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# Acute Toxicity of the Mosquito Larvicide, *Bacillus sphaericus*, to the Grass Shrimp, *Palaemonetes pugio*, and Mummichog, *Fundulus heteroclitus*

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Controlling insect populations with chemical insecticides has proven useful; but limitations due to insect resistance, toxicity to nontarget organisms, and increasing production costs are factors for concern. In addition, increased public awareness of the toxicity hazards posed by many insecticides has led to significant public scrutiny of pest and vector control programs (i.e., Med fly spraying in Southern California). Alternatives to traditional chemical insecticides are, therefore, being investigated. One potential alternative is the use of microbial agents such as bacteria.

In the southeastern United States, controlling salt marsh mosquitoes (*Aedes taeniorhynchus* and *Aedes solicitans*) in their breeding grounds near estuarine tidal creeks is a primary vector control problem. Chemical larvicides applied to these areas present a toxicity hazard to nontarget aquatic organisms (Lee and Scott 1989). To remedy this problem, microbial larvicides have been introduced to lower mosquito populations to tolerable levels without detrimental effects on nontarget organisms (Davidson 1985).

*Bacillus sphaericus* Neide is a bacterial species whose toxic properties on mosquito larvae were first reported by Kellen et al. in 1965. Since then over 30 strains of *B. sphaericus* have been isolated from a variety of mosquito habitats. These strains range from those only slightly insecticidal to those with very toxic spores that kill quickly. Mosquito larvae of *Culex* and *Anopheles* species are generally more vulnerable than *Aedes* species to *B. sphaericus* strains (Davidson 1985). The efficacy of *B. sphaericus* against mosquito larvae has been well documented by Mulla et al. (1984) and Mulla (1985).

The host range of *B. sphaericus* is restricted to mosquito larvae (Davidson 1985). However, there is a lack of information on any effects that might occur in nontarget invertebrate or vertebrate estuarine organisms. The objectives of this study were to determine the acute toxicity of *B. sphaericus* to two common estuarine species, the grass

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shrimp, *Palaemonetes pugio*, and the mummichog, *Fundulus heteroclitus*.

## MATERIALS AND METHODS

Adult *F. heteroclitus* were collected at Bohicket Creek (N $32^0$ 36" ; W $80^0$ 15" ) and adult *P. pugio* were collected from Leadenwah Creek (N $32^0$ 36'12" ; W $80^0$ 07'00"), which are both tidal tributaries of the North Edisto River estuary. Collected shrimp and fish were transported to the laboratory and acclimated for 14 d to $25^0$ C, 20 ‰ salinity, and a 12-hr light-dark cycle. Water for acclimation and toxicity tests was collected from Bohicket Creek. Fish were held in a 300-L circular tank filtered by a 750 L/hr canister filter, while shrimp were held in a 135-L rectangular tank filtered by a 380 L/hr power filter. Fish and shrimp were fed daily with Tetramin® Fish Flakes and newly hatched *Artemia* , respectively.

The 96-hr toxicity tests were static renewal bioassays in which solution changes were made every 24 hr. Shrimp were exposed in 2-L aquaria, and fish were exposed in aerated 5-L aquaria. Toxicity tests were conducted for both fish and shrimp under conditions as described for acclimation. For each exposure concentration and control group, two replicates were used (N=10). During the toxicity tests, fish and shrimp were not fed. Dead organisms were removed from aquaria when discovered.

*B. sphaericus* spores as a technical grade wettable powder (ABG-6184; Abbott Laboratories, North Chicago, Illinois) were used for toxicity tests. Seven concentrations were employed for each test. These concentrations ranged from 56.00 to 1800.00 mg/L for fish and 1.00 to 100.00 mg/L for shrimp. The 96-hr median lethal concentration (LC50) with 95% confidence limits was determined using the Trimmed Spearman-Karber Method (Hamilton et al. 1977). The No Observable Effect Concentration (NOEC) was estimated by calculating the geometric mean of the highest concentration in which no mortality was observed and the subsequent concentration causing mortality. A comparison of the NOEC obtained in this study for each species with the expected *B. sphaericus* water concentration (based upon a 1x surface area application rate of 3.4 kg/ha [3 lb/A] and 30.5 cm [12 in.] of water) provided a measure of relative safety (safety factor = NOEC/expected *B. sphaericus* concentration in 30.5 cm of water).

