EFFECTIVENESS OF BACILLUS THURINGIENSIS SEROVAR. ISRAELENSIS (VECTOBAC 12 AS) AND BACILLUS SPHAERICUS 2362 (ABG-6232) AGAINST CULEX SPP. MOSQUITOES IN A DAIRY LAGOON IN CENTRAL FLORIDA

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Abstract

A wettable powder (WP) formulation of Bacillus sphaericus 2362 (ABG-6232) and an aqueous suspension of Bacillus thuringiensis serovar. israelensis (Vectobac 12 AS) were evaluated against Culex mosquitoes in a dairy wastewater lagoon in central Florida. Culex nigripalpus and Cx. quinquefasciatus inhabited the lagoon; the former species comprised >90% of the total Culex larvae collected during the sampling periods. Vectobac 12 AS (at 1.17 L/ha) and ABG-6232 (at 1.12 kg/ha) were each applied separately to the lagoon on three different occasions during 1987-1988. Vectobac 12 AS caused a maximum 71-88% larval reduction for only one day posttreatment in the three treatments. ABG-6232 (WP) gave an average larval reduction of 84-92% in the three tests for up to 13 days posttreatment with >50% average reduction of the larvae being maintained for beyond 17 days posttreatment.

Resumen

Se evaluó una formulación de polvo humectante de Bacillus sphaericus 2362 (ABG-6232) y una suspensión acuosa de Bacillus thuringiensis serovar. israelensis (Vectobac 12 AS) contra mosquitos Culex en una laguna de agua de desperdicio de una lechería en el centro de la Florida. Culex nigripalpus y Cx. quinquefasciatus habitaron la laguna; la primera especie constituyó >90% del total de larvas de Culex colectadas durante el periodo de muestreo. Vectobac 12 AS (a 1.17 L/ha) y ABG-6232 (a 1.12 kg/ha) fueron separadamente aplicados a la laguna en 3 ocasiones diferentes durante 1987-1988. Vectobac 12 AS causó una reducción máxima de larvas de 71-88% solo un día después...
del tratamiento en los tres tratamientos. ABG-6232 causó un promedio de reducción de larvas de 84-92% en las tres pruebas hasta 13 días después del tratamiento con un promedio de >50% de reducción de larvas mantenidas por más de 17 días después del tratamiento.

Wastewater generated on a daily basis at dairy farms, primarily through washing dairy herds and milking barns, collects into large open retention ponds or lagoons. The impounded water contains high contents of solid and dissolved organic materials, providing ideal conditions for mosquito oviposition and larval development.

In the southeastern United States, the dominant species of mosquito in wastewater ponds and lagoons is usually Culex quinquefasciatus Say (Steelman & Colmer 1970, Rule & Axcell 1978, O'Meara & Evans 1985). However, in some wastewater systems in south Florida, Cx. nigripalpus Theobald is more abundant than Cx. quinquefasciatus (Carlson 1982; the former species is the dominant summer and early fall Culex in peninsular Florida (Edman 1974).

In Florida, as in many other parts of the United States, the dairy industry is being encroached upon by rapid urbanization. This results in increasing human contact with mosquitoes breeding in and around the dairy environments and necessitating mosquito control.

Two bacterial agents, Bacillus thuringiensis serovar. israelensis (B.t.i.) and B. sphaericus, in a number of laboratory and field studies have proven to be excellent larvicides of a variety of mosquito species world-wide (Ali et al. 1981, Ali & Nayar 1986, Davidson et al. 1981, Lacey et al. 1984, Majuri et al. 1987, Mulligan et al. 1980, World Health Organization 1985). For the past several years, B.t.i., in a variety of formulations has been marketed for mosquito control in most parts of the world. However, it has been documented that B. sphaericus, in general, is more toxic (equal potency basis) to some mosquito species than B.t.i., and also has the advantage of longer persistence in the treated habitats (DesRochers & Garcia 1984, World Health Organization 1985). As a consequence, B. sphaericus is presently being enthusiastically developed by the chemical industry as a mosquito larvicide.

