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OPERATIONAL STUDIES WITH VALENT VECTOLEX® WDG, *BACILLUS SPHAERICUS,* IN THREE FLORIDA MOSQUITO CONTROL DISTRICTS

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ABSTRACT. VectoLex® WDG, *Bacillus sphaericus* (Valent BioSciences Corporation), was evaluated in 3 Florida Mosquito Control Districts under operational conditions. Application rates were 0.5 and 1.0 lb/acre and an untreated control in each district. Study sites included woodland ponds, residential areas such as catch basins, and commercial business retention ponds. Pretreatment and posttreatment assessments were made with a standard dipper at designated dipping stations. The most prevalent mosquitoes included *Culex quinquefasciatus, Culex nigripalpus, Culex erraticus, Ochlerotatus infirmatus, Anopheles crucians, Anopheles quadrimaculatus,* and *Psorophora* spp. VectoLex WDG was effective for 24–35 days posttreatment with one application.

KEY WORDS VectoLex® WDG larvicide, mosquitoes, Florida, *Culex nigripalpus, Culex quinquefasciatus, Bacillus sphaericus*

INTRODUCTION

Between the mid-1960s and late 1980s, *Bacillus sphaericus* Neide serotypes gained notice as natural agents exhibiting larvicidal potential. Ongoing research in isolation of wild strains has produced candidate serotypes for commercial application (de Barjac and Sutherland 1990). Lacey et al. (1987) discussed recycling of the pathogen, amplification of the toxin in the host, and subsequent larval control with *B. sphaericus* due to release of the bacterial spores and toxins into the larval habitat from the larval cadavers. Reduced larval vigor and delayed pupation were considered additional benefits of sublethal exposure because those events might lead to increased exposure to predators and/or parasites. The effectiveness of a formulation based on strain 2362 against floodwater mosquitoes was described by Mulla et al. (1988). Mulla et al. (1984) found no adverse effects on associated aquatic macroinvertebrate organisms during studies of several *B. sphaericus* isolates, including 2362 in field culicine larvicide studies. In Florida, larval control utilizing source reduction, larvicides, or a combination of these is the preferred method of mosquito control. Environmentally friendly larvicides lasting several weeks are very useful to Florida mosquito control programs, but few such larvicides exist. Operational studies against natural mosquito larval populations were conducted during 1999 in 3 Florida Mosquito Control Districts to evaluate a *B. sphaericus* formulation produced by Valent BioSciences Corporation, labeled as VectoLex® WDG Biological Larvicide.

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MATERIAL AND METHODS

Study sites were located in Jacksonville (Duval County), Flagler County, and Citrus County, FL. VectoLex WDG, Serotype H5a5b (51.2% active ingredient w/w), strain 2362, was applied at the rate of 0.5 and 1.0 lb/acre at each location. Because the study sites were small, a backpack sprayer was used to apply the larvicide. Three application sites were established in each district and assigned 1 of 3 treatments: control (untreated), a low application rate (0.5 lb/acre), or a high application rate (1.0 lb/acre).

Mosquito larvae were collected and identified and a general description of the mosquito fauna developed for each study site. Larvae/pupae sampling consisted of pretreatment assessments (using a standard larval dipper) and posttreatment assessments (2, 7, 14, 21, 28, and 35 days posttreatment). Larvae were considered small (1st and 2nd stage) and large (3rd and 4th stage). Samples were taken at the same marked dipping station (20/site) each assessment period at each site to establish continuity in collecting. Water quality (eutrophication level) was observed based on the scale of 1 = clear, unpolluted water to 10 = septic and very turbid. The following equation (Mulla et al. 1971) was used to calculate percent reduction:

$$\%\ reduction = 100 - \frac{\#CTRL\ pretrt\ larvae \times \#\mathrm{TRT}\ pt\ larvae}{\#TRT\ pretrt\ larvae \times \#\mathrm{CTRL}\ pt\ larvae} \times 100$$

where # *CTRL pretrtlarvae* is the pretreatment mean number of larvae in the untreated (control) site, # *TRT pt larvae* is the posttreatment mean number of larvae in the treated site, # *TRT pretrtlarvae* is the pretreatment mean number of larvae in the treated site, and # *CTRL ptlarvae* is the posttreatment mean number of larvae in the untreated (control) site. Efficacy was observed as a decrease in numbers of the small larvae progressing to large larvae and pupae and/or reduction of larvae in the treated sites compared with the control sites and pretreatment dips in the treated sites. Efficacy was assessed based on large larvae counts. Reduced numbers of larger larvae is an indication that a product is working.

