Pesticide Resistance:

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VALENT BIOSCIENCES. SUMITOMO CHEMICAL

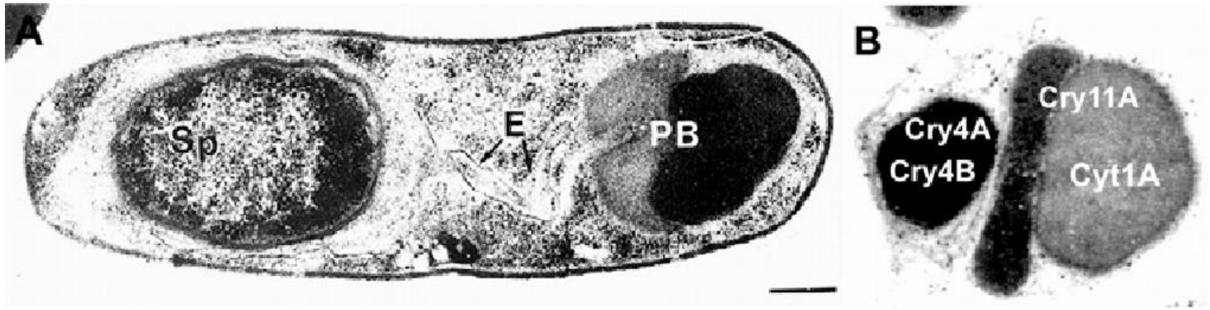
Change and Innovation 3.0
For a Sustainable Future

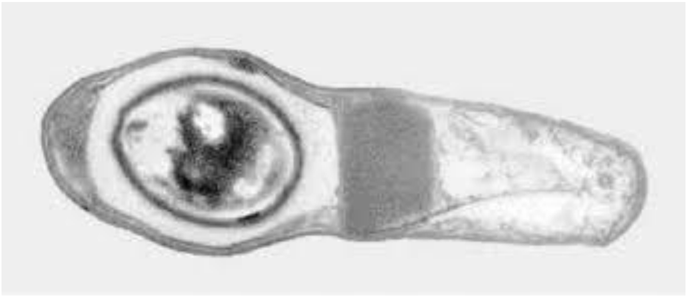
Larvicides-Active Ingredients

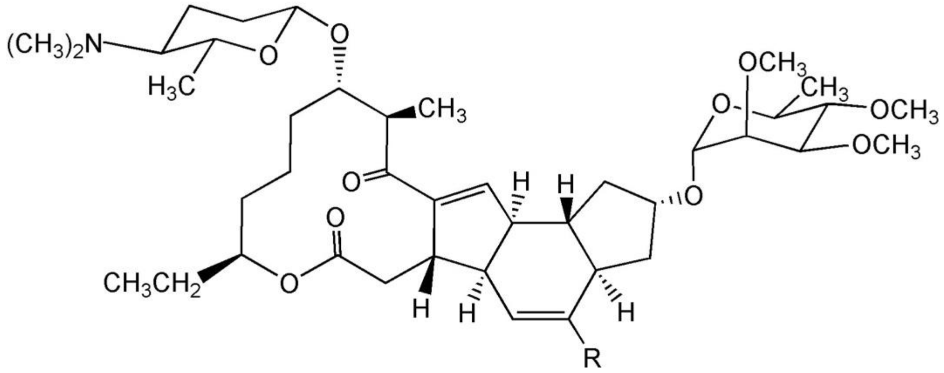
1. Classes of larvicides and modes of action
2. Why is resistance important?
3. How can mosquitoes develop larvicide resistance?
4. What can we do about it?

What are the routes of toxicity for larvicide control?

- Larvicides that must be ingested or eaten
  - *Bacillus thuringiensis israelensis*
  - *Lysinibacillus sphaericus*
- Larvicides that work by contact with mosquito larvae
  - (S)-Methoprene
  - Pyriproxyfen
- Larvicides that work both ways by being ingested or by contact
  - Spinosad
- Larvicides that work by inhibiting respiration
  - Mineral oil
  - MMFs





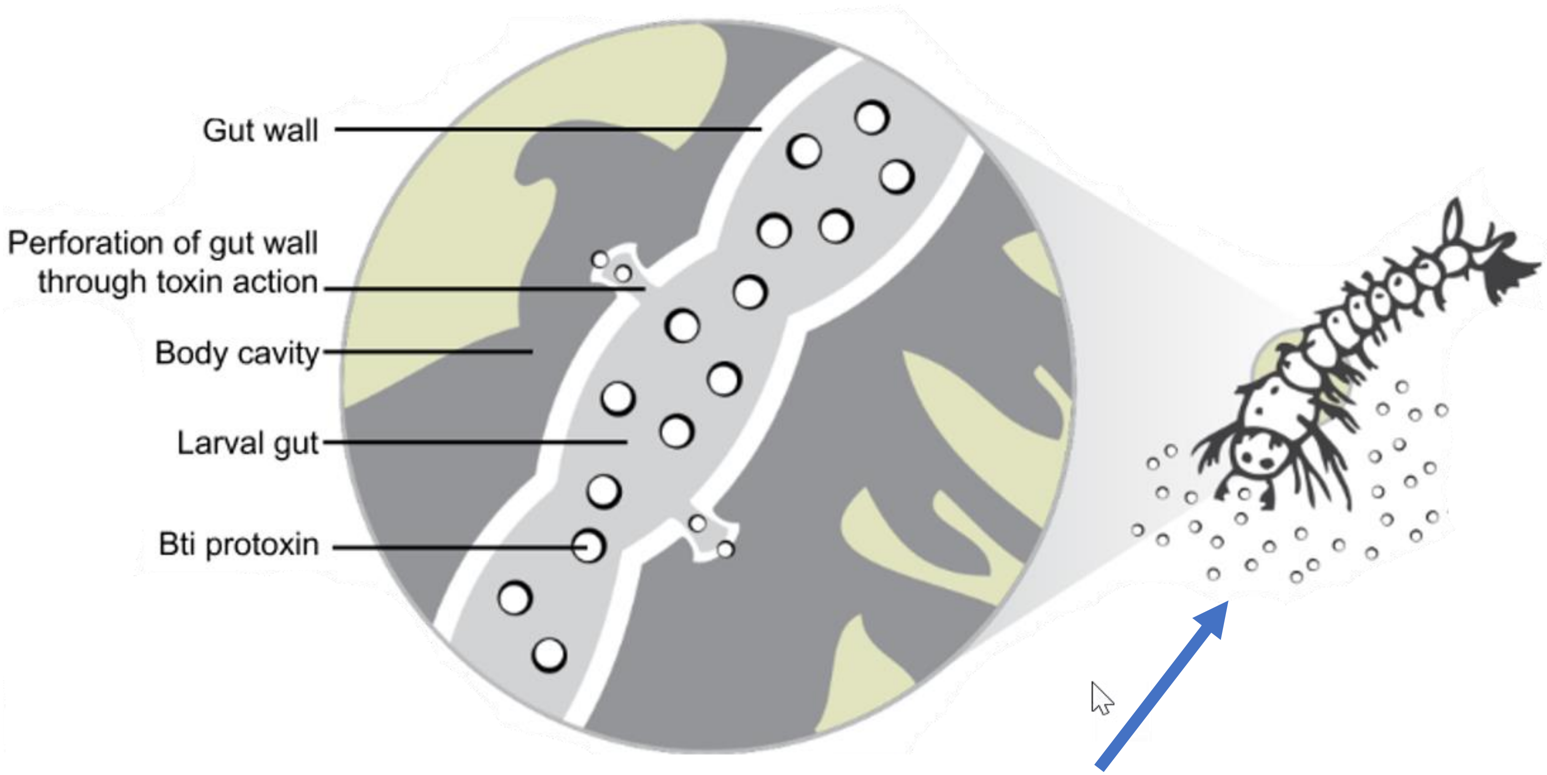


Larvicides- Class and Mode of Action

CLASS GROUP	AI COMPOUNDS	MODE OF ACTION	ACTIVITY SPECTRUM	TARGET SPECIFICITY
Bacterial Larvicide	Fermentation solid sand solubles of whole bacterium containing ICP's	Binds to midgut disrupting digestion and fluid balance		Very High
	Bacillus sphaericus	Bs: Mosquito-specific ICP	Mosquitoes	
	Bacillus thuringiensis subsp. *israelensis*	*Bti*: Nematocera-specific ICP	Mosquitoes, black flies, and closely related flies in Order Diptera: Suborder Nematocera	
Juvenile Hormone	Purified Compound	JH analog is specific to insects that undergo complete metamorphosis; inhibits emergence	Insects that undergo complete metamorphosis	
	(S)-Methoprene			High
	Pyriproxyfen			Moderate
Spinosyns	Metabolites of bacterial fermentation Spinosyn A and Spinosyn D	Neurotoxin specific to invertebrates binds to nicotinic acetylcholine receptors causing excitation of nerves, leading to involuntary muscle contractions	Insects and other invertebrates including some Crustacea and Mollusca	Moderate

Bacillus thuringiensis israelensis (*Bti*) Mode-of-action

- Ingest
- Protoxin activated
- Enzymes break down protoxins
- Polypeptide fractions act on cells
- Cells lyse
- Larvae die



Insecticidal Crystal Proteins

Diagram Origin: Valent Biosciences

Bti Formulations

- **Briquet- 100 ft²**
  - Floating 30-day
  - Non-floating- 45 & 150-day*
  - Small confined areas where water stands or floods

