“*Tuta absoluta* (Meyrick) and insecticide resistance ”

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2 to 4 days

Life cycle:

- 76.3 days at 14°C
- 28.7 days at 25°C
- 23.8 days at 27.1°C

Up to 12 generations per year

Up to 260 eggs/female

4 to 6 days

11 to 13 days

5 to 8 days

Source:**Un nuovo lepidottero segnalato in Italia *Tuta absoluta* (Meyrick),Umberto Bernardo,**2009





Source:**Un nuovo lepidottero segnalato in Italia *Tuta absoluta* (Meyric**

www.tutaabsoluta.com

Control:

- Organophosphates and pyrethroids were used during the 1970’s and 1980’s

- In the 1990’s, new products introduced such as abamectin, spinosad, tebufonzide, and chlorfenpyr. (Lietti *et al.,* 2005)

- up to 36 insecticide applications per single tomato crop cycle (Picanço *et al.,* 1995)

Registered Insecticides on Tomato used to Control *Tuta absoluta*

MOA Group	**Chemical subgroup**	Common Name
Acetylcholinesterase (AChE) inhibitors	1B: Organophosphates	Chlorpyrifos Methamidophos
Sodium channel modulators	3A: Pyrethroids	Deltamethrin
Nicotinic acetylcholine receptor (nAChR) agonists	4A: Neonicotinoids	Imidacloprid Thiacloprid
Nicotinic acetylcholine receptor (nAChR) allosteric activators	5:Spinosyns	Spinosad
Chloride channel activators	6:Avermectins	Abamectin, emamectin
Microbial disruptors of insect midgut membranes	11: *Bacillus thuringiensis*	*Bacillus thuringiensis*
Uncouplers of oxidative phosphorylation via disruption of the proton gradient	13:Chlorfenpyr	Chlorfenapyr
Inhibitors of chitin biosynthesis, type 0	15:Benzoylureas	lufenuron
Voltage-dependent sodium channel blockers	22A : indoxacarb 22B: Metaflumizone	Indoxacarb Metaflumizone
Ryanodine receptor modulators	28: Diamides	Chlorantraniliprole Flubendiamide
Unknown or act on multiple targets	Azadirachtin	Azadirachtin

New Pest Response,GuidelinesTomato Leafminer (*Tuta absoluta)* (USDA,2011)

Control failures :

- Brazil : abamectin, cartap, methamidophos and permethrin (Siqueira et *al*.,2000)
- Chile: deltamethrin, metamidophos, esfenvalerate, lambdacyhalothrin and mevinphos (Salazar and Araya ,1997)
- Argentine: abamectin and Deltamethrin (tolerance reported by Lietti et *al*., 2005)

- Gerson *et al.,* 2011 reported evidence of control failures for bifenthrin, permethrin, diflubenzuron, teflubenzuron, triflumuron and *B.thuringiensis*, moderate levels of resistance to indoxacarb and low resistance levels for bifenthrin and permethrin, abamectin, spinosad, *B. thuringiensis* and the mixture of deltamethrin and triazophos.

Questions

"What is the actual status of insecticide resistance of tuta absoluta in the Mediterranean region and what kind of Resistance Management Model should be implemented to achieve a sustainable control of the insect ?"

What are the levels of resistance of *tuta absoluta* to the existing and used pesticides in the Mediterranean basin?

What are the risks for the appearance of new insecticide resistance?

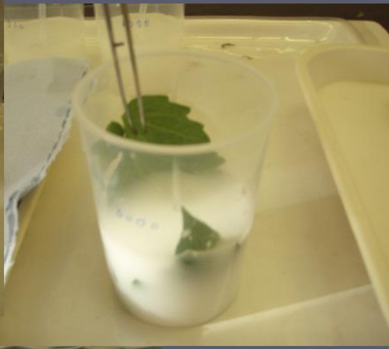
What is the most suitable Resistance Management Model to be adopted for the local context?

How can the model be successfully implemented in different cropping systems?

► Bio-assays: leaf dip

A set of leaf dip bioassays has been carried out to evaluate the susceptibility of the reared strains of *Tuta absoluta*.

- The tomato leaves were immersed in six different concentrations insecticide and allowed to air dry for at least one hour.
- One to two leaves were placed on slightly moistened filter paper inside Petri dishes (90mm diameter × 20 mm height).
- Fifteen to twenty L2 instars were placed on the leaves in each Petri dish then placed in controlled conditions of temperature (26 ± 2 °C) and light (16h/8h).
- Three replicates were used for each concentration of insecticide. Each bioassay was scored after 48 hours.
- The larvae is counted as dead if it shows signs of uncoordinated movements, cannot flip back to the crawling position or cannot craw for a distance at least equal to the double of its length