## RESULTS AND DISCUSSION

*B. sphaericus* ABG-6184 was considerably more toxic to *P. pugio* than to *F. heteroclitus*. Results indicated a 96-hr LC50 value for *P. pugio* of 39.25 mg/L (39,250 international toxic units (ITU/L) with 95% CL ranging from 24.52 to 62.84 mg/L (24,520 to 62,840 ITU/L) (Table 1). The 96-hr LC50 value for *F. heteroclitus* was 423.17 mg/L (423,170 ITU/L) with 95% CL ranging from 314.09 to 570.14 mg/L (314,090 to 570,140 ITU/L) (Table 2).

Table 1. Mortality data for *P. pugio* exposed for 96-hr to *B. sphaericus*

| mg/L | 0 | 1.0 | 5.6 | 10.0 | 18.0 | 32.0 | 56.0 | 100.0 |
|---|---|---|---|---|---|---|---|---|
| number exposed | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| mortality | 0 | 0 | 2 | 1 | 5 | 2 | 4 | 9 |

*B. sphaericus* 96-hr LC50: 39.25 mg/L
95% Confidence Limits: 24.52 - 62.84 mg/L

Table 2. Mortality data for *F. heteroclitus* exposed for 96-hr to *B. sphaericus*

| mg/L | 0 | 56.0 | 100.0 | 180.0 | 320.0 | 560.0 | 1000.0 | 1800.0 |
|---|---|---|---|---|---|---|---|---|
| number exposed | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| mortality | 0 | 0 | 4 | 0 | 0 | 9 | 7 | 10 |

*B. sphaericus* 96-hr LC50: 423.17 mg/L
95% Confidence Limits: 314.09 - 570.14 mg/L

Mulligan et al. (1978) exposed juvenile mosquito fish (*Gambusia affinis affinis*) to *B. sphaericus* 1593 in a 96-hr static test. No adverse effect was noticed at normal field application rates of up to 5.6 kg/ha (1.7 mg/L) (Mulligan et al. 1978). These exposure rates were considerably lower than the concentrations utilized in the present study with *F. heteroclitus* (Table 2) and thus may explain the absence of observable effects.

A comparison of these results to the concentrations effective against mosquito larvae is difficult. With mosquitoes, LC50 values will vary according to the species of mosquito, the instar stage, strain of *B. sphaericus*, and amount of organic matter in the water. For example, in looking at species differences, a 48-hr LC50 for fourth instars of *Culex quinquefasiatus* is 0.044 mg/L using *B. sphaericus* strain 1593. For *Aedes aegypti*, the value is 58.6 mg/L for fourth instars using the same strain (Mulla 1985).

The mode of action of *B. sphaericus*, while not completely understood, involves a toxin or toxins found in the cell wall of the sporulating cell or the spore (Myers and Yousten 1980). This toxin was discovered to be a protein resistant to a variety of proteolytic enzymes. When *B. sphaericus* spores are ingested by mosquito larvae, digestive processes can release the toxin causing the midgut to swell. The gut cells will thus separate, and, depending on the strain of bacteria administered, death of the larvae will follow after 4 to 48 hr (Davidson 1979). Recycling can occur when spores germinate in dead larval tissue and are released to the environment (Davidson 1985).

