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EFFICACY OF *BACILLUS SPHAERICUS* NEIDE AGAINST
LARVAL MOSQUITOES (DIPTERA: CULICIDAE) AND
MIGDES (DIPTERA: CHIRONOMIDAE) IN THE LABORATORY

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ABSTRACT

The primary powders of strains 1593 (IF-119) and 2362 (IF-118 and ABG-6184) of *Bacillus sphaericus* Neide were tested as larvicides of laboratory-reared mosquitoes and field-collected midges, and were compared with the activity of strain 1593-4 (RB-80), the international standard of *B. sphaericus*. Two mosquito species, *Aedes aegypti* (Linn.) and *Ae. taeniorhynchus* (Wiedemann), and two midge species, *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards were insensitive to *B. sphaericus* ($LC_{50} > 50$ ppm). Among the five susceptible mosquito species, *Culex quinquefasciatus* Say, *Cx. nigripalpus* Theobald, *Anopheles albimanus* Wiedemann, and *An. quadrimaculatus* Say, were most susceptible to ABG-6184 (strain 2362) with LC_{90} values of 0.0044, 0.0067, 0.54, and 7.35 ppm, respectively. *Wyeomyia mitchelli* (Theobald) was almost equally susceptible to ABG-6184 ($LC_{90} = 0.276$ ppm) and strain 1593 (IF-119) ($LC_{90} = 0.261$ ppm).

RESUMEN

Los polvos primarios de las razas 1593 (IF-119) y de 2362 (IF-118 y ABG-614) de *Bacillus sphaericus* Neide, fueron probados como larvicidas de larvas de mosquitos criadas en el laboratorio y de larvas de moscas de agua colectadas del campo, y fueron comparadas con la actividad de la raza 1593-4 (RD-80) que es el patrón internacional de *B. sphaericus*. Dos especies de mosquitos, *Aedes aegypti* (Linn.) y *Ae. taeniorhynchus*

(Wiedemann), y dos moscas de agua, *Chironomus crassicaudatus* Malloch y *Glyptotendipes paripes* Edwards, fueron insensitivas a *B. sphaericus* (LC₅₀ 50 ppm). Entre las cinco especies de mosquitos susceptibles, *Culex quinquefasciatus* Say, *Cx. nigripalpus* Theobald, *Anopheles albimanus* Wiedemann, y *An. quadrimaculatus* Say, fueron las más susceptibles a ABG-6184 (raza 2362) con valores de LC₉₀ de 0.0044, 0.0067, 0.54, y 7.35 ppm respectivamente. *Wyeomyia mitchellii* (Theobald) fue casi igualmente susceptible a ABG-6184 (LC₉₀=0.276 ppm) y a la raza 1593 (IF-119) (LC₉₀=0.261 ppm).

The microbial agent, *Bacillus sphaericus* Neide has been known for its mosquito larvicidal activity for many years (Kellen et al. 1965). However, the isolation of more potent strains of this mosquito larval pathogen in recent years has drawn the attention of a large number of researchers, resulting in many articles on the laboratory and field efficacy of several potent strains of this bacterium (Davidson et al. 1981, Lacey and Singer 1982, Lacey et al. 1984, Mulla et al. 1984a,b, Mulligan et al. 1980, and others). Among the different strains of *B. sphaericus* tested against mosquito larvae, strains 1593 and 2362 have shown one of the highest levels of biological activity against several species (Anonymous 1985). The origin, bacteriophage type and serotype of *B. sphaericus* strains 1593 and 2362, and other mosquito-pathogenic *B. sphaericus* strains are summarized in Davidson (1984). At present, there are several experimental primary powders of strains 1593 and 2362 available from government and university laboratories and industry for interested mosquito control researchers to conduct efficacy tests.

Reported here is laboratory response of larvae of seven mosquito species and two chironomid midge species exposed to *B. sphaericus* strains 1593 and 2362 (primary powders) supplied by H. T. Dulmage, USDA, Brownsville, Texas. Larvae of these mosquito and midge species were also simultaneously exposed to the *B. sphaericus* international standard (RB-80) received from H. de Barjac, Institut Pasteur, Paris, France, and to an industrially-produced primary powder (ABG-6184) provided by Abbott Laboratories, N. Chicago, Illinois.

MATERIAL AND METHODS

The mosquito species tested were *Aedes aegypti* (Linn.), *Ae. taeniorhynchus* (Wiedemann), *Anopheles albimanus* Wiedemann, *An. quadrimaculatus* Say, *Culex nigripalpus* Theobald, *Cx. quinquefasciatus* Say, and *Wyeomyia mitchellii* (Theobald). These species were maintained at the Florida Medical Entomology Laboratory at Vero Beach, Florida. For midge bioassays, field-collected 3rd and 4th instars of *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards were used. Larvae of the former species were collected from Lake Monroe, as described in Ali and Baggs (1982), while *G. paripes* was obtained from Lake Jessup located at 5-6 km distance from Lake Monroe, Seminole County, Florida.

