

FINAL REPORT

**Semi-Field Trials of Hunter's Kloak™ Bugg Off Mist
Against *Aedes aegypti***

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Submitted to:

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Abstract

Hunter's Kloak Bugg Off Mist provided 26.2% repellency of *Aedes aegypti* mosquitoes immediately after deployment. Significant variation in mosquito biting activity complicated this study. This was attributed to the diminishing amount of product dispensed over time, differences in evaluator attraction, and prevailing wind conditions.

Study Objective

Determine the repellency of Hunter's Kloak™ Bugg Off Mist (active ingredient: citronella) compared to a negative control (water) in repelling *Aedes aegypti* mosquitoes.

Experimental Protocol

1. This study was performed adjacent to Pine Log Creek about 3 miles south and 2 miles west of Ebro, FL in a swamp creek ecosystem dominated by slash pine, sand pine, laurel oaks, water oaks, tupelo, cypress, and sweet bay. Three trials were conducted on May 11, 18, and 25, 2019. The trials were started at 6:50 a.m. and proceeded through 12:45 p.m. C.T.
2. 3-7 day-old, laboratory-reared *Aedes aegypti* were used in this study. The mosquitoes were starved for several hours to insure avid biting activity prior to each trial.
3. Two 12'W X 24'L X 8'H 28 m³/300 sq. ft. Shelter Logic® greenhouse frames were assembled and covered with 8 ft. rolls of 20X20 mesh screening bound and stapled with 1½ inch black polypropylene webbing (Fig. 1). A ZipWall® zipper was stapled into one end of each house to serve as an entry. The screen houses were positioned 60 ft apart in the shade under oak/pine trees. Each test day, treatments consisting of the Bugg Off Mist (citronella) and a negative control (water mist) were evaluated separately in both screen houses.



Fig. 1. Screen houses constructed for the Hunter's Kloak study.

4. 100 female mosquitoes were stocked into each of two 1 ft³ holding cages placed at the entrance ends of the screen houses. Mosquitoes were released by unlatching the top of the cages and allowed to acclimate for 3 minutes after the misters were turned on before biting counts were performed during Trial #1 and 15 minutes for Trials #2 & #3.
5. Two evaluators (one/screen house) were positioned on the opposing end of the screen houses to conduct timed biting counts (2 minute for Trial #1 and 1 minute for Trials #2 & #3) on an exposed arm at 0, 1, and 2 hours post-release. For Trial #1, the arm from the elbow to the tip of the hand was laid flat against the screen. For Trials #2 & #3, a 9

inch zipper port was installed on the opposite end of the entry and the hand up to the wrist was inserted through the zipper into the screen house.



Fig. 2. Hand-in-cage biting count technique.

6. The treatments (i.e., citronella mist or water mist) were placed halfway between the mosquito holding cages and evaluators and were set on 6-second pulse intervals during Trail #1 and 15-second during Trials #2 & #3. The misters were operated for 15 minutes before releasing the mosquitoes and were allowed to run continuously for 2 hrs.
7. Prior to switching treatments to the opposing screen house, mosquitoes were removed by aspiration and a fresh cohort of 100 females was supplied to each screen house (Fig. 3). The screen house containing the citronella treatment was ventilated with a fan to eliminate lingering vapors, except during Trail #3 when the fan generator malfunctioned. Vapors on that day were allowed to clear by prevailing winds.



Fig. 3. Removing mosquitoes from cage by aspiration.

8. A 2X2 Latin square design was utilized whereby both treatments were tested separately in each of the two screen cages. Each evaluator conducted one test of each product during each of the three trials.
9. Ambient environmental conditions (i.e., temperature, humidity, wind direction and speed) were recorded throughout the study with an Onset Hobo® data logger and Kestrel 5500AG weather meter placed between the two screen houses. The Onset data logger was not available during Trial #1.
10. Biting count means by treatment, screen house, and date were calculated with error variance to facilitate statistical comparisons at $p=0.5$. SAS PC 9.4 software was used to analyze variance by Proc GLM and Tukey and Duncan Multiple Range tests. Percent repellency was determined by subtracting treatment biting means from control means dividing by the control means and multiplying by 100. Biting count means and repellency were charted with standard error.

Results

Environmental Conditions

Warm, humid conditions prevailed throughout the study with lows in the mid 70's and highs ranging from 89-97°F (Table 1). Humidity was high (88-93%) in the early morning and dropped to lows ranging from 36-55% in the early afternoon. Wind conditions were relatively low ranging from 0-4 mph and was predominantly from the S and SW except during Trial #3 when it became more variable and shifted from the NNW and ESE. Temperature and humidity profiles for Trial #2 and #3 were very similar (Fig. 4 & 5).

