

LARVICIDAL ACTIVITY OF NEW ISOLATES OF *BACILLUS SPHAERICUS* AND *BACILLUS THURINGIENSIS* (H-14) AGAINST ANOPHELINE AND CULICINE MOSQUITOES

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ABSTRACT. Two isolates of *Bacillus sphaericus* (2013-4 and 2013-6) and one of *B. thuringiensis* H-14 (2013-9) from Romania were bioassayed in the laboratory against several species of culicine and anopheline mosquitoes. Second instars of *Culex quinquefasciatus*, *Anopheles albimanus*, *An. quadrimaculatus* and *Aedes triseriatus* exposed for 48 hr to the 2013-4 isolate responded with LC₅₀ values of 0.0015, 0.0187, 0.0527 and 0.0941 ppm of lyophilized primary powder respectively. Preliminary tests with 2013-4 against *Psorophora columbiana* indicated an LC₅₀ of 0.0046 ppm. Similar results were obtained for the 2013-6 isolate against *Cx. quinquefasciatus*, *An. albimanus* and *An. quadrimaculatus*.

The successful use of *Bacillus thuringiensis* Berliner var. *israelensis* (H-14) de Barjac as a highly effective larvicide of mosquitoes has been reported by several authors. The specificity for certain families of Nematocera coupled with a high level of efficacy has enabled relatively rapid registration of commercially produced formulations.

Certain strains of another spore forming bacterium, *Bacillus sphaericus* Neide are also potent larvicides of several mosquito species. Although *B. sphaericus* is not as active as *B. thuringiensis* (H-14) against all species of mosquitoes, it persists longer under natural conditions (Hertlein et al. 1979, Mulligan et al. 1980, Hornby et al. 1981) and may provide the added benefit of recycling in nature (Hertlein et al. 1979). Several strains and isolates are known for *B. sphaericus* covering a range

of larvicidal potencies (Singer 1980). New isolates of *B. sphaericus* and *B. thuringiensis* (H-14) from Romania demonstrated high levels of activity against *Culex quinquefasciatus* Say and other mosquitoes (Singer 1980). This paper presents research conducted on the larvicidal efficacy of 2 isolates of *B. sphaericus* and one isolate of *B. thuringiensis* (H-14) against several species of anopheline and culicine mosquitoes.

In comparative tests of Bactimos[®] primary powder, and 2013-9, relative toxicities of 5337, and 2345 ITU/mg were obtained. The *B. sphaericus* isolates compared favorably to other efficacious isolates reported in the literature.

METHODS AND MATERIALS

Lyophilized spore preparations of the 2013-4 and 2013-6 isolates of *B. sphaericus* and the 2013-9 isolate of *B. thuringiensis* (H-14) were used for bioassays. Just prior to exposing larvae to the bacteria, the appropriate spore preparation was suspended in distilled water with a magnetic stirrer for 20 min before serially diluting.

In the bioassay procedure for *B. sphaericus* (2013-4), lab reared 48 hr second instars of *Anopheles albimanus* Wiedemann, *An. quadrimaculatus* Say, *Aedes triseriatus* (Say), *Cx. quinquefasciatus*

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and field collected *Psorophora columbiae* (Dyar and Knab) were utilized. All exposures and controls were conducted with 20 larvae in 100 ml of well water at 27°C in waxed paper cups. No food was added for the first 24 hr of exposure to conform with standard practice and to eliminate the variability that results from the early addition of food. Approximately 20 mg of larval diet (hog supplement) was added to each cup after 24 hr to prevent prolonged stress due to inadequate nutrition and achieve maximum homogeneity in response. Mortality was determined after 48 hr. The same procedures were followed for the 2013-6 isolate of *B. sphaericus* except that *Ae. triseriatus* and *Ps. columbiae* were not tested.

Early fourth instars of *An. albimanus*, *An. quadrimaculatus*, *Ae. aegypti* (L.), *Ae. triseriatus* and *Cx. quinquefasciatus* were used in the bioassay procedure for *B. thuringiensis*. Exposures were made in a manner consistent with the *B. sphaericus* bioassays; however, in these tests no food was added and mortality was determined after 24 hr of exposure following standard practice with this rapidly acting bacterium.

