Larvicide active ingredients and their role in Integrated Mosquito Management

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Early Mosquito Control

- The Integrated Pest Management approach has been a part of mosquito control long before it was a recognized practice
  - 1800's dry rice culture in Georgia
  - The earliest ditching in the NJ salt marshes
  - Open Marsh Water Management practices
- Before pesticides, it was what you did
- Today we are returning to these roots…for many reasons: efficiency, effectiveness, sustainability, public perception

Integrated Mosquito Management

- Education – the public and our workers
- Source Reduction – permanently eliminates sites, improved drainage, habitat manipulation, cleanups
- Surveillance – Knowledge is power! What we have and where do they occur….helps focus our efforts
- Larviciding – targeting habitats that can't be eliminated ….requires effort and resources
- Adulticiding – highly effective, communication becomes essential, need to beware of pollinator concerns and insecticide resistance

University of Georgia, Cooperative Extension Circular 1154

Best Management Practices of Integrated Mosquito Management

Larviciding has many advantages….

- Larvae are concentrated in a confined/defined area, particularly compared to emerging adults
- Larvae are essentially immobile….they're not getting away for a few days
- And they're accessible….(William Horsfall)
- They're susceptible…..
- It's proactive….
- It's more acceptable to the public and it reduces risks to pollinators

Choosing a larvicide

- Wide variety of formulations
- Four types of active ingredients with very different modes of action
- Ideally, the formulation and active ingredient is chosen based on the type of habitat and the mosquito population present
- The more experience and knowledge the operator/program has, and the better our surveillance, the more targeted and efficient the application

Larvicide - Active Ingredients

- Insect Growth Regulators – absorbed through the integument and ingested
  - (S)-methoprene – Insecticide Resistance Action Committee - IRAC 7A
  - Pyriproxyfen – IRAC 7C
- Microbial based products – must be ingested
  - *Bacillus thuringiensis* subsp. *israelensis (Bti)* – IRAC 11
  - *Bacillus sphaericus* (*Lysinibacillus*) – IRAC 11
- Surface oils – physical barrier
  - Highly refined mineral oil today – No IRAC classification
- Spinosad – contact and ingested
  - Biological neurotoxin – IRAC 5

Insect Growth Regulator – (S)-methoprene

- Natural juvenile hormone (J.H. I) that was first identified in 1967, is involved in the regulation of physiological processes….particularly metamorphosis…research quickly followed
- Inhibits ecdysone from initiating the molting processes, particularly disruptive to adult emergence, malformed adults may be observed
- Pupae do not feed, so they eventually deplete body stores of essential nutrients and starve, primarily affects pupal to adult transition
- Effective against all mosquito species

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

Juvenile Hormone Analog

(S) – methoprene Based Products

- Absorbed through the cuticle and ingested
- Concentration must be higher in the larval environment than circulating in the larval body for disruption to occur
- Wide range of applications
- Larvae and pupae DON'T die immediately
- Leaves larvae and pupae in food web
- Efficacy evaluations can be challenging
- Long-time standard with many improvements

Insect Growth Regulator - Pyriproxyfen

- Classified as a Reduced Risk insecticide by EPA
- Recognized by the WHO for the treatment of potable water
- Late 4th instar and pupae just after pupation are most susceptible, eggs can be affected as well
- Newer product line that we're just becoming familiar with….registered in Georgia
- Active at very low concentrations – 1 part/billion
- Good option for storm drains, works well in concrete environments
- Effective against all our common pest species