This study reports the effectiveness of an experimental wettable powder (WP) of B. sphaericus strain 2932 (ABG-6232) against Culex spp. larvae in a dairy lagoon in central Florida. A commercial aqueous suspension of B.t.i. (Vectobac 12 AS) was also tested in the same lagoon to compare the degree and longevity of control given by the two microbial mosquito larvicides.

**Materials and Methods**

The dairy lagoon, rectangular in outline, located at approximately 28° 53' N latitude and 81° 41' W longitude, in Dona Vista, Lake County, Florida, was used for this study. It is 102 m long and 15 m wide, with an average water depth of 1 m. The lagoon receives wastewater effluent daily (through gravity flow) from approximately a 400-cow dairy operation via a series of two small settling ponds (each ca. 8 x 8 m). The ponds retain the bulk of the solids which are allowed to dry through evaporation and percolation and periodically cleaned by dredging. The impounded water in the lagoon is occasionally pumped out for pasture irrigation.

Water in the lagoon is usually highly turbid (>100 NTU) and almost neutral (pH 6.9). The lagoon is lined with a thick natural growth of cattails (Typha spp.) and water-primrose (Ludwigia spp.). Some grasses also border the lagoon. The predominant floating vegetation on the lagoon water consists of Giant duckweed (Spirodela polyrhiza) and Pennywort (Hydrocotyle ranunculoides).
The *B. t.i.* and *B. sphaericus* used in this study were produced and provided by Abbott Laboratories, N. Chicago, IL. On three separate occasions (October 5, 1987, November 16, 1987, and May 31, 1988), the WP ABG-6232 (812 IU/mg, lot no. 08-083-BR) was uniformly applied from a boat to the entire surface of the lagoon at a rate of 1 lb/acre (1.12 kg/ha). Similarly, *V. aspera* 12 AS (1900 IU/mg, lot no. 15-178-BA) was applied to the lagoon on October 26, 1987, June 20, 1988, and July 7, 1988, at a rate of 1 pt/acre (1.17 L/ha). For each treatment the required amount of spray material was thoroughly mixed with about 3 gal (11.4 L) of water in a bucket and transferred to and applied with a 3.5 gal (13.25 L) pressurized spray can (Solo Backpack) (Solo, Inc. Newport News, VA). A max.-min. thermometer was used at one location in the lagoon to record the water temperature range during each field test.

Immediately prior to and periodically after each treatment, samples of mosquito larvae were collected from the sides of the lagoon at 14 predetermined locations using a 500-ml dipper. The middle area of the lagoon was also sampled from a boat on two occasions but no mosquito larvae were found in the open water. Since there was no comparable habitat in the area to use for an untreated control, the posttreatment larval declines had to be compared with the corresponding prevailing pretreatment population levels to elucidate the percent larval reductions and effectiveness of each treatment. The two small retention ponds containing mostly solids or sludge supported insufficient numbers of mosquito larvae and could not be used as controls. The larval samples were brought to the laboratory for taxonomic identifications and counting.

**RESULTS AND DISCUSSION**

Larvae of *Cz. nigricalpous* and *Cz. quinquefasciatus* inhabited the lagoon with the former species comprising over 90% of the total larvae on each sampling occasion. The mean number of larvae per dip during the *B. sphaericus* 2362 (ABG-6232) treatments exceeded 350 larvae at the time of pretreatment on each occasion (Table 1). ABG-6232 at 1.12 kg/ha rate of application produced 64-97, 38-93, and 60-85% reductions of larvae for beyond two weeks (14-17 days) in the treatments 1, 2, and 3, respectively. Overall, ABG-6232 gave larval reductions of 84-92% in the three treatments (combined) for up to 13 days, and a greater than 50% reduction of pretreatment larval numbers was maintained for beyond 17 days. The cumulative trend of larval populations and their