Date	Trial	Time	Temp °F	%RH	Wind Speed(mph)	Wind Direction(°)	Conditions
5/11/19	1	7:10 A.M.	75.0	93.0	1.0	SW 220	Overcast
5/11/19	1	8:10	76.7	89.8	0	--	Cloudy
5/11/19	1	9:10	80.4	78.2	0-2.0	S 175-180	Cloudy
5/11/19	1	10:32	87.0	61.0	1-2.0	S 174	Partly cloudy
5/11/19	1	11:32	89.5	52.7	1-2.5	SSW 183-203	Partly cloudy
5/11/19	1	12:32 P.M.	91.3	55.5	1-2.0	S184	Partly cloudy
5/18/19	2	7:08 A.M.	N/A	88.0	1.6	SSW196	Foggy
5/18/19	2	8:08	74.0	95.0	0	--	Sunny
5/18/19	2	9:08	77.0	81.0	1.5	SSW220	Sunny
5/18/19	2	10:30	87.0	48.0	1.5	SSW203	Sunny
5/18/19	2	11:30	89.0	50.0	1.0	SSW210	Sunny
5/18/19	2	12:30 P.M.	89.0	42.0	3.0	SSW213	Sunny
5/25/19	3	7:25 A.M.	73.4	92.0	1.0	SW215	Sunny
5/25/19	3	8:25	78.0	84.0	2-4.0	SW220	Sunny
5/25/19	3	9:25	83.0	74.9	1-2.0	N/A	Sunny
5/25/19	3	10:40	89.0	55.0	0-2.0	NW	Sunny
5/25/19	3	11:40	90.6	48.0	4.0	ESE123	Sunny
5/25/19	3	12:40	97.0	36.0	1.5-3.0	NNW335	Sunny

Table 1. Kestrel temp/humidity/wind data during testing.

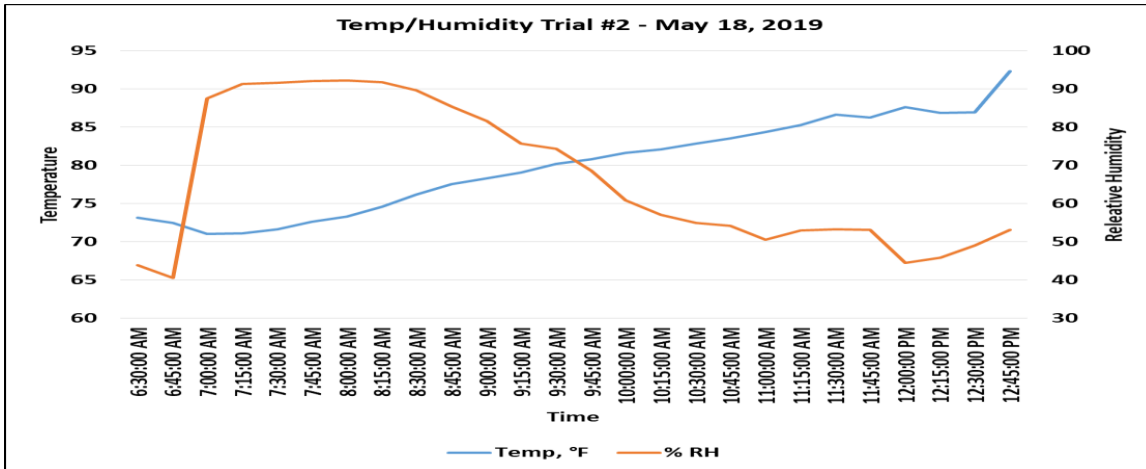


Fig. 4. Data logger temperature and humidity during Trial #2.