The protocol for both bacteria utilized 3 cups per replicate and 3 replicates of each concentration and control over time. Seven concentrations of the isolates were run during each replication such that at least 2 concentrations produced mortality above, and 2 produced mortality below the 50% mortality point. These conditions were only partially fulfilled with *Ps. columbiae*, as there were sufficient larvae for only 2 full replications with 2013-4 and one had only one point below 50%. Data were corrected for control mortality with Abbott's formula and analyzed by probit analysis. Significant differences in species susceptibility to the isolates were determined by nonoverlap of the 95% fiducial limits of the LC_{50} 's.

Comparison of the *B. thuringiensis* isolate was made with the International Standard IPS-78 and the primary powder of Bactimos®, a commercially produced fermentation residue of *B. thuringiensis*

(H-14) used as the base for making the formulated wettable powder. Early fourth instars of *Ae. aegypti* were used for comparing the spore-crystal preparations. Exposures to 5 concentrations ranging from 0.025 to 0.175 ppm were run in the same manner prescribed above for determining the activity of 2013-9. Spore counts were made on the 3 preparations using standard pour plate dilutions and molten (50°C) tryptose blood agar base.

Field exposures of 2013-9 at 0.25, 0.5 and 1.0 ppm were conducted against *Ae. triseriatus* in tree holes in hardwood hammocks in and near Gainesville, Florida. The volume of naturally collected water in each cavity was measured and supplemented with well water to bring each tree hole to full capacity prior to the introduction of inoculum. At least 2 tree holes were used for each concentration and controls during each test. Three replications (tests) were run during a 2 month period using the range of tree hole volumes (750 ml to 3 liters) for each concentration and control. Three hr after the introduction of 2013-9, larvae and tree hole water were collected from each cavity and transported to the laboratory. The number of dead larvae was recorded upon arrival at the laboratory in order to provide data on the immediate effect of the bacteria. Samples of those larvae alive at that time were exposed in the collected water for a further 24 hr; depending on the number of living larvae recovered, up to 60 early 4th instars from each tree hole were divided among 3 waxed paper cups. One hundred ml of water collected from the corresponding tree hole was placed in each cup. The cups were then incubated at 27°C for 24 hr at the end of which time mortality was assessed. The total mortality was then calculated by combining mortality after 3 hr with that observed after the 24 hr incubation period.

RESULTS

Probit analyses of each of the *B. sphaericus* isolates are graphically depicted

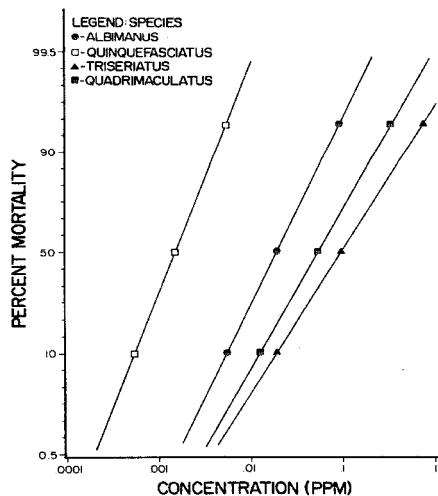


Fig. 1 Probit analysis of *Bacillus sphaericus* (2013-4) against *Culex quinquefasciatus*, *Anopheles albimanus*, *Anopheles quadrimaculatus*, and *Aedes triseriatus*.

in Figs. 1 and 2. The LC_{50} and LC_{95} for each species to each isolate are presented in Table 1. *Culex quinquefasciatus* was clearly the most susceptible of the species. It appeared to be slightly less susceptible to the 2013-6 isolate at higher concentrations ($\frac{LC_{95}}{LC_{50}}$ for 2013-6 = 3.01 vs 2.73

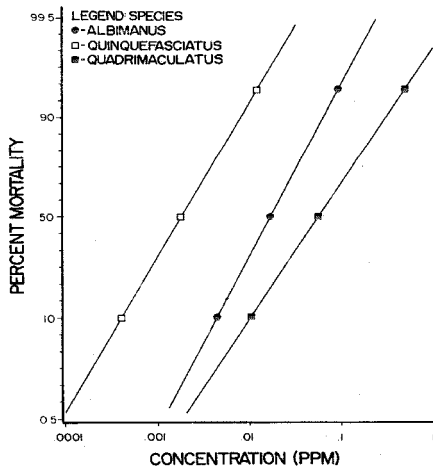


Fig. 2. Probit analysis of *Bacillus sphaericus* (2013-6) against *Culex quinquefasciatus*, *Anopheles albimanus*, and *Anopheles quadrimaculatus*.

for 2013-4), although the LC_{50} did not differ significantly for the 2 isolates.

