AN EXAMINATION OF THE LONG TERM EPIZOOTIC POTENTIAL OF VARIOUS ARTIFICIALLY INTRODUCED MOSQUITO LARVAL PATHOGENS[1]

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ABSTRACT. The potential for long term mosquito larval reduction by *Bacillus thuringiensis* serotype H-14, *B. sphaericus* and *Metarhizium anisopliae* was tested using laboratory simulations of flooded field breeding sites. Results showed that the capability of either bacterial species to reduce larval numbers was lost as early as 1 month after bacterial inoculation. Replicates receiving the fungal pathogen did show reduced mosquito emergence up to 150 days after fungus introduction, but by day 224 mosquito populations were not affected.

Among the advantages historically cited for the incorporation of bio-rational insecticides into pest management systems has been the potential for their perpetuation or long term epizootic potential. Although the utilization of commercially available bacterial and viral insecticides on agricultural crops has generally failed to show 'recycling' to any economic extent, (Ramoska 1980), the situation could be different for medically important pests such as mosquitoes, whose larvae exist in aqueous, often nutrient-rich environments where facultative parasites such as bacteria and fungi might be able to colonize in the absence of the host (Anonymous 1973, Goldberg et al. 1975). The perpetuation of pathogens in breeding sites in the absence of high host densities could provide long-term control by reducing larval populations on an ad hoc basis.

In the following study simulated flooded field breeding ecosystems were used to test the long-term efficacy or recycling potential of mosquito pathogenic microorganisms, specifically the bacteria *Bacillus thuringiensis* serotype H-14 de Barjac, *B. sphaericus* Neide and the fungus *Metarhizium anisopliae* Metchnikoff.

MATERIALS AND METHODS

Larvae from laboratory colonies of *Culex quinquefasciatus* Say were utilized in this study. Third instar larvae were used in the bioassays of leached water and soil core samples.

Strain 1593 of *B. sphaericus* was produced in YEG (1.5% yeast extract, 0.79% H_2PO_4 and 0.2% glucose) broth in the insect pathology laboratory at Kansas State University. The cultures consisted of nearly completely sporulated bacterial cells and reached a density of ca. 1×10^9 c/ml in the broth. The *B. thuringiensis* (H-14) used was a commercial spore crystal wettable powder formulation 6108 II WP (Vectobac™, Abbott Laboratories, N. Chicago, IL).

The strain of *Metarhizium anisopliae* var. *anisopliae* used was strain 'E_9' isolated in Brazil and was supplied on silica gel crystals by Donald Roberts (Boyce Thompson Institute for Plant Research at Cornell University). It was grown in the laboratory on YpSs [yeast extract, potassium phosphate, and soluble starch (Emerson 1941)] agar in enameled baking pans. The agar pans were seeded with *M. anisopliae* spores, foil covered and incubated for 2 weeks at 23°C. Spores were harvested by brushing across the agar surface with a small paint brush. Collected spores were dried for 24 hr on filter paper in darkness and then transferred to glass containers and held at 4°C until needed. Prior to use the spores were filtered through a 147 μ mesh screen to reduce clumping.

In determining the standard dosage levels for the mosquito, bioassays were

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performed using serial log dosages of appropriate preparations following standard bioassay procedures (Ramoska et al. 1977). Each dosage was replicated 4 times with 10 larvae per replicate. The dosage level chosen as indicated in the accompanying data was 10.0 μ g/ml of *B. thuringiensis*, 1.0×10^5 cells/ml *B. sphaericus* and 2.2 mg/cm^2 (= 5.7×10^7 conidia) of *M. anisopliae* for each respective experiment.

FLOODED FIELD EXPERIMENTS. Glass aquaria, 40 liters in volume and measuring 20 × 40 × 50 cm were divided vertically by a glass panel into 2 equal compartments. Taps were installed at the base of each compartment and large pebbles were added to cover the tap hole. Soil collected from a nearby undisturbed field was layered into each compartment in the same vertical stratification in which it had been removed from the field. Total soil depth in each compartment was 40 cm. Soil analysis was performed on each layer (Table 1).

The soil within the compartments was settled and compacted to more closely simulate the undisturbed strata by biweekly flooding with rainwater for approximately 2 months. Kansas prairie grass was seeded into the sod and was grown to 9 cm in height before introducing the mosquito larvae.