Microbial Based Products

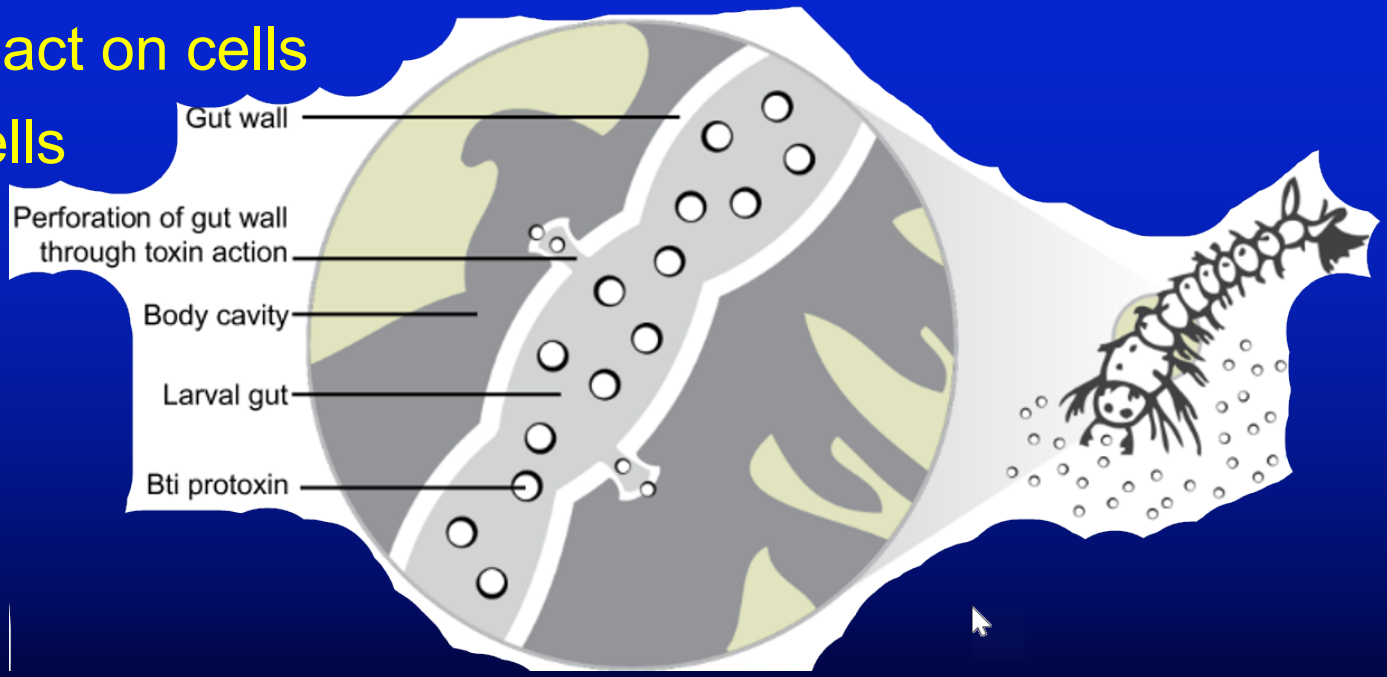
- *Bacillus thuringiensis* subsp. *israelensis* (Bti)
- *Bacillus sphaericus* (*Lysinibacillus*)
- Both of these materials are very common today, with Bti having a more prominent role targeting floodwater mosquitoes
- Bs was more commonly recognized as a material used to target the Culex mosquitoes, particularly for the reduction of West Nile virus transmission, but other uses expanding
- Combination formulations available

Bacillus thuringiensis subsp. *israelensis*

- A bacterium that occurs naturally in soils and aquatic environments worldwide…
- Bti was discovered in a drying pool in a river bed in the Negev Desert of Israel in 1976
- *Bacillus thuringiensis* had long been recognized for having insecticidal properties, the discovery of Bti brought it to the public health arena, there are many serotypes and strains of the Bti bacteria
- Active ingredient in Bti-based formulations are delta endotoxins that are produced at the time of sporulation
- Endotoxins composed of four proteins that are activated by the proteolytic gut enzymes in the alkaline gut environment of Nematoceran Diptera larvae

Bacillus thuringiensis subsp. *israelensis* Mode-of-action

- Ingest
- Protoxin activated
- Enzymes break down protoxins
- Polypeptide fractions act on cells
- Form pores/hole in cells
- Osmotic imbalance
- Cells swell, lyse
- Larvae die



Bti cont.