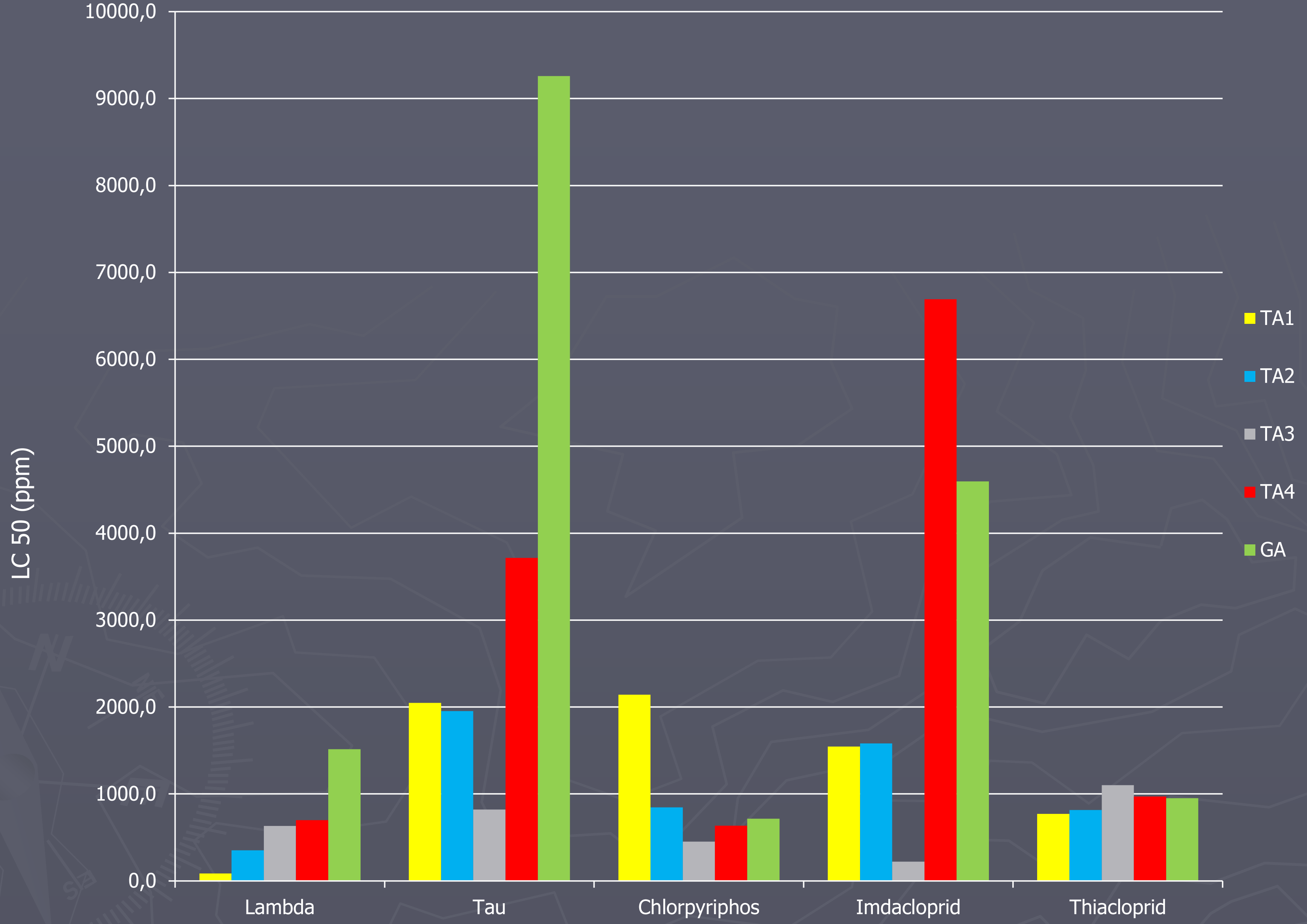




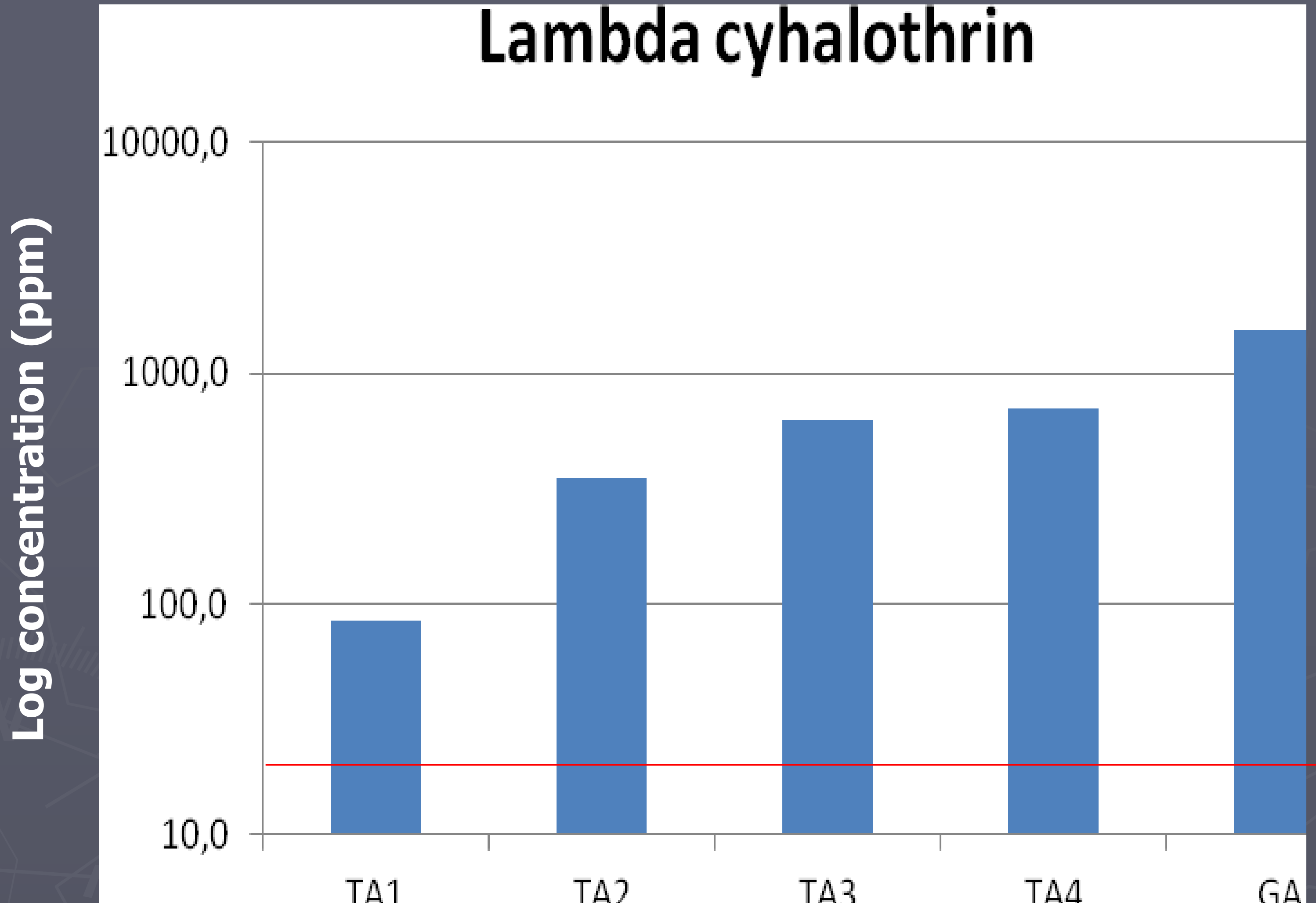
- *Tuta absoluta* strains:
  - TA1, TA2, TA3, TA4, GA
  - No susceptible strain!!!
  - Highest recommended field rates

- Chemicals:

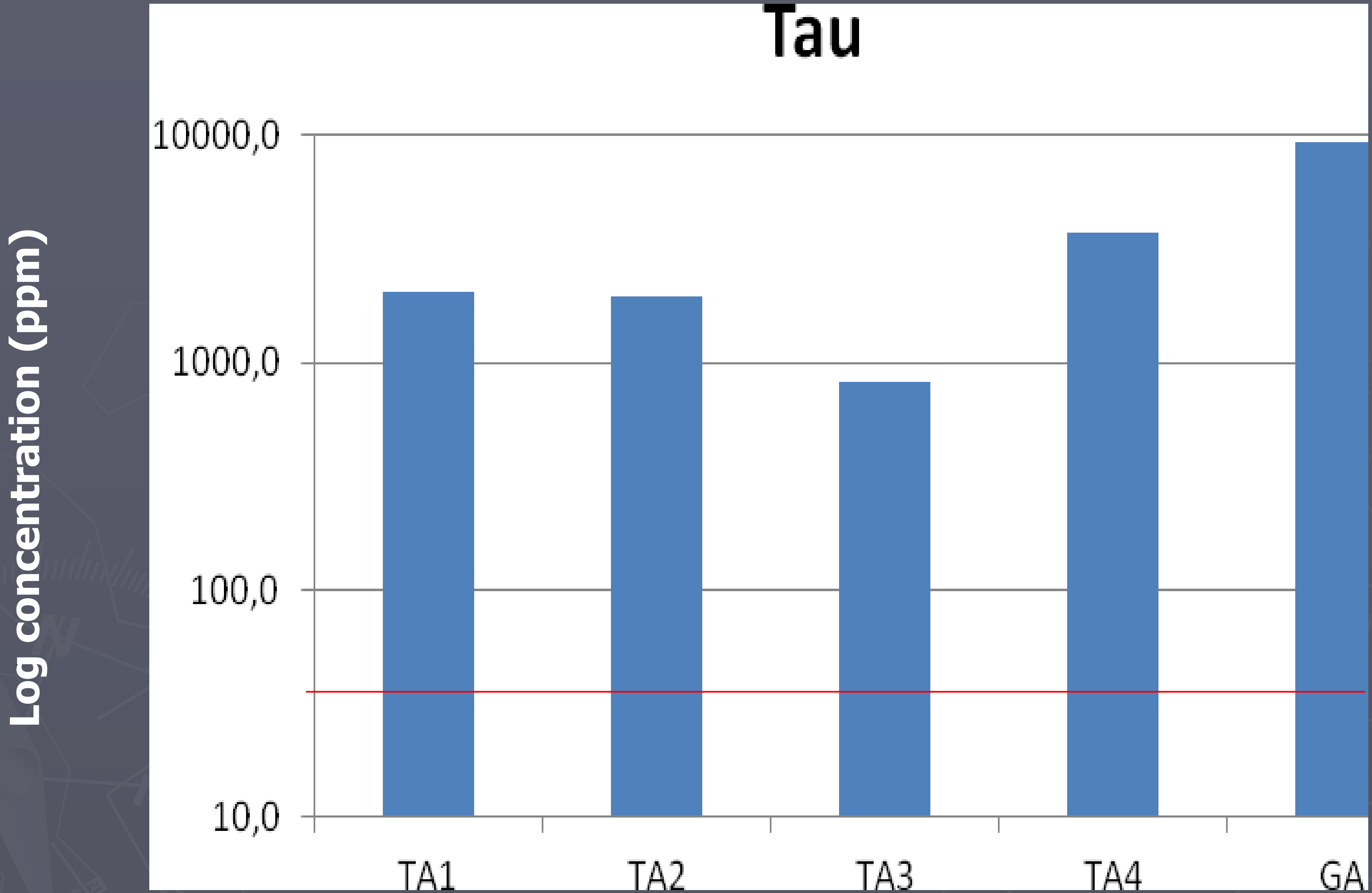
Class	Compound
Pyrethroids	Lambda Cyhalothrin
Pyrethroids	Tau fluvalinate
Organophosphates	Chlorpyrifos
Neonicotinoid	Imidacloprid
Neonicotinoid	Thiacloprid



