EVALUATION OF A NEW TRUCK-MOUNTED ULV SPRAYING MACHINE WITH *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AGAINST LARVAL *CULEX QUINQUEFASCIATUS*

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ABSTRACT

A field study about the effectiveness of a new truck-mounted ultra-low-volume (ULV) machine against larval *Culex quinquefasciatus* Say was conducted at Anastasia Mosquito Control District of St. Johns County, St. Augustine, FL, during the summer of 2017. *Cx. quinquefasciatus* larvae were treated using a ground application at different concentrations of Bti using a new truck-mounted ULV sprayer with a horizontal nozzle. Mortality of larvae was recorded after 24 h, and droplet sizes were measured. Overall, Bti sprayed by the new truck-mounted ULV spraying machine at a concentration of 2.625 mg/L resulted in the highest mortality of mosquito larvae. The results indicate that the Bti concentrations of 0.875 mg/L and 0.065 mg/L resulted in a significant difference in mean larval mortality between each distance from the spray line ($P < 0.05$), while the mortality by the other 3 concentrations (0.477 mg/L, 2.625 mg/L, and 5.25 mg/L) tested did not. The LC50 and LC90 of Bti against larvae were 0.261 mg/L (0.239~0.286) and 1.687 mg/L (1.481~1.922), respectively. The coverage (swath) of the spray by the new ULV machine showed that the Bti could be sprayed at least 33 meters away with a 20 meter width. Therefore, the new truck-mounted ULV spraying machine with liquid Bti could be used to treat a large area effectively and efficiently and as an additional tool for the control of mosquito larvae.

Key Words: *Culex quinquefasciatus*, larvae, mortality, *Bacillus thuringiensis israelensis*, truck-mounted ULV spraying machine

INTRODUCTION

Mosquitoes are a nuisance and many play potential vectors of human diseases, such as malaria, filariasis, and arboviral diseases (dengue fever, Chikungunya, West Nile fever, Zika, and yellow fever) (Mittal 2003). Integrated vector management (IVM), targeting both larval and adult mosquitoes, has been considered the most effective measure for mosquito elimination (Imbahale et al. 2012). Vector control efforts typically target the immature stages and controlling their population is an important task for public health (Lucia et al. 2009). Applying larvicides in breeding sites is still considered a priority for mosquito control (Williams et al. 2014). The traditional approaches to lar-

val control are to find the larval sources and empty/treat those using hand-held sprayers or truck-mounted sprayers with different formulations of larvicides. Previous research illustrated the effectiveness of a hand-pump and gasoline powered backpack sprayer (Sandoski et al. 1985), truck-mounted cold aerosol fogger, Buffalo Turbine mist sprayer (Williams et al. 2014), ULV (Lam et al. 2010, Harwood et al. 2016), and even aerial spraying (Pruszyński et al. 2017) for the control of mosquito larvae.

Bacillus thuringiensis israelensis (Bti), a bacterial larvicide which is highly target specific and appropriate for wide area spraying, is used to control mosquito and black fly larvae (Yap et al. 1997). Different formulations of Bti have been successfully used in

ground applications with hand-pump backpack sprayers/misters, and thermal foggers (Dunford et al. 2014). These applications have a limited utility for large scale control. Truck-mounted cold aerosol foggers have been studied for the large scale control of mosquito larvae over time (Williams et al. 2014). However, their utility has still to be appreciated. Thus, continuous search and evaluation of new effective and economic spray machines for efficient control should concern professionals.

The new truck-mounted ULV spray machine used in this project has been evaluated for barrier treatments (Fulcher et al 2015) but has not been tested for the application of any kind of larvicides. The objectives of this study were to 1) determine whether the new truck-mounted ULV spray machine can be used to apply liquid Bti to control larval *Culex quinquefasciatus* Say in artificial containers, 2) determine the optimal dilution of Bti for larval control by using the new machine, and 3) measure the distance and width (swath) reached by the new machine.

MATERIALS AND METHODS

Study site and mosquito larvae. This study was conducted at the Anastasia Mosquito Control District (AMCD), 120 EOC Drive, St. Augustine, FL, on an unpaved and grassy ground. A susceptible strain of *Cx. quinquefasciatus* Say larvae was provided by the AMCD insectary. Late third- or early fourth-instar larvae were used for the study.