Methods utilized for mosquito bioassays were generally the same as described by Mulla et al. (1982). Twenty larvae (3rd and 4th instars) of a mosquito or midge species were placed in 120-ml disposable paper cups containing 100 ml tap water. Distilled water (pH 6.9 ± 0.2) was used for rearing and testing *Wy. mitchellii* because of the possibility of its larval mortality in tap water (Nayar 1982). In the midge studies, 5 g of sterilized sand per cup was added for the larval acclimatization and to prevent any cannibalism. For treatments, each strain or preparation of *B. sphaericus* was suspended in tap water by using a magnetic stirrer to make its 1% stock suspension (w/v) and, as needed, serial dilutions were made to obtain the appropriate range of concentrations for testing.

Each strain or preparation was tested on at least three different occasions. Each time, 5-6 concentrations (in suspension) of a strain or preparation were applied to each of three cups (replicates) receiving a concentration while three cups were left untreated as controls. The stock suspensions and serial dilutions were freshly prepared on each occasion. The treated and control cups containing mosquitoes or midges were maintained under 14-h photoperiod and $27 \pm 1^\circ\text{C}$ room temperature. After a 48-h exposure period to the pathogen, larval mortality was assessed. The mortality in the treated cups in a test was adjusted against any mortality in the controls (Abbott 1925). The corrected mortality was subjected to log probit regression analysis.

RESULTS AND DISCUSSION

The levels of toxicity of the test strains or preparations of *B. sphaericus* to five mosquito species in the laboratory are presented in Table 1. The data concerning the *Aedes* and the midge species are not included in the table because their LC_{50} values exceeded 50 ppm, thus showing insensitivity to the test strains of *B. sphaericus*. Among the five susceptible species of mosquitoes, *Cx. quinquefasciatus* was the most susceptible. The Abbott's (2362) preparation (ABG-6184) was slightly superior in activity against this species than the Dulmage preparations of strains 2362 (IF-118) and 1593 (IF-119) as indicated by the 0.0044, 0.0052, and 0.0064 ppm LC_{90} values, respectively.

All strains and preparations of *B. sphaericus* showed a high level of biological activity against *Cx. nigripalpus*. ABG-6184 (2362) was nearly 2X more active against *Cx. nigripalpus* than the international standard (1593-4, RB-80). Strains 1593 (IF-119), 2362 (IF-118), and the international standard showed a similar level of activity against *Cx. nigripalpus* (Table 1).

Anopheles albimanus was most susceptible to ABG-6184 ($\text{LC}_{90} = 0.54$ ppm) followed by 1593 (IF-119) ($\text{LC}_{90} = 0.91$ ppm) and 2362 (IF-118) ($\text{LC}_{90} = 1.22$ ppm). By contrast, *An. quadrimaculatus* was several times less susceptible to all the test strains or preparations (Table 1). Against *An. quadrimaculatus*, strain 2362 (IF-118) was the least active ($\text{LC}_{90} = 27.2$ ppm).

Bacillus sphaericus showed good activity against *Wy. mitchellii* with LC_{90} values ranging from 0.261-0.818 ppm. Strains 1593 (IF-119) and Abbott's (2362) preparation showed superior activity against *Wy. mitchellii* than the international standard and the strain 2362 (IF-118). In general, the primary powder, ABG-6184 (strain 2362), proved the most toxic preparation against the mosquito larvae.

Data on the laboratory activity of several toxic strains and preparations of *B. sphaericus* against a number of mosquito species in the genera *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Mansonia*, and *Psorophora* are previously available (Anonymous 1985, Mulla et al. 1984a,b, 1985). The present study adds *Wy. mitchellii* to the list of susceptible species of mosquitoes to *B. sphaericus*. This study also reveals that *C. crassicaudatus* and *G. paripes* midge larvae were insensitive when exposed for 48 h to the test strains and preparations of *B. sphaericus*. The microbial agent was also ineffective against *Ae. aegypti* and *Ae. taeniorhynchus* at exposure rates exceeding 50 ppm. The insensitivity of *Ae. aegypti* to the available most toxic strains of *B. sphaericus* is already documented (Anonymous 1985, Mulla et al. 1984b).

Among the susceptible species of mosquitoes, *Cx. nigripalpus* and *Cx. quinquefasciatus* were highly susceptible; the LC_{90} values of *Cx. quinquefasciatus* achieved by different strains and preparations of *B. sphaericus* used in the present study were 6-20 times lower than those reported by Mulla et al. (1984b) for *B. sphaericus* strains 2362 (IF-97 AP) and 1593 (IF-94) against *Cx. quinquefasciatus*. In contrast, lower LC_{90} values of *An. quadrimaculatus* were observed by Mulla et al. (1984b) than those

TABLE 1. SUSCEPTIBILITY OF LABORATORY REARED LARVAE (3RD AND 4TH INSTARS) OF FIVE SPECIES OF MOSQUITOES^a TO VARIOUS STRAINS AND PREPARATIONS OF *BACILLUS SPHAERICUS* IN THE LABORATORY.