Since *B. sphaericus* must enter the digestive tract to be effective, the dose reaching the target site will vary according to the feeding mechanism of the organism and the availibility of the bacteria in the feeding zone (Mulla 1985). Filter feeders may ingest *B. sphaericus* directly, but interference with ingestion of lethal doses of bacteria will occur if other filterable particles are present (Davidson 1985). Grass shrimp feed mainly on detritus along with its associated bacteria (Welsh 1975). This leads to the possibility of direct ingestion of *B. sphaericus* by *P. pugio*. In fish, *Bacillus* species have been isolated from the digestive tract and external surfaces; however, these bacteria are considered non-pathogenic to fish (Sanders and Fryer 1988). In the present study, shrimp and fish were confined to aquaria which contained *B. sphaericus* suspended in the water column thus forcing contact with the bacteria.

Field studies have examined the effects of *B. sphaericus* to non-target aquatic macroinvertebrates. No harmful effects were observed when *B. sphaericus* 2362 was applied to a pond at 0.23 kg/ha [0.2 lb/A]. Non-target organism (diving beetle adults and larvae, mayfly naiads, and ostracods) numbers remained constant or increased while achieving over 90% control of mosquito larvae (Mulla et al. 1984). Certain non-estuarine vertebrates (lizards and rats) have been exposed to *B. sphaericus* and monitored for infections. Different tissues of the tested animals were examined and no bacilli were found after exposure ended (Hudson 1981; Shadduck et al. 1980).

*Bacillus thuringiensis* var. *israelensis* (Bti) is a microbial mosquito larvicide similar to *B. sphaericus* in method of application and mode of toxicity (Mulla 1985). Several studies have been conducted with Bti to ascertain its effects to nontarget aquatic organisms. Lee and Scott (1989) reported a 96-hr LC50 for adult *F. heteroclitus* of 980 mg/L (95% CL=730-1330 mg/L) with a NOEC of 22.36 mg/L for Bti. This was the least toxic among five tested larvicides (temephos, fenoxycarb, diflubenzuron, methoprene, and Bti). Dee (1988) tested the same 5 larvicides against *P. pugio* and reported a 96-hr LC50 of 1080 mg/L (95% CL= 980-1190 mg/L) with a NOEC of 27.39 mg/L for Bti. This was also the least toxic of the 5 larvicides. It appears that *B. sphaericus* is considerably more toxic than Bti to grass shrimp and somewhat more toxic to mummichogs.

The NOEC of *B. sphaericus* for *P. pugio* was 2.37 mg/L compared with74.83 mg/L for *F. heteroclitus* (Table 3). The safety factors ranged from 18 for *P. pugio* to 573 for *F. heteroclitus*. Earlier reported safety

Table 3. NOEC and safety factors for *B. sphaericus*

| Application Rate (kg/ha) | Species | Expected Conc. in 30.5 cm of Water (mg/L) | NOEC (mg/L) | Safety Factor[1] |
|---|---|---|---|---|
| 3.4 | *P. pugio* | 0.131 | 2.37 | 18 |
| 3.4 | *F. heteroclitus* | 0.131 | 74.83 | 573 |

[1]Safety Factor = NOEC/Expected Conc. in 30.5 cm of water

factors for Bti ranged from 1242 for *F. heteroclitus* (Lee and Scott 1989) to 1521 for *P. pugio* (Dee 1988). These findings clearly indicate that while both Bti and *B. sphaericus* larvicides have large margins of safety, the margin of safety is significantly lower for *B. sphaericus*.

Crustaceans appear to be much more sensitive to *B. sphaericus* ABG-6184 than fish. This difference in sensitivity was not observed with Bti (Lee and Scott 1989; Dee 1988). Additional toxicity studies with crustacean species, focusing on life history stages, are needed for a more complete evaluation of this compound. This is particularly important given the wide range in *B. sphaericus* toxicity observed among different mosquito species (Mulla 1985). Future studies should also be focused on different fish and invertebrate species to gain a better understanding of *B. sphaericus* toxicity in estuarine organisms. This would provide environmental managers with the information necessary for the appropriate selection and use of this larvicide in safely controlling salt marsh mosquitoes.

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