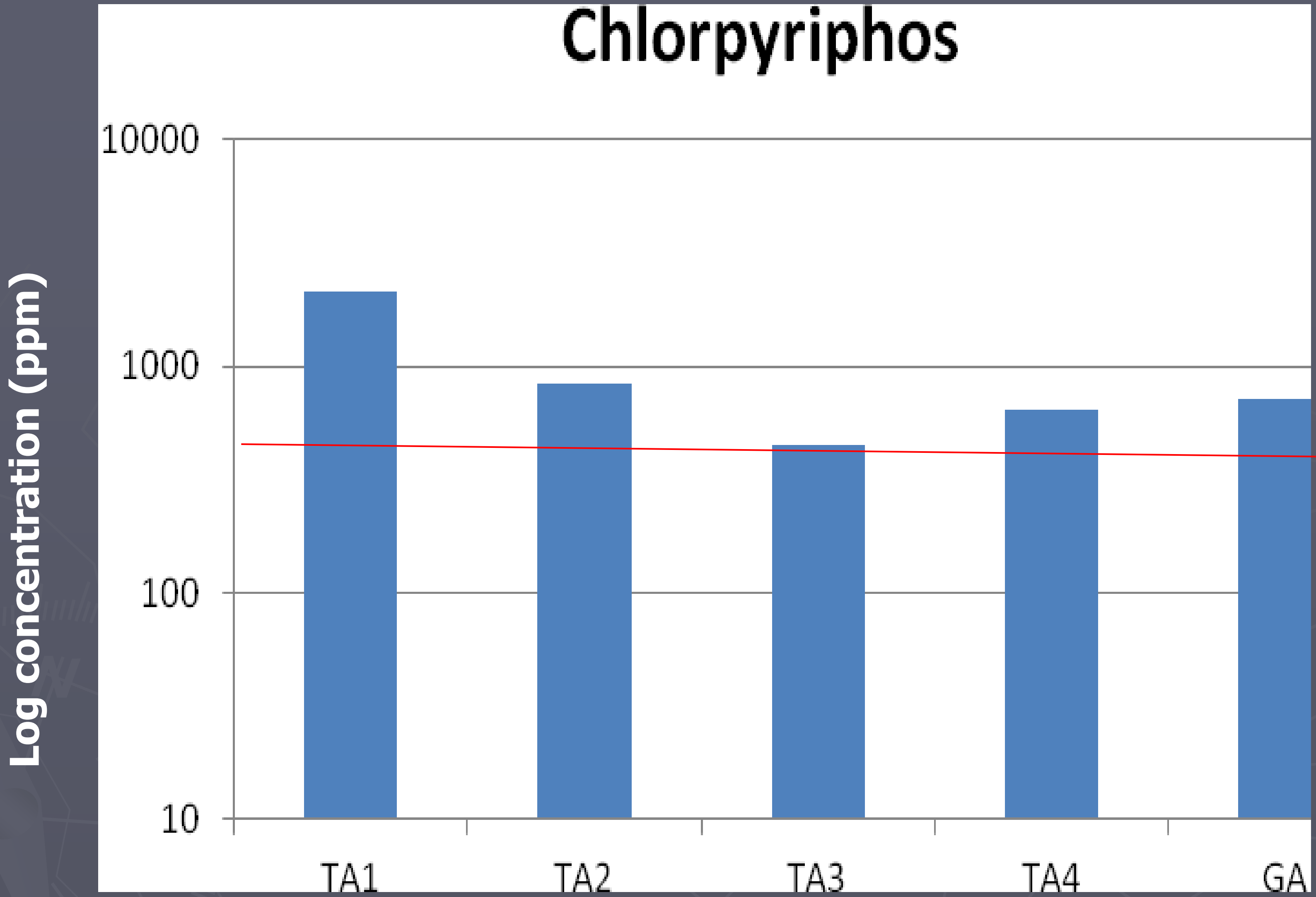
LC 50 (ppm)
10000,0
9000,0
8000,0
7000,0
6000,0
5000,0
4000,0
3000,0
2000,0
1000,0
0,0
Lambda
Tau
Chlorpyriphos
Imdacloprid
Thiacloprid
TA1
TA2
TA3
TA4
GA



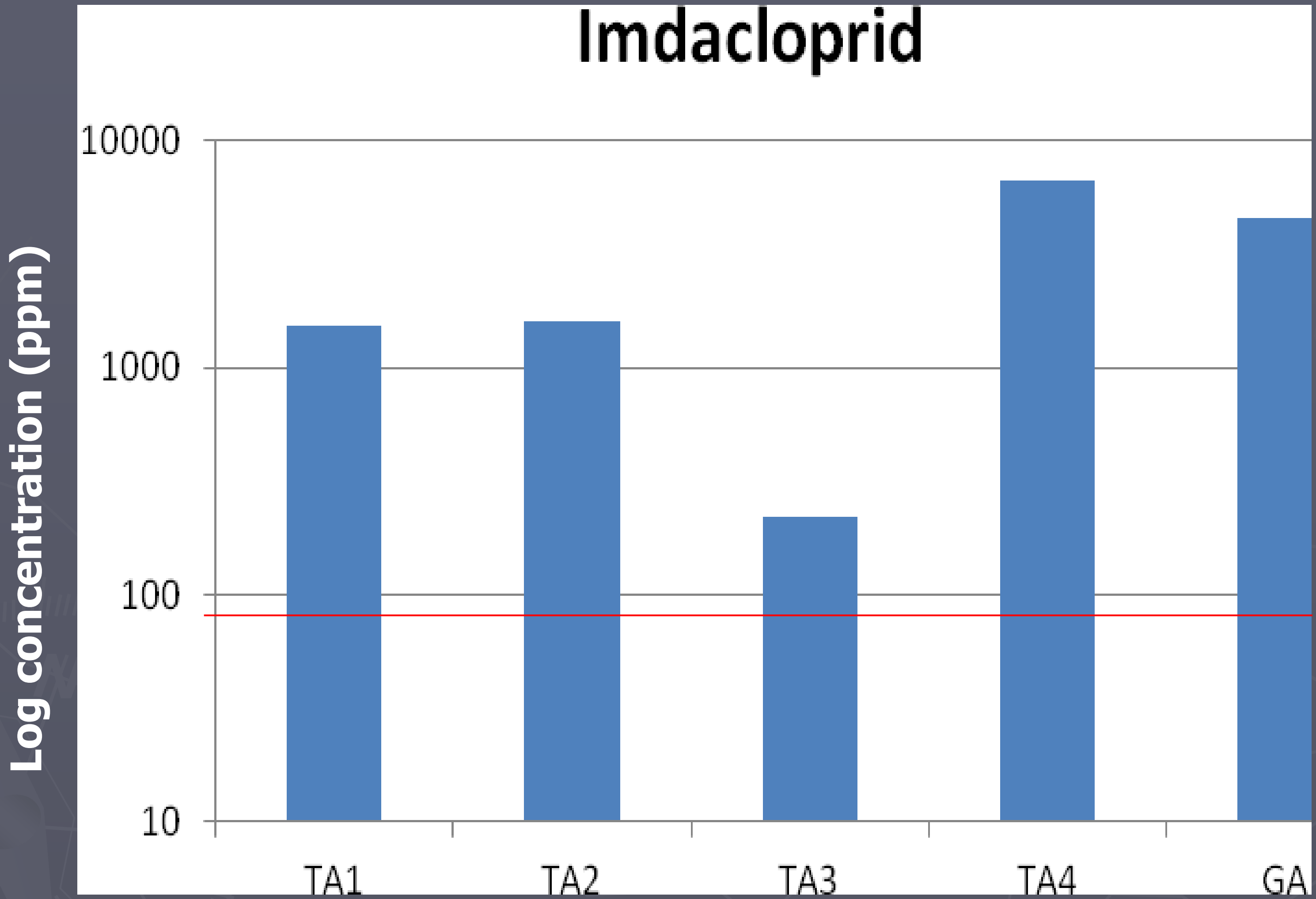
Lambda cyhalothrin
LC50 estimates (Log concentrations)
for five different *T. absoluta* strains.