- **Liquid Bti**
  - Larvae must be present
  - Cannot pre-treat
  - Single brood control to 1 wk
  - Often used on wide areas and/or ditches
-

- **Flash Release Granule**
  - Larvae must be present
  - Cannot pre-treat
  - Single brood control to 1 wk
  - Wide area applications or spot treatments

- **Controlled Release Granule**
  - Can pre-treat (shaded areas)
  - 40-day control
  - 4 floodings*
  - Multiple broods
  - Wide area applications or spot treatments

Water-Dispersible granule

- Larvae must be present
- Cannot pre-treat
- Single brood control to 1 wk
- Wide area applications or spot treatments

Lysinibacillus sphaericus
Mode-of-Action

- Ingest
- Feeding ceases
- Bacteria is suspended in the midgut during digestion
- BIN and Cry toxins bind to midgut receptors
- Cells lyse
- Larvae die

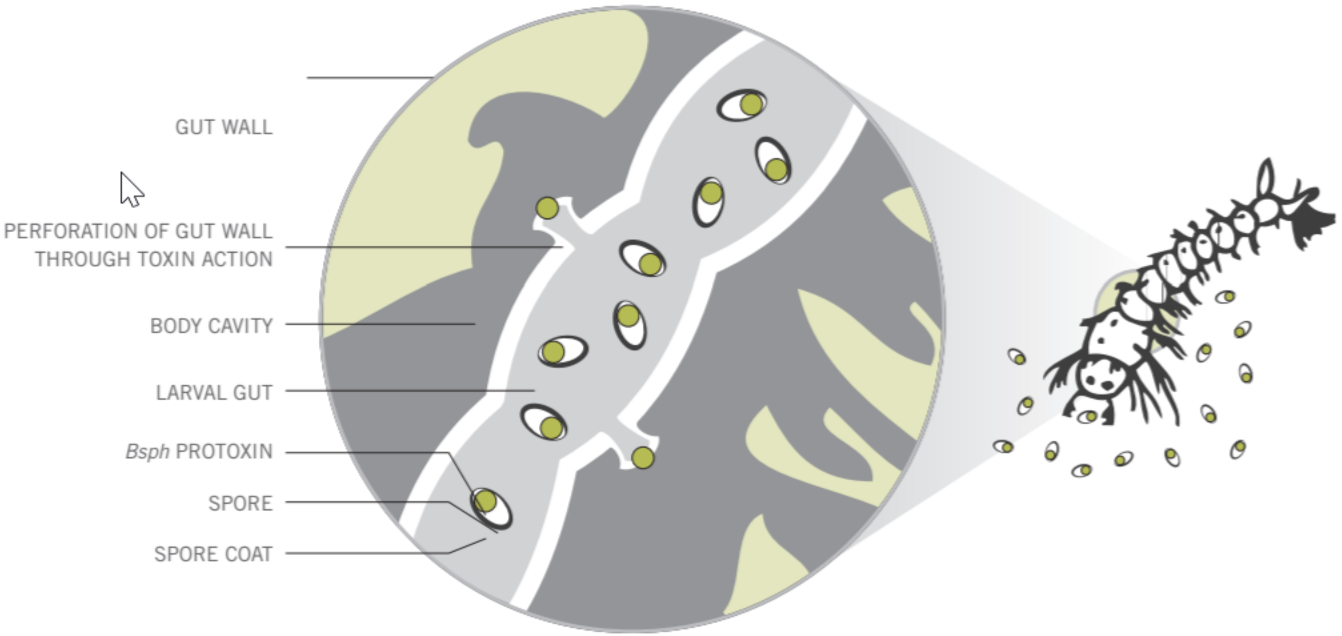


Diagram Origin: Valent Biosciences

Bs formulations

Briquet- 100 ft²

- Non-floating- 45,90,180-day
- Small confined areas where water stands or floods

WSP- 50 ft²

- Storm drains
- Smaller permanent water sites

WDG (water-dispersible granule)

- Larvae must be present
- Cannot pre-treat
- ~30 day control
- Often used on wide areas and/or ditches

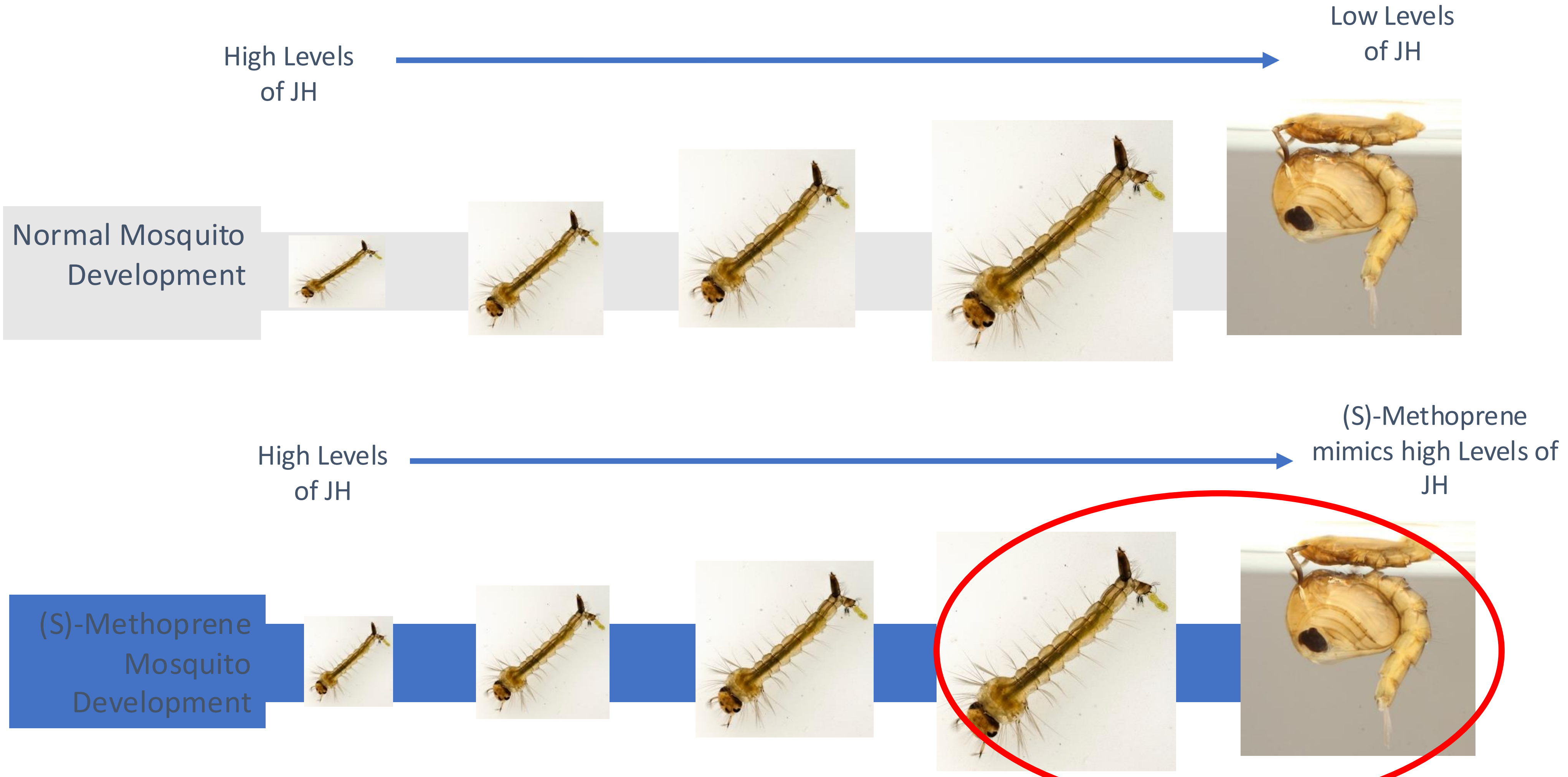
Flash Release Granule

- <u>Larva</u> must be present
- Cannot pre-treat
- ~30 day control
- Wide area applications or spot treatments
- Corncob substrate

Controlled Release Granule

- Can pre-treat (shaded areas)
- 60-day control
- 4 floodings
- Multiple broods
- Wide area applications or spot treatments

(S)-Methoprene: Insect Growth Regulator Mode-of-Action



- Juvenile Hormone Analog

Methoprene Formulations

Briquets- 100 ft²

- 30 & 150 day
- 150 day
- Must compensate for water depth (swimming pools)

WSP- 100 &135 ft²

- 30 day- 100 control (pellets)

Pellets

- 30-day control (flooded)
- Can pretreat
- Low application rates
- Often used in wide area flood sites

Granules

- Single brood
  - Sand granule for dense foliage
  - Cannot pretreat, flash release
- Extended release
  - Can pretreat
  - 21-42 day control (flooded)
  - Several wet-dry cycles
  - Sand granule substrate
  - UV inhibitor

Liquids

- Larvae should be present
- Duplex tank mix is common
- Aerial applications

Spinosad Mode-of-Action

- Alters the function of the nicotinic and GABA-gated ion channels causing rapid excitation of the insect nervous system, leading to involuntary muscle contractions, tremors, paralysis and death.

