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SR450 AND SUPERHAWK XP APPLICATIONS OF *BACILLUS THURINGIENSIS ISRAELENSIS* AGAINST *CULEX QUINQUEFASCIATUS*[1]

JAMES C. DUNFORD,[2] CRAIG A. STOOPS,[3] ALDEN S. ESTEP,[4] SETH C. BRITCH,[5] ALEC G. RICHARDSON,[4] TODD W. WALKER,[4] MUHAMMAD FAROOQ,[4] DAVID F. HOEL,[2] RAYMOND R. PLATT,[4] VINCENT L. SMITH,[4] ROBERT A. WIRTZ[6] AND JERRY D. KERCE[7]

ABSTRACT. Sprayer comparisons and larval morality assays were conducted following SR450 backpack mist blower and Superhawk XP thermal fogger applications of Vectobac® WDG *Bacillus thuringiensis israelensis* (*Bti*) against *Culex quinquefasciatus*. *Bacillus thuringiensis israelensis* was applied at maximum label rate in a 232.26-m^2 field plot located in north-central Florida with containers placed at 2 heights (ground level and 1.52 m above ground) on stakes positioned 3.04, 6.09, 9.14, 12.19, and 15.24 m from the spray line. Results indicated that there was no significant ($P > 0.05$) difference in 24- and 48-h larval mortality between the 2 sprayers or between the 2 heights. There was significant difference ($P < 0.05$) among the 5 rows, with mortality continuously decreasing with increasing distance from sprayer. Both sprayers provided on average >70% larval mortality 3.04–9.14 m from the spray line, and <60% mortality at 12.19 and 15.24 m. The data suggest that the SR450 and Superhawk XP may be comparable sprayers for use with *Bti* to control mosquito larvae.

KEY WORDS Larval control, container-inhabiting, backpack sprayer, thermal fogger

INTRODUCTION

Bacillus thuringiensis israelensis de Barjac (*Bti*) is known for its efficacy and selectivity against mosquito larvae (Garcia et al. 1980, Margalit and Dean 1985, Seleena et al. 2001). However, application technology and improved application methodologies are still needed for treating mosquitoes with microbial agents (Seleena et al. 2001, Yap et al. 2002, Tan et al. 2012). The magnitude of initial and persistent mosquito control using *Bti* also depends on several environmental factors as well as the concentrations/flow rates applied (Becker et al. 1992, Yap et al. 1997, Seleena and Lee 1998, Tan et al. 2012). Establishing long-term management programs to reduce the source of container-inhabiting mosquitoes is difficult, but the distribution of *Bti* via application methods such as ultra-low volume (ULV) and thermal fogging could provide an economical and rapid means to control larvae (Stoops 2005, Tan et al. 2012).

The effectiveness of *Bti* dispersed by a variety of sprayers has been assessed in a number of studies (Lee et al. 1996, Seleena et al. 1996, Yap et al. 1997, Seleena and Lee 1998, Sharma et al. 2008, Tan et al. 2012, Jacups et al. 2013). ULV application of *Bti* was effective in controlling *Aedes*, *Culex*, and *Anopheles* spp. in field trials when satisfactory droplet profiles provided good coverage and penetration of the target area (Lee et al. 1996). Yap et al. (1997) achieved high larval mortalities of *Aedes* and *Culex* spp., utilizing foggers with an aqua-suspension *Bti* formulation. VectoBac® WDG *Bti* has been applied using calibrated fire trucks in Kabul, Afghanistan, providing control of *Anopheles* and *Culex* spp. in complex contingency environments (Faulde et al. 2008). Similar studies suggest that vegetational cover and *Bti* formulation directly affect the efficacy of *Bti* for larval control (Kelly and Henley 1995, Yap et al. 2002). Additionally, achieving adequate penetration with *Bti* may be difficult for some ULV or thermal fog sprays, whereas coverage area may be restricted using granular *Bti* under certain environmental conditions. Results provided by Yap et al. (2002) indicated water-dispersible granule formulations provided better larval control than liquid formulations when used in thermal fog applications.

Recent studies have revealed that applying *Bti* (VectoBac WDG) with a Stihl® SR420 mist blower provided high mortality (>80%) against *Aedes* spp. for up to 11 wk, with applications penetrating 16 m of dense Australian bushland

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[2] Navy and Marine Corps Public Health Center Detachment, Centers for Disease Control and Prevention, Atlanta, GA 30329.