Each of 8 compartments (4 aquaria) was flooded to 9 cm above the top of the sod and seeded with 100 first instar larvae. After the larvae had grown to the third instar, the appropriate pathogen was added to two replicate compartments at the dosage level indicated above. Third instar larvae were chosen as targets for the introductions because this is a common instar treated in actual mosquito control stations. Following this initial treatment the simulations were subjected to 12 hr photoperiods illuminated by overhead aquaria lighting (27 cm Westinghouse F8T5 bulbs). The water temperature was maintained at 20°C. Water in the simulations was undisturbed (except for evaporation) for 10 days, after which it slowly leached through the tank via the taps. The leached effluent from the top, middle, and bottom soil layers was bioassayed against 3d instar *Cx. quinquefasciatus* for larvicidal activity. Periodically (as indicated in Table 2), the tanks were reflooded and first instar larvae added. Adults that emerged were counted as they stuck to tank covers coated with a sticky substance (Tac trap ™, Animal Repellents, Inc., Griffin, GA). Larvae that died were not removed. After all the insects had either emerged or died, the water was again percolated through the soil layers. This cycle was repeated 3 times throughout the year. After the final cycle, core samples of the strata were taken from the tanks using a 70 cm PVC tube with a 5 cm inside diameter. The samples were divided into 5 cm sections and were suspended in 100 ml of demineralized water. Each suspension was equally divided into 2 cups and tested for larvicidal

Table 1. Soil analysis for field simulation.

Layer	% sand	% silt	% clay
1 (top)	31.4	52.4	16.2
2 (middle)	23.8	51.6	24.6
3 (middle)	25.8	57.6	16.6
4 (bottom)	24.8	62.2	13.0

Table 2. Mortality and adult emergence of *Cx. quinquefasciatus* larvae in simulated field microcosms previously treated with mosquito pathogens.

Pathogen	Days post-microbial inoculation	% emergence[1]	% mortality
B. thuringiensis	7	0.0	100.0
(H-14) 10.0 mg/ml	34	90.0	10.0
	227	95.6	4.4
	355	90.1	9.9
B. sphaericus	7	0.0	100.0
1.0×10^5 c/ml	34	93.6	6.4
	227	97.8	2.2
	355	97.2	2.8
M. anisopliae	7	0.0	100.0
2.2 mg/cm^2	150	67.3	32.7
	224	97.2	2.8

[1] Corrected according to Abbott's formula (Finney 1952).

activity against 3d instar *Cx. quinquefasciatus* larvae.

RESULTS AND DISCUSSION

Mortality among the initial group of larvae subjected to the 3 treatments was complete as had been anticipated by the experimental design (Table 2). Host survival, however, did increase significantly in the bacterial treatments 34 days after tank inoculation even though the original dead larvae were kept *in situ*. The *M. anisopliae* seeded aquaria continued to suppress approximately ⅓ of the total larval test population after 150 days, but by day 225 it had ceased to exert any control on the test populations. Both *B. sphaericus* and *B. thuringiensis* (H-14) had ceased to exert control by the 34 day bioassay (Table 2). The *B. sphaericus* and *B. thuringiesis* (H-14) aquaria were again bioassayed at day 355 and as might be expected there was no significant mortality.

These data indicate *M. anisopliae* to be the most capable in maintaining efficacy in this type of environment. The results presented here are comparable to earlier findings which indicated that *B. thuringiensis* (H-14) and *B. sphaericus* were significantly effective for no longer than 10–14 days in artificial container breeding sites while *M. anisopliae* suppressed populations for nearly one month (Ramoska unpublished, Ramoska et al. 1981).

Of the effluent leached through the aquaria strata 8 days after introduction of *B. thuringiensis* (H-14), only the water that had been in the middle strata of the aquaria exhibited any pathogenicity and it was relatively low. The middle effluent from the *B. sphaericus* treated aquaria after 48 days of incubation also demonstrated some pathogenicity (Table 3). Effluent from the *M. anisopliae* treated aquaria showed no significant levels of pathogenicity.

Earlier investigators studying the selective retrieval and recycling potential of *B. sphaericus in situ* within ditches found viable *B. sphaericus* spores in the strata of the soil up to 9 mo. after inoculations (Hertlein et al. 1979). Findings in these tests from the leached water tend to support their data. No one, however, has demonstrated that *B. sphaericus* is naturally available to the host after a period of incubation or that the host populations are affected to any degree by the presence of the bacillus in the soil.

Bioassays of the core samples taken at the conclusion of the experiment (Table 4) indicate that *B. sphaericus* was the most active in the top core sample 0–5 cm and then decreased to insignificant levels of pathogenicity. All cores samples of *B. thuringiensis* were not significantly different from the untreated replicates while *M. anisopliae* showed highly significant activity in the bottom 2 core layers.

The lack of pathogenicity in either core samples or water samples from the *B.*

Table 3. Mosquito larval bioassay of water leached from flooded field microcosms in which pathogens have been applied.

