CHARACTERIZATION AND EFFICACY OF VECTOBAC® WDG APPLICATIONS TARGETING CONTAINER-INHABITING MOSQUITOES USING AN UNMANNED AERIAL VEHICLE

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ABSTRACT

Application of *Bacillus thuringiensis israelensis* (*Bti*)-based liquid larvicide for the control of container inhabiting mosquito species, such as *Aedes aegypti*, is typically performed through the use of portable sprayers, truck-mounted mist systems, or manned fixed and rotary-wing aircraft. Recently, unmanned aerial vehicles (UAV) have provided a new avenue for control material applications. Here we report the characterization and efficacy of Wide Area Larvicide Sprays (WALS™) applications of Vectobac® WDG using UAV technology. Collier Mosquito Control District's PrecisionVision 13 UAV was outfitted with the PrecisionVision Liquid Application System using four flat-fan TeeJet nozzles capable of producing fine/extra fine droplets for WALS applications of Vectobac WDG at a rate of 0.5 lb/A. Droplet characterization and mortality assays indicated that we achieved nearly 100% efficacy within 30-40 ft swaths. Furthermore, semi-field tests indicated delivery of the control material with six to seven adjacent swaths of 30 ft to open bioassay containers at the desired application rate within a 1 A treatment block, which was supported by reduction of natural populations of container inhabiting mosquitoes in the treatment area.

Key Words: *Aedes aegypti*, *Bacillus thuringiensis israelensis* (*Bti*), containers, larvicide, Unmanned Aerial Vehicles (UAV)

Unmanned aerial vehicle (UAV) technology represents a novel tool for mosquito control operations. The potential for UAV usage has rapidly expanded to different operational activities, including enhanced larval and mosquito surveillance methods (Hass-Stapleton et al. 2019), habitat mapping (Hardy et al. 2017, Carrasco-Escobar et al. 2019) and precise application of control materials. Small-scale applications of control materials using UAVs has successfully been used to target agricultural pests and pathogens (Qin et al. 2016, Hunter et al. 2019, Wang et al. 2019, Xiao et al. 2019), as well as adult mosquito populations (Li et al. 2016). Recently, the Collier Mosquito Control District (the District) began incorporating UAVs into their Integrated Pest Management Program, including the adoption of the PrecisionVision 13 (PV13) UAV (Leading Edge Aerial Technologies, Fletcher, NC) outfitted with the PrecisionVision Liquid Application System (Leading Edge Aerial Technologies, Fletcher, NC).

Application of *Bacillus thuringiensis israelensis* (*Bti*)-based liquid larvicide for the control of container inhabiting mosquito species is typically performed through the use of portable sprayers, truck-mounted mist systems, or manned fixed and rotary-winged aircraft. Here we report the characterization and efficacy of the proprietary Wide Area Larvicide Spray (WALS™, Valent Biosciences, Libertyville, IL) applications of the *Bti*-based water dispersible granule, Vectobac® WDG (Valent Biosciences, Libertyville, IL), using UAV technology in targeting container-inhabiting mosquito species. The District's PV13 UAV Liquid Application System was equipped with four flat-fan TeeJet nozzles (#800067) capable of producing fine/extra fine droplets for WALS applications of a 12% VectoBac WDG suspension in water at an application rate of 0.5 lb/A (0.6 kg/Ha). A flow rate of 40 oz/min (1.2 L/min), at 10 mph (16.1 km/h) and an application height of 30 ft (9.1 m) was suitable for delivery of 0.5 lb/A at a theoretical swath of 30 ft.

Initial calibrations and swath characterizations included the use of VectoBac WDG mixed with food-grade red dye (FD&C Red Number 40 Granular) at a rate of approximately 1 oz/gal (7.8 ml/L). The system was calibrated and flow-checked to achieve 40 oz/min of the 12% VectoBac® WDG suspension. For droplet characterization and application efficacy, two sampling lines were established – one setup to assess droplet distribution into the wind (narrowest swath) and another taking into account cross wind (widest swath).