Site descriptions for each district are now given.

Flagler County, East Flagler Mosquito Control District

These sites were typical East Coast woodland pools located near residential/shopping areas and are routinely flooded during seasonal rains. Once flooded, these pools may continue to produce nuisance mosquitoes for several weeks. The control site (29°37′86″N, 81°13′52″W) average water depth was 8–10 in., with little floating vegetation or debris, and covered 0.25 acres. The pollution level was 2 with vegetation along the water border. This woodland pool had a canopy of longleaf pine (*Pinus palutris*) (Radford et al. 1964). Predominant mosquitoes were *Psorophora* spp., *Culex nigripalpus* Theobald, *Ochleratatus infirmatus* Dyar and Knab, *Anopheles* spp., and *Uranotaenia* spp. The low-application site (treated at 0.05 lb/acre) (29°37′82″N, 81°13′50″W) had a canopy of longleaf pine but little or no floating vegetation. Water depth was 6–8 in., with a pollution level 2, and covered 0.5 acres. Predominant mosquitoes were *Cx. nigripalpus, Oc. infirmatus, Anopheles* spp., and *Uranotaenia* spp. The 1.0-lb/acre-treated site (high) (29°37′90″N, 81°13′51″W) was 8–10 in. deep, with some floating debris and emergent vegetation along the sides, and covered 0.5 acres. The pollution index was 3. Torpedo grass (*Panicum* sp.) bordered all sides and extended into the water several inches. These sites received 1 rainfall event of over 5 in. due to Hurricane Floyd during the study.

Duval County, Jacksonville Mosquito Control

Sites in Jacksonville were residential. These were primarily catch basin stormwater runoff sites that held water most of the time because of daily grass irrigation and garden watering runoff from nearby homes. Such sites were rich in nutrients, with grassy substrates and sides, and were subjected to irregular fluctuations in water depth and quality. The ones in this study held water at the outflow of several catch basins. These sites also received substantial rain (>5 in.) from Hurricane Floyd. Predominant species was *Culex quinquefasciatus* Say. Egg rafts were usually present. Adult *Ochlerotatus taeniorhynchus* (Wiedemann) were often associated with these areas because of nearby breeding areas. Daytime landing rates of 25/min were observed. The control site (30°26′61″N, 81°36′60″W) was essentially a large hole in the ground approximately 3 ft in diameter and 2 ft deep, located approximately 200 ft from the St. Johns River in a residential area. The site was surrounded by woody vegetation. The low-application site (treated at 0.05 lb/acre) (30°22′44″N, 81°30′98″W) was a neighborhood storm drain system spillway, emptying into a large retention area (100 ft × 200 ft). The site was constructed of concrete, 5 ft wide by 15 ft long and approximately 1.5 ft deep. Neighboring trees and some waist-high shrubbery shaded the site. It was centered approximately midway between two homes. The high-application site (treated at 1.0 lb/acre) (30°21′95″N, 81°34′62″W) was also a catch basin storm drain spillway, emptying into a retention area (40 ft × 80 ft × 10 in.), located on the corner of the block and shaded by 1 oak tree. The breeding area was created by erosion and was approximately 2 ft wide × 3 ft long × 1.5 ft deep and contained a significant amount of decaying vegetation. The closest home was approximately 50 ft from the site. All these sites had a pollution index of 5.

Citrus County, Citrus County Mosquito Control District

Sites here were retention ponds on commercial properties subject to heavy organic pollution and organic runoff from well-maintained lawns. Predominant mosquitoes were *Cx. nigripalpus, Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann, and *Culex erraticus* (Dyar and Knab). The control site (28°52′12″N, 82°29′27″W) was a settling pond, approximately 30 ft by 60 ft, with cattails, algae, grasses (emergent and along the edge), and willows (*Saururus* sp). It was about 12–14 in. deep in the middle with floating debris and had a pollution index of 5. The low-application site (treated at 0.05 lb/acre) (28°52′47″N, 82°29′40″W) was 10 ft by 16 ft at the time of treatment. Vegetation includes duckweed (*Lemna* sp.), algae, emergent grass, and cattails (*Typha* sp). It was 12 in. deep, with floating debris and a pollution index of 6. The high-application site (treated at 1.0 lb/acre) (28° 53′29″N, 82°32′76″W) covered approximately 0.25 acres with floating debris, algae, shore, and emergent grasses and willows. The water depth was approximately 10 in. in the middle. The pollution index was 6.