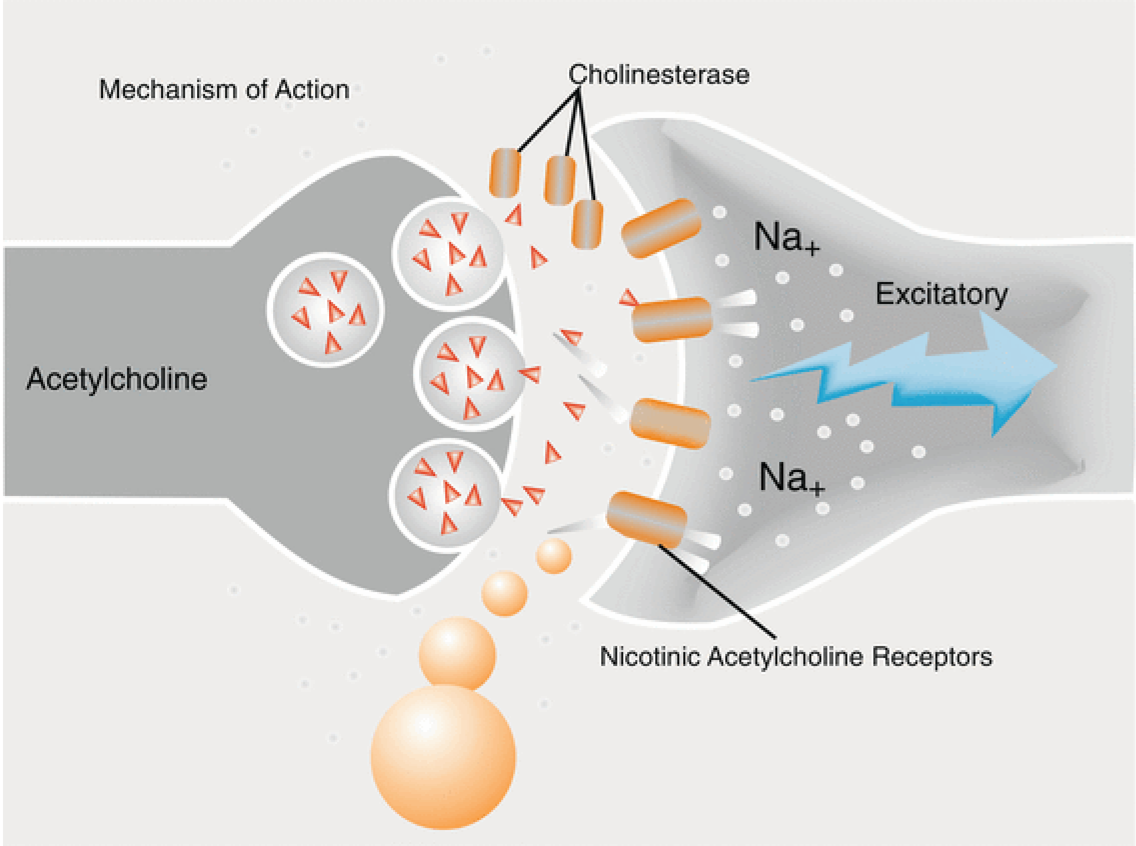


Figure: Shivanandappa T., Rajashekar Y. (2014) Mode of Action of Plant-Derived Natural Insecticides. In: Singh D. (eds) Advances in Plant Biopesticides. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2006-0_16

Spinosad Formulations

Tablets (Briquets)

- DT- treats 55 gallons
    - Storm drains
- T30- treats 100 ft^2, 30 days
- XRT- treats 100 ft^2, 180 days

Liquid- 2EC

- Emulsifiable concentrate
- Cannot pretreat
- ~1 week control
- Often used for wide area applications and ditches

G Granules

- 1 week control
- Cannot pretreat

G30 Granules

- ~30 days control
- Can pretreat
- Single flooding
- Must allow for water depth

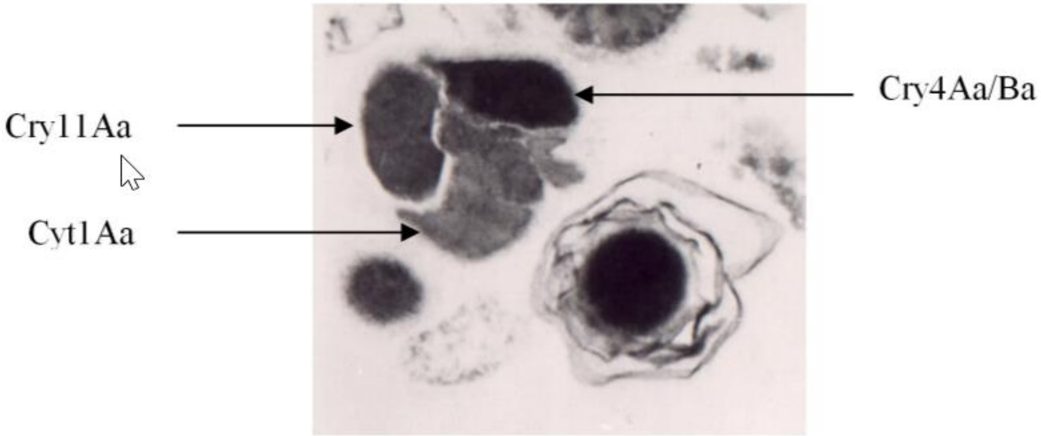
Bacillus thuringiensis israelensis Resistance

- According to the EPA, “there is no documented resistance to *Bti* as a larvicide.”

Monitoring resistance to *Bacillus thuringiensis* subsp. *israelensis* in the field by performing bioassays with each Cry toxin separately

Guillaume Tetreau,+ Renaud Stalinski, Jean-Philippe David, and Laurence Després

This study confirms previous works showing a lack of *Bti* resistance in field mosquito populations treated for decades with this bioinsecticide. It also provides a first panorama of their susceptibility status to individual *Bti* Cry toxins. In combination with bioassays with *Bti*, bioassays with separate Cry toxins allow a more sensitive monitoring of *Bti*-resistance in the field



Lysinibacillus sphaericus Resistance

azelis A&ES US

- There have been several instances of resistance *to L. sphaericus* throughout the United States.

Journal of the American Mosquito Control Association, 11(1):1–5, 1995

DEVELOPMENT OF A HIGH LEVEL OF RESISTANCE TO *BACILLUS SPHAERICUS* IN A FIELD POPULATION OF *CULEX QUINQUEFASCIATUS* FROM KOCHI, INDIA

D. R. RAO, T. R. MANI, R. RAJENDRAN, A. S. JOSEPH,
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Post Box No. 11, Madurai 625 002 India*

ABSTRACT. Field resistance to *Bacillus sphaericus* was observed in a population of *Culex quinquefasciatus* in Kochi, India, exposed to 35 rounds of spraying with a formulation of *B. sphaericus* 1593M over a 2-year period. Larvae from the sprayed area gave LC_{50} and LC_{90} values that were 146 and 180 times greater than corresponding values for a susceptible strain from an unsprayed locality. When the resistant strain was colonized in the laboratory and subjected to moderate selection pressure at each generation, resistance rapidly increased and by the 18th generation it was 6,223 and 31,325 times greater at the LC_{50} and LC_{90} levels in comparison with the susceptible strain. There were no significant differences among 6 susceptible strains tested. Tests were repeated and validated using the standard primary powder SPH88, *B. sphaericus* 2362. No cross resistance was observed against *B. thuringiensis* H-14.

Resistance to *Lysinibacillus sphaericus* and Other Commonly Used Pesticides in *Culex pipiens* (Diptera: Culicidae) from Chico, California

Tianyun Su ✉, Jennifer Thieme, Chris Ocegueda, Matthew Ball, Min-Lee Cheng

Journal of Medical Entomology, Volume 55, Issue 2, March 2018, Pages 423–428,

Published: 19 December 2017 **Article history** ▾

since invasion of West Nile virus. This report documents the first occurrence of high-level resistance to *L. sphaericus* in a natural population of *Culex pipiens* L. in Chico, CA, where resistance ratio was 537.0 at LC_{50} and 9,048.5 at LC_{90} when compared with susceptible laboratory colony of the same species. Susceptibility profile to other groups of pesticides with different modes of action was also determined. Various levels of resistance or tolerance were noticed to abamectin, pyriproxyfen, permethrin, and indoxacarb. Resistance management and susceptibility monitoring strategies are discussed and recommended.

Susceptibility to Other Common Pesticides in *Culex pipiens* (Diptera: Culicidae) from Salt Lake City, UT

Tianyun Su ✉, Jennifer Thieme, Gregory S White, Taylor Lura, Nadja Mayerle, Ary Faraji, Min-Lee Cheng, Michelle Q Brown

Journal of Medical Entomology, Volume 56, Issue 2, March 2019, Pages 506–513,

Published: 01 November 2018 **Article history** ▾

Abstract

Bacillus sphaericus Neide (recently *Lysinibacillus sphaericus* Meyer and Neide), are

How to manage resistance of *Lysinibacillus sphaericus*?