	Mortality					
	8 day post-introduction			48 day post-introduction[1]		
		Strata			Strata	
Pathogen	Upper	Middle	Lower	Upper	Middle	Lower
B. thuringiensis (H-14)	5.0 b[2]	17.5 a	2.5 b	12.5 c	21.2 c	15.0 c
B. sphaericus	5.0 b	5.0 b	0.0 b	57.5 ab	61.2 a	17.5 c
M. anisopliae		NO DATA		37.5 abc	25.0 c	25.0 c
No treatment	2.5 b	5.0 b	0.0 b	17.5 c	12.5 c	30.0 c

[1] $\bar{x}$ of 4 replicates of 25 larvae/rep.

[2] Numbers followed by the same letters are not significant at the P = .05 level.

Table 4. Bioassay of strata of soil from test aquaria treated with mosquito larval pathogens.

Pathogen	Experimental period (Days)	% mortality Sample depth top to bottom (cm)									
		0–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50
B. thuringiensis (H-14)	335	10.0 cfed	7.5fed	5.0 fed	3.7 fe	1.2 fe	3.7 fe	2.5 fe	1.2 fe	3.7 fe	2.5 fe
B. sphaericus 1593	335	30.0 b	11.2 cfed	2.5 fe	0.0 f	5.0 fed	2.5 fe	10.0 cfed	7.5 fed	0.0 f[2]	22.5 cbd[2]
M. anisopliae	224	16.2 ced[3]			8.67 fed[3]	5.0 fed	8.7 fed	7.5 fed	23.7 cb	51.2 a	46.2 a
No treatment	335	3.7 fe	3.7 fe	10.0 cfed	1.6 fed	1.2 fe	3.7 fe	5.0 fed	5.0 fed	3.7 fed	2.5 fe

[1] Numbers followed by the same letters are not significant at the P = .05 level of significance.
[2] n = 3 in all others n = 4. 25 larvae/rep.
[3] These two core samples compacted, thus fall between 0–10 and 10–20 cm respectively.

thuringiensis (H-14) aquaria is not surprising in light of recent independent findings by Ramoska et al. (1982) and Ignoffo et al. (1981), which showed that silt and clay both appear to have a detrimental effect on *B. thuringiensis* (H-14) efficacy that may be related to adsorption.

Although simulations such as those presented in this paper do have limitations when correlated to natural environmental situations, they do permit a degree of control over the experiment that is not attainable in the natural state. Based upon simulation of a flooded field mosquito breeding site, it is felt that none of the pathogenic microorganisms used is capable of recycling to a degree that would foster epizootic levels of disease within their respective populations. This is not because the organisms are necessarily inactivated or dead but rather they are for the most part unavailable to the mosquito due to either the effects of adsorption, drainage, or leaching into the substratum. Whatever the reason, it is obvious that the viable microorganisms are not in the same place as the hatching larvae as soon as a month after introduction of the pathogen. These findings indicate that the possibility of these pathogens initiating epizootics regularly after artificial introduction into mosquito populations breeding in the flooded field environment is remote.

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References Cited

Anonymous. 1973. Mosquito control: Some perspectives for developing countries. Natl. Acad. Sci. Report, Washington, DC, 63 pp.

Emerson, R. 1941. An experimental study of the life cycle and taxonomy of allomyces. Lloydia. 4:77–144.

Finney, D. J. 1952. Probit analysis. 2nd. ed. Cambridge Univ. Press, Cambridge.

Goldberg, L. J., I. Ford and S. Singer. 1975. *Bacillus sphaericus* var. *fusiformis* as a potential

pathogen against *Culex tarsalis* and *Culex pipiens*. Proc. Ann. Conf. Calif. Mosq. Control Assoc. 42:81–82.

Hertlein, B. C., R. Levy and T. W. Miller. 1979. Recycling potential and selective retrieval of *Bacillus sphaericus* from a mosquito habitat. J. Invertebr. Pathol. 33:217–221.

Ignoffo, C. W., C. Garcia, M. J. Kroha, T. Fukuda and T. L. Couch. 1981. Laboratory tests to evaluate the potential efficacy of *Bacillus thuringiensis* var. *israelensis* for use against mosquitoes. Mosq. News 41:85–93.

Ramoska, W. A. 1980. Recent advances in the study of epizootology of bacterial diseases of insects with particular emphasis on mosquito pathogenic bacteria. XVI Int. Congr. Entomol. Kyoto, Japan Aug. 1980.

Ramoska, W. A., C. Pacey and S. Watts. 1981. Tests of the pathogenicity and persistence of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* Neide against larvae of *Culex restuans* Theobold. J. Kans. Entomol. Soc. 54:56–60.

Ramoska, W. A., S. Watts and R. E. Rodriguez. 1982. Influence of suspended particulates on the activity of *Bacillus thuringiensis* serotype H-14 against mosquito larvae. J. Econ. Entomol. 75:1–4.

Ramoska, W. A., S. Singer and R. Levy. 1977. Bioassay of three strains of *Bacillus sphaericus* on field collected mosquito larvae. J. Invertebr. Pathol. 30:151–154.

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