Anopheles albimanus was significantly more susceptible to the 2 isolates than *An. quadrimaculatus*. No significant difference was shown between isolates for either of the anophelines, although *An. albimanus* was slightly less susceptible to 2013-6 at higher concentrations.

Table 1. LC_{50} and LC_{95} values of the 2013-4 and 2013-6 isolates of *Bacillus sphaericus* against *Culex quinquefasciatus*, *Anopheles albimanus*, *An. quadrimaculatus* and *Aedes triseriatus*.

Species	LC	Concentration (ppm) and 95% Fiducial Limits*	
		2013-4	2013-6
<i>Cx. quinquefasciatus</i>	50	0.0015 (0.0013-0.0017) ^a	0.0018 (0.0013-0.0025) ^a
	95	0.0054 (0.0043-0.0074)	0.0122 (0.0073-0.0299)
<i>An. albimanus</i>	50	0.0187 (0.0171-0.0203) ^b	0.0168 (0.0154-0.0184) ^b
	95	0.0909 (0.0786-0.1076)	0.0932 (0.0797-0.1119)
<i>An. quadrimaculatus</i>	50	0.0527 (0.0480-0.0576) ^c	0.0558 (0.0437-0.0700) ^c
	95	0.3379 (0.2824-0.4193)	0.4935 (0.3128-1.0205)
<i>Ae. triseriatus</i>	50	0.0941 (0.0798-0.1107) ^d	—
	95	0.7466 (0.5477-1.1225)	—

* LC_{50} followed by the same letter are not significantly different at the .05 level. Each isolate contained ca 3.0×10^7 spores/mg of lyophilized primary powder.

The mortality of *Ae. triseriatus*, the least susceptible of the species tested, was significantly different from that of *An. quadrimaculatus*. *Psorophora columbiae* responded with 0 to 87.2% corrected mortality to 7 concentrations of 2013-4 ranging from 0.0005 to 0.01 ppm. An approximate LC_{50} determined by interpolation was 0.0046 ppm.

The effects of the 2013-9 isolate of *B. thuringiensis* (H-14) on fourth instars of the 5 species tested are shown in Fig. 3. The LC_{50} , LC_{95} and their respective fiducial limits for 2013-9 against the 5 species are presented in Table 2. The steep regression lines (low LC_{95} to LC_{50} ratios) indicate a fairly uniform susceptibility of the species tested. A slightly broader response curve is shown for *An. albimanus*. The difference in species susceptibility is less distinct than was observed for the *B. sphaericus* isolates.

The LC_{50} values for the Bactimos primary powder, 2013-9 and IPS-78 were 0.0380, 0.0865 and 0.2028 ppm respectively against fourth instars of *Ae. aegypti*. Arbitrarily assigning IPS-78 with 1000 International Toxicity Units (ITU)/mg and dividing the LC_{50} of IPS 78 by those of Bactimos and 2013-9 multiplied by 1000 yields the relative toxicities of 5337 and 2345 ITU/mg, respectively. The viable spore counts were $7.93 \pm 0.13 \times 10^7$;

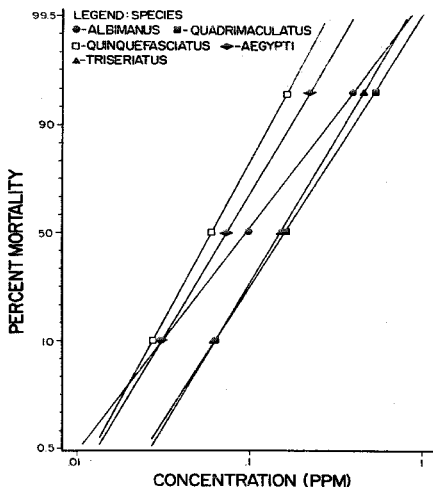


Fig. 3. Probit analysis of *Bacillus thuringiensis* (H-14; 2013-9) against *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles albimanus*, *Anopheles quadrimaculatus*, and *Aedes triseriatus*.