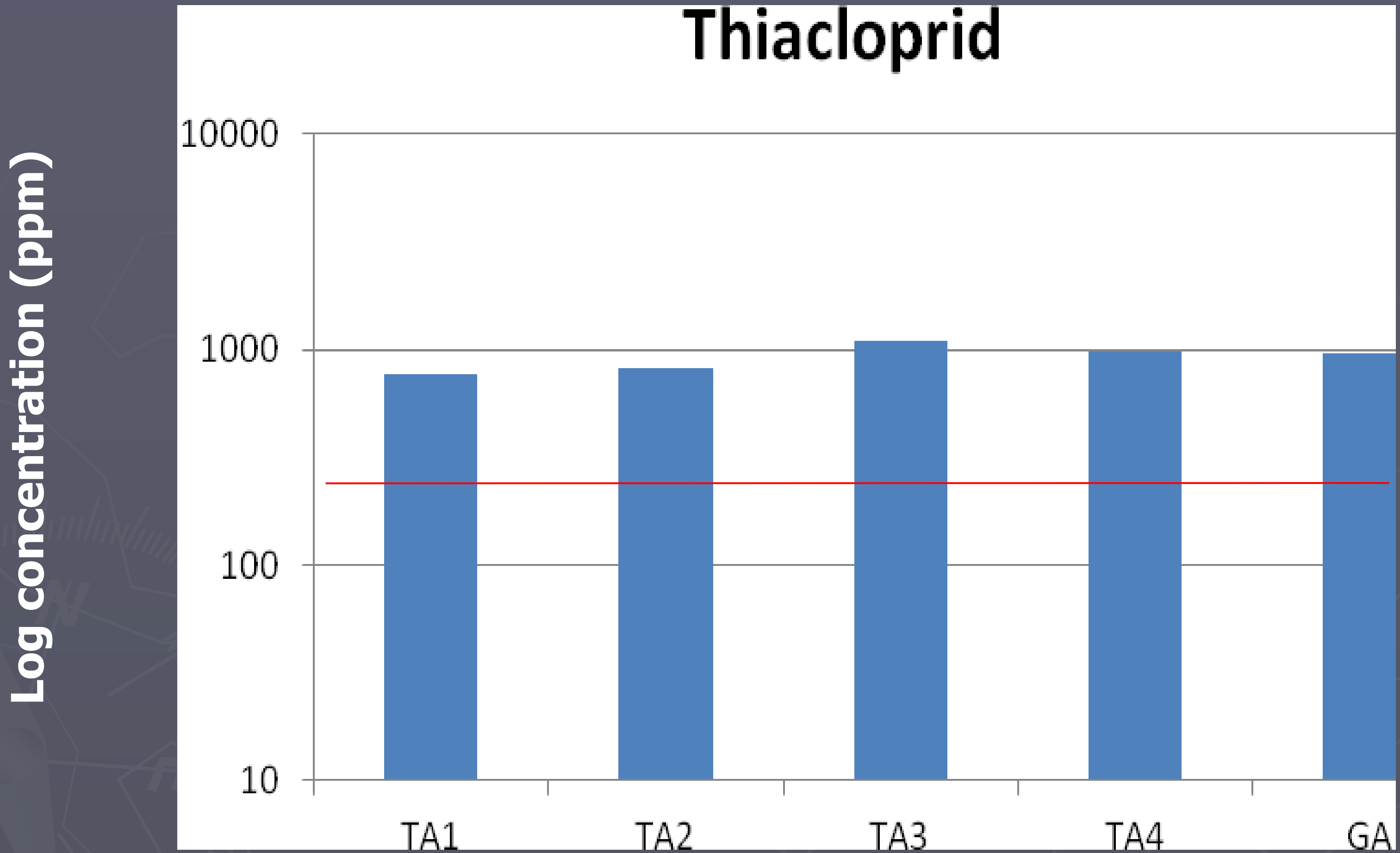
Tau fluvalinate
LC50 estimates (Log concentrations)
for five different *T. absoluta* strains.



Chlorpyriphos
LC50 estimates (Log concentrations)
for five different *T. absoluta* strains.



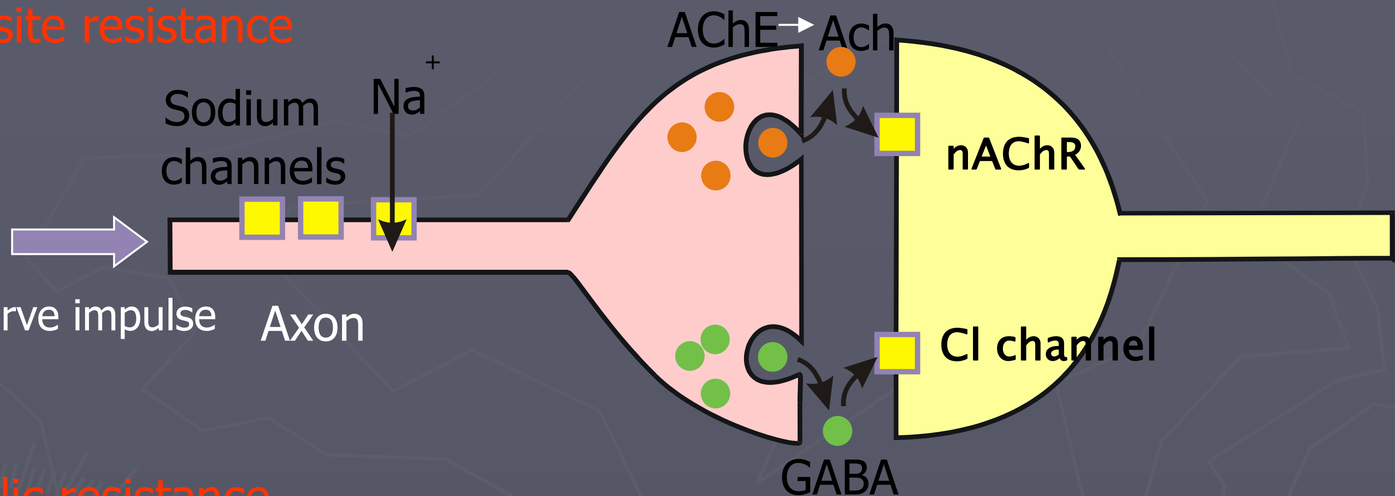
Imidacloprid
LC50 estimates (Log concentrations)
for five different *T. absoluta* strains.



Thiacloprid
LC50 estimates (Log concentrations)
for five different *T. absoluta* strains.