Strain and/or preparation	48-h lethal concentration (ppm)			
	LC ₅₀	95% CL	LC ₉₀	95% CL
	<i>Anopheles albimanus</i>			
1593 ^b	0.31	0.26 - 0.35	0.91	0.81 - 0.98
2362 ^c	0.50	0.44 - 0.57	1.22	0.99 - 1.37
Abbott (2362) ^d	0.19	0.16 - 0.22	0.54	0.48 - 0.61
	<i>Anopheles quadrimaculatus</i>			
1593	2.88	2.50 - 3.40	9.58	8.16 - 11.20
2362	8.20	7.14 - 9.74	27.20	24.20 - 30.64
Standard (1593-4) ^e	2.42	2.25 - 2.60	7.86	7.06 - 8.69
Abbott (2362)	2.29	2.09 - 2.50	7.35	6.86 - 7.82
	<i>Culex nigripalpus</i>			
1593	0.0052	0.0049 - 0.0056	0.016	0.012 - 0.019
2362	0.0048	0.0046 - 0.0050	0.014	0.011 - 0.018
Standard (1593-4)	0.0071	0.0066 - 0.0077	0.012	0.011 - 0.013
Abbott (2362)	0.0026	0.0021 - 0.0029	0.0067	0.0061 - 0.0073
	<i>Culex quinquefasciatus</i>			
1593	0.0023	0.0020 - 0.0025	0.0064	0.0057 - 0.0073
2362	0.0016	0.0014 - 0.0019	0.0052	0.0042 - 0.0064
Abbott (2362)	0.0017	0.0015 - 0.0019	0.0044	0.0040 - 0.0049
	<i>Wyeomyia mitchellii</i>			
1593	0.052	0.045 - 0.060	0.261	0.245 - 0.278
2362	0.269	0.242 - 0.294	0.818	0.719 - 0.920
Standard (1593-4)	0.129	0.116 - 0.145	0.442	0.437 - 0.448
Abbott (2362)	0.069	0.062 - 0.076	0.276	0.266 - 0.290

^aMaintained at the Florida Medical Entomology Laboratory at Vero Beach, FL.

^bIF 119, spray dried.

^cIF 118, spray dried.

^dA8G-6184, lot 76-828-BD, spray dried.

^eInternational Standard (RB-80), lyophilized.

achieved for the same species in the present study. Variations in activity levels in different studies could be attributed to several factors such as larval strain, larval age (instar) and vigor at the time of testing, and procedures used in bioassays. The type of formulation, percent of active ingredient in a formulation, and the production of the bacterium under different conditions would also attribute to the toxicity potential of different preparations of *B. sphaericus*.

This study confirms the usefulness of *B. sphaericus* in the biological control of mosquitoes. The host range of available isolates of this microbial mosquito larvicide reported in several laboratory and field studies (e.g., Anonymous 1985, Mulla et al. 1984a,b, 1985, Ramoska et al. 1978, and others) covers species of *Culex*, *Culiseta*, *Anopheles*, *Mansonia*, *Psorophora*, *Wyeomyia* (present study), and even some aedine species, such as *Ae. melanimon*, *Ae. nigromaculis*, and *Ae. triseriatus*. The mosquito host range of *B. sphaericus* is considered narrower than that of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*). However, in general, *B. sphaericus* is more toxic to many susceptible species of mosquitoes than *B.t.i.* and also has the advantage of longer persistence in the treated habitats (Anonymous 1985, Des Rochers and Garcia 1984) although the persistence phenomenon of *B. sphaericus* is not as evident in highly polluted environments (Mulla

et al. 1984a). Further research on isolation of more toxic strains, development of new and more effective formulations, and genetic manipulation to enhance the toxicity of this microbial insecticide (Lacey 1984, and others) may broaden the host range and ultimate usefulness of this microbial agent in vector control.

ACKNOWLEDGMENTS

This is Florida Agricultural Experiment Stations Journal Series No. 7224. Gratitude is expressed to Drs. H. de Barjac (Institut Pasteur, Paris) and H. T. Dulmage (USDA, Brownsville, Texas), and Abbott Laboratories (N. Chicago, Illinois) for provision of samples of *Bacillus sphaericus*. Drs. M. S. Mulla (University of California, Riverside) and L. A. Lacey (USDA, Gainesville, Florida) kindly reviewed the paper which led to its improvement.

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LIFE 48: A BASIC COMPUTER PROGRAM TO CALCULATE LIFE TABLE PARAMETERS FOR AN INSECT OR MITE SPECIES

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ABSTRACT

A computer program in BASIC was developed following methods used by Birch (1948) to calculate life table parameters for an arthropod from experimental data. Actual data were presented as an example and data previously used by Birch (1948) were included in this paper for verification. This program was written to run on VAX minicomputer or IBM microcomputer compatibles.

RESUMEN

Se desarrolló un programa de computadoras en BASIC siguiendo los métodos usados por Birch (1948) usando datos experimentales para calcular los parámetros de un cuadro sobre la vida de un artrópodo. Se presentó datos actuales como ejemplo y se incluyó datos usados por Birch (1948) como verificación. Este programa se escribió para ser usado en microcomputadoras VAX o con microcomputadoras compatibles con IBM.