The "into wind" format included a 75 ft (22.9 m)sampling line placed perpendicular to the wind with UAV applications occurring toward the wind. Twenty-five card sampling stations were set at 3 ft (1 m) intervals. At each station, one 7 x 9 cm Kromekote® card (CTI Paper USA Inc, Sun Prairie, WI) was secured to a CD Jewel Case and placed flat on the ground. A larval assay cup (8 oz soup container; Good Start Packaging, Bedford, NH) was placed every 6 ft (1.8 m) starting at 15 ft (4.8 m) to 69 ft (21 m) for a total of 10 cups across 54 ft (16.5 m). Three replicate single-pass flights at the 36 ft (11 m) station with an application rate of 0.5 lb/A were performed using the PV13 UAV – after each replicate, cards and larval assay cups were replaced. Applications were made prior to sunrise – between 6:04 AM and 6:38 AM – to maximize ground deposition of small droplets. Kromakote cards were analyzed for droplet measurements using BacDrop™ (Valent Biosciences, Libertyville, IL) and larval assay cups were brought back to the District's laboratory for mortality assays. Larval assays were performed by adding 100 mL of deionized water and approximately 20 late-2nd and early-3rd instar laboratory reared *Aedes aegypti* L. larvae (Orlando 1952 Strain) to each assay cup. Non-treatment assay cups, placed in untreated area at the District headquarters, supplemented with 100 mL of deionized water were used as controls. Larval mortality was scored at 0 hr, 1 hr, 24 hr, and 48 hr post-treatment, and determined by the absence of movement upon disturbance. Percent mortality was determined by dividing the number of dead larvae by the total number of larvae and multiplying by 100, and an average was produced between the three replicates.

The "cross wind" design included a 200 ft (61 m) sampling line placed parallel to expected wind pattern with UAV applications occurring perpendicular to the card line. Forty sampling stations were established starting from 0 to 200 ft at 5 ft (1.5 m) intervals along the sampling line. At each station one Kromekote card secured to a CD Jewel Case was placed flat on the ground as described above. A larval assay cup was placed every 10 ft (0.9 m) starting at 20 ft (6.1 m) to 120 ft (36.6 m) for a total of 10 cups across 100 ft (30.5 m). Three replicate single-pass flights commenced as described above; however, the PV13 UAV crossed the card line at the 30 ft station to allow drift of the product down the sampling line – after each replicate, cards and larval assay cups were replaced. Applications were made prior to sunrise – between 7:05 AM and 7:46 AM – to maximize ground deposition of small drops. Droplet analysis and larval assays were performed as described above.

The "into the wind" design, representing the narrowest swath, resulted in an average droplet median diameter ($Dv_{0.5}$) of 216.67 ± 4.78 µm across all three replications (Table 1, Figure 1A). Droplet size was greatest

Table 1. Droplet Characterization.

	N	Dv 0.1 (um)	Dv 0.5 (um)	Dv 0.9 (um)	Mean Total Droplets	Mean Drop Density (cm^2)
Into Wind	3	150.33 ± 6.13	216.67 ± 4.78	285 ± 15.12	190.39 ± 43.89	3.07 ± 0.72
Cross Wind	3	131.33 ± 9.74	235.67 ± 22.95	332.33 ± 25.95	203.75 ± 36.13	3.78 ± 0.18