[3] Navy and Marine Corps Public Health Center Detachment, USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608.

[4] Navy Entomology Center of Excellence, Naval Air Station, Jacksonville, FL 32212.

[5] USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608.

[6] Centers for Disease Control and Prevention, Atlanta, GA 30329.

[7] Camp Blanding Joint Training Center, Starke, FL 32091.

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14. ABSTRACT
Sprayer comparisons and larval morality assays were conducted following SR450 backpack mist blower and Superhawk XP thermal fogger applications of VectobacH WDG Bacillus thuringiensis israelensis (Bti) against Culex quinquefasciatus. Bacillus thuringiensis israelensis was applied at maximum label rate in a 232.26-m2 field plot located in north-central Florida with containers placed at 2 heights (ground level and 1.52mabove ground) on stakes positioned 3.04, 6.09, 9.14, 12.19, and 15.24mfrom the spray line. Results indicated that there was no significant (P . 0.05) difference in 24- and 48-h larval mortality between the 2 sprayers or between the 2 heights. There was significant difference (P,0.05) among the 5 rows, with mortality continuously decreasing with increasing distance from sprayer. Both sprayers provided on average .70% larval mortality 3.04?9.14 m from the spray line, and ,60% mortality at 12.19 and 15.24 m. The data suggest that the SR450 and Superhawk XP may be comparable sprayers for use with Bti to control mosquito larvae.

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without decline in efficacy over distance (Jacups et al. 2013). The residual efficacy of *Bti* (VectoBac WGD) has also been recently examined. Ritchie et al. (2010) conducted studies using 10×, 20×, and 50× the recommended rate of 8 mg/liter and observed that *Bti* provided >90% control for 8, 8, and 23 wk, respectively. Pretreatment of dry containers with megadoses (10×, 20×, and 50× the recommended rate) of dry-formulated *Bti* remained viable up to 8 wk before flooding and did not significantly decrease efficacy through 11 wk against *Aedes aegypti* (L.).

The use of ULV and thermal foggers with effective *Bti* formulations/dosages may increase the ability to penetrate various natural and artificial barriers while reducing costs and work hours. VectoBac WDG (National Stock Number 6840-01-565-8241) is a water-dispersible granule formulation of *Bacillus thuringiensis* subsp. *israelensis* (strain AM65-52) for control of mosquito larvae that has been demonstrated to be effective against *Anopheles, Culex,* and *Aedes* larvae (Fillinger and Lindsay 2006, Aldemir 2009, Tan et al. 2012). Backpack mist blowers designed by Stihl have shown to be an effective tool to dispense *Bti* formulations (Lee et al. 2008, Rydzanicz et al. 2009, Jacups et al. 2013). We provide a comparison of the Stihl SR450 model mist blower (National Stock Number 3740-01-463-0147) to a previously untested (using *Bti*) thermal fogger designed by Curtis Dyna-Fog, Ltd. (Superhawk XP) (National Stock Number 3740-01-480-3040) using VectoBac WDG *Bti* against *Culex quinquefasciatus* Say. The cosmopolitan distribution of *Cx. quinquefasciatus* and its close association with anthropogenic environments enables it to successfully vector several arboviruses as well as filarial nematodes to humans and domestic animals. The SR450 and Superhawk XP sprayers were evaluated to assess their ability to apply *Bti* and to achieve sufficient control of mosquito larvae at specific distances. The studies herein also represent an attempt to identify potentially useful sprayers for use with VectoBac WDG *Bti*, given a National Stock Number in 2010 and currently approved by the Armed Forces Pest Management Board (2013), during contingency or humanitarian military/civilian vector control operations.