Synergy between Toxins of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*

Margaret C. Wirth, Joshua A. Jiannino, Brian A. Federici, William E. Walton

Journal of Medical Entomology, Volume 41, Issue 5, 1 September 2004, Pages 935–941,
https://doi.org/10.1603/0022-2585-41.5.935
Published: 01 September 2004 **Article history ▾**

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Abstract

Synergistic interactions among the multiple endotoxins of *Bacillus thuringiensis* subsp. *israelensis* de Barjac play an important role in its high toxicity to mosquito larvae and the absence of insecticide resistance in populations treated with this bacterium. A lack of toxin complexity and synergism are the apparent causes of resistance to *Bacillus sphaericus* Neide in particular *Culex* field populations. To identify endotoxin combinations of the two *Bacillus* species that might improve insecticidal activity and manage mosquito resistance to *B. sphaericus*, we tested their toxins alone and in combination. Most combinations of *B. sphaericus* and *B. t.* subsp. *israelensis* toxins were synergistic and enhanced toxicity relative to *B. sphaericus*, particularly against *Culex quinquefasciatus* Say larvae resistant to *B. sphaericus* and *Aedes aegypti* (L.), a species poorly susceptible to *B. sphaericus*. Toxicity also improved against susceptible *Cx. quinquefasciatus*. For example, when the Cyt1Aa toxin from *B. t.* subsp. *israelensis* was added to Bin and Cry toxins, or when native *B. t.* subsp. *israelensis* was combined with *B. sphaericus*, synergism values as high as 883-fold were observed and combinations were 4–59,000-fold more active than *B. sphaericus*. These data, and previous studies using cytolytic toxins, validate proposed strategies for improving bacterial larvicides by combining *B. sphaericus* with *B. t.* subsp. *israelensis* or by engineering recombinant bacteria that express endotoxins from both strains. These combinations increase both endotoxin

Strategies for the Management of Resistance in Mosquitoes to the Microbial Control Agent *Bacillus sphaericus*

Nayer S. Zahiri, Tianyun Su, Mir S. Mulla

Journal of Medical Entomology, Volume 39, Issue 3, 1 May 2002, Pages 513–520,
https://doi.org/10.1603/0022-2585-39.3.513
Published: 01 May 2002 **Article history ▾**

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Abstract

Bacillus sphaericus (*Bsph*) strain 2362 has been recognized as a promising mosquito larvicide, and various preparations of this strain have been tested and used in mosquito control programs worldwide. This control agent has advantages of high efficacy, specificity, persistence, and environmental safety. However, resistance in *Culex pipiens* complex mosquitoes to *Bsph* has occurred in both laboratory and field populations, necessitating development of resistance management strategies. Studies were initiated aiming at reversing previously established *Bsph* resistance in a laboratory colony of *Culex quinquefasciatus* Say by selections with *Bti* alone, *Bti* and *Bsph* in rotation, or mixture. Partial restoration of susceptibility to *Bsph* was achieved by selection of resistant colony for 10 generations with *Bti* alone at LC_{80}. After this colony was switched back to *Bsph* selection for 20 generations, resistance to *Bsph* partially increased to a stable level. Selections of *Bsph*-resistant colonies with *Bti* and *Bsph* in rotation or mixture resulted in steady decline of resistance over 30 generations, with rapid decline in resistance noted in the initial 10–15 generations. It is interesting to note that selections with *Bti* and *Bsph* in rotation increased susceptibility to *Bti* in *Bsph*-resistant colony. It is promising that

(S)-Methoprene Resistance

> Pest Manag Sci. 2002 Aug;58(8):791-8. doi: 10.1002/ps.521.

High level methoprene resistance in the mosquito Ochlerotatus nigromaculis (Ludlow) in central California

Anthony J Cornel [1], Matthew A Stanich, Rory D McAbee, F Steve Mulligan 3rd

Affiliations + expand

PMID: 12192903 DOI: 10.1002/ps.521

Abstract

In the summer of 1998, failures of methoprene field applications to control the mosquito Ochlerotatus nigromaculis (Ludlow) were noticed in several pastures in the outskirts of Fresno, California, USA. Effective control with methoprene had been achieved for over 20 years prior to this discovery. Susceptibility tests indicated that the Fresno Oc nigromaculis populations had developed several thousand-fold higher LC50 and LC90 tolerance levels to methoprene compared with methoprene-naïve populations. The synergists piperonyl butoxide (PBO), S,S,S-tributyl phosphorotrithioate and 3-octylthio-1,1,1-trifluoro-2-propanone had little synergistic effect, suggesting that the mechanism of methoprene tolerance was not mediated by P450 monooxygenase or carboxylesterase enzyme degradation. As part of initiating a resistance management strategy, partial reversion back to methoprene susceptibility was achieved in a resistant population after six consecutive applications of Bacillus thuringiensis israelensis Goldberg & Marga coupled with two oil and two pyrethrum + PBO applications.

Spinosad Resistance

Cross Resistances in Spinosad-Resistant *Culex quinquefasciatus* (Diptera: Culicidae)

Tianyun Su ✉, Min-Lee Cheng

Journal of Medical Entomology, Volume 51, Issue 2, 1 March 2014, Pages 428–435,
https://doi.org/10.1603/ME13207
Published: 01 March 2014 **Article history** ▾

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Abstract

A *Culex quinquefasciatus* Say colony was selected for 45 generations at LC_{70-90} levels using Natular XRG, a granular formulation of 2.5% spinosad for induction of spinosad resistance. Resistance to spinosad was noticed in early generations (F_1–F_9). Resistance levels increased gradually from generations F_{11}–F_{35}, and elevated significantly from generation F_{37} through F_{47}, when resistance ratios reached 2,845–2,907-fold at LC_{50} and 11,948–22,928-fold at LC_{90}. The spinosad-resistant *Cx. quinquefasciatus* colony was found not to be cross-resistant to *Bacillus thuringiensis israelensis* (Bti), a combination of Bti and *Bacillus sphaericus*, methoprene, pyriproxyfen, diflubenzuron, novaluron, temephos, or imidacloprid. However, it showed various levels of cross-resistance to *B. sphaericus*, spinetoram, abamectin, and fipronil. Conversely, a

What can we do about larvicide resistance?

- Rotate your chemistries!!
  - Utilize what we have in the toolbox
  - Seasonality and exposure can affect the timing of your rotations
  - When resistance is present, switch to *Bti*
- Use other methods of IPM when applicable including source reduction, physical control, and adulticide control when you have flying, biting adults.

Adulticide Resistance Management

1. IR definition, influencers, and development
2. Types of resistance mechanisms
3. Detoxification of insecticides
4. Importance of resistance monitoring
5. Insecticide modes of action
6. Resistance monitoring techniques (mostly CDC BBA)
7. Review of insecticide resistance monitoring for operations

What is insecticide resistance?

- “A **<u>heritable</u>** change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species.”

- – Insecticide Resistance Action Committee (IRAC)

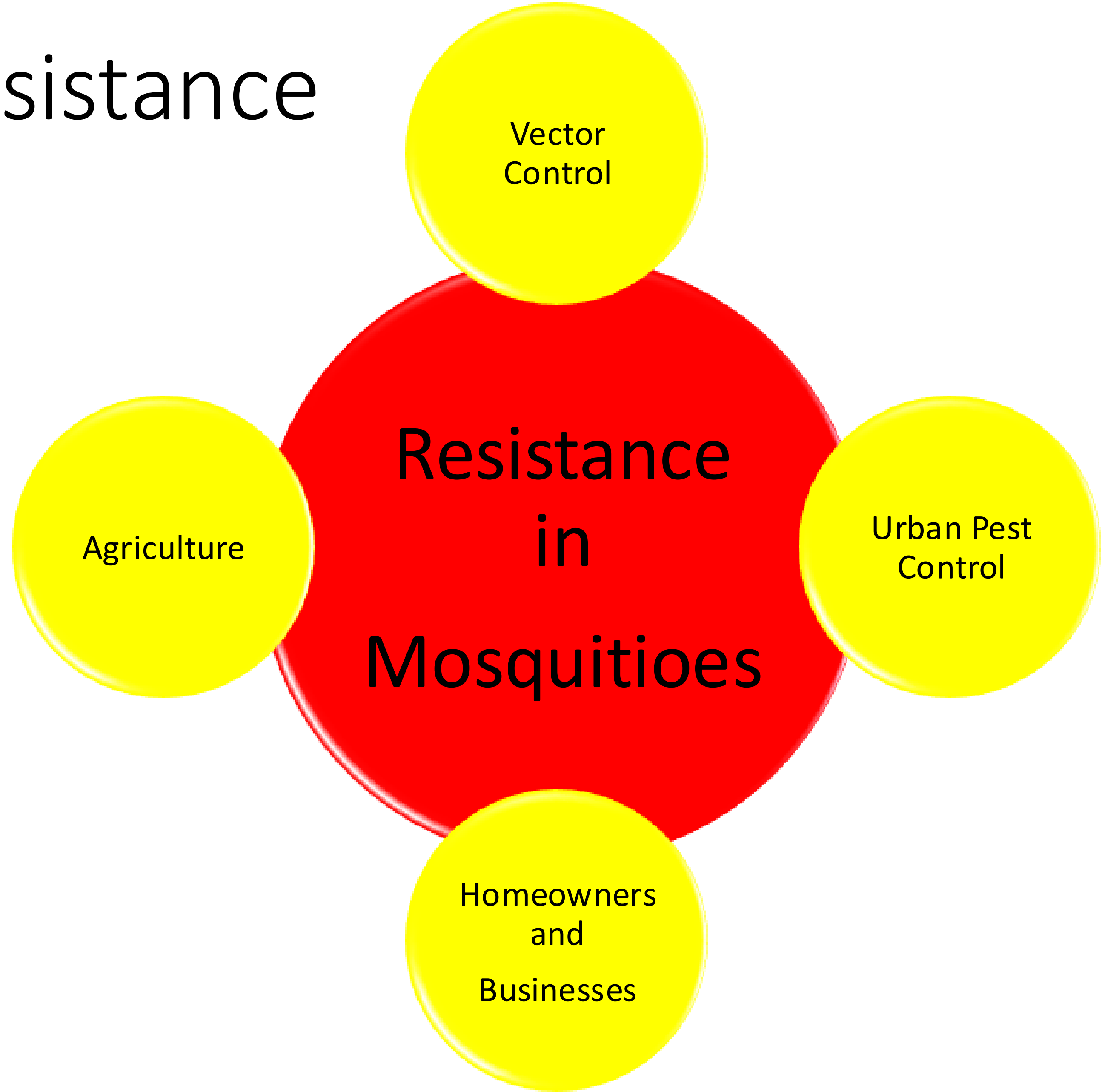
Traditional Definition

"**Insecticide resistance**" describes the ability of strains of insects to survive "**normally**" lethal doses of insecticide, the ability having resulted from selection of tolerant individuals in populations exposed to the toxicant for several generations.

