Evaluation of efficacy of ESS-GPS sprayer versus a non-electrostatic sprayer when applying TalstarOne at varying rates to bushes and panels and exposing to *Aedes aegypti* mosquitoes.

Eric Snell and David Weiner
Snell Scientifics, LLC
472 Cannafax Road
Barnesville, GA 30204
770.358.4591

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472 Cannafax Rd ~ Barnesville, GA 30204 ~ 770.358.4591

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Objectives:

To determine the efficacy of ESS-GPS electrostatic sprayer versus WTS sprayer when applying TalstarOne at varying rates to bushes and aluminum panels and exposing the treated bushes and panels to adult *Aedes aegypti* mosquitoes.

Treatments:

1. TalstarOne 0.444% applied to bushes by ESS-GPS sprayer
2. TalstarOne 0.06% applied to bushes by WTS sprayer

Materials and Methods:

There are two (2) different evaluations for this series of tests: (1) A release room test using treated bushes from 2 separate treatment methods, and, (2) a panel test evaluating the efficacy of the same 2 treatment methods when applied to aluminum panels and exposing mosquitoes to each method. The applications of both methods were applied at the same time and the same 2 methods were applied to both bushes and panels.

I. Application Methods to Bushes and Panels:

A total of four (4) bushes *(Osmanthus fragrans)* were treated at Day 0 with two different sprayers:

Two (2) bushes were treated with the ESS-GPS unit with electrostatic technology. The sprayer was calibrated to deliver 0.077 gal/min. The material was applied at 7.4 oz of active ingredient/gal**.** which equals 0.44% solution. Two (2) separate bushes were treated with a non-electrostatic sprayer. The sprayer was calibrated to deliver 0.57 gals/min. The material was applied at 1.0 oz of active ingredient/gal which equals 0.06% solution. The bushes were treated at walking speed to ensure accuracy and full exposure. The differences in the mixture rates were calculated to equate for the lower flow rate of the ESS technology compared to the non-electrostatic sprayer. Based on spraying each technology for the same amount of time but at different flow rates, the rate mixed in the ESS-GPS was increased in order to apply equal amounts of active ingredients.

A total of sixteen (16) aluminum panels 5”x8” were treated at Day 0 with the same two different sprayers and rates. Eight (8) panels were treated with the ESS-GPS unit with electrostatic technology. The sprayer was calibrated to deliver 0.077 gals/min. The material was applied at 7.4 oz of active ingredient/gal which equals 0.44% solution. Eight (8) panels were treated with the Liquid-Only sprayer. The sprayer was calibrated to deliver 0.57 gals/min. The material was applied at 1.0 oz of active ingredient/gal which equals 0.06% solution.

The panels were fixed to a wooden frame and positioned 30” above (and parallel) to the ground. This method was used to ensure adequate exposure to all surfaces during treatment. The panels were treated at walking speed to ensure accuracy and full exposure. After treatment the bushes and panels were removed to a protected area and allowed to dry.

II. Environmental Aging of Bushes and Panels:

The bushes were placed under protected conditions until 15 days after treatment (DAT). At 15 DAT, the bushes were removed from cover and exposed to prevailing weather conditions. At 7 DAT and 15 DAT the panels were removed from the frames, transported to the testing area, and arranged on the laboratory counter for evaluation as described below. At the end of the 15 DAT panel test, the panels were moved out of the protected area and exposed to the prevailing weather conditions. At 30 DAT, the panels were removed from the rack and transported to the testing area and arranged on the laboratory counter for evaluation as described below.

