified as being important resistance

standing the molecular basis of imidacloprid resistance, five nAChR subunits (Nlα1–Nlα4 and Nlβ1) have been cloned from *N. lugens*. A comparison of nAChR subunit genes from imidacloprid-sensitive and imidacloprid-resistant populations has identified a single point mutation at a conserved position (Y151S) in two nAChR subunits, Nlα1 and Nlα3. A strong correlation between the frequency of the Y151S point mutation and the level of resistance to imidacloprid has been demonstrated by allele-specific PCR. By expression of hybrid nAChRs containing *N. lugens* α and rat β2 subunits, evidence was obtained that demonstrates that mutation Y151S is responsible for a substantial reduction in specific [^{3}H]imidacloprid binding. This study provides direct evidence for the occurrence of target-site resistance to a neonicotinoid insecticide.

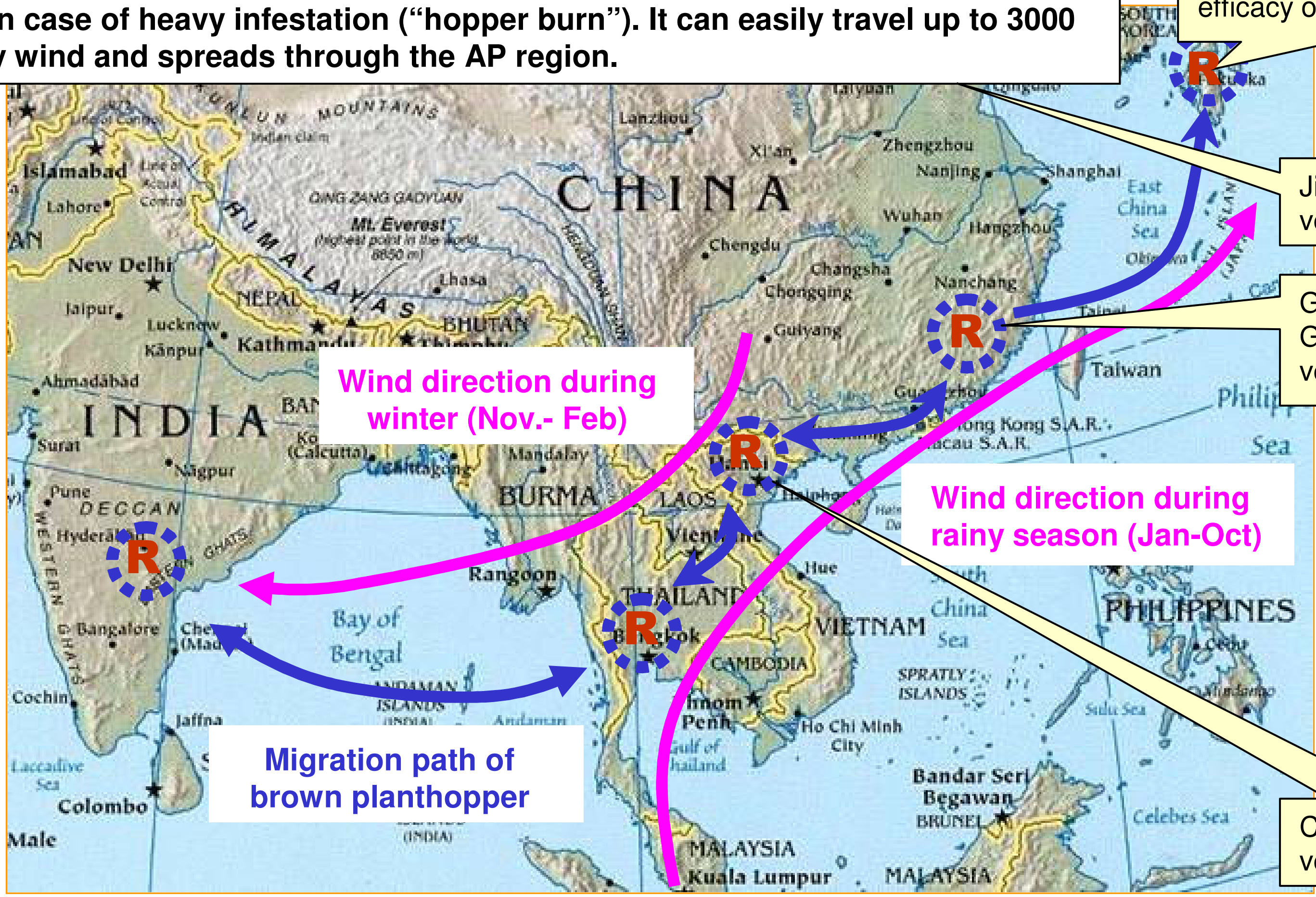
dacloprid-susceptible and resistant *N. lugens* strains, a mutation