<table>
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<tr>
<th>Table 1. Efficacy of Bacillus sphaericus 2362 wettable powder (ABG-6232) applied at 1.12 kg/ha against Culex spp. larvae in a dairy wastewater lagoon in Central Florida, 1987-1988.</th>
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<tr>
<td>Mean no. larvae/dip pretreatment</td>
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<tr>
<td>Treatment 1 (October 5, 1987)≤</td>
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<td>Treatment 2 (November 16, 1987)≤</td>
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<td>Treatment 3 (May 31, 1988)≤</td>
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*≤812 IU/mg
*≥Mixture of *Cz. nigricalpous* and *Cz. quinquefasciatus* (>90% *Cz. nigricalpous*).
≤Water temperature: 21-24°C; 41-48°C; 21-28°C.
declines due to the three *B. sphaericaeus* treatments combined are shown in Fig. 1. In the pretreatment samples, 3rd and 4th instar larvae were predominant but after the treatments, mature larvae (3rd and 4th instars) declined considerably while the young larvae (1st and 2nd instars) predominated for more than two weeks posttreatment. The prevalence of the young larvae was probably due to their continuous addition as a result of continuous oviposition and egg hatching in the lagoon. These larvae were not exposed to the pathogen for sufficient time to suffer mortality. A similar field observation on asynchronously developing mosquito larvae exposed to *B. sphaericaeus* was reported by Majori et al. (1987).

Vectobac 12 AS at 1.17 L/ha rate of treatment produced a maximum of 71% (treatment 1), 88% (treatment 2), and 83% (treatment 3) reduction of mosquito larvae within a day after each treatment (Table 2). In treatment 1, the number of larvae was reduced by 14-67% for up to 3 days. In treatment 2, 10-80% reductions of the larval numbers were recorded during the 2-4 days of posttreatment sampling, while in treatment 3, the larval populations returned to the pretreatment levels within 2 days. Overall, for the three treatments combined, *B.t.i.* induced a maximum larval reduction of 84% in one day posttreatment and <36% after 2 days. Third and 4th instar larvae were reduced considerably after the treatments but 1st instar larvae, due to their continuous recruitment, predominated during the pre-, and posttreatment periods (Fig. 1).

This study suggests that appreciable larval reduction (84%) of *Cz. nigricalus* and *Cz. quinquefasciatus* provided by *B.t.i.* (Vectobac 12 AS) at 1.17 L/ha in the dairy wastewater lasted only for one day posttreatment. Although Vectobac 12 AS had a higher ITU/mg (1200) as compared to the 812 ITU/mg for *B. sphaericaeus* 2362 (ABG-6232), on equal wt/vol basis, Vectobac 12 AS appeared to produce slightly lower levels of larval control and for a much shorter time than ABG-6232. These data on *B.t.i.* are compatible with some previous mosquito control studies in polluted waters where *B.t.i.* in different formulations had caused 91-100% larval reductions of *Cz. quinquefasciatus* for 1-3 days after treatments at rates ranging from 0.65 to 5.6 kg/ha (Majori et al. 1987). Also, Mulla et al. (1982) reported *Cz. quinquefasciatus* larval reductions of 0, 81 and 91% one day after treatment with Baetimos (WP, 3500 ITU/mg) applied at 0.56, 1.12, and 2.24 kg/ha, respectively, to dairy lagoons in southern California.

The field activity of some potent strains of *B. sphaericaeus* (including strain 2682) against larvae of a large number of mosquito species in different parts of the world has

**TABLE 2. EFFICACY OF BACILLUS THURINGIENSIS SEROVAR. ISAELENSIS AQUEOUS SUSPENSION (VECTOBAC 12 AS)* APPLIED AT 1.17 L/HA' AGAINST CULEX spp. b LARVAE IN A DAIRY WASTEWATER LAGOON IN CENTRAL FLORIDA, 1987-1988.**