The time to act is before resistance reaches this level.

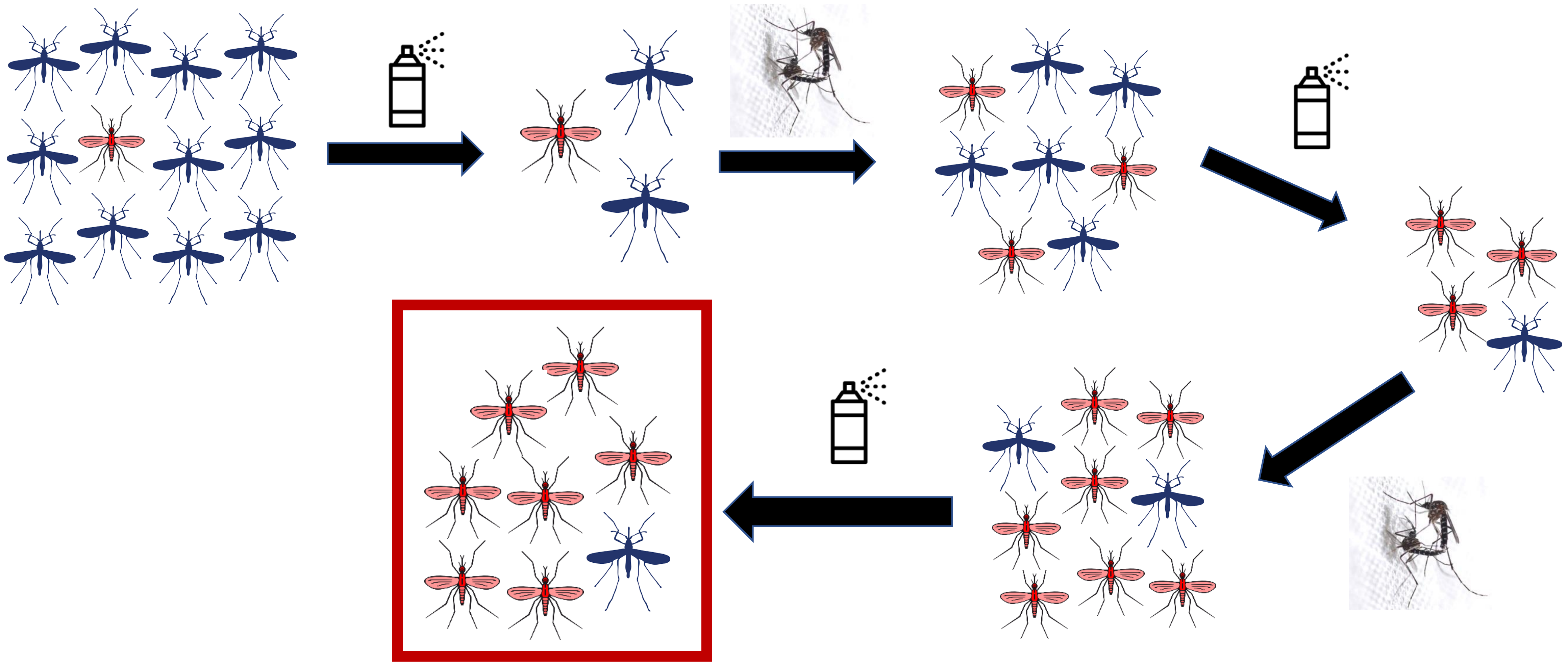
Influencers of Resistance

- Insecticides
- Herbicides
- Fungicides
- Fertilizers
- Non-point source runoff

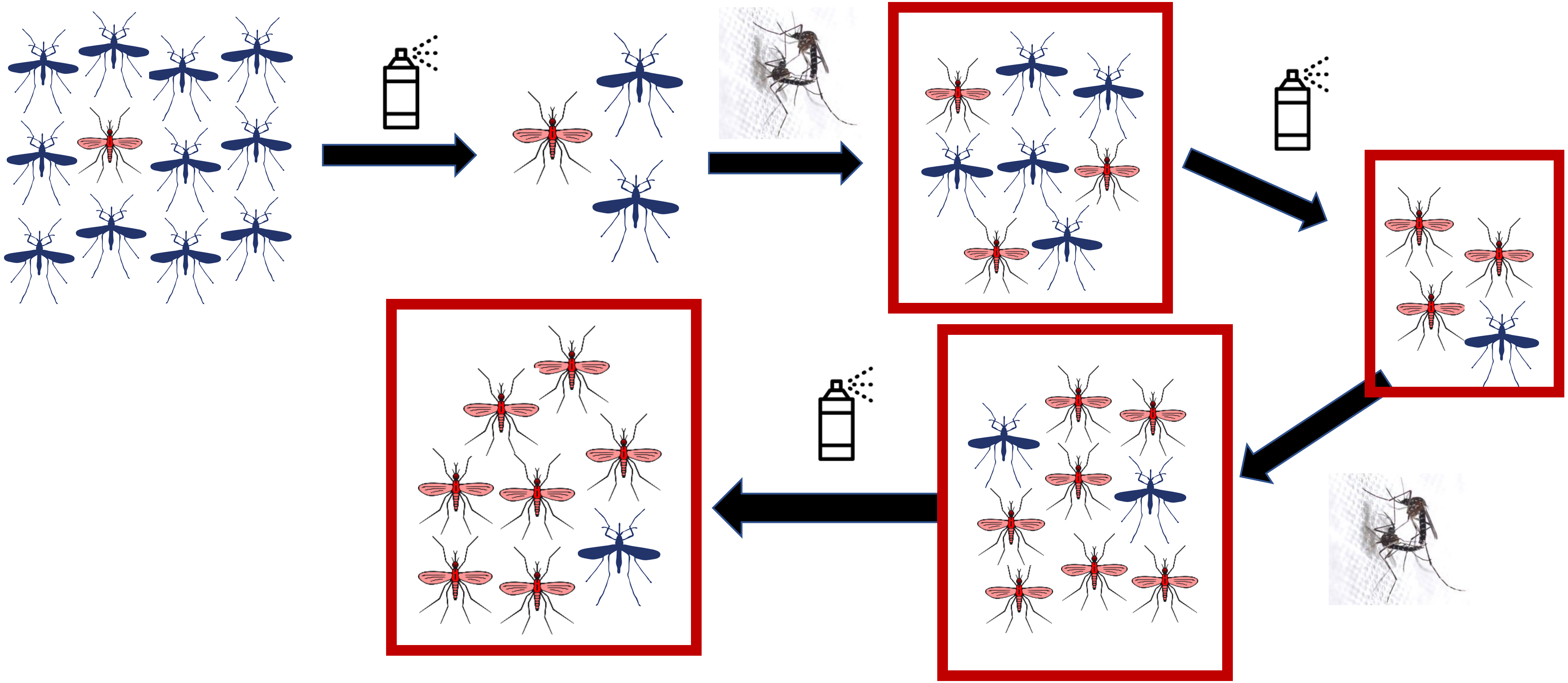


Slides from Janet McAllister, CDC

Resistance Development

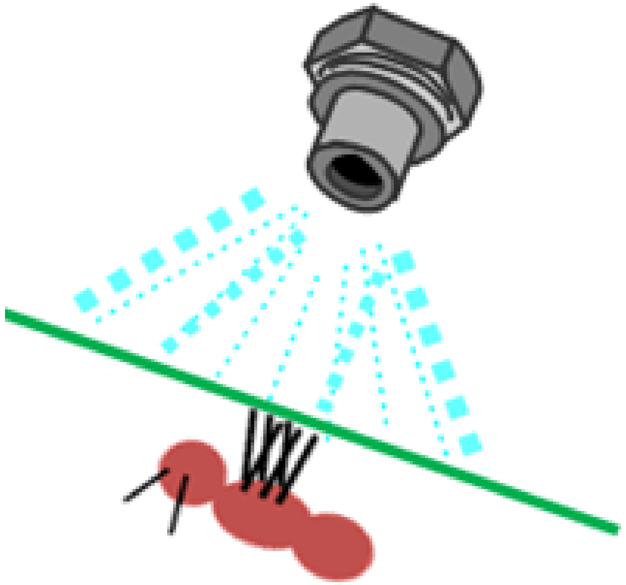


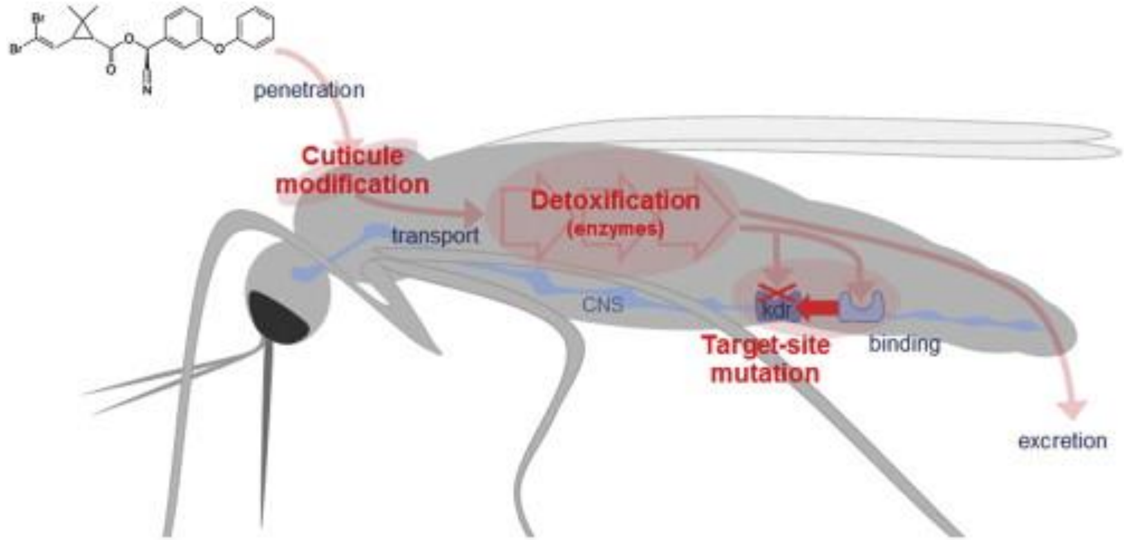
Which one of these is classified as resistant?



Types of Resistance

- Behavioral resistance – avoid toxin
- Penetration resistance – cuticular barriers to toxin penetration