$4.53 \pm 0.25 \times 10^7$; and $4.24 \pm 0.18 \times 10^7$ spores/mg, respectively.

The average laboratory assessed mortality of *Ae. triseriatus* treated *in situ* with 0.25, 0.5 and 1.0 ppm of 2013-9 was 33.8, 87.6 and 96.3% respectively. Little

Table 2. LC_{50} and LC_{95} values of the 2013-9 isolate of *Bacillus thuringiensis* against *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles albimanus*, *An. quadrimaculatus* and *Ae. triseriatus*.

Species	LC	Concentration (ppm) and 95% Fiducial Limits*	
		LC_{95}	LC_{50}
<i>Cx. quinquefasciatus</i>	50	0.0607 (0.0582-0.0633) ^a	2.73
	95	0.1656 (0.1530-0.1812)	
<i>Ae. aegypti</i>	50	0.0743 (0.0628-0.0883) ^{ab}	3.01
	95	0.2236 (0.1684-0.3569)	
<i>An. albimanus</i>	50	0.0945 (0.0830-0.1060) ^b	4.13
	95	0.3907 (0.3194-0.5138)	
<i>An. quadrimaculatus</i>	50	0.1605 (0.1453-0.1775) ^c	3.26
	95	0.5235 (0.4287-0.6888)	
<i>Ae. triseriatus</i>	50	0.1509 (0.1362-0.1690) ^d	3.01
	95	0.4542 (0.3626-0.6297)	

* LC_{50} followed by the same letter are not significantly different at the 0.05 level.

or no mortality was observed after the 3 hr *in situ* exposure period with the 0.25 ppm treatment. However, an average of 30% and 51.7% of the larvae were dead upon arrival in the lab with the 0.5 ppm and 1.0 ppm treatments respectively. Combining the mortality after 3 hr with that observed in the lab after another 24 hr results in respective totals of 91.3 and 98.2% mortality.

DISCUSSION

The larvicidal activity of both isolates of *B. sphaericus* is equal to or greater than most previously investigated isolates. Closest in activity to the 2013 isolates is the 1593 strain. Ramoska et al. (1977), Mulligan et al. (1978), Myers et al. (1979), and Wraight et al. (1981a) report similar or lower activity for 1593 against several species of culicine mosquitoes.

The most susceptible species in our tests, *Cx. quinquefasciatus*, has previously been reported highly susceptible to other isolates of *B. sphaericus* including 2013-4 and 2013-6 (Myers et al. 1979, Singer 1980). The lower mortality response in anophelines exposed to *B. sphaericus* compared to that of culicines has been documented in other investigations and may be due in part to lower innate feeding rates (Ramoska and Pacey 1979, Ramoska and Hopkins 1981).

The least susceptible species to *B. sphaericus* (2013-4), *Ae. triseriatus*, was considerably more susceptible than *Ae. aegypti* (Ramoska and Hopkins 1981), *Ae. melanimon* Dyar (Mulligan et al. 1978) and *Ae. taeniorhynchus* Wiedemann (Ramoska et al. 1977). Other *Aedes* species have responded in a fairly susceptible manner to the 1593-4 strain of *B. sphaericus* (Mulligan et al. 1978, Wraight et al. 1981b).

The calculated 2345 ITU/mg of the 2013-9 isolate of *B. thuringiensis* compares favorably to commercial formulations which range in activity from 286 to 3482 ITU/mg (Dame et al. 1981). The 2.28-fold difference in potency of the Bactimos primary powder over that of 2013-9 may be due in part to the 1.61-

fold difference in spore count, assuming a corresponding difference in parasporal crystalline inclusions for the 2 preparations. Difference in potency could also be due to methods and media used in fermentation as well as the inherent potency of the 2 preparations against *Ae. aegypti*. Side by side comparisons of the 2 grown and preserved under identical conditions and bioassayed against several other species of mosquitoes and black flies will provide a clearer picture of their relative potencies.