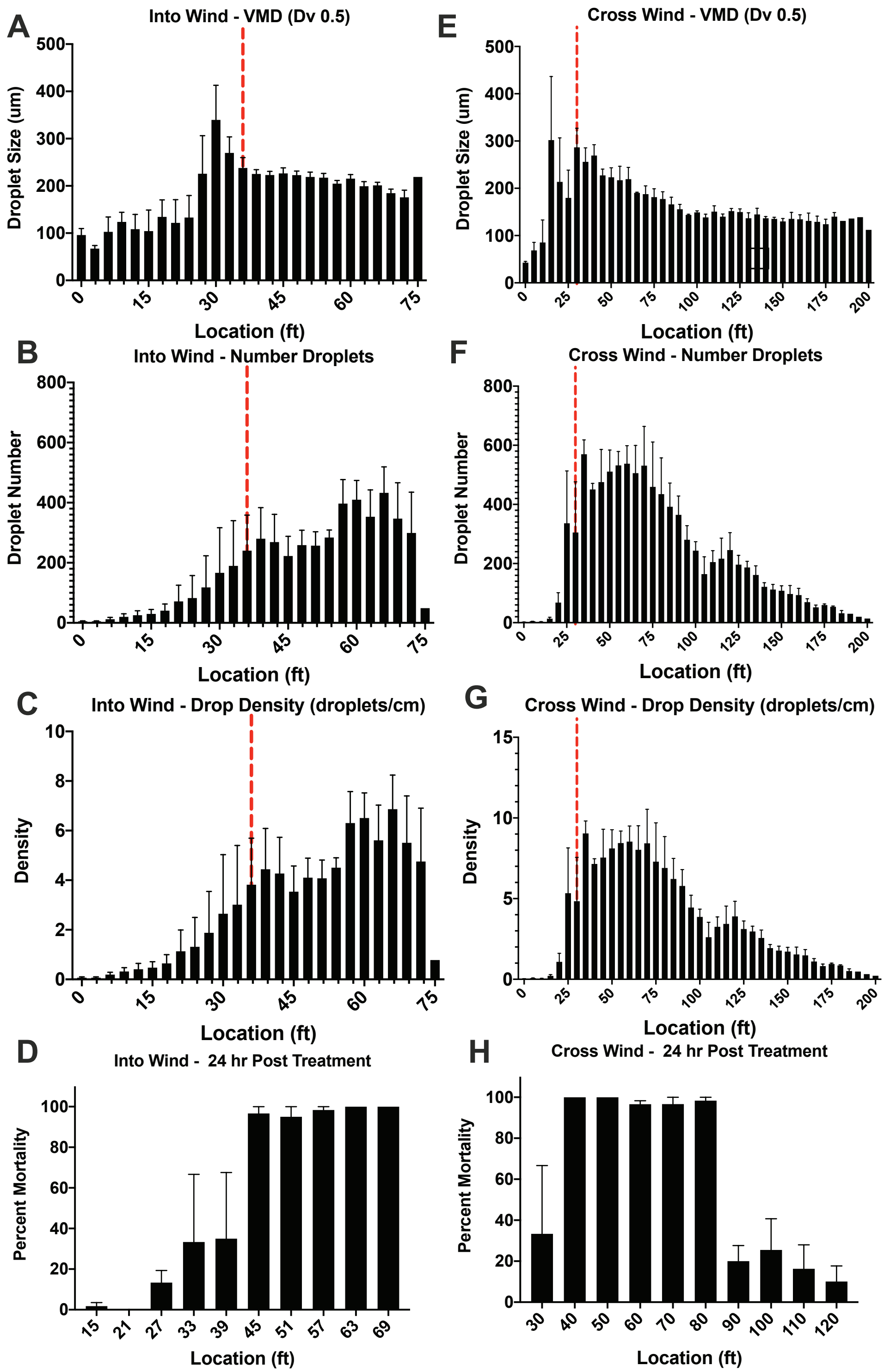

Figure 1. Droplet characterization and larval mortality for into wind (A-D) and cross wind (E-H) designs. Red dashed line indicates flight line. Data represent three replicates and are shown as mean ± SEM. (A) Average Volume Median Diameter (VMD), (B) average number of droplets, and (C) average droplet density, and (D) larval mortality across collection stations. (E) Average VMD, (F) average number of droplets, (G) average droplet density, and (D) larval mortality across collection stations of cross wind characterization.

near the flight line (36 ft station); however, droplets were relatively larger in size from 30-75 ft (9.1-22.9 m) compared to 0-24 ft (0-7.3 m) collection stations (Figure 1A), which may represent drift of the product to changing wind direction or variable flow between the spray system booms. Average droplet number and droplet density followed a similar pattern (Figure 1B-C). Larval assays displayed mortality of greater than 90% between the 45-69 ft (13.7-21 m) stations, indicating an effective swath of approximately 24 ft on average based on mortality rates at 24 hr post-treatment (Figure 1D). Mortality was not observed in the non-treatment control cups. The narrowest effective swath may be larger than captured in mortality assays, as the drift pattern appears to have extended beyond the last larval assay cup placed at the 69 ft station (Figure 1A-D). Temperature averaged 78.6 °F (25.9 °C) with an average relative humidity of 95% and a wind speed of 1.7 mph (0.76 m/s) NE.