- Target-site resistance – modified target-site
- Metabolic resistance – detoxification



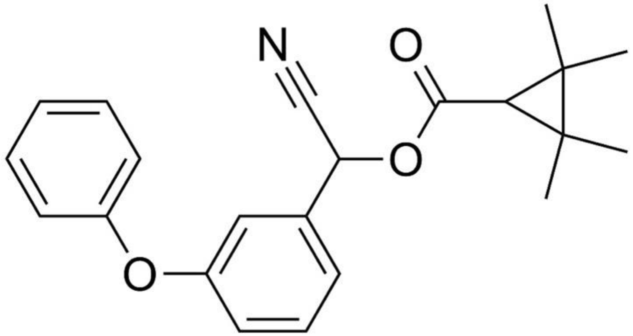


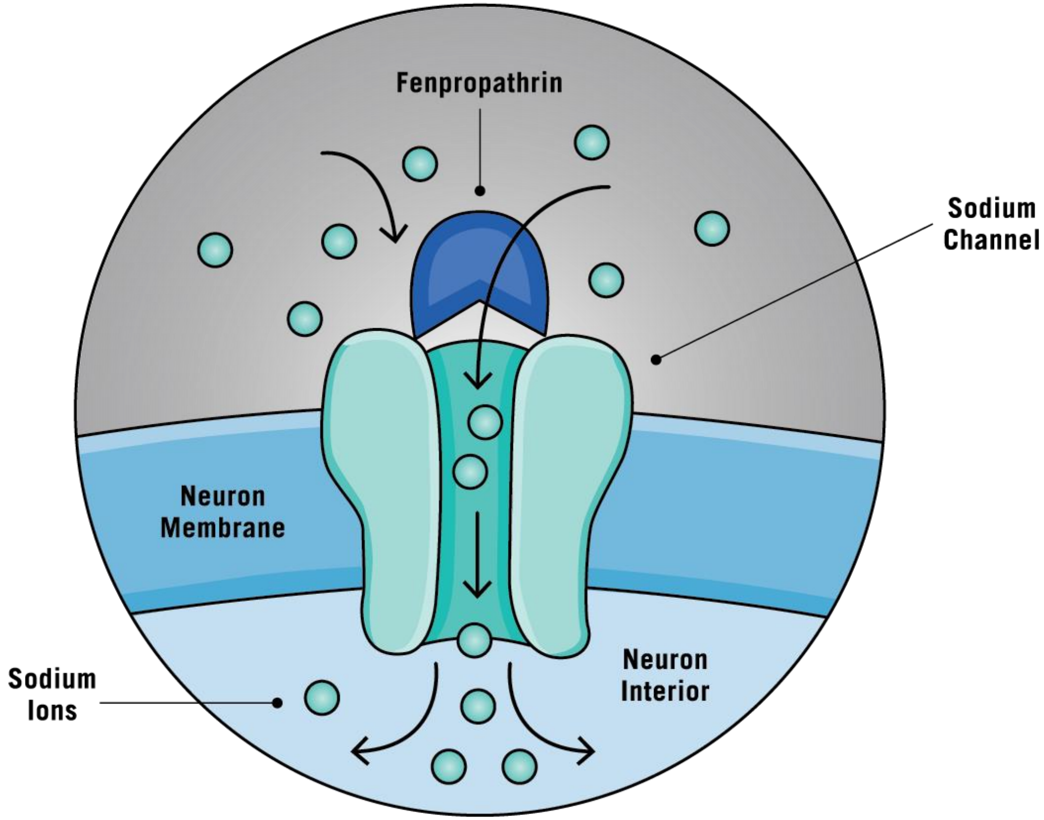
Resistance-The Problem

- Only 3 chemical classes available to public health vector control
  - Pyrethroids (Group 3A)
  - Organophosphates (Group 1B)
  - Avermectins/milbemycins (Group 6)
- Rotation ability is limited
- Can result in resistance development
- Another consideration: public opinion

Pyrethroids - Mode of Action

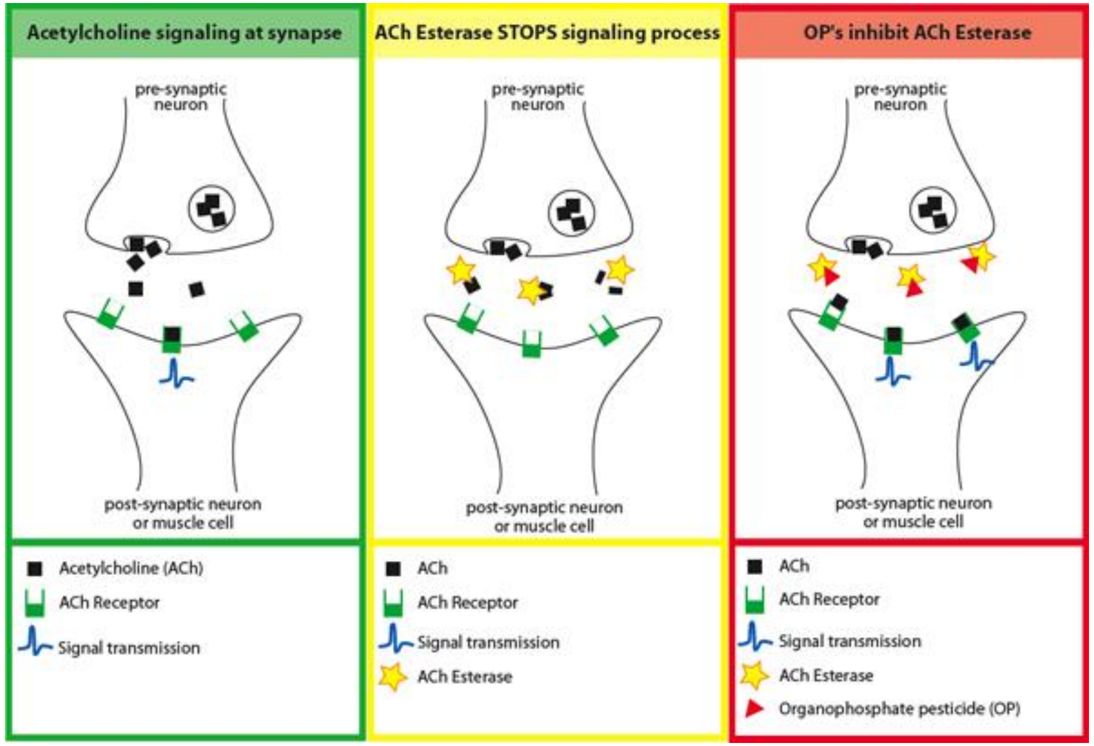
- Voltage-gated sodium channel modulator
- Impacts the nervous system



