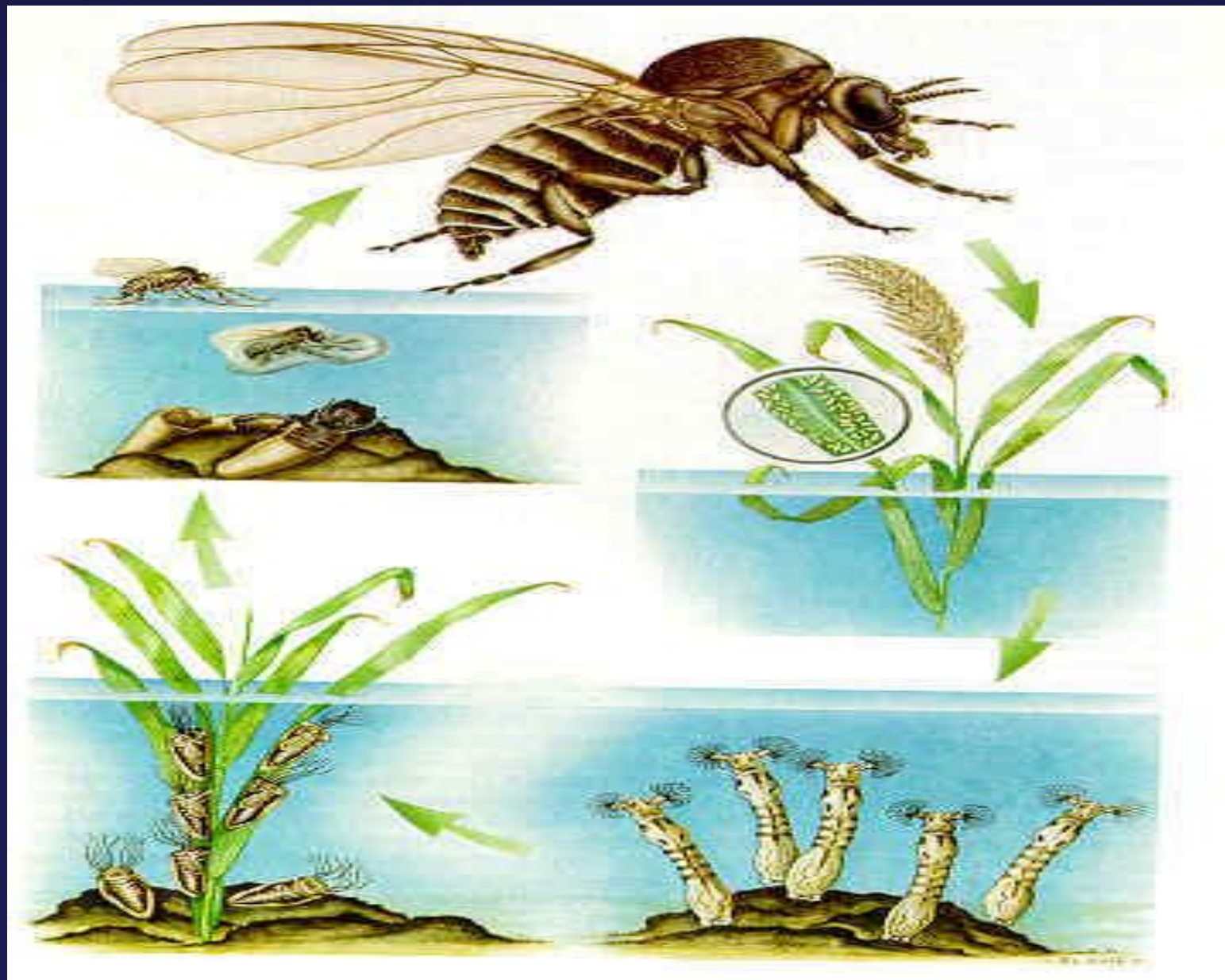


Influence of Antibiotics on Black Fly Susceptibility to *Bti*

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Black Flies

- Develop in flowing waters
- Transmit diseases
- Nuisances
- Populations suppressed through larvicide application



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Photo by M. Clapp

Bti Susceptibility

- Nematocera, same as mosquitoes
- Both susceptible to *Bti* ICPs
- Rapid mortality (30min-3hrs)
- Black fly control vs. Mosquito control
- Parameters - flow rate, pooling, turbidity



Control problems?

- Occasional less than optimum control
- West Africa, South Africa, Pennsylvania
- Attributed to turbidity, possibly algal counts
- Could there be something else?



(photo © 2006 Arlen Thomason.)

Broderick *et al.*

- Reared lepidopteran larvae with antibiotics in diet
- Reduced enteric bacteria = ↓ *Btk* efficacy
- Reintroduce enteric bacteria = ↑ *Btk* efficacy
- Efficacy related to septicemia
- Delayed mortality (12 hrs+)

Broderick, N.A., Raffa, K.F., Handelsman, J., 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proc. Natl. Acad. Sci. USA 103, 15196–15199.