II. Evaluation of Residual Applied to Bushes In Enclosed Release Room:

Room conditions were established prior to the introduction of the bushes by carefully washing all wall, ceiling and floor surfaces with plain, room-temperature water. A heater was placed in the room to bring the ambient temperature to an optimum level. At 7 DAT the bushes were brought into the release room and placed equidistant from the walls and each other. 50 Adult mosquitoes *(Aedes aegypti)* were released and left undisturbed for 24 hours. During the test the release room lights were turned on at 8:00 am and off at 6:00 pm. At the end of the 24 hour period, the room was opened and the bushes were examined for live and dead mosquitoes and returned to the protected area. Dead mosquitoes were gathered and counted, and any live mosquitoes were captured by net and removed from the release room. The number found dead in the room were compared to the live individuals caught in the nets in order to factor the percentage of kill.

At 30 DAT, the bushes were again placed in the release room and an additional 50 Adult mosquitoes *(Aedes aegypti)* were released in the room for 24 hours. During this cycle, the release room lights were left on for the full 24 hour period. At the end of the test cycle, the room was opened and the bushes were examined for live and dead mosquitoes and removed to the exterior of the building. Dead mosquitoes were gathered and counted and any live mosquitoes were captured.

III. Evaluation of Residual Applied Panels (Top and Bottom):

At 7 DAT the panels were removed from the frames and transported to the testing area and arranged on the laboratory counter. As show in the illustration below, the panels were inverted with the treated surface facing down into the test arena to expose the mosquitoes. Five (5) Adult mosquitoes were introduced to each arena. Counts were taken at 15min, 30 min, 1 hour, 4 hours, and 24 hours and results recorded as “alive”, “knocked down”, and “dead”. “Alive” were counted as mosquitoes which could fly. “Knocked down” were recorded as any mosquitoes capable of movement, but not flight. “Dead” were recorded as any mosquitoes incapable of any movement. At the end of the recording period, the panels were transferred back to the wooden frame and store in the protected area.

At 15 DAT, the panels were removed from the frame and transported to the testing area and arranged on the laboratory counter. Four (4) of the panels treated by each sprayer were designated as “tops” and inverted over the arena as before. The remaining four (4) panels treated by each sprayer were

designated as “bottoms” and were not inverted, but placed over the arenas as they came off the rack to test the “wrap-around” effect of the GPS unit. Five (5) adult mosquitoes were introduced into each arena. The counts were taken as before and the panels replaced on the rack at the end of the recording period.

At the end of the 15 DAT panel test, the panels were moved out of the protected area and exposed to the prevailing weather conditions. At 30 DAT, the panels were removed from the rack and transported to the testing area and arranged on the laboratory counter. The same four (4) panels were again designated as “tops” and inverted over the arena. The same four (4) panels were again designated as “bottoms” and were not inverted to again test the “wrap-around” effect. Five (5) adult mosquitoes were introduced to each arena. Counts were again taken at the appropriate intervals and the test was concluded.

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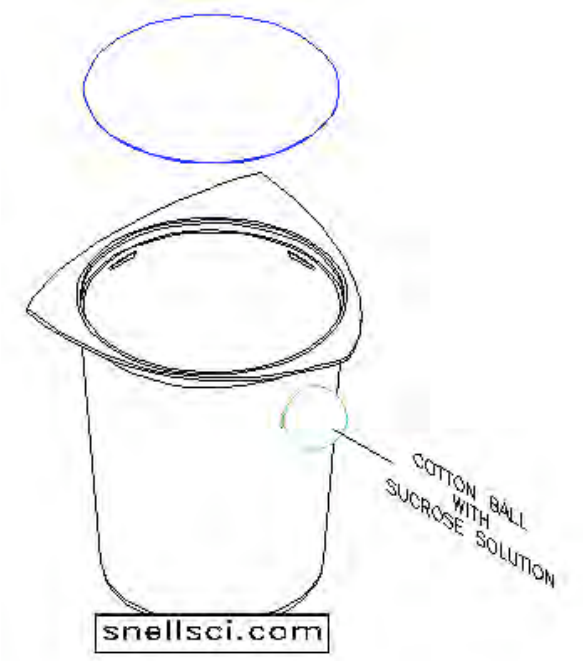
Illustrations:

Container with Inverted Treated Panel on Top



Results / Discussion:

The data is summarized in Tables 1-15. The weather data for the testing period is summarized in Table 16. The panel data is presented in Tables 1-11. Throughout the panel evaluations, the mosquitoes were observed crawling or resting on the treated panels or the walls of the arenas and were readily exposed to the treatment at all times. Tables 2-3 show that there were no appreciable differences in the speed of kill with the panel top surfaces at Day 7. By Day 15, the residual on the top surface of the panels killed mosquitoes faster at the 1 hour when treated with the ESS-GPS technology than with standard liquid technology. The residual on the bottom of the panels showed even greater contrast with the electrostatic technology compared to liquid-only as illustrated in Tables 5 and 7. Tables 8 and 10 compare the 2 treatment methods at Day 30 and the top surface residuals. At this point the top residual is essentially equal with the two technologies. However, the electrostatic technology which wrapped around to the underside of the panels provided significantly higher levels of control (approx 3x better at 24 hours) than the liquid without electrostatic.

Tables 12-15 illustrate the data from placing the bushes into the release room with approximately 50 mosquitoes each time. At Day 7, the bushes killed 100%, regardless of the treatment type. Again at Day 30 both methods provided close to complete kill.

Based on the panel data, the ESS electrostatic technology clearly provided a far superior residual to the bottom surfaces of the panels, which is evident at the 15 day and 30 day periods. This added residual to the protected underside of a leaf will likely provide 45+ days of residual from an area of the leaf that should get a reasonable amount of mosquito landings. Without this added charge to the particles, only the mosquitoes landing on the top of the leaves after the first few days will likely die from the exposure. The ESS-GPS provided 360 degree coverage and essentially doubled the killing power of the residual applied to surfaces. By adjusting the mixture ratio of active ingredient applied, the applicator can spend the same amount of time, apply a fraction of the total liquid, and equal amount of active, and get 30+ days of killing power from all surfaces of the leaves.

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Tables:

Table 1.

Efficacy of water as control applied to panel and exposed to *Aedes aegypti*						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	5	4	4	1
	Kd	0	0	1	1	0
	Dead	0	0	0	0	4
B	Alive	5	5	5	3	4
	Kd	0	0	0	2	1
	Dead	0	0	0	0	0
C	Alive	5	5	4	3	2
	Kd	0	0	1	2	0
	Dead	0	0	0	0	3
D	Alive	5	5	4	0	0
	Kd	0	0	1	0	0
	Dead	0	0	0	5	5
	Avg Dead	0	0	0	1.25	3

Table 2.

Efficacy of TalstarOne 0.444% (7DAT} applied with ESS-GPS electrostatic spray technology to aluminum panels wherein 5 mosquitoes (*Aedes aegypti*) were exposed to the top of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	0	0	0	0
	Kd	0	1	0	0	0
	Dead	0	4	5	5	5
B	Alive	3	0	0	0	0
	Kd	2	1	1	0	0
	Dead	0	4	4	5	5
C	Alive	4	1	0	0	0
	Kd	1	1	1	0	0
	Dead	0	3	4	5	5
D	Alive	5	1	0	0	0
	Kd	0	3	1	0	0
	Dead	0	1	4	5	5
	Avg Dead	0	3	4.25	5	5

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Table 3.

Efficacy of TalstarOne 0.06% (7 DAT) applied by Liquid-Only sprayer to aluminum panels wherein 5 mosquitoes (A*edes aegypti*) were exposed to the top of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	1	0	0	0
	Kd	0	1	1	0	0
	Dead	0	3	4	5	5
B	Alive	5	0	0	0	0
	Kd	0	1	2	0	0
	Dead	0	4	3	5	5
C	Alive	5	1	0	0	0
	Kd	0	1	1	0	0
	Dead	0	3	4	5	5
D	Alive	4	1	0	0	0
	Kd	1	1	2	0	0
	Dead	0	3	3	5	5
	Avg Dead	0	3.25	3.5	5	5

Table 4.