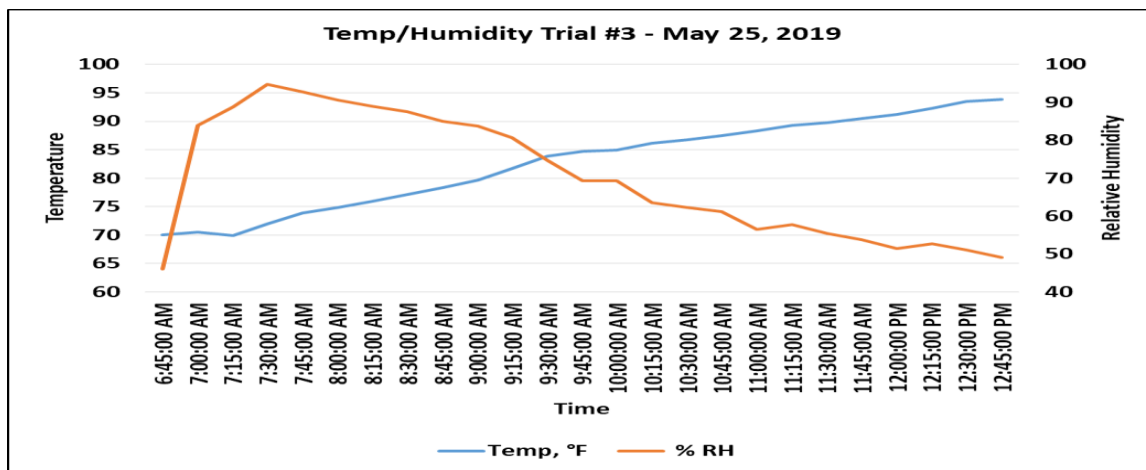


Fig. 5. Data logger temperature and humidity during Trial #3.

Biting Counts

The only statistically significant difference detected in biting count means was between the evaluators (i.e., testers; $p < 0.0158$) (Table 2). The female evaluator averaged 13 bites out of 18 biting count readings (6 tests X 3 time intervals) compared to 6.9 for the male evaluator. There were no significant differences in biting counts observed by trial, treatment, time interval, and the interactive effects of treatment*time or tester*treatment*time. There were slightly more bites in the negative control (i.e., water) compared to the Bugg Off repellent, although these differences could not be substantiated due to large variability in biting counts (Fig. 6).

Bugg Off repellency maxed at 26.2% at the beginning of the test trials (i.e., time 0) and dropped to 5.7% at 1 hr post-release and then increased to 20.7% at 2 hrs (Fig. 7).

The SAS System
The GLM Procedure

Class Level Information		
Class	Levels	Values
TRIAL	3	1 2 3
TESTER	2	Ann John
TRT	2	CIT CON
TIME	3	0 1 2

Number of Observations Read	36
Number of Observations Used	36

The SAS System
The GLM Procedure
Dependent Variable: COUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	772.3611111	59.412393	1.23	0.3233
Error	22	1062.611111	48.300505		
Corrected Total	35	1834.972222			

R-Square	Coeff Var	Root MSE	COUNT Mean
0.420912	69.69215	6.949856	9.972222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRIAL	2	10.72222222	5.36111111	0.11	0.8954
TESTER	1	330.0277778	330.0277778	6.83	0.0158
TRT	1	34.0277778	34.0277778	0.70	0.4103
TIME	2	243.3888889	121.6944444	2.52	0.1035
TRT*TIME	2	7.7222222	3.8611111	0.08	0.9234
TESTER*TRT*TIME	5	146.4722222	29.2944444	0.61	0.6957

Table 2. Statistical analysis of biting count means by variable.