IRAC Public Health Team

CropLife International

Migration of BPH populations across Asia

Brown planthopper (BPH) is a serious pest in rice which can cause 100% yield loss in case of heavy infestation ("hopper burn"). It can easily travel up to 3000 km by wind and spreads through the AP region.



IRAC Public Health Team

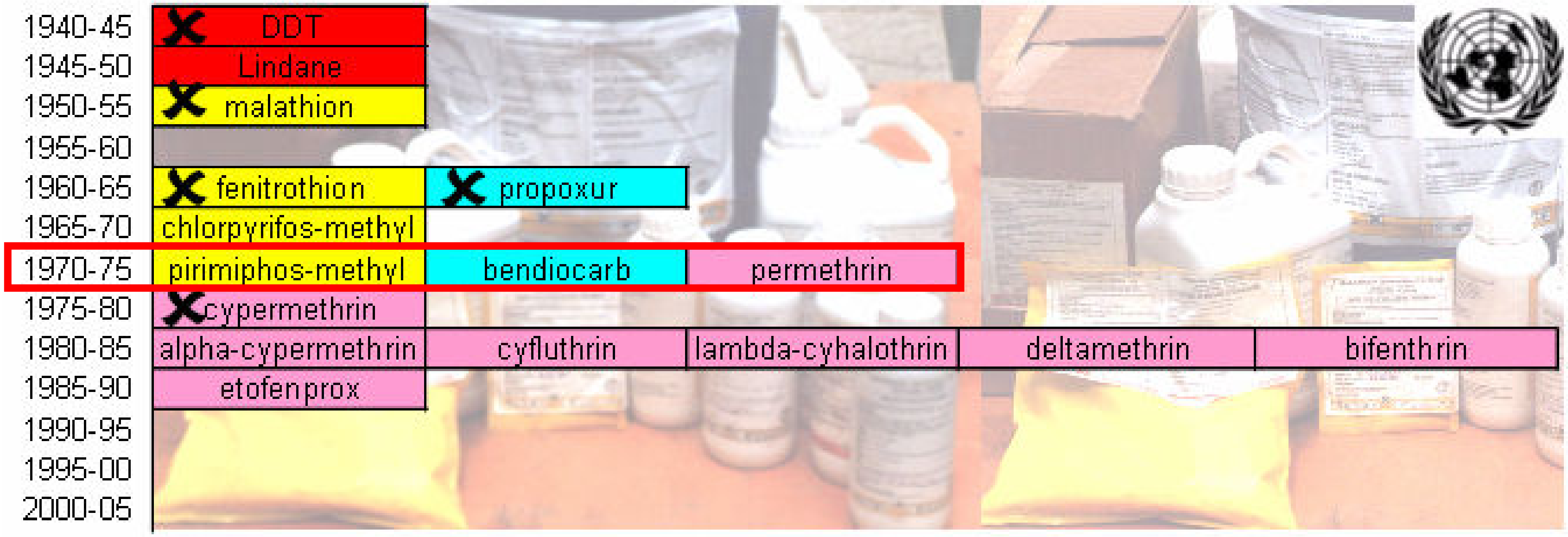
Chemical options for RM in brown planthoppers