Likewise, the “cross wind” format resulted in a $Dv_{0.5}$ of 235.67 ± 22.95 µm across all three replications (Table 1, Figure 1E), with the greatest number of droplets accumulating between the 30-90 ft (7.1-27.4 m) stations (Figure 1F-G). Droplet size was also largest near the flight line (30 ft station) with droplets extending beyond the 200 ft station (Figure 1E). Larval assays displayed mortality of greater than 90% between the 40-80 ft (12.1-24.3 m) stations, indicating an effective swath of 40 ft on average based on mortality rates at 24 hr post-treatment (Figure 1H). Mortality was not detected in the non-treatment control cups. Together these results suggest that the UAV liquid larvicide spray system equipped with the four flat-fan TeeJet nozzles were sufficient to deliver 0.5 lb/A at an effective droplet spectrum recommended for aerial WALS applications of a 12% suspension of VectoBac WDG. Temperature averaged 78.0 °F (25.6 °C) with an average relative humidity of 95%, and a wind speed of 1 mph (0.45 m/s) NE.

We then conducted two semi-field trials to determine efficacy within a treatment block using two different design methods. The first semi-field trial was performed by placing 10 larval assay cups within a 1 A treatment block located within an open area at the District headquarters (Figure 2A). Three replicate multi-swath treatments were performed using the PV13 UAV with an application rate of 0.5 lb/A with the 12% suspension of VectoBac WDG at an application height at 10 ft above canopy (40-50 ft above ground) and a speed of 10 mph. After each replicate treatment of 6 adjacent swaths, cups were replaced. Control cups were placed in an untreated area at District headquarters during trials. Applications were made prior to

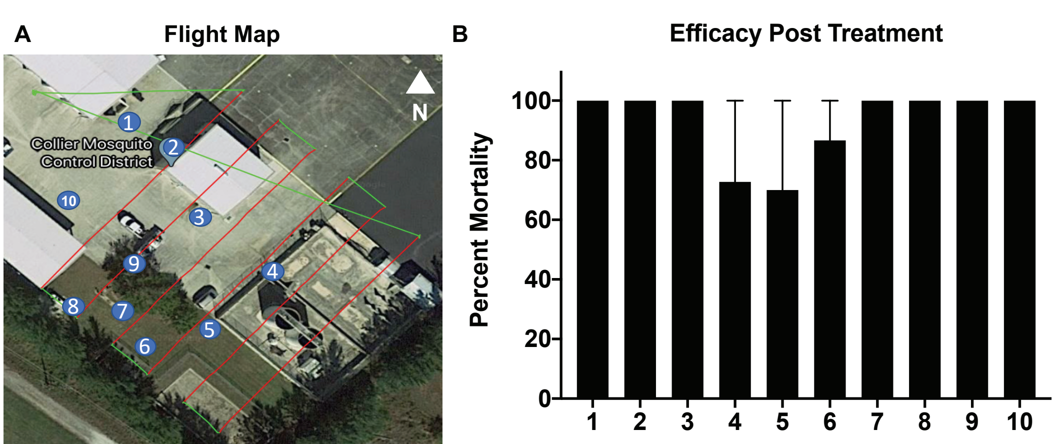

Figure 2. (A) A flight map showing six 30 ft swaths and bioassay sentinel cup placements. (B) Percent mortality for each cup placement at 24 hr post treatment. Graphical analysis was performed using GraphPad Prism 8. Data represent three replicates and are shown as mean ± SEM.

sunrise – between 6:45 AM and 7:10 AM – to maximize ground deposition of small drops. Temperature averaged 78.1 °F with an average relative humidity of 96% and a wind speed of 3 mph (1.3 m/s) ENE. Treatment was manually offset to account for drift. Larval assay cups were brought back to the District's laboratory for mortality assays as described above. Nearly 100% efficacy was achieved within 24 hr post-treatment when compared to non-treatment controls, with the exception of the final replicate displaying reduced efficacy in cups 4-6 (Figure 2B). Reduced efficacy in the final replicate is likely to have been caused by an increase in wind speeds and change in wind direction toward the end of the field trials.