Insecticide. A commercial biolarvicide named AQUABAC®xt (a.i. *Bacillus thuringiensis* var. *israelensis* (Bti), Becker Microbial Products, Inc. Plantation, FL), which contained 8% *Bacillus thuringiensis* subspecies *israelensis* Strain BMP 144 solids, spores and insecticidal toxins, was used for the experiment. The formulation of the biolarvicide is an aqueous suspension. The label application instructions specify the rate of AQUABAC®xt required for control with a sufficient amount of water to provide thorough coverage of the target area when it is applied in conventional aerial and ground application equipment. The formulation was

diluted in purified water at 1:0, 1:1, 1:5, 1:10 and 1:80 (Bti:Water) ratios to provide active ingredient concentrations of 5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L, respectively.

Equipment. The new truck-mounted ULV spray machine (model: LR-4P, 4 nozzles installed on 4 spray heads, American LongRay, Inc., San Francisco, CA) was evaluated in this study. The machine has excellent atomization, quick diffusion, and creates a dense fog that penetrates into spaces and lingers in the air for a longer time. Also, the spray machine heads can be adjusted to swivel 360 degrees horizontally and 180 degrees vertically by manual or remote control. This Truck-mounted cold fogger can spray a variety of chemicals used for public health protection, disinfection, vector control, pest control, and crop protection. This equipment produced droplets at a volume median diameter (Dv0.5) of 14.3 µm and 90th percentile (Dv0.9) of 30.4 µm while spraying water.

Field test. Between 20-30 mosquito larvae were collected using a plastic aspirator from a stock plastic pan (50 cm L × 38 cm W × 7 cm H) and transferred to each of nine white plastic containers (30 cm L × 18 cm W × 10 cm H) with 2 L purified water. The dilutions were sprayed with the new truck-mounted ULV spray machine at a flow rate of 92 ml/min. At a travel speed of 16 Km/h, the certified pesticide sprayer applied the different Bti dilutions at an application rate of 37 ml/ha.

For the optimal concentration in each treatment, all four nozzle heads were angled horizontally (parallel to the ground). The effectiveness of each application was assessed by the mortality of *Cx. quinquefasciatus* larvae at 24 h post treatment.

Three rows of larval containers (from No 1 to No 9) at 2 m apart were placed up to 15 m perpendicular to the spray line. In each row, 3 containers were separated at 5, 10, and 15 m away from the spray line. Alongside treatment container No. 3, 7, and 9, a Florida Latham Bond spinner (model 319; John W. Hock Company, Gainesville, FL) using a 3 mm × 75 mm Teflon® coated slide was deployed to determine spray droplet

sizes. Larval containers were placed on the ground and the spinners were hung at 1.2 m height on the posts. Spray application of each concentration was replicated three times at the same site, with at least one week interval between trials. For each replication, three control larval containers were deployed for 15 minutes at the first row of the spray zone (5 m from the spray line) and were removed before start of the spray. Test larval containers and spinner slides were placed in the field immediately before spray. The truck-mounted ULV sprayer was operated at a speed of 16 Km/h from 17 m before the test area and ended 17 m past the test area. Spinner slides and test larval containers were removed from the field at 5 min and 15 min, respectively post-treatment. The air temperature (25-28 °C), relative humidity (65-90%), wind speed (3-15 Km/hr), and wind direction were recorded at 1.5 m above ground during the field tests. After applications, all larval containers were transferred to the AMCD laboratory and larval mortality counts were taken at 24 h post treatment. All slides were transported to the laboratory for droplet size measurements. Droplets on the slides were measured with DropVision system (Leading Edge, Fletcher, NC) and droplets statistics were determined with the associated software.

The distance and width (swath) reached by the new truck-mounted ULV sprayer was tested using circular plastic disks set in an array at fixed distances. The machine's tank was filled with a mixture of water and red dye to visually detect the swath on the disks. Four rows of collectors, 7 m apart, were set up to 33 m perpendicular to the sprayer on either direction. In each row, four, 4.72 cm diameter circular plastic disks, each with a piece of white test filter paper, were held on the ground at 13, 20, 27, and 33 m from the sprayer. The sprayer was oriented towards the test array and was stationed in the center of the array. Two tests were conducted. The first test was for all four nozzle heads sprayed toward the same direction horizontally (parallel to the ground). The second test divided the four nozzles into two groups, one set spraying horizontally (parallel to the

ground) to one direction and the other oriented toward the opposite direction. Each test spray lasted for 10 seconds. All the white test filter papers were collected and examined under the microscope for the red dye to assess the distance and width (swath) which the sprayer can reach.