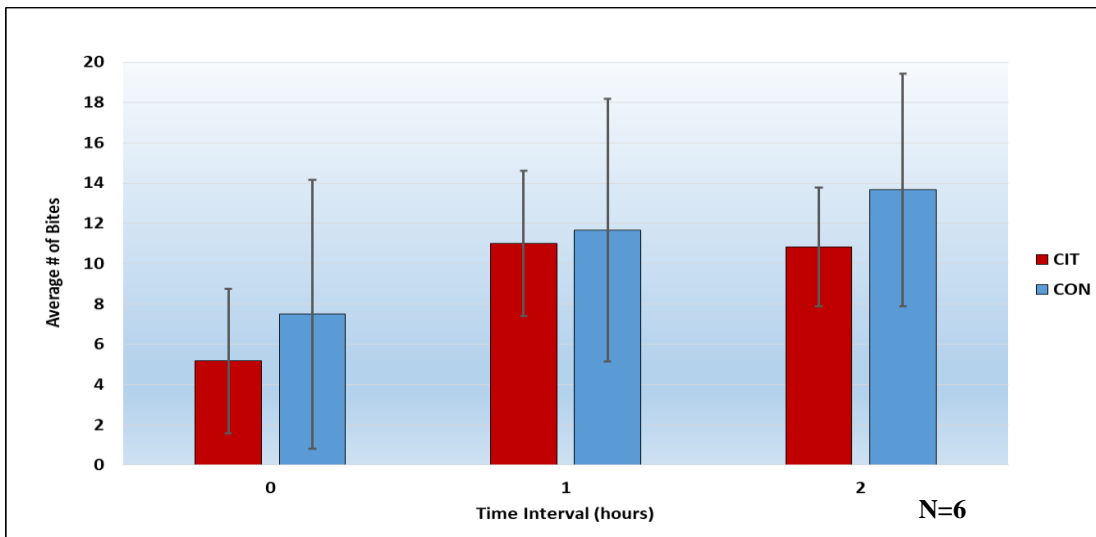


Fig. 6. Mean biting count and standard error by time interval for Bugg Off (CIT) and the negative control (CON).

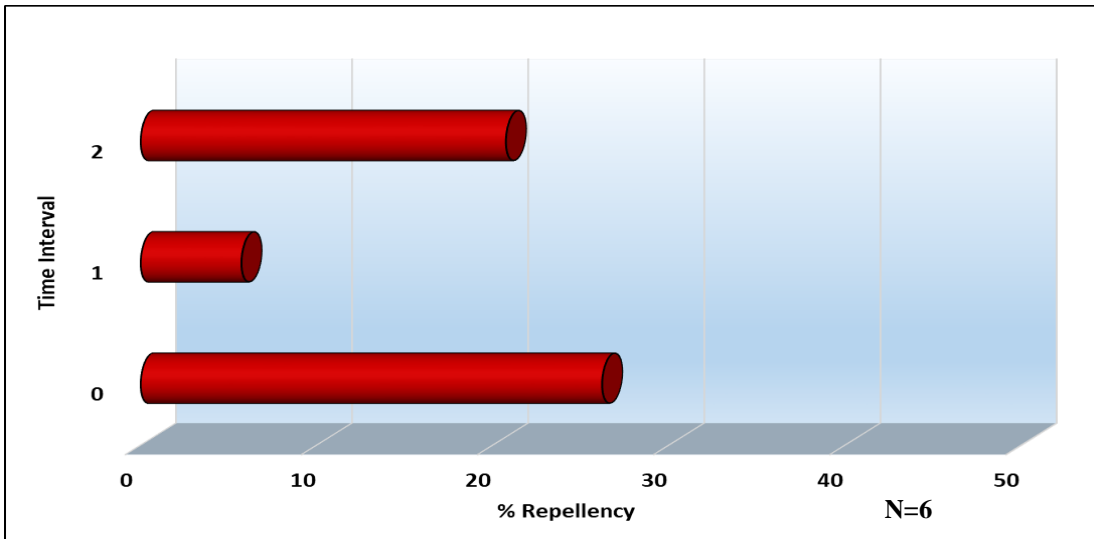


Fig. 7. Percent repellency of Hunter’s Kloak Bugg Off mister at three post mosquito release time intervals.

Discussion and Conclusions

Inconsistencies in biting counts were attributed to confounding factors occurring as the experimental protocol progressed. These are discussed below.

1. The misters released different volumes than anticipated even though both were initially set at 6-second dispense intervals. The reservoir for the control mister consistently emptied before 2 hrs. The Bugg Off treatment dispensed a very noticeable, smaller plume than the water (about ½ the height) and lasted the entire test period although diminished substantially near the end of each trial. Efforts were made to correct for this by adjusting the misting intervals from 6 to 15 seconds for Trials #2 and #3. With this change, Bugg Off lasted 2 hrs during the early morning tests with about 1/3 of the liquid remaining in the misting container. When the air temperature rose during the later morning tests, the liquid dwindled to about 5% remaining. The water treatment emptied before completing the 2-hr test period regardless of the interval setting.
2. The Bugg Off treatment did not produce a strong citronella odor. In fact, it was hardly noticeable compared to recently tested citronella candles.
3. The small plume emanating from the misters appeared to be easily blown away by low wind conditions (1-4 mph) (Fig. 8). Any chance of significant repellency would likely require the mister to be placed adjacent to the user. With that in mind, a better evaluation of the treatments would be to place them in a corner and taking biting counts directly from that location.
4. To improve the misting device for mosquito control, I would recommend trying a more potent and proven repellent such as geraniol. Geraniol is an essential oil that has good efficacy at 5% concentration. Whatever active is used, it would be helpful if the misting device could dispense greater volume and sustain the release rate at or near the same level throughout the 2-hr. test period.



Fig. 8. Hunter Kloak mister dispensing water. The Bugg Off formula misted about half this amount.