Determining the susceptibility of *Culiseta melanura* to *Bacillus sphaericus* (Serotype H5a5b Strain 2362) in a laboratory bioassay

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Introduction

There is a close association between Eastern Equine Encephalitis virus and cedar swamps, in particular white cedar swamps along the East coast of the United States. The main reason for this association is the mosquito *Culiseta melanura*, principal enzootic vector of EEEv, utilizes these cedar swamps for their larval habitat. Larvae tend to favor crypts under mature cedar trees where the pH is 5.0 or lower. The adaptation to living in this environment provides protection from pathogens and predators. In many regions, cedar wetlands are refugia for species that are rare, endangered, or threatened locally or nationally (Laderman 1989). The need for biorational mosquito control products for use in these environmentally sensitive areas is important.

Figure 1. Distribution of Chamaecyparis thyoides. Records were compiled from field observations, herbarium records, published sources, and personal communications. Counties in which Atlantic white cedar has been found are inked in black (from Laderman 1982).

Purpose

A series of experiments were conducted to determine if *Cs. melanura* 4th instar larvae are susceptible to the bacteria *Bacillus sphaericus* and at what concentration.

Materials and methods

***Culiseta melanura* Susceptibility Test Protocol**

Test System:

Bioassay will be carried out in standard bioassay cups (120 ml plastic). Each cup will first be filled with 80 ml of field collected crypt water (pH 4.3). Twenty 3rd instar or early 4th instar *Cs. melanura* in de-ionized distilled water (no more than 5 ml) will be added to each cup. One drop of larval food (2 g of mortar and pestle ground TetraMin® fish food in 20 ml distilled water) will also be added to each cup.

Appropriate quantities of the serial dilutions of test material will be added to produce the desired concentrations. De-ionized, distilled water will then be added to produce a final volume of 100 ml per cup. All concentrations tested in the final bioassays, including the untreated controls, will be replicated three times.

Test Material:

VectoLex® WDG – 650 *B. sphaericus* ITU Water Dispersible Granule will be used in performance of the bioassays.

Test Concentrations:

Test concentrations should be selected following a non-replicated, range finding assay using the following concentrations of test material: 0.25 ppm, 0.2 ppm, 0.15 ppm, 0.1 ppm, 0.05 ppm, 0.025 ppm, and 0.00 ppm (UTC). Results of this assay will be used to select a range of five concentrations for the final assays with the objective of having the LC_{50} and LC_{90} fall within the selected range.

Materials and methods (cont.)

Range-finding Stock Suspensions and Dilutions:

Initial suspension (1%) will be prepared by adding 1 g of test material to 99 ml of de-ionized, distilled water and vigorously shaking the suspension in a flask for 1 minute. Gentle agitation using a magnetic stirrer will be maintained to keep this and subsequent dilutions in suspension. A second serial dilution will be made by adding 1 ml of the first suspension to 99 ml of de-ionized, distilled water under moderate agitation to produce a concentration of 100 ppm (mg/L). A final range-finding stock suspension with a concentration of 2.5 ppm will be produced by adding 1 ml of the second dilution to 39 ml of deionized, distilled water and maintaining under similar agitation.

Sample Weight
1 Gram

	GMS or ml	INTO	FINAL CONC.
FIRST DILUTION (1:99)	1	100 ml	10000 mg/L
SECOND DILUTION (1:99)	1	100 ml	100 mg/L
STOCK CONC. (1:39)	**1**	**40 ml**	**2.5 mg/L**

Preparation of Range-finding Dilutions:

A single, non-replicated set of cups containing the range-finding concentrations will be prepared as described above under "Test System". Concentrations to be used are shown below.

TOTAL VOLUME	100 ml
HIGHEST CONC.	0.25 mg/L
MAX. STOCK APPLIED	10 ml
STOCK CONC.	2.5 mg/L

CONC. mg/L	mg/CUP	STOCK APPLIED
0.250	0.0250	10.0 ml
0.200	0.0200	8.0 ml
0.150	0.0150	6.0 ml
0.100	0.0100	4.0 ml
0.050	0.0050	2.0 ml
0.025	0.0025	1.0 ml
0	0	0.0

Bioassay Conditions:

Treated cups with larvae will be held in low-light conditions at 15 to 20°C between treatment and taking mortality readings.