Antibiotics in Black Fly Habitats

- Found in most surface waters (ppb)
- Increase in sensitivity of analytical equipment
- Increased use throughout society
- USGS - Antibiotics in Susquehanna River



Photo from: binalshah.wordpress.com

Antibiotics and *Bti*

- Evaluation of four common antibiotics
- Enrofloxacin - broad spectrum, agriculture
- Tylosin – Macrolide, infections, agriculture
- Sulfamethoxazole - human and veterinary
- Trimethoprim - synergist of sulfamethoxazole

CCTT

- Technique
 - 195 mL larval medium (antibiotic and control)
 - 30 larvae (*S. vittatum* IS-7)
 - 1 hr acclimation
 - 43 or 67 hours antibiotic exposure
 - 5 mL dose of *Bti*
 - 10 min exposure
 - 5 hr hold
 - Mortality assessment



The response of larvae to *Bti* ICPs after exposure to an antibiotic mixture for 48 and 72 hrs (Experiment 1).

| Treatment | Mortality (%) at 48 hrs. | Mortality (%) at 72 hrs. |
|---|-----------------------------|-----------------------------|
| Control | 2.4 ± 0.9 ^a | 5.1 ± 1.3 ^a |
| Antibiotic mixture only ^c | 2.4 ± 0.7 ^a | 6.5 ± 1.5 ^a |
| <i>Bti</i> ICP only ^d | 89.5 ± 2.6 ^b | 93.5 ± 2.0 ^b |
| Antibiotic mixture ^c + <i>Bti</i> ICP ^d | 93.8 ± 3.5 ^b | 93.9 ± 1.4 ^b |

^{a,b} Values (Mean±SEM) with different superscripts differ significantly within a column (P≤0.05).

^c The antibiotic concentrations in the larval medium were: tylosin, 0.05 µg/L, SMZ:TMP, 1.34 µg/L:0.268 µg/L, enrofloxacin, 0.05 µg/L.

^d The concentration of *Bti* ICPs was 1.08 ITU/200 mL.

The response of larvae to *Bti* ICPs after exposure to enrofloxacin for 48 and 72 hrs (Experiment 2).

| Treatment | Mortality (%) at 48 hrs. | Mortality (%) at 72 hrs. |
|---|-----------------------------|-----------------------------|
| Control | 2.0 ± 0.6 ^a | 2.0 ± 0.5 ^a |
| Enrofloxacin only ^c | 3.4 ± 0.7 ^a | 2.7 ± 1.2 ^a |
| <i>Bti</i> ICP ^d only | 80.4 ± 2.8 ^b | 88.4 ± 1.3 ^b |
| Enrofloxacin ^c + <i>Bti</i> ICP ^d | 83.0 ± 3.0 ^b | 92.9 ± 1.6 ^b |

^{a,b} Values (Mean±SEM) with different superscripts differ significantly within a column (P≤0.05).

^c The concentration of enrofloxacin in the larval medium was 0.5 mg/L.

^d The concentration of *Bti* ICPs was 1.08 ITU/200 mL.

The response of larvae to *Bti* ICPs after exposure to tylosin for 48 and 72 hrs (Experiment 3).

| Treatment | Mortality (%) at 48 hrs. | Mortality (%) at 72 hrs. |
|--|-----------------------------|-----------------------------|
| Control | 3.4 ± 0.9 ^a | 1.3 ± 0.7 ^a |
| Tylosin only ^d | 3.4 ± 1.1 ^a | 2.3 ± 1.2 ^a |
| <i>Bti</i> ICP only ^e | 76.4 ± 3.8 ^b | 78.2 ± 2.8 ^b |
| Tylosin ^d + <i>Bti</i> ICP ^e | 76.8 ± 3.3 ^b | 85.80 ± 1.2 ^c |

^{a,b,c} Values (Mean±SEM) with different superscripts differ significantly within a column (P≤0.05).

^d The concentration of tylosin in the larval medium was 8.0 mg/L.

^e The concentration of *Bti* ICPs was 1.08 ITU/200 mL.

The response of larvae to *Bti* ICPs after exposure to SMZ:TMP for 48 and 72 hrs (Experiment 4).

| Treatment | Mortality (%) at 48 hrs. | Mortality (%) at 72 hrs. |
|--|-----------------------------|-----------------------------|
| Control | 3.4 ± 0.8 ^a | 2.5 ± 1.2 ^a |
| SMZ:TMP only ^d | 5.0 ± 1.4 ^a | 4.0 ± 1.0 ^a |
| <i>Bti</i> ICP only ^e | 75.5 ± 2.6 ^b | 81.8 ± 5.7 ^b |
| SMZ:TMP ^d + <i>Bti</i> ICP ^e | 92.8 ± 2.3 ^c | 93.5 ± 2.4 ^c |

^{a,b,c} Values (Mean±SEM) with different superscripts differ significantly within a column (P≤0.05).

^d The concentration of SMZ:TMP in the larval medium was 25.0 mg/L:5.0 mg/L.

^e The concentration of *Bti* ICPs was 1.08 ITU/200 mL.

Summary

- Environmental concentrations of four commonly found antibiotics had no impact on *Bti* efficacy.
- Concentrations of antibiotics, 10,000-80,000 times higher than those found in contaminated water, had no negative impact on *Bti* efficacy.
- Black flies exposed to high tylosin concentrations prior to exposure to *Bti* exhibited an increase in mortality after 72 hours exposure to the antibiotic, but not after 48 hours.
- Black flies exposed to high SMZ:TMP concentrations, prior to exposure to *Bti*, exhibited an increase in mortality after 48 and 72 hours of exposure to the antibiotics.