<table>
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<th>Mean no. larvae/dip pretreatment</th>
<th>Percent larval reduction posttreatment (days)</th>
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<td>1</td>
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<tr>
<td>111 Treatment 1 (October 26, 1987)**</td>
<td>71</td>
</tr>
<tr>
<td>410 Treatment 2 (June 20, 1988)**</td>
<td>88</td>
</tr>
<tr>
<td>41 Treatment 3 (July 7, 1988)**</td>
<td>83</td>
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</tbody>
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*1200 ITU/mg
bPredominantly *Cz. nigricalus* (*Cz. quinquefasciatus* <5%)
**Water temperature: 19-24°C, 21-27°C, 21-28°C.
Fig. 1. Pre- and posttreatment larval trends of Culex spp. (predominantly Cx. nigripalpus) in a dairy wastewater lagoon treated at 1.12 kg/ha with a wettable powder formulation of Bacillus sphaericus strain 2362 (ABG-6232), and at 1.17 L/ha with an aqueous suspension of Bacillus thuringiensis serovar. israelensis (Vectobac 12 AS), Dona Vista, Lake County, central Florida, 1987-1988.
been documented (World Health Organization 1985). A flowable concentrate (BSP-1, containing 12% primary powder of strain 2362) applied at 20 g/m² provided a satisfactory control of Cx. quinquefasciatus for 6 to 10 weeks in cesspits and latrines in the United Republic of Tanzania, while a WP of B. sphaericus 2362 applied at 0.25 kg/ha produced 90% larval reduction of Cx. quinquefasciatus in polluted waters in Ivory Coast (World Health Organization 1985). Recent studies of Mulla et al. (1988) in dairy wastewater lagoons in California indicated that two primary powder preparations of B. sphaericus (ABG-6184) at rates of 0.26 and 0.56 kg/ha gave mediocre and short-term control of Culex mosquitoes (Cx. peus and Cx. quinquefasciatus). However, the level of control and persistence greatly increased as the dosages were increased to 1.12, 2.24, and 4.48 kg/ha. The lower two rates yielded almost 100% control for 4 weeks while the 4.48 kg/ha rate yielded control (99%) for up to 49 days or longer. A flowable concentrate preparation of B. sphaericus (BSP-2) yielded complete initial and persistent control of Culex larvae for 14-21 days at 2.24, 4.49, and 5.6 kg/ha rates of treatment.

The present study confirms the superiority of B. sphaericus 2362 over B. t. i. in controlling Culex mosquitoes in polluted waters. Only one treatment rate (1.12 kg/ha) of B. sphaericus was employed in this study; higher rates of this microbial mosquito larvicide may produce better initial and longer-lasting control as shown by the studies of Mulla et al. (1988). The increased application rate(s) of B. sphaericus is feasible and justified in view of the long-lasting control obtained with one treatment, which would save on the costs of site inspections and repeated treatments when less potent and less persistent larval control agents are employed (Mulla et al. 1988). Thus, rapid development of B. sphaericus leading to its availability for commercial use in mosquito control programs is deemed necessary.

Acknowledgements

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References Cited


LIFE HISTORY PARAMETERS OF BARK BEETLES
(COLEOPTERA: SCOLYTIDAE) ATTACKING
WEST INDIAN PINE IN THE DOMINICAN REPUBLIC

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ABSTRACT

An outbreak of pine bark beetles (Coleoptera: Scolytidae) occurred in the central highlands of the Dominican Republic during 1986-1987. Initiation of the outbreak coincided with a period of severe drought. Thousands of native West Indian pines, Pinus occidentalis Sw., were killed by beetles attacking the trunk and branches. Ips calligraphus (Germar) was the principal mortality agent. Two other bark beetles infested the smaller branches (Pityophthorus antillicus Bright) and shoots (Pityophthorus pinivoros Bright). Spatial attack pattern, harem size, egg gallery length, egg density, and sex ratio of Dominican I. calligraphus populations were similar to values reported from the southeastern United States. However optimal pheromone blends differed between the two populations. Of five pheromone blends tested, the 50% (+)-ipsdienol:50% (-)-ipsdienol plus cis-verbenol attracted the most Dominican beetles. Ips calligraphus adults were collected throughout the year in pheromone-baited traps and theoretically could complete 11 to 12 generations per year in the Dominican highlands. No other species of Ips nor any species of Dendroctonus were collected in traps baited with pheromones of the pine bark beetle complex of the southeastern United States. Average tree diameter, tree height, and stand basal area from several infestation sites are presented. Infested pines ranged from 5 to 50 cm in diameter and from 6 to 20 m in height.