MATERIALS AND METHODS

Sprayer applications

Applications of VectoBac WDG (Valent BioSciences, Libertyville, IL) *Bti* using an SR 450 backpack mist blower (Stihl Inc., Virginia Beach, VA) and Superhawk XP thermal fogger (Curtis Dyna-Fog, Dayton, OH) were conducted at Camp Blanding Joint Training Center, Clay County, FL, on October 20, 2011. The field plot was located along Avenue B on the installation (29°58.230′N, 81°58.733′W) and consisted of sparse to moderate grassy vegetation (Fig. 1). Field plot configuration consisted of a 232.26-m^2 area containing 5 rows of 1.52-m stakes (Tipper Tie Fencing Systems, Dothan, AL) positioned 3.04, 6.09, 9.14, 12.19, and 15.24 m from the spray line. Rows were spaced 3.04 m apart. One-half pint size cups (92.1-mm diam, 47.6-mm height with lid) (Neptune Paper Cup Co., Ft. Lee, NJ) were prelabeled following this scheme for each sprayer: REP (1–3): ROW (A–E): DISTANCE (10, 20, 30, 40, 50 ft): LOCALITY (ground, top). Two empty cups were fastened to each stake facing upward using rubber bands and Velcro® strips (Fig. 1). One cup was located near the ground at the base and one at 1.27 m near the top of each stake. Three replicate spray applications were conducted using the Superhawk XP thermal fogger and 3 with the SR450 backpack mist blower using the maximum label rate (14.01 oz/acre = 874 g/ha) rate of Vectobac WDG mixed in a water suspension for a 232.26-m^2 area. Specific sprayer application rates and weather conditions are summarized in Table 1. After each application, cups were capped and collected from the stakes approximately 15 min postapplication. New cups were installed on the stakes, and the next application was conducted. As a negative control for each replicate, 3 cups were handled as above but were maintained at a location 75 m upwind during the actual spray. These controls were capped at 15 min postapplication and held under the same conditions as cups exposed to spray.

After completion of field work, cups were returned to the Navy Entomology Center of Excellence (NECE) and were stored under laboratory conditions for 5 days under normal indoor heating/cooling (room temperature: 20.3°C) cycles before adding water and *Cx. quinquefasciatus* larvae to conduct bioassays.

Bioassays

Each cup taken from the field was filled and swirled with 100 ml of untreated well water and allowed to reach room temperature (20.3°C). Each cup was then seeded with ten 2nd-stage and 3rd-stage instar *Cx. quinquefasciatus* from the colony at the USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology. Cups were maintained in incubators (14L:10D photoperiod) at 27°C, and mortality counts were conducted at 24 and 48 h.

Statistical methodology

The data set consisted of percent larval mortality (24 and 48 h) measured for 2 sprayers (Superhawk XP and Stihl SR 450), 2 heights

Fig. 1. Satellite view of field site located at Camp Blanding Joint Training Center (CBJTC), Starke, FL, showing the configuration of collecting cups, the spray line and direction, and direction of prevailing winds. Upper right inset shows overview of the CBJTC installation with location of study indicated. Lower left inset shows upper collection cup fitted to pole, and lower cup on ground next to pole. Lower right inset shows arrangement of the 25 poles in the field site, looking northwest from the terminus of the spray line; the patch of sand in the background is visible in the upper left of the satellite photograph.

(ground and 1.52 m), 5 rows, 5 distances (3.04, 6.09, 9.14, 12.19, 15.24 m), and 3 replications. For this analysis, a 3-way ANOVA or Kruskal-Wallis (K-W) hypothesis test is appropriate (Zar 1999). Sampling among the 3 replicates was assumed randomized, hence the base sample size $n_{\text{base}} = 3$. For each sprayer and replicate, 3 controls were measured. These 3 control values were averaged and subtracted from the percent mortalities (Abbott 1925), the latter being calculated as the ratio of the measured mortalities to the organism counts at the beginning of the study, for each of the 2 sprayers and each of the 3 replicates.

A preliminary analysis was also conducted to determine whether the dataset conformed to normal (Gaussian) or non-normal behavior. Results of the Kolmogorov-Smirnov test for normality and the Bartlett test for homoscedacity (homogeneity of variances; Zar 1999) showed non-normal behavior, hence the nonparametric 3-way K-W test was used to assess differences in 24- and 48-h larval mortalities between the 2 sprayers, between the 2 heights, among the 5 rows, the 2-way interactions (Sprayer × Height, Sprayer × Row, Height × Row), and the 3-way Sprayer × Height × Row interaction (Zar 1999).

Table 1. Treatment details for *Bacillus thuringiensis israelensis* applications (3 replicates conducted for each sprayer model).