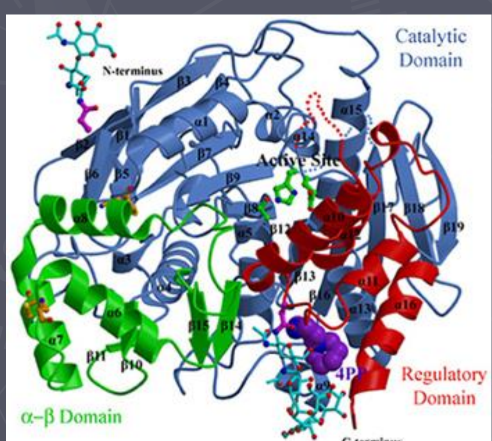
mechanisms of resistance

Target-site resistance

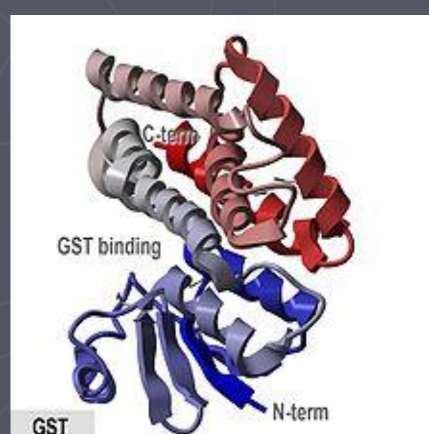


Metabolic resistance

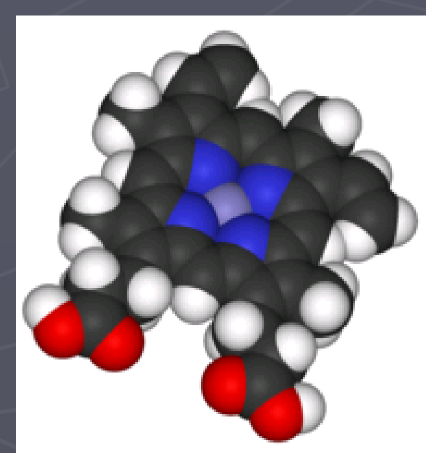
Carboxylesterases

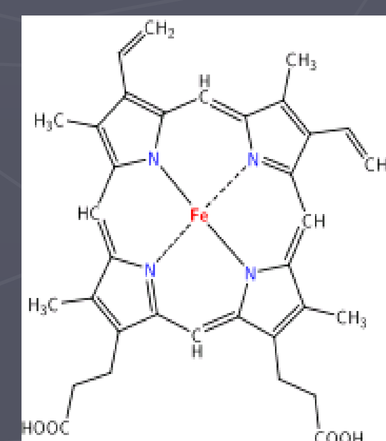


Glutathione S-transferases

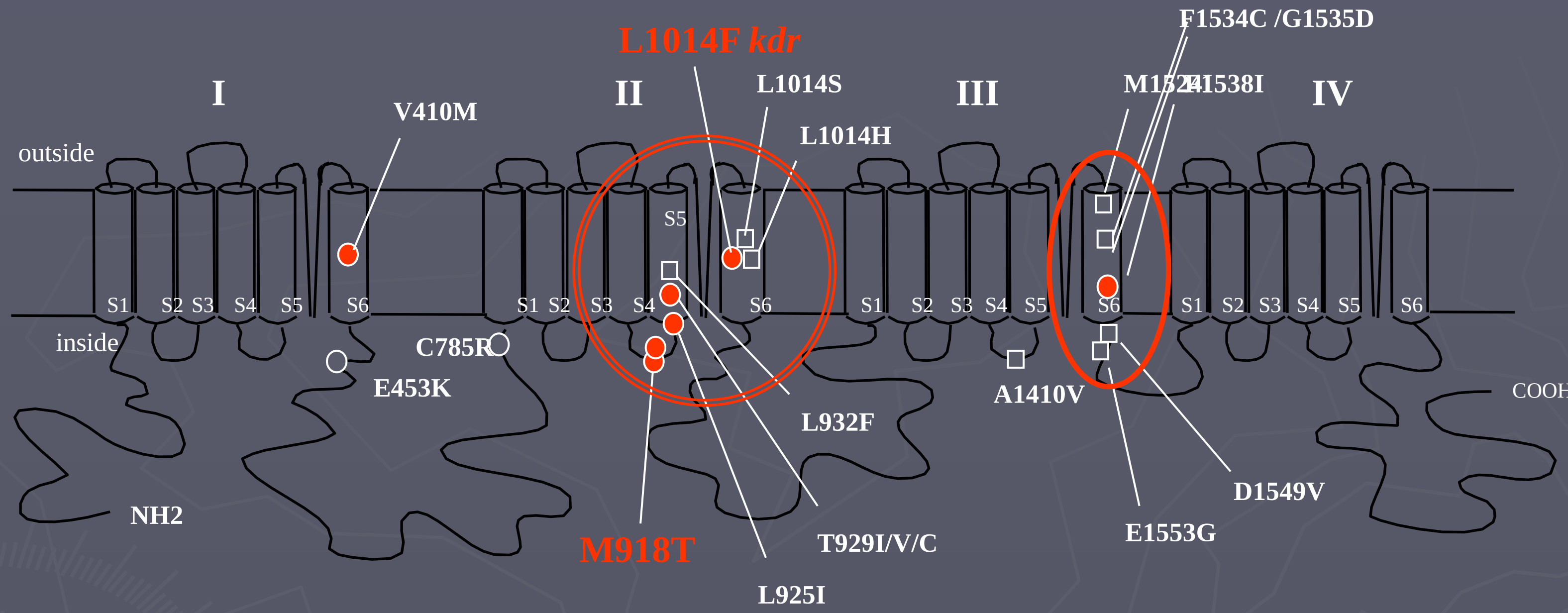


Cytochrome P450s



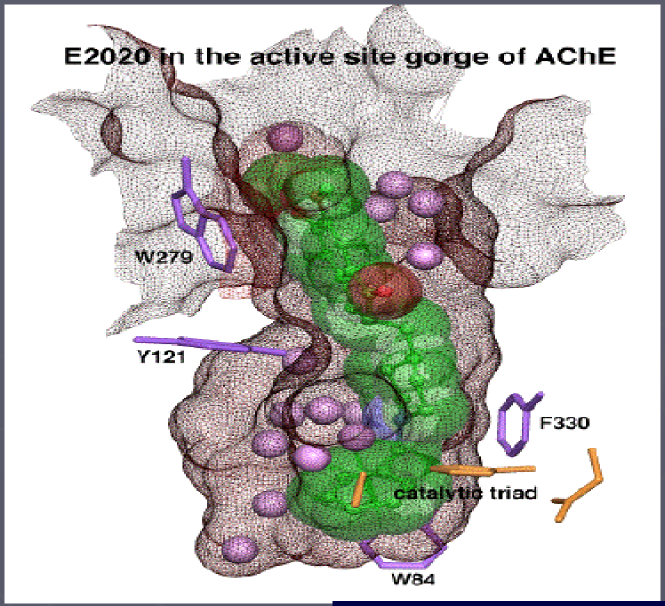


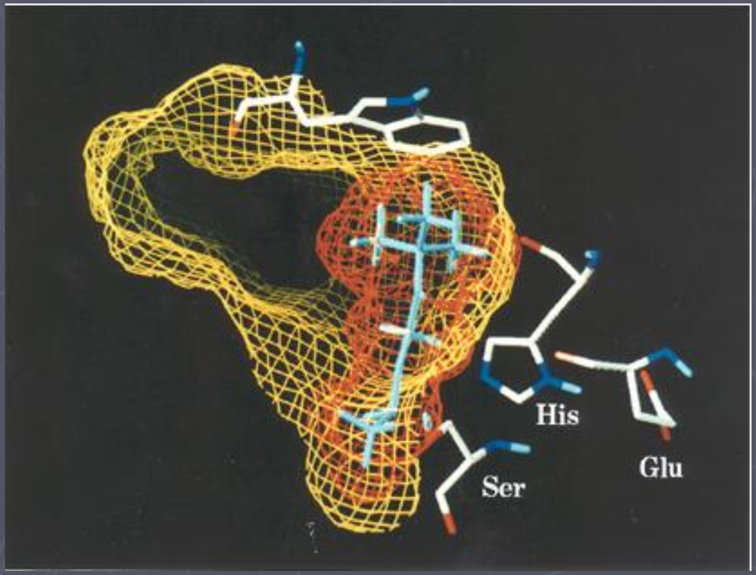
Sodium channel and kdr/super-kdr mutations



Anopheles gambiae
Aphis gossypii
Bemisia tabaci
Blattella germanica
Boophilus microplus
Ctenocephalides felis
Culex pipiens
Drosophila melanogaster
Aedes aegypti
Liriomyza huidobrensis

Helicoverpa armigera
Heliothis virescens
Hematobia irritans
Leptinotarsa decemlineata
Musca domestica
Myzus persicae
Pediculus capitis
Plutella xylostella
Frankliniella occidentalis
Sarcoptes scabiei





Torpedo acetylcholinesterase structure

Mutations	Position	Insect
E to G	73	only in one drosophila population
• F to L • F to S	78	• *Aphis gossypii* • has been expressed in *Drosophila melanogaster*, *Lucilia cuprina* and *Aedes aegypti* recombinant enzymes
E to K	82	found only once, in a drosophila population
• G to S • G to S	119	• *Culex pipiens, Anopheles gambiae* and *Anopheles albimanus* • *Tetranychus urticae*
D to E	128	*Tetranychus urticae*
I to V	129	Drosophila, *Bractocera oleae,* expressed in *Lucilia cuprina* and drosophila recombinant enzymes
V to L	151	House fly
A to S	201	*Aphis gossypii, Chilo suppressalis, Plutella xylostella, Tetranychus urticae, Bactrocera dorsalis*
G to A G to V	227	• Drosophila, • *Musca domestica,*has been expressed in *Drosophila melanogaster*, *Lucilia cuprina,Aedes aegypti* and *Musca domestica* recombinant enzymes
S to G	238	*Leptinotarsa decemlineata,*
F to Y	290	Drosophila, Musca domestica, has been expressed in Drosophila, house fly, Lucilia cuprina and Aedes aegypti recombinant enzymes
G to A	328	House fly and drosophila
S to F S to W S to C	331	• *Aphis gossypii,* • *Culex tritaeniorhynchus* • *Tetranychus urticae*
G to S	396	*Bactrocera oleae*
		(From Fournier, 2005 with amendments)

Tuta absoluta	SWPTFNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFIFAVMGMQL
Plutella	SWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFIFAVMGMQL
Heliotis	SWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFIFAVMGMQL
Droso	SWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFIFAVMGMQL
Musca	SWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFIFAVMGMQL
Myzus	SWPTLNLLISIMGRTIGALGNL TFVLCIIIFIFAVMGMQLFIFAVMGMQL
Blattella	SWPTLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFIFAVMGMQL
Tutaabsoluta	FGKNYVDNVDRFPDGDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCML
Plutella	FGKNYVDHVDRFPDGDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCML
Heliotis	FGKNYVDYVDRFPDGDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCML
Droso	FGKNYHDHKDRFPDGDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMY
Musca	FGKNYIDHKDRFKDHELPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMY
Myzus	FGKNYTEKMYMFKDHELPRWNFTDFLHSFMIVFRVLCGEWIESMWDCLH
Blattella	FGKNYYDNVERFPDGDMPRWNFTDFMHSFMIVFRVLCGEWIESMWDCML
Tutaabsoluta	VGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSTPTADNDTN
Plutella	VGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSTPTADNETN
Heliotis	VGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSTPTADNETN
Droso	VGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSAPTADNDTN
Musca	VGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSAPTADNDTN
Myzus	VGEPTCIPFFLATVVIGNLVVLNLFLALLLSNFGSSNLSVPTADNETN
Blattella	VGDWSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSNLSAPTADNETN

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AGCACCGGCTGCAAATTAAATGAGTAGAGCTAATGCCCATCCTGCTACCCACGGAACCCG

-N--L--T--F--V--L--C--I--I--I--F--I--F--A--V--M--G--M--Q--L-
AACCTGACCTTCGTATTGTGCATCATTATATTCATATTTGCCGTGATGGGTATGCAACTA  120
TTGGACTGGAAGCATAACACGTAGTAATATAAGTATAAACGGCACTACCCATACGTTGAT

-F--G--K--N--Y--V----D--N--V--D--R--F--P--D--G--D--L--P--R--W-
TTTGGGAAAAATTATGTGGACAACGTTGATCGTTTTCCCGATGGACACCTACCACGATGG  180
AAACCCTTTTTAATACACCTGTTGCAACTAGCAAAAGGGCTACCTCTGGATGGTGCTACC

-N--F--T--D--F--M--H--S--F--M--I--V--F--R--V--L--C--G--E--W-
AACTTTACGGATTTCATGCATAGCTTCATGATTGTGTTTAGAGTACTCTGCGGAGAATGG  240
TTGAAATGCCTAAAGTACGTATCGAAGTACTAACACAAATCTCATGAGACGCCTCTTACC

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-A--T--V--V--I--G--N--F--V----V--L--N--L--F--L--A--L--L--L--S-
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CGGTGGCAGCAGTAACCATTAAAACACCATGAATTGGAGAAGAATCGAGACAATGACAGT

-N--F--G--S--S--S--L--I--T--P--T--A--D--N--D--T--N--K--I-
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TTGAAACCAAGCAGCTCAAATAGCTGTGGATGACGGCTATTGCTGTGGTTAAACTAT
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M918T

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T929I

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862 nt

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TCTAGGCAAAATACTAATATAATGTTTCTTATATACAGACAACGTTGATCGTTTTCCCGATGGAGACCTACCAC

GATGGAACTTTACGGATTTCATGCATAGCTTCATGATTGTGTTTAGAGTACTCTGCGGAGAATGGATTGAGA

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TTTTGTGGTTCGTATCTTCTTTATATTCAGCTTTTTATTGCAATATACCAGTAAAAGCGTTTTTGTAATTGTGTGA

106 nt

ATTTAAAGATTCACCAAGATCTTCATTCGCATTTCAGGTACTTAACCTCTTCTTAGCTCTGTTACTGTCAAACTT

TGGTTCGTCGAGTTTATCGACACCTACTGCCGATAACGACACCAATAAG

L1014F

AAA: Introns　　AAA : Exons　　AAA : Mutations sites

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                      1                                                                           75
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TA 2           (2)    TCGTGGCCGACGTTTAATTTACTCATCT    ACGGGTAGGACGATGGGTGCCTTGGGCAACCTCACC
TA 3           (2)    TCGTGGCCGACGTTTAATTTACTCATCT    ACGGGTAGGACGATGGGTGCCTTGGGCAACCTCACC
TA 4           (2)    TCGTGGCCGACGTTTAATTTACTCATCT    ACGGGTAGGACGATGGGTGCCTTGGGCAACCTCACC
GA             (1)    TCGTGGCCGACGTTTAATTTACTCATCT    ACGGGTAGGACGATGGGTGCCTTGGGCAACCTCACC

Consensus      (2)    TCGTGGCCGACGTTTAATTTACTCATCT    ACGGGTAGGACGATGGGTGCCTTGGGCAACCTCACC
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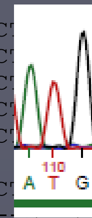

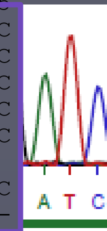


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                      76                                                                          150
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TA 2          (77)    TTCGTATTGTGCATCATTATATTCATATTTGCCGTGATGGGTATGCAACTATTTGGGAAAAATTATGTGGACAAC
TA 2          (77)    TTCGTATTGTGCATCATTATATTCATATTTGCCGTGATGGGTATGCAACTATTTGGGAAAAATTATGTGGACAAC
TA 4          (77)    TTCGTATTGTGCATCATTATATTCATATTTGCCGTGATGGGTATGCAACTATTTGGGAAAAATTATGTGGACAAC
GA            (71)    TTCGTATTGTGCATCATTATATTCATATTTGCCGTGATGGGTATGCAACTATTTGGGAAAAATTATGTGGACAAC

Consensus     (77)    TTCGTATTGTGCATCATTATATTCATATTTGCCGTGATGGGTATGCAACTATTTGGGAAAAATTATGTGGACAAC

                      -F--V--L--C--I--I--I--F--I--F--A--V--M--G--M--Q--L--F--G--K--N--Y--V--D--N-

                      151                                                                         225
TA 1         (152)    GTTGATCGTTTTCCCGATGGAGACCTACCACGATGGAACTTTACGGATTTCACGCATAGCTTCATGATTGTGTTT
TA 2         (152)    GTTGATCGTTTTCCCGATGGAGACCTACCACGATGGAACTTTACGGATTTCATGCATAGCTTCATGATTGTGTTT
TA 2         (152)    GTCGATCGTTTTCCCGATGGAGACCTACCACGATGGAACTTTACGGACTTCATGCATAGCTTCATGATTGTGTTT
TA 4         (152)    GTTGATCGTTTTCCCGATGGAGACCTATCACGATGGAACTTTACGGATTTCATGCATAGCTTCATGATTGTGTTT
GA           (146)    GTTGATCGTTTTCCTGATGGAGACCTCCCACGATGGAACTTTACGGATTTCATGCATAGCTTCATGATTGTGTTT

Consensus    (152)    GTTGATCGTTTTCCCGATGGAGACCTACCACGATGGAACTTTACGGATTTCATGCATAGCTTCATGATTGTGTTT

                      -V--D--R--F--P--D--G--D--L--P--R--W--N--F--T--D--F--M--H--S--F--M--I--V--F-

                      226                                                                         300
TA 1         (227)    AGAGTACTCTGCGGAGAATGGATTGAGAGTATGTGGGACTGTATGTTGGTCGGAGATGTATCGTGTATTCCATTC
TA 2         (227)    AGAGTACTCTGCGGAGAATGGATTGAGAGTATGTGGGACTGTATGTTGGTCGGAGATGTATCGTGTATTCCATTC
TA 2         (227)    AGAGTACTCTGCGGAGAATGGATTGAGAGTATGTGGGACTGTATGTTGGTCGGAGATGTATCGTGTATTCCATTC
TA 4         (227)    AGAGTACTCTGCGGAGAATGGATTGAGAGTATGTGGGACTGTATGTTGGTCGGAGATGTATCGTGTATTCCATTC
GA           (221)    AGAGTACTCTGCGGAGAATGGATTG          GGACTGTATGTTGGTCGGAGATGTATCGTGTATTCCATTC

Consensus    (227)    AGAGTACTCTGCGGAGAATGGATTG          GGACTGTATGTTGGTCGGAGATGTATCGTGTATTCCATTC
             (226)
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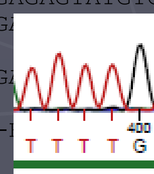


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                      301                                                                         375
TA 1         (302)    TTCTTAGCCACCGTCGTCATTGGTAATTTTGTGGTACTTAACCTCTTCTTAGCTCTGTTACTGTCAAACTTTGGT
TA 2         (302)    TTCTTAGCCACCGTCGTCATTGGTAATTTTGTGGTACTTAACCTCTTCTTAGCTCTGTTACTGTCAAACTTTGGT
TA 2         (302)    TTCTTAGCCACCGTCGTCATTGGTAATTTTGTGGTACTTAACCTCTTCTTAGCTCTGTTACTGTCAAACTTTGGT
TA 4         (302)    TTCTTAGCCACCGTCGTCATTGGTAATTTTGTGGTACTTAACCTCTTCTTAGCTCAGTTACTGTCAAACTTTGGT
GA           (296)    TTCTTAGCCACCGTCGTCATTGGTAATTTTGTGGTACTTAACCTCTTCTTAGCTCTGTTACTGTCAAACTTTGGT