- **Nervous system**
  - **Acetylcholinesterase**
    - Organophosphates
    - Carbamates
  - **vg Sodium channel**
    - Pyrethroids
    - DDT
    - Indoxacarb
  - **Acetylcholine receptor**
    - NeoniXtinoids
    - Nicotinoids
    - Spinosyn
    - Nereistoxin analogues
  - **GABA-gated Cl channel**
    - Fiproles
    - Cyclodienes
    - Avermectins
- **Development**
  - **Chitin biosynthesis**
    - Benzoylphenylureas
    - Buprofezin
  - **Ecdysone receptor agonists**
    - Dibenzoylhydrazines
  - **Endocrine system**
    - Methoprene
    - Pyriproxyfen
    - Fenoxycarb
    - Azadirachtin
- **Energy production**
  - **NADH dehydrogenase**
    - METI acaricides
    - Rotenone
  - **Complex III**
    - Fluacrypyrim
    - Acequinocyl
  - **Uncouplers**
    - Chlorfenapyr
  - **ATPase**
    - Diafenthiuron
- **Others**
  - **Lipid metabolism**
    - Tetronic acids
  - **Gut function**
    - Bt toxins
  - **Feeding disruption**
    - Pymetrozine
    - Flonicamid

5 alternative modes of action to choose from to control BPH

It is possible to manage resistance properly – but tool box diversity is necessary

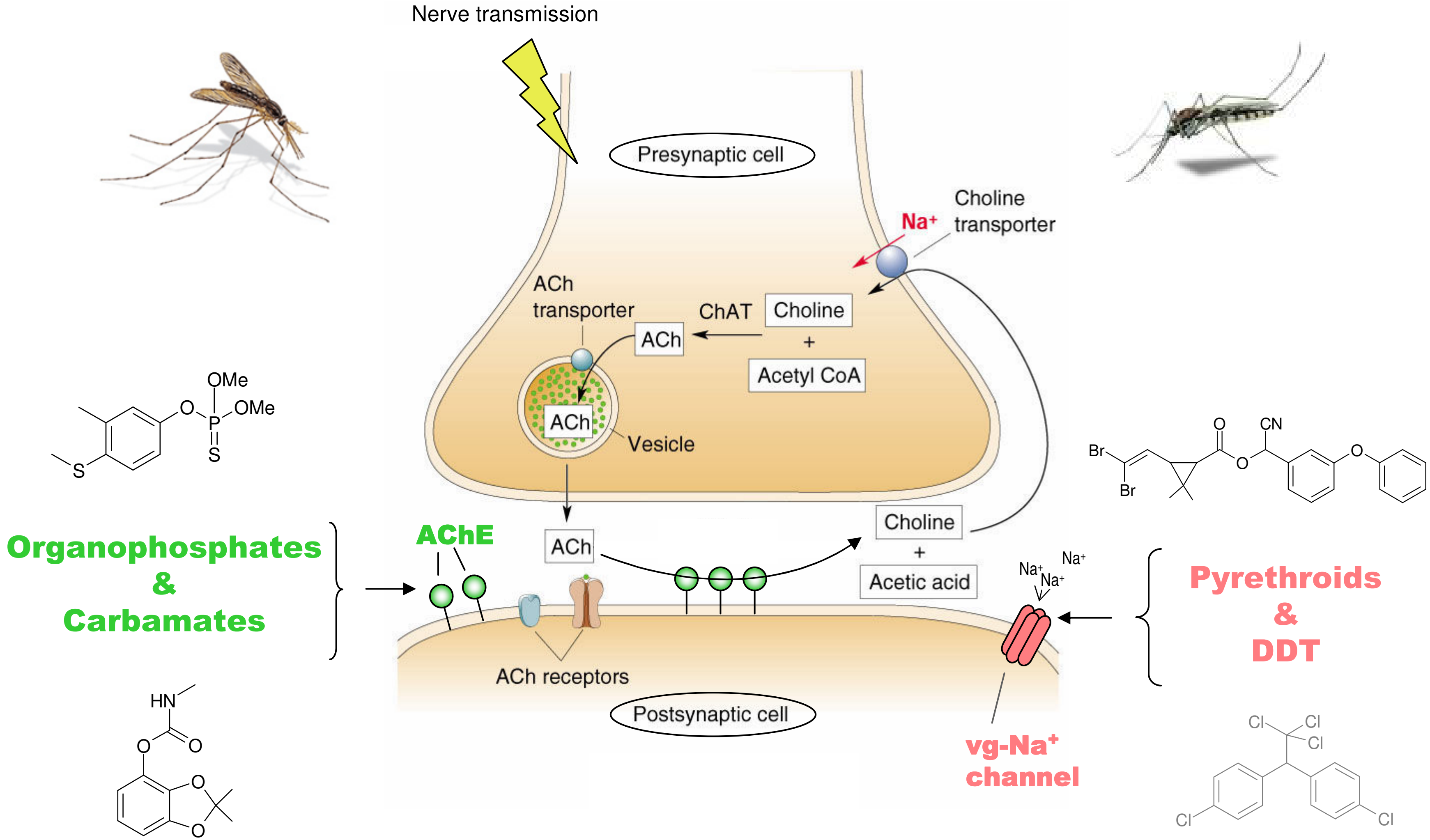
No new insecticidal mode of action class introduced for adult mosquito control since more than 30 years !