	SR450	Superhawk XP
Dose rate	14.01 oz/acre (0.874 g/ha)	14.01 oz/acre (0.874 g/ha)
Mix rate	179 g WDG/17.0 liter water	393 g WDG/1.89 liter water
Application rate	1280.8 fl oz/acre (85.1 liter/ha)	64.7 fl oz/acre (42.5 liter/ha)
Flow rate	89.27 oz (2.5 liter)/min	9.8 oz (0.28 liter)/min
Swath width	15.24 m	15.24 m
Walking pace	0.69 mph (2.4 km/h)	1.5 mph (5.3 km/ha)
	Replicate 1, 2, 3	Replicate 1, 2, 3
Time	0930, 0951, 1017 h	0815, 0846, 0907 h
Temperature	11.44°C, 12.61°C, 13.16°C	8.77°C, 10.61°C, 10.61°C
Wind speed	0–1.5 mph, 1.8 mph, 0.7 mph	1.1 mph, 0 mph, 0–1.9 mph
Application duration	1 min 48 sec, 1 min 38 sec, 1 min 15 sec	1 min 2 sec, 45 sec, 45 sec

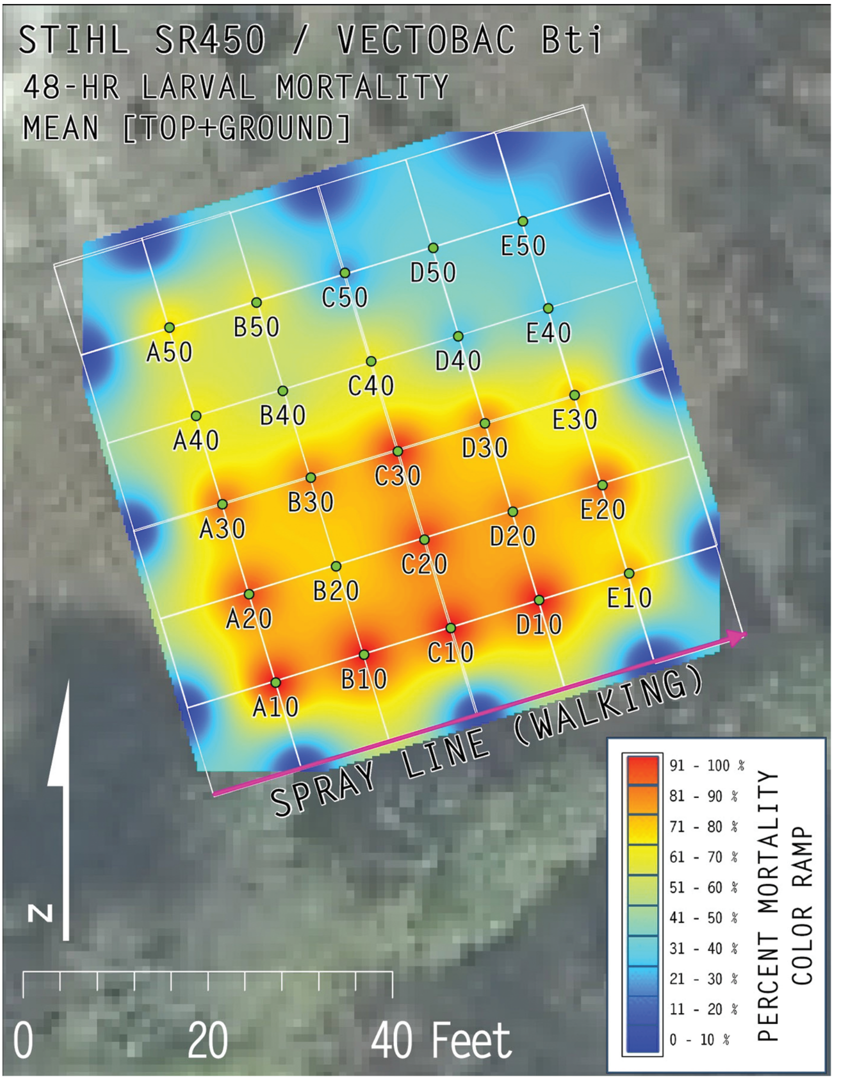

Fig. 2. Interpolated larval mortality surface from VectoBac WDG *Bti* applied with the Stihl SR450 backpack mist blower. Larval mortality is shown as a mean of 48 h mortality in the top- and ground-level cups across the 3 trials at each of the 25 sentinel pole locations. Mortality was interpolated in a GIS across all pole locations to generate the estimated mortality surface, scaled as shown in the percent mortality color ramp key.