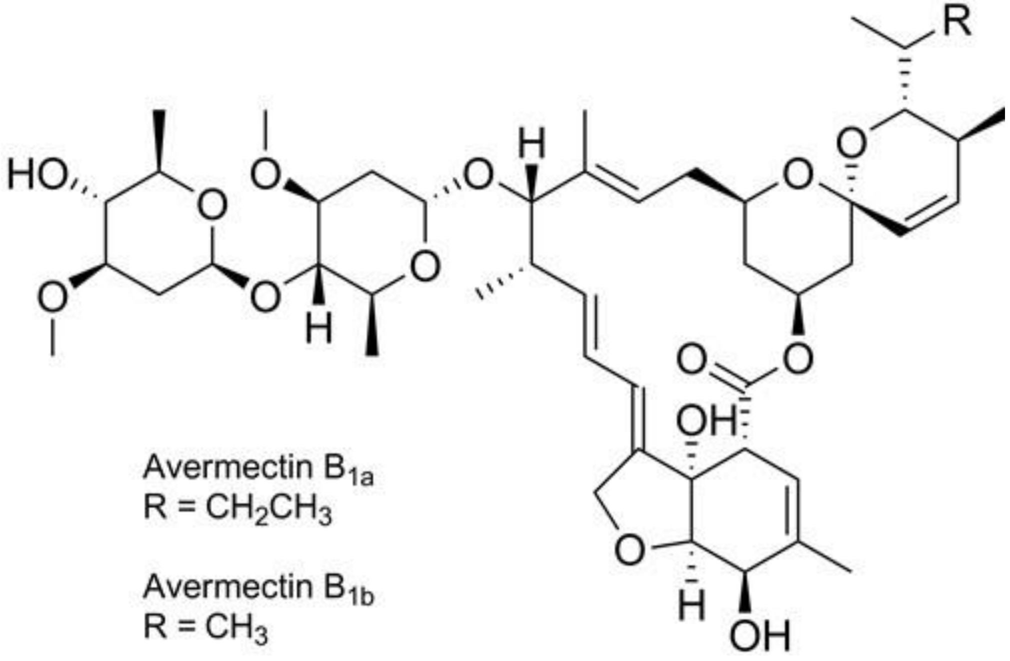


Organophosphate - Mode of Action

- Acetylcholinesterase inhibitor
- Impacts the nervous system



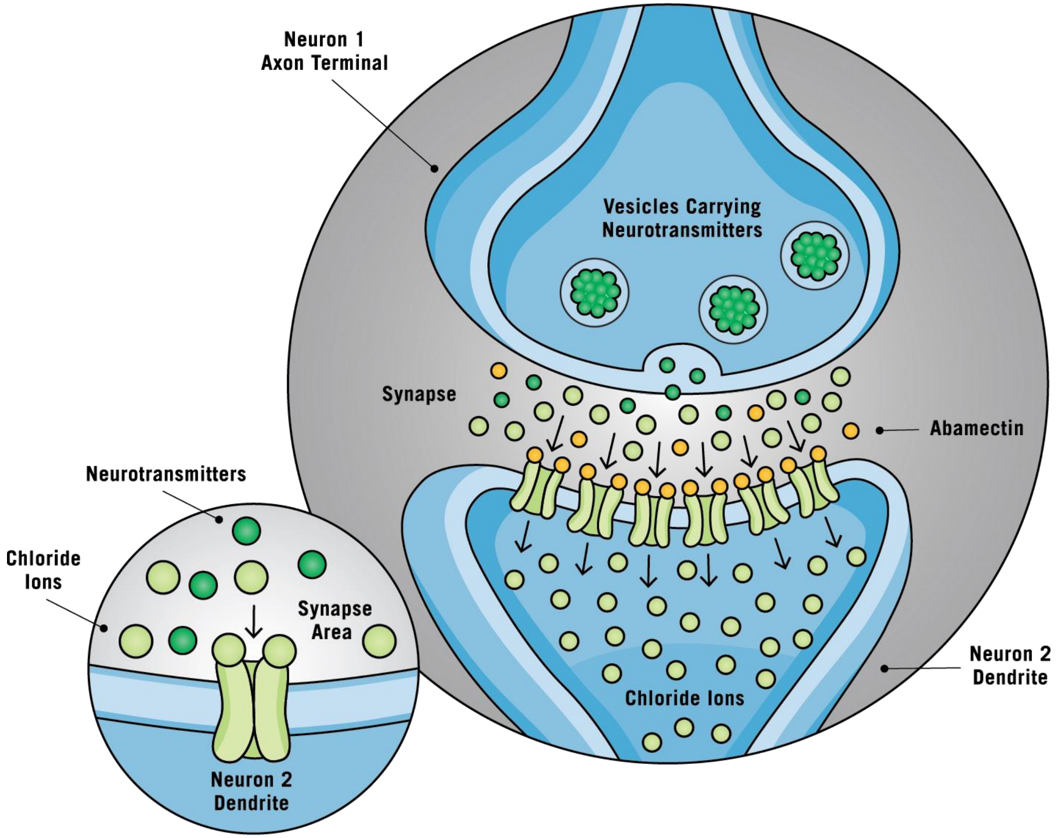
Avermectins - Mode of Action



Avermectin B_{1a}
$R = CH_2CH_3$

Avermectin B_{1b}
$R = CH_3$

Glutamate-gated channels



Active Ingredients

- **Synthetic Pyrethroids**
  - Permethrin
  - Etofenprox
  - Deltamethrin
  - Resmethrin
  - Prallethrin
  - Fenpropathrin

- **Organophosphates**
  - Malathion
  - Naled
  - Chlorpyrifos

- **Avermectins**
  - Abamectin

Additional Definitions

Cross-resistance results from a common detoxification system or from target-site insensitivity.

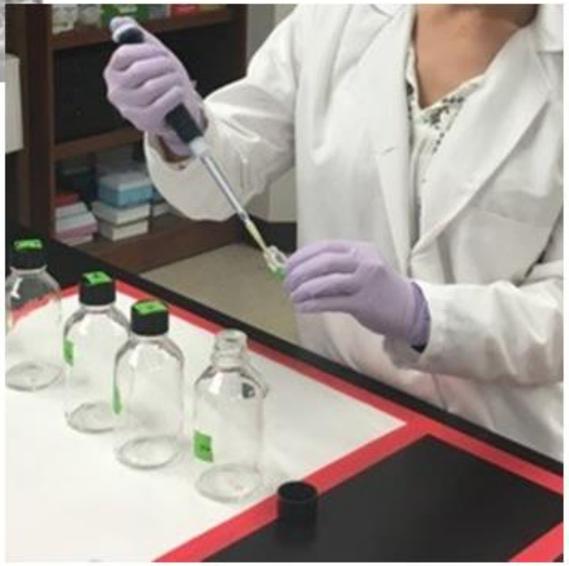
Multiple-resistance extends to a variety of classes of insecticides with differing modes of action and different detoxification pathways.

Negative cross-resistance occurs when resistance to one class of insecticide enhances the toxicity of another class of insecticide.

Insecticide Resistance Testing Methods

VALENT BIOSCIENCES
Protecting Human Potential.

- CDC bottle bioassay
- Topical assay
- WHO adult bioassay kit
- WHO larval bioassay
- Bucket bioassay
- *Kdr* testing
- Enzyme testing
- Wind tunnel
- Field trials
    - Caged
    - Operational

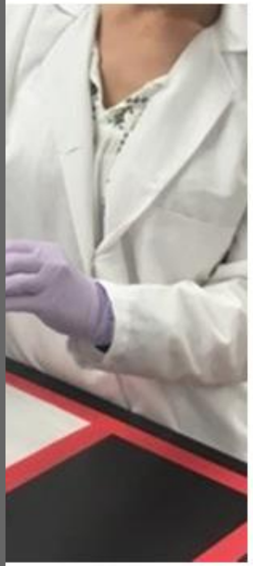


Insecticide Resistance Testing Methods

VALENT BIOSCIENCES
Protecting Human Potential.

- CDC
- Topi
- WHO
- WHO
- Buck
- *Kdr*
- Enzy
- Win
- Fiel
  - Cag
  - Ope

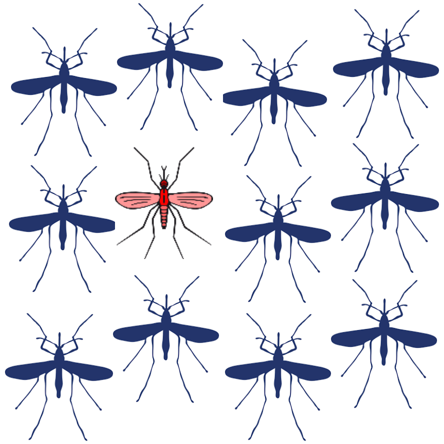
- There are multiple assays used to detect resistance.
- Assays do not correlate with operational control parameters. i.e. doses in assays ≠ label rates.
- Only caged field tests on adulticides mimics operational control but are difficult to interpret unless done with susceptible mosquitoes to detect resistance.



CDC Bottle Bioassay

1. Treat bottles
2. Add mosquitoes to insecticide-treated bottles
3. Record mortality at designated time points

- Diagnostic time – time at which there is 100% mortality of a susceptible population at a specific active ingredient concentration (Abbott's formula can be used)



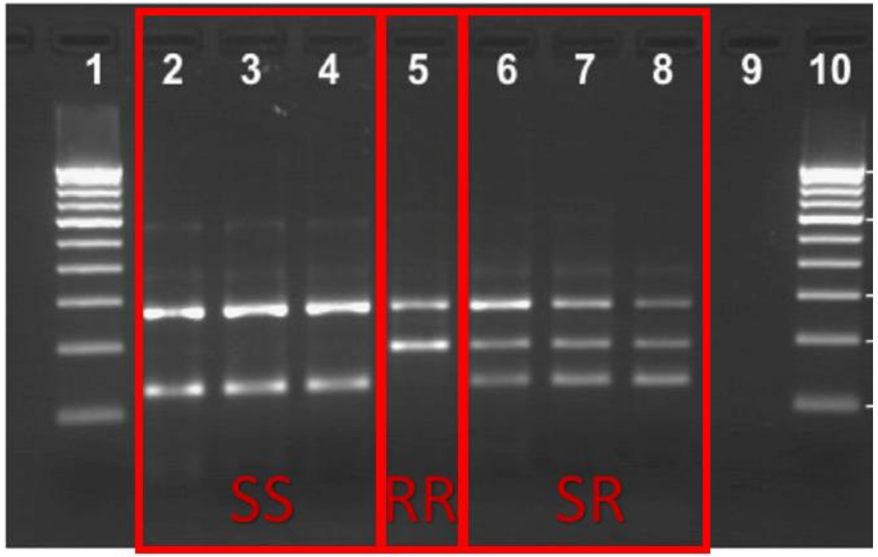
- CDC Susceptibility Definitions
  - Susceptible: > 97% Mortality
  - Developing Resistance: 90-96% Mortality
  - Resistant: < 90% Mortality