Date analysis. The larval mortality was corrected by Abbott's formula (Abbott 1925) when a 5% or more mortality was detected in the control group. The values for larval mortality of each replicate in each test were calculated and the dose-mortality response was assessed using the R-script BioRssay package, which computes the doses of insecticides killing 50% (LC50) and 90% (LC90) of the tested larvae. For the comparison of 24 hour mortality between the 5 different concentrations (5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L) and 3 distances (5 m, 10 m, and 15 m) from the spray line with droplet Dv0.5, nonparametric Kruskal-Wallis analysis and multifactor analysis of ANOVA were conducted, respectively. A posteriori of Tukey HSD means multiple comparison tests was conducted by using Software STATGRAPHICS Plus for Windows Version 4.0-CorpsGWIN P®. Analysis was conducted with R software (Version 3.4.1). A p-value of <0.05 was considered statistically significant.

RESULTS

The Bti dilution ratio by water (1:0, 1:1, 1:5, 1:10, and 1:80) was converted to the actual active ingredient concentrations of 5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L, respectively. There was a significant difference in 24 h larval mortality between each test and control at all test concentrations ($P < 0.05$) (Fig. 1).

As shown in Table 1, there was a significant difference in 24 hr mortality between different Bti concentrations ($\chi^2 = 77.98$, $P < 0.001$). The concentration of 2.625 (1:1) mg/L resulted in the highest larval mortality (100.00%), while 0.065 mg/L resulted in the lowest larval mortality (24.26%). Tukey multiple comparison tests identified that the mortalities from different test concentrations were significantly

Fig. 1. Mean mortality (% $\pm$ SD) of larvae caused by Bti at different concentrations sprayed by the new truck-mounted ULV spraying machine, compared with mortality in control group

different from each other. The mortality from 0.875 mg/L and 2.625 mg/L was generally similar and were significantly higher than those of the other three concentrations tested (0.065 mg/L < 5.250 mg/L < 0.477 mg/L). The LC50 and LC90 of the larvae for Bti were 0.261 mg/L (0.239~0.286 mg/L) and 1.687 mg/L (1.481~1.922 mg/L), respectively, computed by the equation $Y = 1.658 + 2.713 \cdot X$ which was assessed by the dose-mortality response regression analysis.

The comparison of mean larval mortality between distances from the spray line from

each concentration (Table 2) indicated that distance significantly affected mortality from the concentrations of 5.250 mg/L and 0.875 mg/L ($P < 0.05$). However, from the other 3 concentrations (0.477 mg/L, 2.625 mg/L, and 0.065 mg/L) distance did not significantly affect mortality. The concentrations of 0.875 mg/L and 2.625 mg/L at all distances produced 100% larval mortality except for the 15 m distance of 0.875 mg/L concentration, which produced 67.43% larval mortality only. Other concentrations at

Table 1. Mean larval mortality at 24 hr exposure to different concentrations of Bti sprayed by the new truck-mounted ULV spraying machine.

Concentration	N	Mean mortality%	SD	SE	95%CI	K-W(χ^2 value)	P
0.065 mg/L	27	24.26	30.15	5.80	12.33~36.19	77.979	0.000
0.477 mg/L	27	35.44	38.36	7.38	20.26~50.62		
0.875 mg/L	27	89.14	24.73	4.76	79.36~98.92		
2.625 mg/L	27	100	0.00	0.00	100~100		
5.250 mg/L	27	31.85	38.55	7.42	16.6~47.1		

Table 2. Comparison of larval 24h mortality at different distances from the spray line after exposure to different concentrations of Bti sprayed by the new truck-mounted ULV sprayer.

Concentration	Distance from spray line	N	Mean mortality	SD	SE	CI	K-W(χ^2 value)	P
0.065 mg/L	5m	9	39.34	31.08	10.36	15.45-63.23	4.970	0.083
	10m	9	10.91	13.71	4.57	0.37-21.45		
	15m	9	22.52	36.63	12.21	0-50.68		
0.477 mg/L	5m	9	39.91	38.63	12.88	10.21-69.61	0.088	0.916
	10m	9	33.92	41.20	13.73	2.25-65.59		
	15m	9	32.51	39.47	13.16	2.17-62.85		
0.875 mg/L	5m	9	100	0.00	0.00	100-100	11.661	0.003
	10m	9	100	0.00	0.00	100-100		
	15m	9	67.43	34.52	11.51	40.9-93.96		
2.625 mg/L	5m	9	100	0.00	0.00	100-100	—	—
	10m	9	100	0.00	0.00	100-100		
	15m	9	100	0.00	0.00	100-100		
5.250 mg/L	5m	9	44.62	44.21	14.74	10.63-78.61	0.751	0.482
	10m	9	23.44	36.61	12.2	0-51.58		
	15m	9	27.49	35.37	11.79	0.30-54.68		

all the distances produced similar mortality, which were lower than 50%.