Interpolation and analysis of mortality data

Larval mortality data were mapped on a georeferenced grid of 25 poles on a background satellite image of the study area using ArcGIS (Environmental System Research Institute, Redlands, CA), shown in Fig. 1. Pole locations were linked with an attribute table containing fields for Abbott-corrected (Abbott 1925) percent larval mortality data at 24 and 48 h for top- and ground-level cups for each sprayer and for each trial, as well as per-trial mortality data averaged between top- and ground-level cups at each pole. The attribute table also contained fields for mean mortality data averaged across all 3 trials for each sprayer for top and ground level and mean top-ground at both 24 and 48 h. The inverse distance weighting (IDW) interpolation command with default settings and a fixed search radius of 15.2 m in ArcGIS was used to derive estimated percent mortality surfaces across the 25-pole spray grid for each field in the attribute table (examples shown in Figs. 2 and 3). Interpolated mortality surfaces provide a quantified and comparable means to visualize spatially heterogeneous mortality data from aerosol pesticide applications (Britch et al. 2010, 2011).

Statistical analysis of tabulated mortality frequencies as described earlier is a powerful method to compare performance of the SR450 and the Superhawk XP; however, visualization and

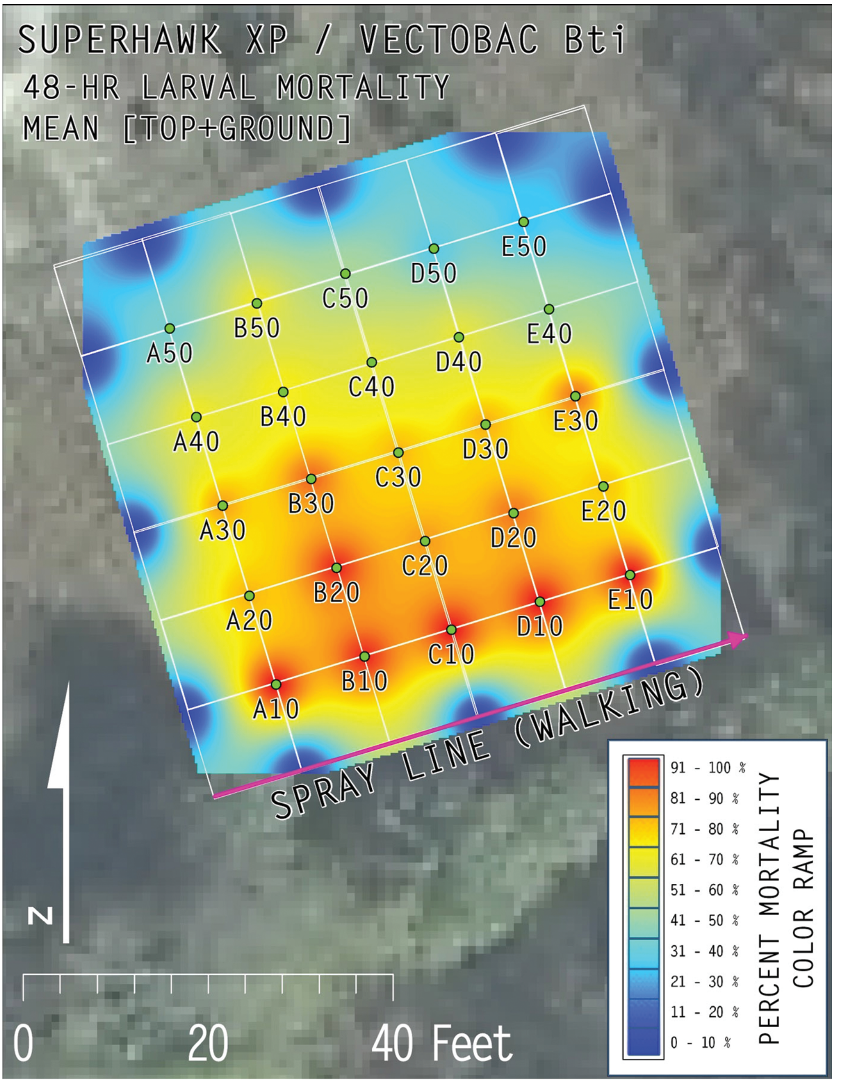

Fig. 3. Interpolated larval mortality surface from VectoBac WDG *Bti* applied with the Superhawk XP thermal fogger. Larval mortality is shown as a mean of 48 h mortality in the top- and ground-level cups across the 3 trials at each of the 25 sentinel pole locations. Mortality was interpolated in a GIS as described in Fig. 2.