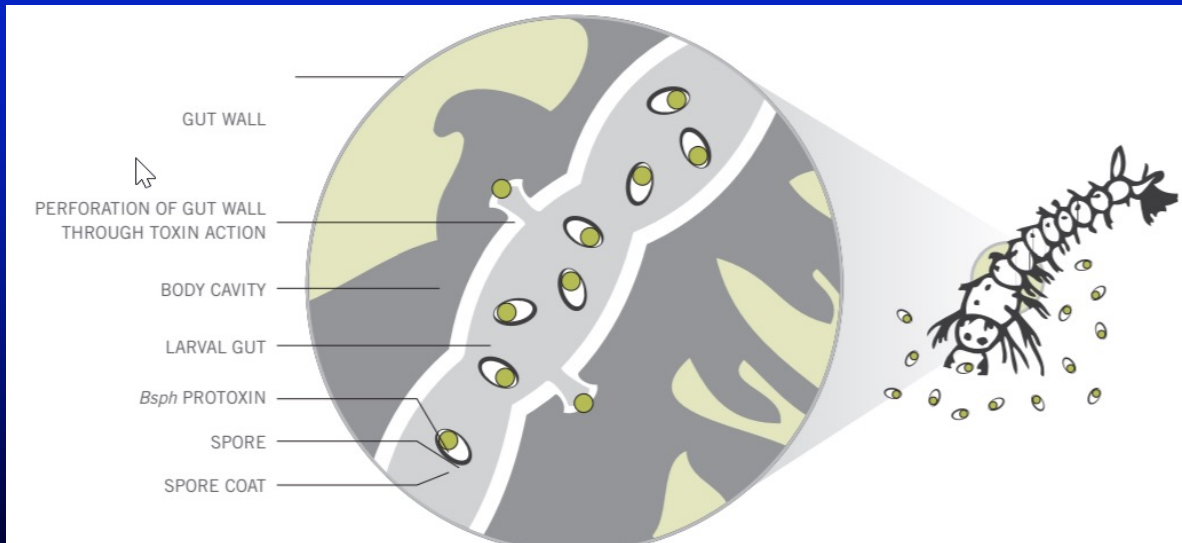
- Must be ingested to be effective, larval feeding absolutely critical, larvae very susceptible
- Kills larvae quickly that are exposed to high concentrations, slower for those larvae exposed to lower concentrations
- Four proteins (27,65,128,135 kDa) associated with larvicidal activity, consequently, there is a reduced risk of resistance
- Organic Material Review Institute (OMRI) approved, no mammalian toxicity, non-target impacts minimal

Bacillus sphaericus (also known as *Lysinibacillus sphaericus*)

- Another bacterium that occurs naturally in soils and aquatic environments worldwide…first isolated in 1964 from dead *Culiseta incidens* larvae in California rock pools
- Several serotypes with larvicidal activity, but has a narrower spectrum of activity, only two proteins involved
- Similar, mode of action to Bti, but the toxins are attached to a living bacterial spore, aids the residual aspects
- Consequently, there can be some "recycling" of toxicity associated with *sphaericus,* the greater the larval population at initial treatment the greater the residual effect
- Up to 28 days of residual control depending on conditions
- Approved by the National Organic Program (NOP)

Bacillus sphaericus Mode-of-Action

- Ingest spore and associated protoxin
- Feeding ceases
- Spore coat dissolves releasing protoxins
- Protoxins (proteins) cleaved by enzymes, activated and bind to gut wall creating pores
- Cells swell & lyse
- Larvae die, some recycling will occur



Surface Oils

- Petroleum oils were the first chemicals used in efforts to control mosquito larvae….
- Today most are highly refined mineral oil
- ONLY pupacide, extremely important aspect
- Effectiveness limited to mosquito larvae and pupae that breathe air at the water's surface
- Lower rates will minimize non-target impacts
- Higher rates required for polluted water and areas with significant vegetation

Surface Oils cont.