There was a significant difference in droplet size Dv0.5 between different test concentrations ($P < 0.05$). At the 0.065 mg/L concentration, the droplet size Dv0.5 was the biggest (31.49 μm), but the smallest (18.42 μm) was at the concentration of 2.625 mg/L (Table 3).

During the first spray test for distance and width (swath), all four nozzles sprayed in the same direction at the same time under SE 1 mph wind. After analysis using light microscopy, 10 out of 16 (62.50%) plates with white filter paper in this test had been stained with red dye. The number of disks with white filter paper stained in each row were 3, 2, 2 and 3 from 13, 20, 27, and 33 m away from the truck sprayer, respectively.

When four nozzles were divided into two groups (two in each group) and sprayed

towards two opposite direction under SE 2 mph wind, 11 out of 16 (68.75%) plates with white filter paper at the downwind side had been stained and the number of disks with white filter paper stained in each row were 4, 3, 3 and 1 from 13, 20, 27, and 33 m away from the truck sprayer, respectively. None of plates with white filter paper had been stained at the upwind side. These results indicate that spray reached up to 33 m in the downwind of the spray and did not travel much to the upwind side.

DISCUSSION

When liquid is used to treat mosquito breeding sites, such as tidal water, salt marshes, catch basins, and storm water retention areas, we usually use conventional spraying equipment or apply our treatments directly in the breeding sites (Xue and Doyle 2005).

Table 3. Droplet size (Dv0.5) of the liquid Bti at different concentrations sprayed by the new truck-mounted ULV spraying machine.

concentration	N	Droplet size(Dv0.5)	SD	SE	CI	chi-square	P
5.250 mg/L	27	31.92	1.48	0.28	0.90~2.06	90.29	0.000
0.477 mg/L	27	24.72	2.77	0.53	1.67~3.87		
0.875 mg/L	27	22.58	4.01	0.77	2.42~5.60		
2.625 mg/L	27	18.42	4.82	0.93	2.91~6.73		
0.065 mg/L	27	31.49	4.00	0.77	2.42~5.58		

Our findings indicate that all 5 Bti concentrations (5.250 mg/L, 2.625 mg/L, 0.875 mg/L, 0.477 mg/L and 0.065 mg/L), resulted in 24.26%-100% larval mortalities after 24 hours of exposure to liquid Bti (1,200 ITU/mg). The significant difference in larval mortality from all concentrations and control and > 90% mortality in some concentrations suggested that the new cold ULV spraying machine could be used to spray liquid Bti to kill mosquito larvae.

The new ULV spray machine with the highest and the lowest dilution ratios of Bti didn't result in significant mosquito larval mortality. The concentrations of 0.785 mg/L and 2.625 mg/L resulting from 1:5 and 1:1 dilutions, respectively were the suitable for the new machine to spray a large area.

The analysis of the larval mortality at different distances from the spray line suggested that the acceptable larvae mortality of >80% within a 15 m distance from spray could be obtained at the dilution ratio of 1:1 (2.625 mg/L) or up to 10 m with dilution ratio of 1:5 (0.875 mg/L).

Droplet size is a very important factor (Harburguer et al. 2012) for killing larvae using Bti (Seleena et al. 1996). In our study, we set three groups of teflon-coated slides atop spinners for droplet size analysis at different distances from the spray line to check the DV0.5. The droplet size data analysis and mortality relationship indicated that the smaller DV0.5 spray resulted in higher larval mortalities.

Additionally, the results of the distance and width reached by the new ULV spray suggests that the farthest distance (swath) should be no more than 33 m along wind when the new truck-mounted ULV spray machine is fixed at a central position spraying towards one side or two sides with nozzles oriented horizontally. Also, it was shown that the droplets could travel further and were more effective with all four nozzles sprayed towards same direction at the same time than that of subgroups (two nozzles for each group) sprayed towards two opposite direction at the same time. Also the distance and width can be dramatically affected by wind speed and direction from the two opposite

directions. But this test was conducted by using dyed water only, and the actual effective distance and width (swath) of the ULV spray machine needs to be addressed further. Finally, the study was conducted in a simulated field with a laboratory-reared sensitive strain of mosquitoes. A large-scale community field test needs to be carried out in the future.

The new truck-mounted ULV spray machine with liquid Bti provided efficient control for mosquito larvae in containers placed on the ground in the field trails. The new ULV spray machine may cover at minimum a 33 m distance x 20 m width. Bti sprayed with the new truck-mounted ULV spray machine is practical and could be considered as an alternative method for treatment for the large scale control of larval mosquitoes.

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