analysis of mortality surfaces from IDW also account for the proximity of high-mortality areas in the treatment zone. To assess the relative efficacy of each sprayer, each mortality surface was analyzed by tallying pixels with values of ≥90% mortality, and the tallies were evaluated between sprayers (i.e., SR450 compared to Superhawk XP) and within sprayers (i.e., 24-h mortality compared to 48-h mortality, and top- compared to ground-level mortality) with a *t*-test in SigmaStat (Systat Software Inc., San Jose, CA).

RESULTS

Results of the 3-way nonparametric Kruskal-Wallis test at the 95% confidence level showed no significant difference in the 24-h larval mortality between the 2 sprayers or between the 2 heights, and likewise no significant difference in the 48-h larval mortality. However, the 24 h ($DF_1 = 4$, $DF_2 = 299$, $X^2 = 21.3629$, $X^2_{crit} = 2.4002$, $P < 0.0001$) and 48 h ($DF_1 = 4$, $DF_2 = 299$, $X^2 = 17.5879$, $X^2_{crit} = 2.4002$, $P < 0.0001$) mortalities were significantly different among the 5 rows, with the Tukey Multiple Comparisons test showing significant differences among all 10 pairwise combinations of the 5 rows, with mortality continuously increasing with decreasing distance from the sprayer. The 24-h mortality increased in the order Row E (farthest from sprayer) << Row D << Row C << Row B << Row A (closest to sprayer). The 48 h mortality likewise increased in the order Row E ≪ Row D ≪ Row C ≪ Row B ≪ Row. For 24-h mortality, the Sprayer × Height, Sprayer × Row, Height × Row, and Sprayer × Height × Row interactions were all insignificant. Likewise, for 48-h mortality, the Sprayer × Height, Sprayer × Row, Height × Row, and Sprayer × Height × Row interactions were all insignificant. Among the 3-way combinations, the 24- and 48-h larval mortalities were highest for the Superhawk+ Ground+Row A combination and lowest for the Stihl + 1.52 m + Row E combination. Based on power analysis of the desired power and precision, the sample sizes used in this study were statistically adequate.

Both sprayers provided on average >70% larval mortality 3.04–9.14 m from spray line and <60% mortality at 12.19 and 15.24 m. Maps of representative results of interpolation of percent larval mortality data are shown in Figs. 2 and 3. Statistical analysis of interpolated percent larval mortality data with *t*-tests on tallies of pixels with ≥90% mortality revealed no significant differences ($P > 0.05$) among the following comparisons: SR450 vs. Superhawk XP applications (3 trials each, top cups) at 24 or 48 h; SR450 vs. Superhawk XP applications (3 trials each, ground-level cups) at 24 or 48 h; SR450 vs. Superhawk XP applications (3 trials each, mean percent mortality between top and ground-level cups) at 24 or 48 h; SR450 applications (3 trials, top cups) vs. SR450 applications (3 trials, ground-level cups) at 24 or 48 h; Superhawk XP applications (3 trials, top cups) vs. SR450 applications (3 trials, ground-level cups) at 24 or 48 h; SR450 applications (3 trials, mean percent mortality between top- and ground-level cups) 24 vs. 48 h; Superhawk XP applications (3 trials, mean percent mortality between top- and ground-level cups) 24 vs. 48 h; or SR450 vs. Superhawk XP applications (pooled top- and ground-level mortality across all 3 trials for each machine) at 24 or 48 h.