The laboratory data for 2013-9 against both the culicine and anopheline larvae indicate a wide spectrum of larvicidal activity within the Culicidae. The relatively reduced susceptibility of *Anopheles* species to *B. thuringiensis* (H-14) has previously been documented by other investigators using the Israeli isolates of *B. thuringiensis* (H-14) (Goldberg and Margalit 1977, de Barjac and Coz 1979, Dame et al. 1981). As with *B. sphaericus*, these differences in susceptibility are probably due, at least in part, to differences in feeding rates and feeding behavior. Although *Ae. aegypti* and other *Aedes* species are susceptible to low concentrations of *B. thuringiensis* (H-14), *Ae. triseriatus* was one of the least susceptible of the species utilized in our tests. A number of factors including feeding rate could be responsible for the observed higher tolerance in this study.

The organically rich tree hole habitat and *Ae. triseriatus* were selected to provide a maximum field challenge for the 2013-9 isolate. Under somewhat similar conditions, the 1897 Israeli isolate was considerably less efficacious against *Ae. sierrensis* (Ludlow), the western treehole mosquito (Garcia and Des Rochers 1979). The observed disparity between the laboratory determined LC_{95} of 0.4542 ppm for 2013-9 against *Ae. triseriatus* and the *in situ* LC_{95} of ca 0.93 ppm is probably due to settling of the inoculum, a factor of no consequence in the shallow cups in the laboratory, and to interaction of the parasporal crystals and the type of water employed. Under laboratory conditions, Ignoffo et al. (1981) detected rapid set-

ting of *B. thuringiensis* (H-14) as well as highly decreased activity when assays were made in pond water as opposed to distilled water. Similar observations were made by Mulligan et al. (1980) using *B. thuringiensis* (H-14) in raw and autoclaved sewage effluent vs. tap water.

The ultimate evaluation of both *B. sphaericus* (2013-4 and 6) and *B. thuringiensis* (2013-9) will be made under field conditions. The successful use of other isolates of *B. sphaericus* and *B. thuringiensis* (H-14) against field populations of mosquitoes is well documented (Davidson et al. 1981, Mulligan et al. 1978, 1980; Ramoska et al. 1978, Dame et al. 1981, Engler et al. 1980, Hembree et al. 1980). Results obtained in these studies indicate that additional research on formulated material against field populations of mosquitoes is warranted.

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DISPERSAL OF *CULEX SALINARIUS* IN SOUTHWESTERN LOUISIANA

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ABSTRACT. The dispersal of post-teneral female *Culex salinarius* was studied near a brackish water breeding source in southwestern Louisiana. An estimated 4,000, 8,000 and 22,000 mosquitoes were marked with fluorescent pigments in 3 mark-release-recapture experiments conducted in 1980 and 1981. Mosquitoes were collected over a 26-hr period fol-

lowing release. Thirteen marked *Cx. salinarius* were recovered in all directions from the release point at distances of up to 2,000 m on the night of release and 1,000 m on the following night. Single specimens of *Aedes vexans* and *Anopheles* sp. were recovered at 1,000 m on the night of release.

INTRODUCTION

Insect dispersal by flight may be divided into 3 categories, migratory, appetitive and consumatory. Kennedy (1975) defined migration as "adaptive travel" involving persistent locomotion in an oriented fashion with a depression of vegetative responses. In the case of mosquitoes, this type of movement usually involves newly emerged adults. Localized movements within the immediate habitat are termed trivial or appetitive and occur as a result of active search for stimuli related to feeding, mating or oviposition (Craig 1918, Matthews and Matthews

1978). These movements usually involve reproductively active members of the population and may include both horizontal and vertical movement. Reception of certain stimuli related to feeding, mating or oviposition leads to consumatory flight which results in the insect approaching its objective (Craig 1918, Tinbergen 1951). The last 2 categories of dispersal are therefore closely related.

This study concerns the appetitive and consumatory flight of *Culex salinarius* Coquillett, a dominant component of the mosquito community in coastal marshes along the U.S. Gulf coast. Information concerning the long distance flight of *Cx.*