Mortality Readings:

Mortality of larvae will be read at 24, 48, 96, 144 and 192 hours after treatment. Moribund larvae that do not surface to breathe or elicit an evasive response when probed will be counted as dead. Both live and dead larvae will be counted and recorded, but only live larvae will be considered in calculations, to avoid errors due to potential larval cannibalism.

Selection of Final Concentrations:

Range-finding bioassay results will be plotted in order to estimate in which range the LC_{50} and LC_{90} will be found. It may be necessary to repeat the range-finding assay before selecting the final concentrations.

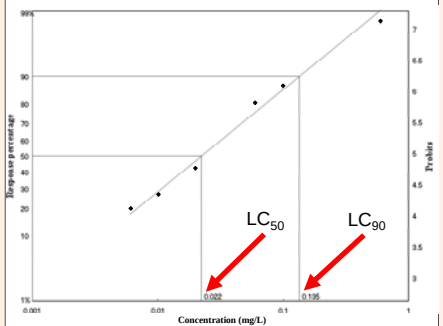
Final Bioassays:

Final bioassays will be carried out similarly to the range-finding assays replicating each treatment three times simultaneously. It will be important to attempt to complete final bioassays with larvae of approximately the same age/instar as the range-finding assays.

Data Analysis:

Mean percent mortality will be calculated for each concentration tested, and data will be subject to log/dose/probit analysis to estimate LC_{50} and LC_{90} values and associated 95% confidence intervals for the materials tested.

Results



DATA

NUMBER	TREATED	CONC. * 1000	% OBSERVED RESPONSE	% LINEAR RESPONSE	LOG (Con.* 1000)	LINEAR PROBITS
1	60	6	20.00	17.48	0.78	4.064
2	60	10	26.67	28.38	1.00	4.428
3	60	20	41.67	46.89	1.30	4.922
4	60	60	80.00	75.95	1.78	5.705
5	60	100	86.67	85.73	2.00	6.068
6	60	600	98.33	99.05	2.78	7.345

STATISTICS

Slope	1.64	+/- 0.162
Chi	1.934	tabulated 9.5
p	0.748	
h	0.483	
g	0.038	
Cycles	5	
r	0.995	tabulated 0.811

LETHAL CONCENTRATION (95 % CI)

LC	CONC. (mg/L)	LOWER LIMIT (mg/L)	UPPER LIMIT (mg/L)
25	0.009	0.006	0.011
50	0.022	0.018	0.028
75	0.058	0.045	0.079
90	0.135	0.096	0.217
95	0.225	0.149	0.404
99	0.585	0.336	1.303

Conclusions

Culiseta melanura 4th instar larvae are susceptible to the biorational *Bacillus sphaericus* (Serotype H5a5b Strain 2362). We screened field collected 4th instar *Cs. melanura* from 0.6 mg/L to 0.006 mg/L. We found that this mosquito has a LD_{50} of 0.022 mg/L and a LD_{90} of 0.135 mg/L. Mortality could not be determined until day 7 at an average temperature of 16.2°C. These concentrations are 10 to 100 times lower when compared to application rates for control of *Culex pipiens*, and well within the recommended application rates.

Performing the laboratory experiment with field collected crypt water provided additional information. The pH of 4.3 did not adversely effect the efficacy of VectoLex® WDG.

To date, there are only a few successful products shown to control *Cs. melanura* in field studies. Methoprene (2 lbs/acre) (Woodrow et al. 1995), granular heptachlor (1 lb/acre), and granular dieldrin (1 lb/acre) (Hayes 1962) were effective in controlling the larvae. Wettable DDT dust was unsuccessful as a winter prehatch control measure at a rate of 1 to 2 lbs/acre (Hayes 1962).

Future field studies would include placing quantified amounts of *B. sphaericus* in *Cs. melanura* crypts. The deposition and diffusion of the larvicide into the larval crypts would be measured. The bioassay technique employed in this study could be used to determine developing resistance of *B. sphaericus* in other mosquito species, such as *Cx. pipiens* and *Cx. tarsalis*.

Literature cited

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For further information

Please contact *brismosqwa@comcast.net* More information on this and related projects can be obtained at *www.nmca.org/AMCA09poster.pdf*