DISCUSSION

The results of this study demonstrate that VectoBac *Bti* can be applied at concentrations sufficient to induce larval mortality with both the SR450 mist blower and Superhawk XP thermal fogger at a distance up to 15.24 m across open ground in a warm temperate region. Although there was a significant decline in larval efficacy beyond 12.19 m for either sprayer, weather conditions were less than favorable on the days of application, including little or no wind, shifts in wind direction during some spray replicates, and temperatures below 15.5°C. Britch et al. (2010) noted that thermal fog applications create turbulent clouds of very small heated droplets that in low or zero wind speeds will disperse over a greater area than ULV-generated droplet clouds. The cooler, larger droplets from a ULV application possess less internal energy and greater inertia and require a relatively higher magnitude of turbulence from wind energy for dispersal. Thus, little or no wind during a ULV application could cause the majority of droplets to drift to the ground more rapidly compared to a thermal fog application. The Stihl most blower, although capable of creating ULV droplets, typically dispenses low-volume droplets and not true ULV. Additional evaluation of these sprayer models should include complete droplet profiles along with larval efficacy trials.

This study represents the first time the Superhawk XP thermal fogger has been used with a *Bti* formulation and, to the best of our knowledge, the first time a direct comparison has been conducted between thermal fog and cold mist application of *Bti*. The results of this study suggest that a thermal fog application with the Superhawk XP may be comparable to a standard cold mist application with the SR 450 using VectoBac WDG *Bti* for larval control. The Superhawk XP is compact and lightweight and provides a dense fog. In addition, the unit's injection point and heat exposure interval apparently do not denature *Bti* toxins, thus allowing *Bti* dispersal without degradation of efficacy to this biological insecticide. The only potential drawback observed during field trials was a slight buildup of *Bti*/water mixture at the tip of the nozzle.

Statistical analyses of both standard and map-derived larval mortality data indicate no significant difference in 24- and 48-h mortality between the 2 sprayers or between the 2 heights. We included 48-h mortality counts in this study to mimic previous studies evaluating *Bti* applications to determine if longer exposure time to *Bti* would provide an increase in larval mortality. Our results should be compared to results of future studies to further evaluate if 48-h mortality should be considered when evaluating aerosol applications of larvicides. *Bacillus* crystal spots persisted in cups without water for 5 days before assays were conducted during this study. Although the crystal spots (=evaporated droplets) were not exposed to outdoor conditions, VectoBac WDG *Bti* has been observed to persist in natural environments up to 8 wk before inundation (Ritchie et al. 2010). The duration that VectoBac may persist in the environment suggests it would be effective in "preemptive" larvicide applications. One advantage of the VectoBac formulation integrity for the current and future studies evaluating sprayer equipment for delivering *Bti* is the elimination of the need to transport and preposition sentinel larvae in the field, which introduces substantial logistical difficulties to experimental design and associated larval mortality.

Several considerations should be made before initiating and incorporating larviciding into a vector control management scheme. Field conditions such as wind speed and direction, temperature, and humidity, as well as larval microhabitat conditions, must be considered because environmental factors can affect the efficacy of *Bti*. Control operations should choose a *Bti* dose rate determined by budget and operational logistics, with a higher dose appropriate for situations where technicians have infrequent access to treatment sites (Jacups et al. 2013). Decisions on treatment swath width should be assessed for each treatment site depending on vegetation or structure density and site access. Optimization of a spray technology should include trials with a range of nozzles, dilutions or formulations, timing and speed of delivery, and machine settings (Britch et al. 2010). Further evaluation of variable application rates (i.e., application duration) should also be made. Application duration, wind speed, and temperature varied slightly for each replicate (Table 1) during this study, and the amount of product applied during each replicate may have also varied. Achieving exact replicates during field trials is challenging; however, even with slightly shorter application durations (Table 1) the thermal fogger provided similar results to the mist blower.

This study was an initial attempt to standardize the larval mortality associated with the swath width of vector control equipment, which would be useful data for all application equipment evaluated under similar environmental conditions. The goals of NECE and the Armed Forces Pest Management Board's Deployed War Fighter Protection program are to develop and evaluate user-friendly, deployable methods to control disease-transmitting mosquitoes. Contingency operations, especially humanitarian-based missions, are becoming more common for US military forces. Field conditions for personnel deployed during contingency operations are often less than favorable, operational entomology personnel are few, and opportunities for vector control pesticide treatments are irregular; hence smaller, lighter, low-maintenance application equipment is preferred. Application methodologies implemented during this project may provide insight into the efficacy of larval control near US troop encampments and troops operating in urban environments. Additional research and testing should attempt to identify and adapt currently available portable sprayer models and *Bti* formulations in environments where both natural and artificial barriers may be present. Integrating adulticides with *Bti* should also be investigated with both standard and electrostatic cold mist and thermal fog application equipment.

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