Consensus    (302)    TTCTTAGCCACCGTCGTCATTGGTAATTTTGTGGTACTTAACCTCTTCTTAGCTCTGTTACTGTCAAACTTTGGT
                      -F--L--A--T--V--V--I--G--N--F--V--V--L--N--L--F--L--A--L--L--L--S--N--F--G-
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                   1        10         20         30         40         50         60         70         80         90        100
» TA3 10 (c)      (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA8(c)           (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGACCTTCGTATTGTGCATCATTATATTCATAT
» TA42(c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 3(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA20(c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA19(c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA32(c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA7 (c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA4(c)           (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 8 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA12(c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA11 (c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 7(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 9(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA3 7HETR (2)    (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 3 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCATGGGCAACCTGACCTTCGTATTGTGCATCATTATATTCATAT
» TA4 10 (c)       (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCATGGGCAACCTGACCTTCGTATTGTGCATCATTATATTCATAT
» TA2 4HETR (c) ...(1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 5_HETR (2)   (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 5 HETR(c) ...(1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA3 6HETR (2)    (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 6HETR (c) ...(1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA3 5 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 4(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 7(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA21(c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» A31(c)           (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA22 (c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA3 (c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 7 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» GA6 (c)          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 23(c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 9 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 9 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 6 HETR (c)...(1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 6 HETR (c)   (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA3 3 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA3 7HETR (c)    (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 4HETR(c)     (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 5HETR(c)     (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA1 5HETR(c)     (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA3 6HETR (c)    (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 3 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGACCTTCGTATTGTGCATCATTATATTCATAT
» TA4 6HETR (c)    (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA4 8(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 6(c)         (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
» TA2 5 (c)        (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTACGGGTAGGACGATGGGTGCCTTGGGCAACCTGACCTTCGTATTGTGCATCATTATATTCATAT
» GA5(c)           (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
Consensus          (1) GAAGTCGTGGCCGACACTTAATTTACTCATCTCGATTATGGGTAGGACGATGGGTGCCTTGGGCAACCTGATCTTCGTATTGTGCATCATTATATTCATAT
                                                             +                    +            +
                        -K--S--W--P--T--L--N--L--L--I--S--I--M--G--R--T--M--G--A--L--G--N--L--I--F--V--L--C--I--I--I--F--I--
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		M918T	T929I			M918T	T929I			M918T	T929I
TA1	1	S/S	R/R	TA3	1	S/S	R/R	GA	1	S/S	R/R
	2	S/S	R/R		2	S/S	R/R		2	S/S	R/R
	3	R/S	R/S		3	S/S	R/R		3	R/S	R/S
	4	R/S	R/S		4	S/S	R/R		4	S/S	R/R
	5	R/S	R/S		5	S/S	R/R		5	S/S	R/R
	6	R/S	R/S		6	R/S	R/S		6	R/S	R/S
	7	S/S	R/R		7	R/S	R/S		7	S/S	R/R
	8	S/S	R/R		8	R/S	R/S		8	R/S	R/S
	9	R/S	R/S		9	S/S	R/R		9	S/S	R/R
	10	R/R	S/S		10	R/S	R/S		10	R/S	R/S
TA2	1	S/S	R/R	TA4	1	R/S	R/S				
	2	S/R	S/R		2	R/S	R/S				
	3	R/R	S/S		3	R/S	S/S				
	4	R/S	R/S		4	S/S	R/R				
	5	R/R	S/S		5	R/S	R/S				
	6	S/S	R/R		6	R/S	R/S				
	7	S/S	R/R		7	R/S	R/S				
	8	R/S	R/S		8	R/S	R/S				
	9	R/S	R/S		9	R/S	R/S				
	10	R/R	S/S		10	R/S	S/S				