Statistical analysis

Analyses of variance (ANOVA) of treatment (trt or T) and treatment × posttreatment dipping times (PT) were made using PROC GLM (SAS Institute 1990, v 6.12). All data were subjected to a Student–Neuman–Keuls (SNK) means separation test and differences were considered significant at $P < 0.05$ (Sokal and Rohlf 1981).

Table 1. VectoLex® WDG evaluation study against natural mosquito populations in East Flagler County Mosquito Control District for 29 days posttreatment.[1]

Application rate	Days PT	Number/dip Small larvae	Number/dip Large larvae + pupae	Percent reduction[2]
Control[a]	prttrt	8	22	—
	1	12	19	—
	5[3]	0	0	—
	15	154	28	—
	22	103	56	—
	29[4]	53	30	—
0.5 lb/acre[b]	prttrt	105	184	—
	1	3	21	86.80
	5[3]	0	14	0.00
	15	23	1	99.60
	22	30	5	99.90
	29[4]	11	2	99.20
1.0 lb/acre[b]	prttrt	23	38	—
	1	0	2	93.90
	5[3]	0	0	0.00
	15	57	12	75.20
	22	47	5	94.80
	29[4]	20	4	92.30

[1] PT, posttreatment; prttrt, pretreatment. Application rates followed by a different lowercase letter represent significant differences ($P < 0.05$) between application rates using Student–Neuman–Keuls (SNK) means separation test (Sokal and Rohlf 1981).
[2] Percent reduction after Mulla et al. (1971).
[3] Rain event >5 in. in 24 h.
[4] Sites drying up.

Table 2. VectoLex® WDG evaluation study against natural mosquito populations in Jacksonville Mosquito Control District for 32 days posttreatment.[1]

Application rate	Days PT	Number/dip Small larvae	Number/dip Large larvae + pupae	Percent reduction[2]
Control[a]	prttrt	2,001	63	—
	2	34	51	—
	7	3	0	—
	15	854	5	—
	26[3]	1,285	29	—
	32[4]	11	4	—
0.5 lb/acre[ab]	prttrt	147	20	—
	2	313	14	13.50
	7	1	0	0.00
	15	0	0	100.00
	26[3]	620	53	0.00
	32[4]	39	2	50.00
1.0 lb/acre[b]	prttrt	1,979	83	—
	2	10	5	92.60
	7	0	0	100.00
	15	1	0	100.00
	26[3]	49	7	81.70
	32[4]	0	0	100.00

[1] PT, posttreatment; prttrt, pretreatment. Application rates followed by a different lowercase letter represent significant differences ($P < 0.05$) between application rates using Student–Neuman–Keuls (SNK) means separation test (Sokal and Rohlf 1981).
[2] Percent reduction after Mulla et al. (1971).
[3] Rain event >5 in. in 24 h.
[4] Retreated.

RESULTS AND DISCUSSION

VectoLex WDG was expected to last several weeks and exhibit excellent control. Tables 1–4 present results for each study area. The East Flagler Mosquito Control District data (Table 1) represents a 29-day study where VectoLex WDG demonstrated effective control throughout the study at the 0.5 lb/acre rate with the exception of one assessment period. No larvae were obtained in the control site though larvae were observed in the treated pools during this assessment. Effective control was observed 1 day posttreatment in the study, with larval reduction of more than 86%. Significant differences were shown between treatments ($P = 0.0004$, df = 2, $F = 8.11$) and between treatments and posttreatment dipping periods ($P = 0.0001$, df = 15, $F = 12.30$). Observations using Mulla's formula suggested VectoLex WDG might be effective at lower application rates against *Oc. infirmatus* and *Cx. nigripalpus* as well as *Cx. quinquefasciatus,* as seen in other test areas. At 1.0 lb/acre, effective control was maintained throughout the study.