The second semi-field trial was performed in conjunction with an operational treatment of a 1 A treatment block in an urban industrial park known to harbor large number of container inhabiting mosquitoes on September 26, 2019. The habitat designated for treatment contained several larval habitats including tires, pallets, paint buckets, storage containers, PVC and large metal pipes, and trash bins. The habitat contained abundant garbage and debris, with emergent vegetation. Because of its high production rate of container-inhabiting mosquito species, the industrial park is routinely on rotations for larvicide treatment using VectoBac WDG from the District's Buffalo Turbine sprayer (Buffalo Turbine, Springville, NY); however, treatment to this area had not been made in over 6 wk prior to UAV applications. No adulticide treatments had been performed in the area in 2019. Ten larval assay cups were placed in the 1 A treatment block to simulate containers in cryptic habitats (Figure 3A). In addition, pre-treatment larval dips, human landing rates[1], and BG-trap (Biogents AG, Regensburg, Germany) data were collected (Figure 3C-F).

A single replicate multi-swath application was made at the industrial park using the PV13 UAV at a rate of 0.5 lb/A of the 12% suspension of VectoBac WDG at an application height of 30 ft above canopy/buildings (75 ft [22.9 m] above ground). The application of 7 adjacent swaths was made prior to sunrise – at 7:11 AM – to maximize ground deposition of small drops and minimize disruption to industrial park employees. Temperature was 71° F with a relative humidity of 93% and a wind speed of 1 mph NE. Larval assay cups were brought back to the District's laboratory for mortality assays as described above. Control cups were placed in untreated area at the District headquarters during trials. Nearly 100% efficacy was achieved within 24 hr ($t = 60.45$, $df = 9$, $P < 0.0001$) and 48 hr ($t = 64.29$. $df = 9$, $P < 0.0001$) post-treatment in larval assay cups (Figure 3B) compared to non-treatment controls.

Post-treatment larval dips, landing rates, and BG-trap data were collected each week for four weeks following VectoBac WDG treatment at the industrial park. Larval density appeared lowest at 2-wk post-treatment ($t = 2.672$, $df = 8$, $P = 0.0283$) when compared to pre-treatment dips, with larval density reestablishing by 4-wk post-treatment (Figure 3C). By 1-wk post-treatment, the percent of positive containers in the area reduced by 60% and remained low during the duration of surveillance activity in the area (Figure 3D). Larvae inhabiting tires were most difficult to control, possibly due to the difficulty of the material to penetrate the standing water hiding within the tires (data not shown). Human landing rates (*Ae. aegypti*) were significantly reduced by 1-wk post-treatment ($t = 7.780$, $df = 4$, $P = 0.0015$) and remained low for 3 wk (2-wk: $t = 8.795$, $df = 4$, $P = 0.0009$; 3-ws: $t = 4.908$, $df = 4$, $p = 0.0080$) compared to pre-treatment landing rates, with the population reestablishing within 4-wk post-treatment (Figure 3E). Trap collections using BG-Sentinel traps baited with CO_2 and BG-lure depicted a decreased trend in the container-inhabiting species *A. aegypti* and *Culex quinquefasciatus* (Say) by 2-wk post-treatment, with the population reestablishing within 3-wk post-treatment (Figure 3F). *Culex nigripalpus* (Theobald), previously identified in containers in the area, also depicted a decreased trend. Comprehensive data for larval dips, human landing rates, and

[1]Landing rates were performed as defined by CMCD SOP#12 (effective date: January 15, 2018) in accordance with F.S. Chapter 388 and 5E-13.