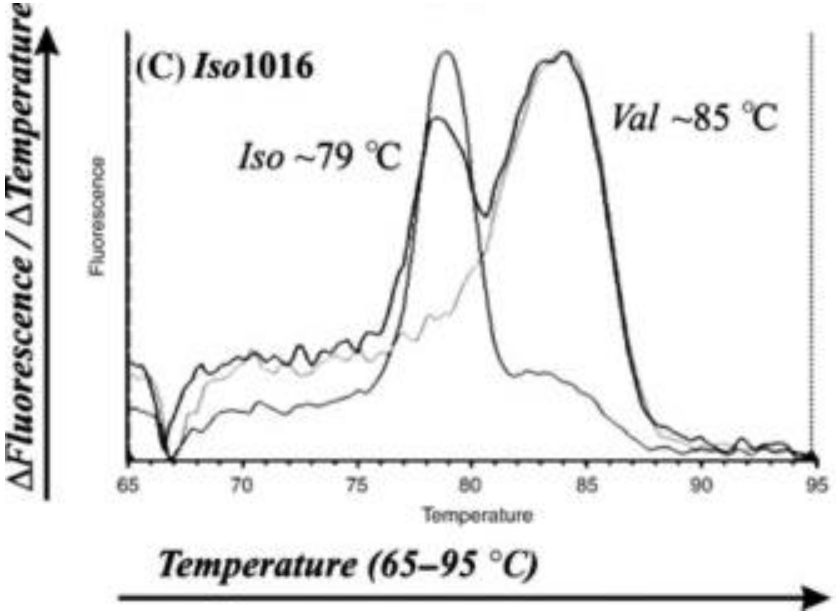
Topical Assay

- Microsyringe used to apply small drop of insecticide directly to thorax of mosquito
- Variety of doses used with ~10 mosquitoes/ dose
- LC50 and LC90 can be calculated
- Requires minimal supplies

Molecular Methods

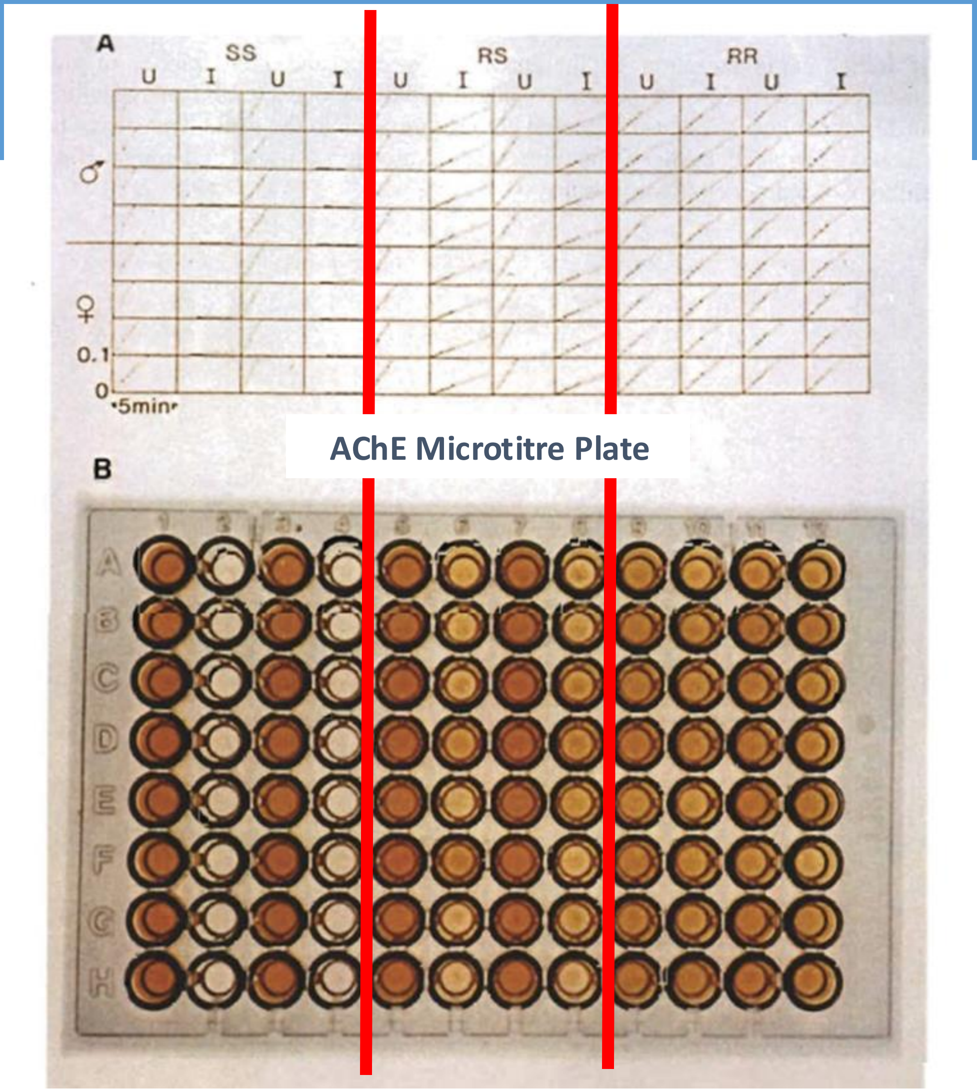
- Mutations to target site can be detected using multiple methods
  - GABA receptor
  - <u>Sodium channels (kdr)</u>
  - AChE





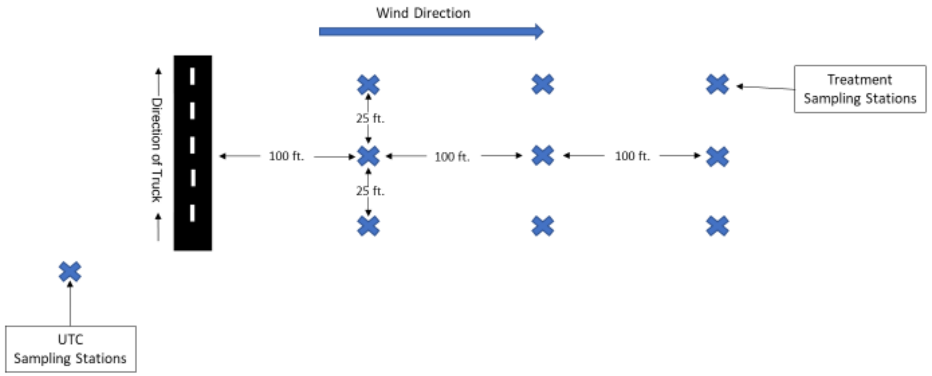
Enzyme Testing

- Can detect increased activity in:
  - Esterases
  - Monooxygenases
  - Glutathione-S transferases
- Can measure AChE insensitivity



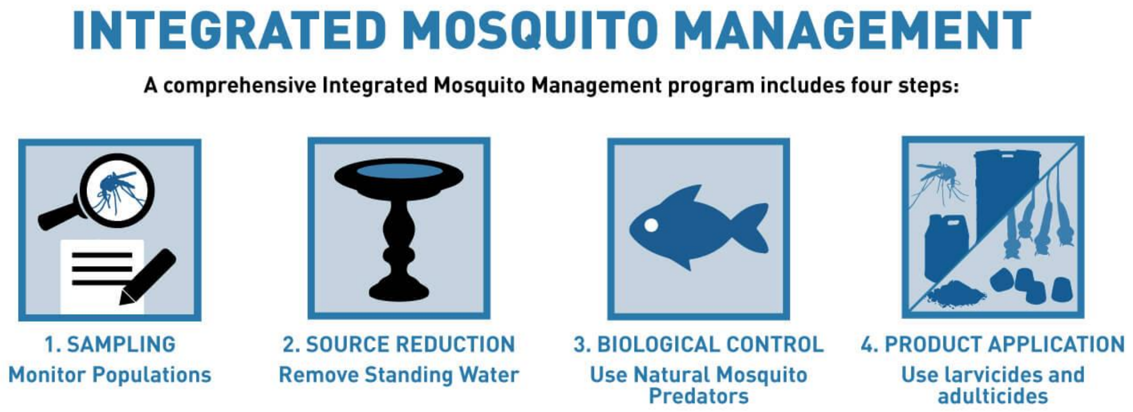
Caged Mosquito Field Trials

- Controlled cage trials with formulated product
- Efficacy easily related to label rates
- Aerial or ground applications
- Multiple species/ populations can be tested
- Collect droplets
- Monitor for 24 hours for recovery



What do we do with this information?

- Rotate chemicals?
- More emphasis on other control methods?
- Change decision points to reduce use?
- Areas with resistance:
  - Chemical Rotation
  - IPM
    - Utilize larvicides and adulticides



Resistance Monitoring

- Early detection of resistance can protect existing chemistries
- Allows public health professionals to be <u>proactive</u> instead of <u>reactive</u>
- Knowledge of existing resistance and mechanisms helps us choose the best strategy to combat further development
- Clearest picture of resistance comes when multiple monitoring tools are used

Thank you!

Any questions?

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