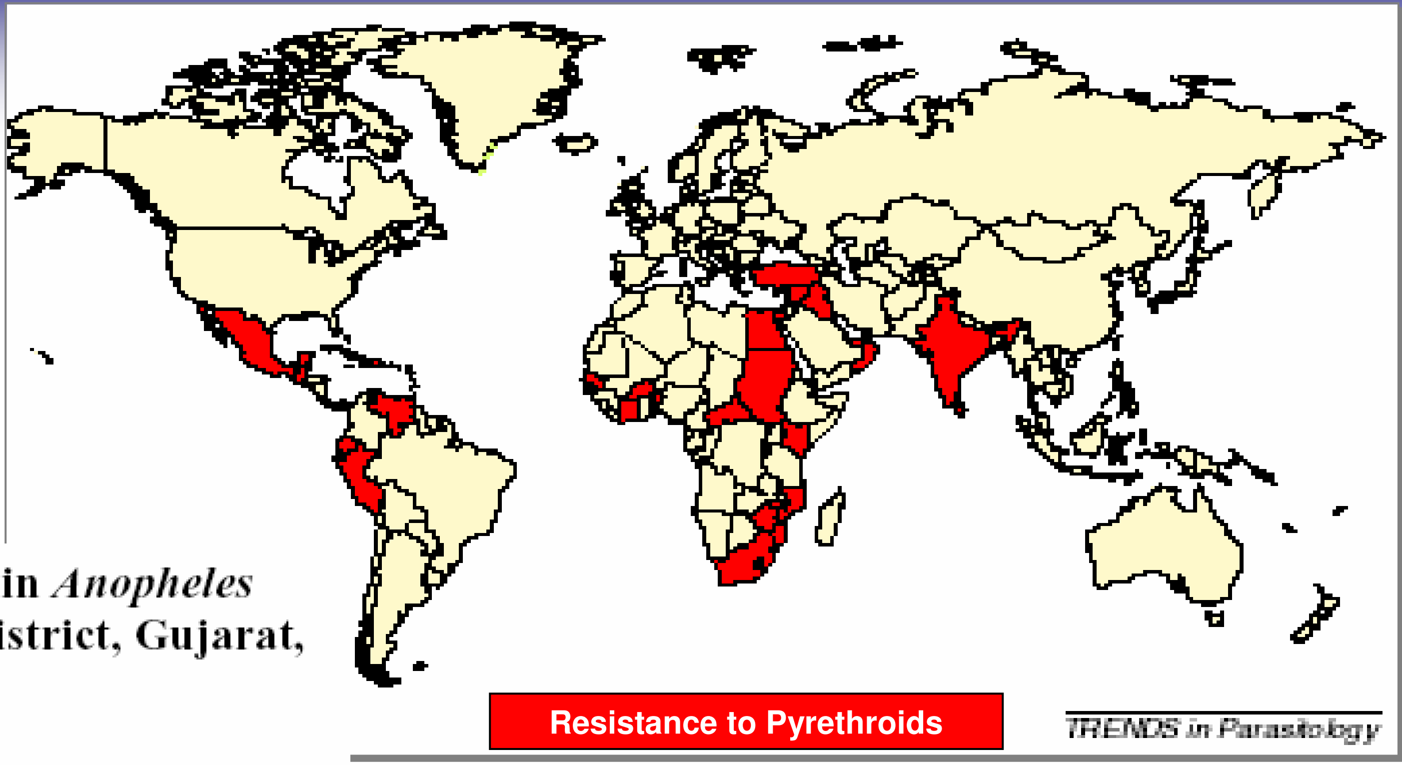
Loss of insecticides due to:

1. **Resistance issues**
2. **Regulatory hurdles**
3. **Economic reasons**

Biochemical sites of action of important disease vector control agents



Abbreviations: AChE = Acetylcholinesterase; Ach = Acetylcholine; ChAT = Choline Acetyl-Transferase; vg-Na+ channel = voltage-gated sodium channel



Pyrethroid resistance in *Anopheles culicifacies* in Surat district, Gujarat, west India

O. P. Singh, K. Raghavendra, N. Nanda, P. K. Mittal and S. K. Subbarao*

Malaria Research Centre (ICMR), 22 Sham Nath Marg, Delhi 110 054, India. December 2001.

A focus of deltamethrin resistance in *Anopheles culicifacies*, the major vector of malaria in India, was identified in Surat district, Gujarat, western coast of India, where two synthetic pyrethroids, deltamethrin and cyfluthrin are being used under the public health programme since 1996, as a selective vector control measure. The per cent mortalities in *An. culicifacies* after one-hour exposure to 0.05% deltamethrin varied from 60 to 78.

The development of pyrethroid resistance in *An. culicifacies* is of great concern to the malaria control programme because SPs are being used in public health programmes to control multiple-resistant vectors and tackle epidemic outbreaks. Also, this is the only group of insecticides currently used for bed-net impregnation for malaria control.

Major biochemical mechanisms conferring resistance to important classes of insecticides in adult mosquitoes

	Biochemical mechanism of resistance				
	Metabolic			Target-site	
	Esterases	Monooxygenases	GSH S-Transferases	kdr	MACE
Pyrethroids	•	●		●	
DDT		•	●	●	
Carbamates	•				●
Organophosphates	●	•			●

WHO urged for new chemicals

Alternative insecticides: an urgent need

Morteza Zaim and Pierre Guillet

Most insecticides used against pests and vectors of human disease (e.g. fleas, flies and mosquitoes) are spin-offs from agrochemical research and development. The arsenal of safe and cost-effective public health insecticides is being depleted by restrictions for various reasons (e.g. insecticide resistance, unacceptable side effects and non re-registration) and the number of new products launched is dwindling. Mobilizing public resources and establishment of partnerships to support research and development of public health insecticides is crucial in the post-DDT and post-pyrethroid era.

WHO investigation in all the Pesticide Industries in 2001: No new compounds collected

Thanks for your attention!

Time for coffee…