1824 nt

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CACTGAAGGATGGGGTGAAGAAATACTGAATACAACGACACTGCCACACTCGT
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GAAGCCGCGTCCCAAGAATGCGGCAGTAATGTTATGGGTGTTCGGCGGAGGGT
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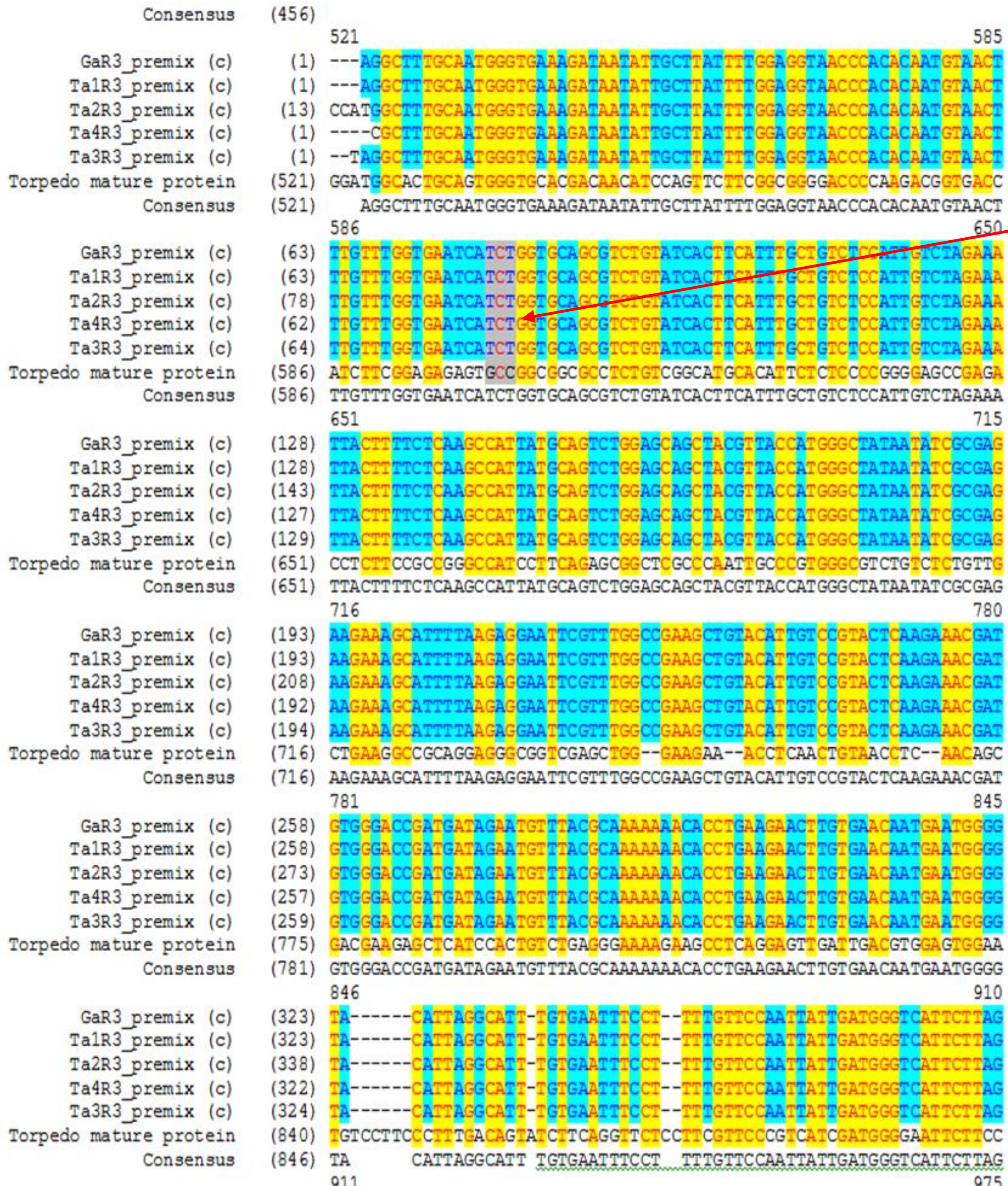
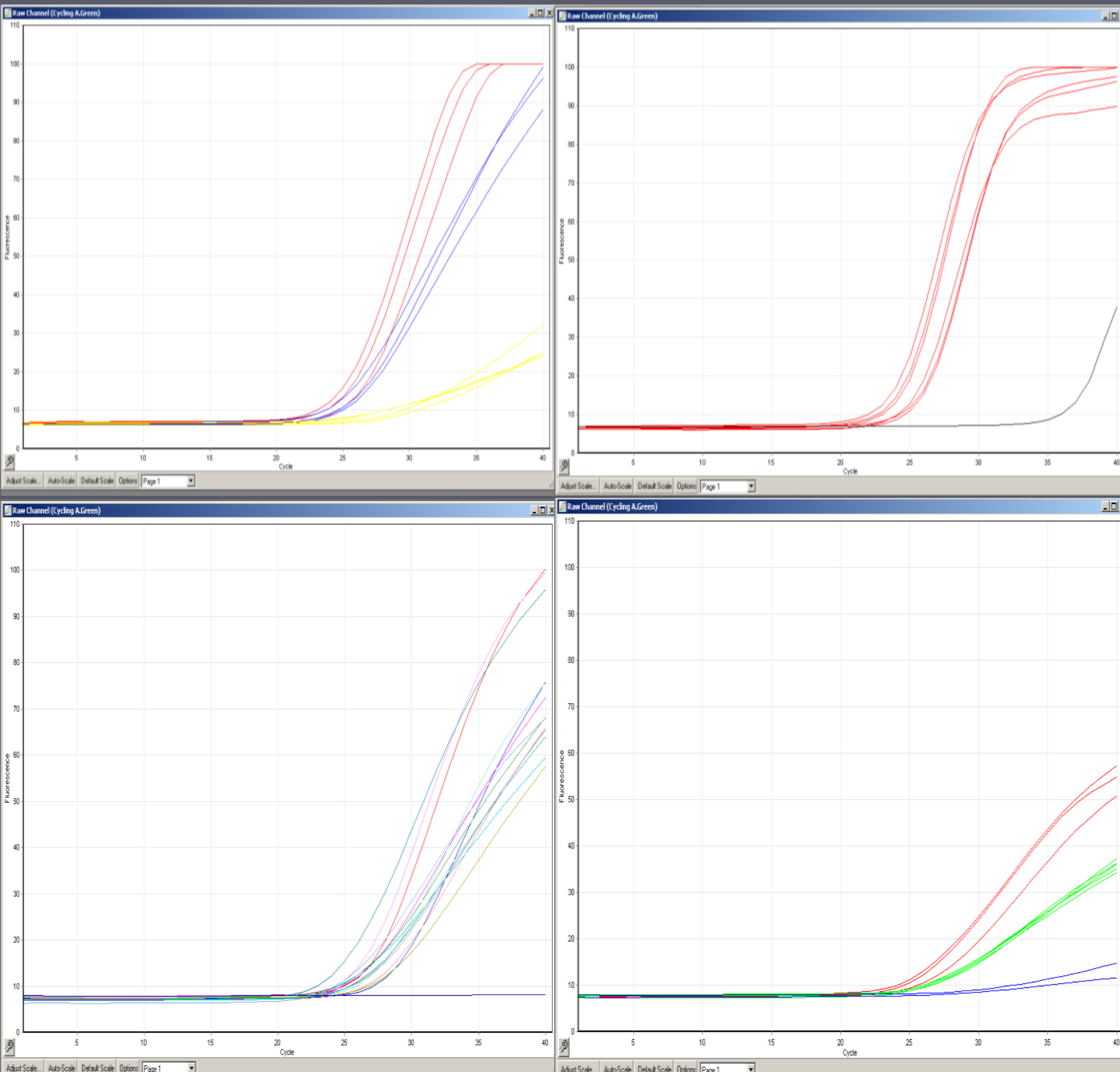
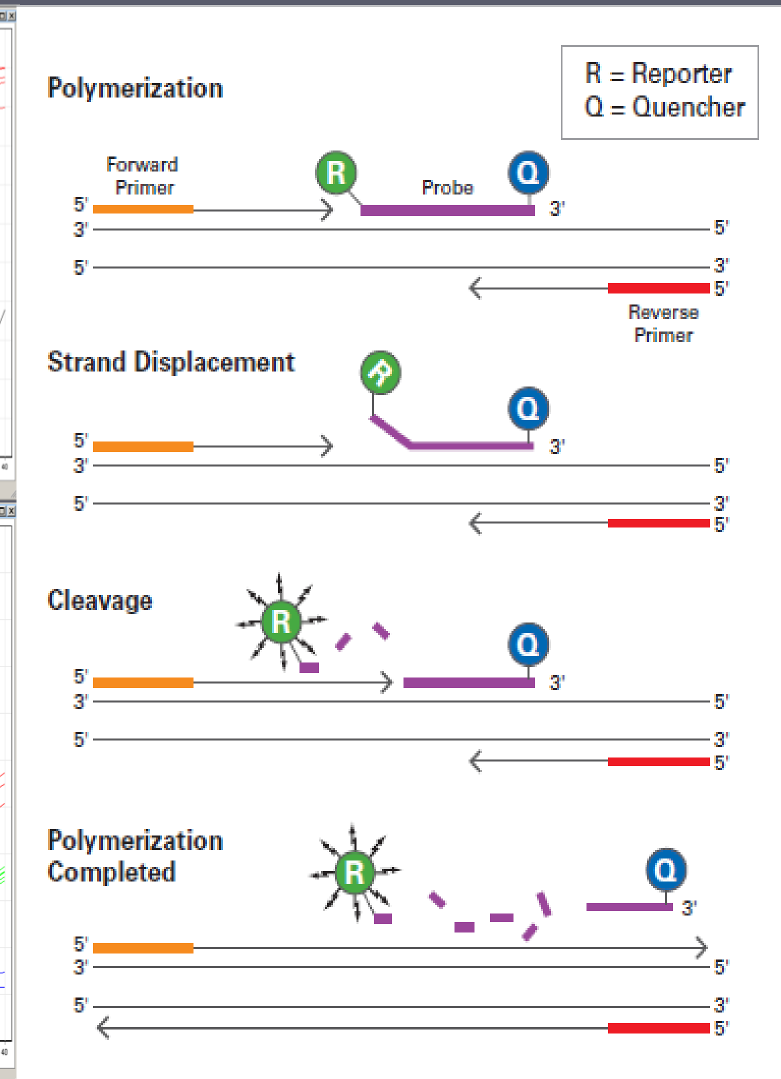


Ala ⇨ Ser (position 201):

Aphis gossypii
Chilo suppressalis,
Plutella xylostella,
Tetranychus urticae,
Bactrocera dorsalis





R = Reporter
Q = Quencher
Polymerization
Forward Primer
Probe
R
Q
5'
3'
Reverse Primer
Strand Displacement
Cleavage
Polymerization Completed

Sample ID Nº	Origin	Sampled from
1	Tejina, (La Laguna) Tenerife**,. Canary Islands**	tomato
2	Guía de Isora, Tenerife, **Canary Islands**	tomato
3	Granadilla, Tenerife, **Canary Islands**	tomato
4	Arico,Tenerife, **Canary Islands**	tomato
5	Teulera, Mallorca, Is Baleares	tomato
6	San Fangos, Mallorca, Is.Baleares	tomato
7	Mostaganem, **Algeria**	Tomato under-protection
8	Mostaganem, **Algeria**	Tomato under-protection
9	Turín **Italy**	Unknown
10	La Tola, Pichincha, **Ecuador**	tomato
11	Tudela, Navarra, **SPAIN**	tomato
12	Barrancas,Santa Fé, **Argentina**	Tomato under-protection
13	LaPrimavera, Mendoza, **Argentina**	tomato
14	La Plata, Bs.As. **Argentina**	Tomato under-protection
15	Cagliari.S.Margherita di Pula/ **ITALY**	Tomato under-protection
16	La Palma, **Canary Islands**	Unknown
17	Sicilia **ITALY**	Aubergine
18	Ramonete/Lorca, MU, **SPAIN**	tomato
19	(Curicó costa), **CHILE**	tomato
20	Antioquía/Rionegro/**Colombia**	tomato
21	Heraklion/Creta- **Greece**	wild plants
22	Mazarrón, Murcia, **SPAIN**	tomato
23	Canelones; **URUGUAY**	tomato
24	Chulacanas, Piura. **Perú**	tomato
25	Valencia, **SPAIN**	Unknown
26	Maresme, Cataluña, **SPAIN**	tomato
27	Silveira. Concello Torres Vedres, **Portugal**	tomato
28	Sele valley.Salerno.Campania. ITALY	tomato
29a	beit hashita, **israel valley** (1)	Unknown
29b	ein hmifraz, **western galilee** (2)	Unknown

Conclusions:

- All the five strains showed differences in susceptibility to the compounds used with LC50 far higher than the recommended field rates.
- A 420 b fragment of sodium channel (domains IIS5/IIS6) and 1824 b fragment of the acetylcholinesterase of *Tuta absoluta* were sequenced for the first time.
- All the five strains have the kdr (L1014F) mutation in combination with either super kdr(M918T) or T929I mutation.
- A rare novel mutation L925M was detected in 2 individuals from one strain.
- All the strains showed the Ala to Ser mutation at the position 201 of acetylcholinesterase.
- A TaqMan was designed to screen field collected samples of diverse geographic origin and to examine the frequency and distribution of the reported mutations.

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- Kevin Gorman
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Thank You