- Probably least studied larvicide….
- Mode of action related to the effect of the product to the surface of the water
- Suffocation of both larvae and pupae
- Mode of action completely different from other larvicides
- Resistance difficult to envision
- Coverage is important, mortality rapid

Spinosad – Biological Neurotoxin

- One of our newest active ingredients
- In 1982 a vacationing scientist took a soil sample from a drum that was used to make rum in the Caribbean….
- From that sample, a new species of bacteria was identified in 1986, *Sacchrapolyspora spinosa*, translates to “spiney sugar”
- During fermentation of the bacteria, the two most prominent and active compounds in the fermentation broth are Spinosyn A & Spinosyn D, hence “Spinosad”

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.

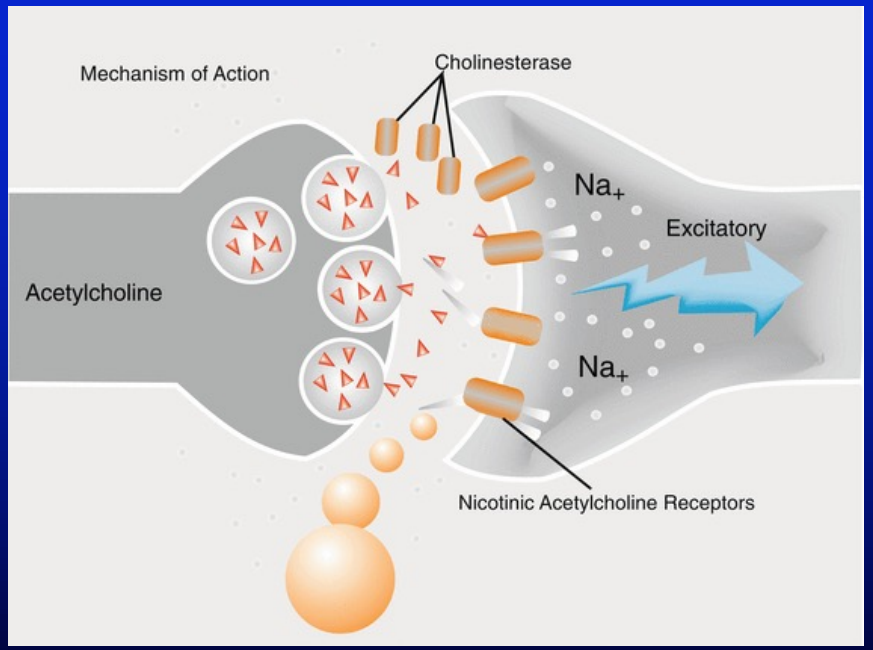


Figure: Shivanandappa T., Rajashekar Y. (2014) Mode of Action of Plant-Derived Natural Insecticides. In: Singh D. (eds) Advances in Plant Biopesticides. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2006-0_16

Spinosad cont.

- Neurotoxin specific to invertebrates, binds to nicotinic acetylcholine receptors causing excitation of nerves, leading to involuntary muscle contractions….. Reduced Risk by EPA
- Breaks down rapidly in sunlight and in soils, microbes break it down readily
- Organic Materials Review Institute (OMRI) approved for use in organic production
- Effective against all larval instars, mortality most rapid when ingested, but some contact toxicity
- Is a unique larvicidal A.I. whose use is growing, excellent part of a rotation

Larvicide Options

- Many different formulations available
- Combinations of active ingredients provide more application flexibility – wider treatment window
- Matching the optimum formulation and active ingredient(s) will maximize the efficiency and effectiveness of a larvicide application
- Discuss the strengths and weaknesses of formulations with our commercial representatives and other control specialists – use your resources!
- **Product rotation remains an important aspect**

Integrated Mosquito Management

- **Has never been more important!**
- Use all aspects: education, source reduction, surveillance, larviciding and adulticiding
- Larviciding can be highly effective in preventing all types of mosquito populations
- Larval surveillance and knowledge of the physical parameters of the larval habitat is critical to choosing the proper product and optimizing the timing of the application
- Be cognizant of non-target impacts