Efficacy of TalstarOne 0.444% (15DAT} applied with ESS-GPS electrostatic spray technology to aluminum panels wherein 5 mosquitoes (*Aedes aegypti*) were exposed to the top of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	3	2	0	0	0
	Kd	2	2	2	0	0
	Dead	0	1	3	5	5
B	Alive	2	2	0	0	0
	Kd	3	1	2	0	0
	Dead	0	2	3	5	5
C	Alive	4	3	0	0	0
	Kd	1	1	1	0	0
	Dead	0	1	4	5	5
D	Alive	4	3	0	0	0
	Kd	1	1	1	0	0
	Dead	0	1	4	5	5
	Avg Dead	0	1.25	3.5	5	5

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Table 5.

Efficacy of TalstarOne 0.444% (15 DAT) applied with ESS-GPS electrostatic spray technology to aluminum panels wherein 5 mosquitoes (A*edes aegypti*) were exposed to the bottom of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	4	2	1	0	0
	Kd	1	1	1	1	0
	Dead	0	2	3	4	5
B	Alive	3	1	1	0	0
	Kd	2	1	1	0	0
	Dead	0	3	3	5	5
C	Alive	4	1	0	0	0
	Kd	1	2	2	0	0
	Dead	0	2	3	5	5
D	Alive	4	4	2	0	0
	Kd	1	1	2	0	0
	Dead	0	0	1	5	5
	Avg Dead	0	1.75	2.5	4.75	5

Table 6.

Efficacy of TalstarOne 0.06% (15 DAT) applied by Liquid-Only sprayer to aluminum panels wherein 5 mosquitoes (A*edes aegypti*) were exposed to the top of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	5	4	0	0
	Kd	0	0	1	0	0
	Dead	0	0	0	5	5
B	Alive	5	5	1	0	0
	Kd	0	0	1	1	0
	Dead	0	0	3	4	5
C	Alive	4	4	3	0	0
	Kd	1	1	2	1	0
	Dead	0	0	0	4	5
D	Alive	5	4	2	0	0
	Kd	0	1	2	1	0
	Dead	0	0	1	4	5
	Avg Dead	0	0	1	4.25	5

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Table 7.

Efficacy of TalstarOne 0.06% (15DAT) applied by Liquid-Only sprayer to aluminum panels wherein 5 mosquitoes (*Aedes aegypti)* were exposed to the bottom of the panels.

Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	5	5	4	5
	Kd	0	0	0	1	0
	Dead	0	0	0	0	0
B	Alive	4	4	4	3	2
	Kd	1	1	1	2	1
	Dead	0	0	0	0	2
C	Alive	4	3	3	3	2
	Kd	1	2	1	2	0
	Dead	0	0	1	0	3
D	Alive	4	4	4	3	2
	Kd	1	1	1	2	0
	Dead	0	0	0	0	3
	Avg Dead	0	0	0.25	0	2

Table 8.

Efficacy of TalstarOne 0.444% (30DAT) applied with ESS-GPS electrostatic spray technology to aluminum panels wherein 5 mosquitoes (*Aedes aegypti*) were exposed to the top of the panels.

Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	5	4	1	0
	Kd	0	0	1	0	0
	Dead	0	0	0	4	5
B	Alive	5	5	4	0	0
	Kd	0	0	0	1	0
	Dead	0	0	1	4	5
C	Alive	5	5	5	4	2
	Kd	0	0	0	1	0
	Dead	0	0	0	0	3
D	Alive	5	5	3	0	0
	Kd	0	0	1	1	0
	Dead	0	0	1	4	5
	Avg Dead	0	0	0.5	3	4.5

Table 9.