The Jacksonville Mosquito Control VectoLex WDG study was ongoing for more than 50 days (Tables 2 and 3). This study was initiated mid-September (Table 2), retreated October 11th, and completed November 4th (Table 3). In the Jacksonville study, the 0.5 lb/acre rate was not as effective as it was in the other 2 studies. The low-treatment site here had a high organic content from lawn and garden runoff as well as road debris and runoff. *Culex quinquefasciatus* were the only mosquito larvae found in this site. The high-treatment site exhibited excellent control, with 3 of 5 assessment periods in

Table 3. VectoLex® WDG evaluation study against natural mosquito populations in Jacksonville Mosquito Control District for 24 days posttreatment.[1]

Application rate	Days PT	Number/dip Small larvae	Number/dip Large larvae + pupae	Percent reduction[2]
Control[a]	prttrt	1,285	29	—
	10[3]	11	4	—
	18	1,004	35	—
	24	26	9	—
0.5 lb/acre[b]	prttrt	620	53	—
	10[3]	39	2	72.64
	18	35	83	0.00
	24	3	2	87.84
1.0 lb/acre[b]	prttrt	49	7	—
	10[3]	0	0	100.00
	18	2	2	76.33
	24	8	0	100.00

[1] PT, posttreatment; prttrt, pretreatment. Application rates followed by a different lowercase letter represent significant differences ($P < 0.05$) between application rates using Student–Neuman–Keuls (SNK) means separation test (Sokal and Rohlf 1981).
[2] Percent reduction after Mulla et al. (1971).
[3] Rain event >4 in. between this and next dipping date.

Table 4. VectoLex® WDG evaluation study against natural mosquito populations in Citrus County Mosquito Control District for 35 days posttreatment.[1]

Application rate	Days PT	Number/dip		Percent reduction[2]
		Small larvae	Large larvae + pupae	
Control[a]	prttrt	59	21	—
	3	69	0	—
	7	26	51	—
	14	136	66	—
	21	206	3	—
	28	34	8	—
	35	83	72	
0.5 lb/acre[b]	prttrt	147	46	—
	3	0	0	0.00
	7	4	0	100.00
	14	2	1	99.90
	21	0	0	100.00
	28	0	0	100.00
	35	0	0	100.00
1.0 lb/acre[b]	prttrt	46	15	—
	3	0	0	0.00
	7	10	0	100.00
	14	85	30	97.00
	21	0	1	97.80
	28	0	1	99.20
	35	0	0	100.00

[1] PT, posttreatment; prttrt, pretreatment. Application rates followed by a different lowercase letter represent significant differences ($P = 0.05$) between application rates using Student–Neuman–Keuls (SNK) means separation test (Sokal and Rohlf 1981).
[2] Percent reduction after Mulla et al. (1971).

the 32-day study showing 100% control (no live larvae or pupae present) (Table 2) and 2 of 3 assessment periods of the 2nd test (Table 3) showing the same. In both tests, no significant differences were observed between treatments ($P = 0.0111$, df $= 2$, $F = 4.69$ and $P = 0.1302$, df $= 2$, $F = 2.09$, respectively), but significant differences were observed between treatments and posttreatment dipping periods ($P = 0.0001$, df $= 10$, $F = 13.18$ and $P = 0.0001$, df $= 10$, $F = 13.14$, respectively). Student–Neuman–Keuls means separation ranked treatments together in both tests. In addition, SNK means separation ranked the low-application treatment with the control (Table 2). This might be a result of the inconsistency in the data in the 0.5 lb/acre treatment site. Based on percent reduction data, the 1.0 lb/acre WDG application rate was effective against *Cx. quinquefasciatus* larvae in this district.

The Citrus County Mosquito Control study was started late in the season and continued for over 35 days, until one site dried up and the study was stopped. Both the low and high application rates were very successful in Citrus County based on the percent reduction formula (Table 4). An anomaly of this study was that, on 1 assessment day, no larvae were recovered in the control site, leading to an error in the analytical equation; otherwise, mortality was above 97% throughout the study. Although ANOVA showed significant differences ($P = 0.0005$, df $= 2$, $F = 8.12$) between treatments, SNK means separation did not. *Culex nigripalpus* was the prevalent species in this study. The same observations of fewer small larvae maturing to large larvae and a reduction in numbers were made in Citrus County as in the other study sites.

In these operational studies, VectoLex WDG was a successful larvicide. Effective control was quickly achieved and sustained over several weeks. One district retreated and continued to get control above 90% each assessment period. Test sites in each district were typical of their respective control needs. Characteristics of the 9 sites (3 in each area) varied among counties, making the results applicable to many natural Florida Mosquito Control District settings.

Operational studies conducted by district personnel under actual conditions provide valuable information on effectiveness of larvicides. VectoLex WDG proved effective for 3 or more weeks in natural conditions and will become another useful tool in the Florida mosquito control arsenal.

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