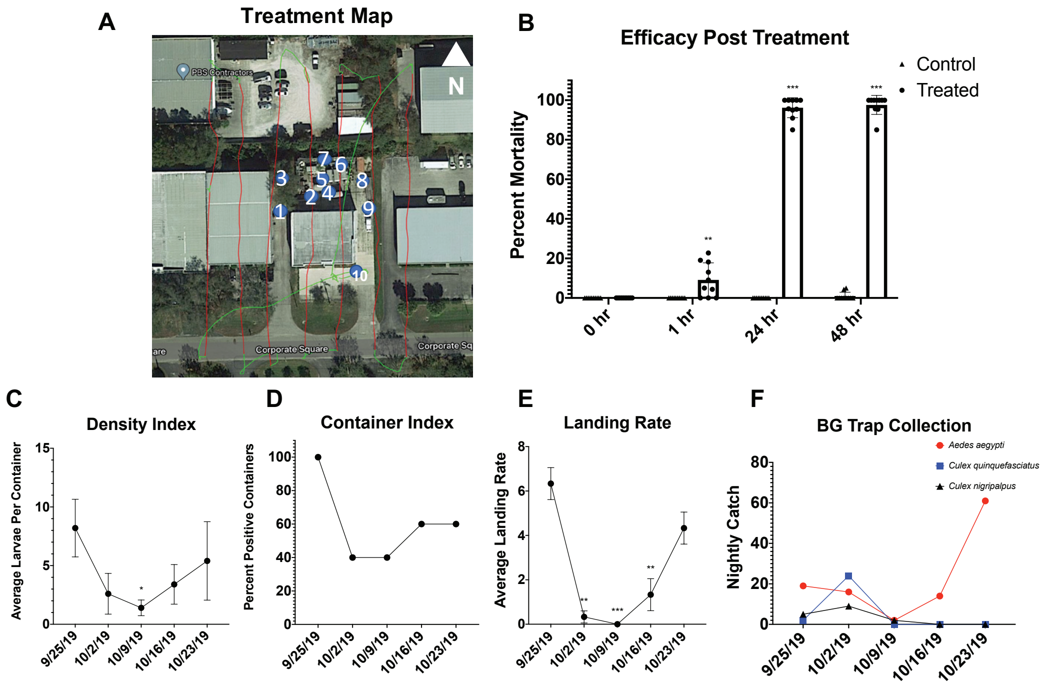

Figure 3. (A) 1 A flight map showing seven 30 ft swaths and cup placements within industrial park. (B) Percent mortality at 0 hr, 1 hr, 24 hr, and 48 hr post-treatment. (C) Average larvae per container (Density Index), (D) percent positive containers (Container Index), (E) average human landing rate, and (F) BG-trap collections determined weekly for 4 wk post-treatment. Graphical and statistical analysis were performed using GraphPad Prism 8. Data represent three replicates and are shown as mean ± SEM where appropriate. A two-tailed student's t-test was performed to indicate statistical significance where appropriate; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

BG-Sentinel traps were not collected for a non-treatment control site; however, weather patterns and larval habitat (water-holding containers) remained consistent throughout the study.

This study indicated that the District's PV13 UAV equipped with the PrecisionVision Liquid Application System was suitable for delivery of a 12% VectoBac WDG suspension using the WALS application strategy. Four tee-jet nozzles capable of producing fine/extra fine droplets, a flow rate of 40 oz/min, at 10 mph, and either 6 or 7 adjacent swaths of 30 ft effectively delivered 0.5 lb/A of VectoBac WDG into cryptic containers and reduced juvenile and adult container-inhabiting mosquito populations for up to 21 days. For adequate control of container-inhabiting mosquitoes using the PV13 UAV and VectoBac WDG, the District will plan to conduct multi-swath applications every 14-21 d in areas suitable for UAV use.

Although these results with our UAV system are highly encouraging and point to broader future adoption of UAVs in operational vector control, tank capacity of the larvicide system, acreage needing treatment, labor available, and applicator skill must be taken into consideration when deliberating incorporation of UAVs in a mosquito control operation. For example, the District's current PV13 application system has a tank capacity of 2.5 gal (9.5 L) which supports delivery of VectoBac WDG to 5 A (2 Ha) per tank requiring returns and refills for larger areas. Also, the operator must maintain visual line of sight with the UAV either directly or via communication with another employee. Both of these aspects often require additional labor. Furthermore, regulations set by the Federal Aviation Administration (FAA) and the Florida Department of Agriculture and Consumer Services (FDACS) require UAV operators conducting mosquito control pesticide applications to obtain a Part 107 Remote

Pilot Certification, a Public Health Pest Applicator License, and an Aerial Pesticide Applicator License.

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