Efficacy of TalstarOne 0.444% (30DAT) applied with ESS-GPS electrostatic spray technology to aluminum panels wherein 5 mosquitoes (A*edes aegypti*) were exposed to the bottom of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	4	3	2	0	0
	Kd	1	1	1	1	0
	Dead	0	1	2	4	5
B	Alive	4	4	4	2	1
	Kd	1	0	0	0	1
	Dead	0	1	1	3	3
C	Alive	4	4	4	1	2
	Kd	1	1	1	1	1
	Dead	0	0	0	3	2
D	Alive	3	1	2	1	0
	Kd	2	3	1	1	0
	Dead	0	1	2	3	5
	Avg Dead	0	0.75	1.25	3.25	3.75

Table 10.

Efficacy of TalstarOne 0.06% (30DAT) applied by Liquid-Only sprayer to aluminum panels wherein 5 mosquitoes (A*edes aegypti*) were exposed to the top of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 hrs	24 Hrs
A	Alive	4	3	3	1	0
	Kd	1	1	1	1	2
	Dead	0	1	1	3	3
B	Alive	4	4	1	2	0
	Kd	1	1	3	1	0
	Dead	0	0	1	2	5
C	Alive	3	3	3	1	1
	Kd	2	1	1	0	0
	Dead	0	1	1	4	4
D	Alive	4	4	3	0	0
	Kd	1	0	1	0	0
	Dead	0	1	1	5	5
	Avg Dead	0	0.75	1	3.5	4.25

Table 11.

Efficacy of TalstarOne 0.06% (30DAT) applied by Liquid-Only sprayer to aluminum panels wherein 5 mosquitoes (*Aedes aegypti)* were exposed to the bottom of the panels.						
Rep	Condition	15 Min	30 Min	1 Hrs	4 Hrs	24 Hrs
A	Alive	5	4	5	4	4
	Kd	0	1	0	1	1
	Dead	0	0	0	0	0
B	Alive	5	4	3	2	1
	Kd	0	1	1	1	1
	Dead	0	0	1	2	3
C	Alive	5	4	5	4	3
	Kd	0	1	0	1	0
	Dead	0	0	0	0	2
D	Alive	5		5	5	5
	Kd	0	1	0	0	0
	Dead	0	0	0	0	0
	Avg Dead	0	0	0.25	0.5	1.25

Table 12.

Efficacy of TalstarOne 0.445% when applied by ESS-GPS electrostatic sprayer technology to bushes (Osmanthus fragrans) and exposed to 50 Adult Mosquitoes (Aedes aegypti) for 24 hours (7 DAT)			
Rep	**# released**	**# alive at end**	**% mortality**
1	50	0	100%
1	50	0	100%
2	50	0	100%

Table 13.

Efficacy of TalstarOne 0.06% when applied by Liquid-Only sprayer to bushes (Osmanthus fragrans) and exposed to 50 Adult Mosquitoes (Aedes aegypti) for 24 hours (7 DAT)			
Rep	**# released**	**# alive at end**	**% mortality**
1	50	0	100%
2	50	0	100%

Table 14.

Efficacy of TalstarOne 0.445% when applied by ESS-GPS electrostatic sprayer technology to bushes (Osmanthus fragrans) and exposed to 50 Adult Mosquitoes (Aedes aegypti) for 24 hours (30 DAT)			
Rep	**# released**	**# alive at end**	**% mortality**
1	57	0	100%
2	56	4	91%

Table 15.

Efficacy of TalstarOne 0.06% when applied by Liquid-Only sprayer to bushes (Osmanthus fragrans) and exposed to 50 Adult Mosquitoes (Aedes aegypti) for 24 hours (30 DAT)			
Rep	**# released**	**# alive at end**	**% mortality**
1	59	0	100%
2	58	3	95%

Table 16.

Date	**5/1-5/7**	**5/8-5/14**	**5/15-5/21**	**5/22-5/28**	**5/29-6/4**
Avg Temp	63	71.8	73.1	72.2	73.1
Avg Rainfall	0	0.05	0.15	*0	0.19
*Local thunderstorm produced 1.8" rain.					