# Bite-Lite® Candle Repellency Evaluation

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ABSTRACT: From February  $27^{th}$  – May  $1^{st}$ , 2011 Field testing was conducted using Mosquito Magnet Liberty® Traps to evaluate the repellency of new Bite-Lite® candles with Cloak & Scatter® technology. The new candles significantly (P = 0.0003) decreased the number of mosquitoes attracted to the Mosquito Magnet trap. On average, the addition of candles to the traps resulted in an 84.1% reduction in attractancy.

Evaluation of Repellency of New Bite-Lite® Candles with Cloak & Scatter® Technology Utilizing Mosquito Magnet Liberty® Traps in # Florida, April 2011.

#### Introduction

Repellent candles are frequently used to decrease the occurrence of biting insects in and around human gathering areas. Citronella is, by far, the most commonly used essential oil for these candles even though it has not, historically, tested very well. In some cases, showing only a 35% reduction in attractancy (Muller et al 2008). However, people continue to purchase citronella candles because they are non-toxic and have aesthetic appeal. New candles are being evaluated each year with improved efficacy; with offerings such as geraniol and linalool that show greater success, up to 81.5% reduction in attraction (Muller et al 2008). This study was commissioned to look at a new candle made with a mixture of 3.1% Spearmint Oil, 3.1% Lemongrass Oil and 0.33% Citronella Oil to create a 6.53% active Bite-Lite® candle with Cloak & Scatter® technology¹.

#### Methods

<u>Materials.</u> Mosquito Magnet Liberty® traps² were purchased in October of 2010 to begin evaluation of candles as soon as mosquitoes were available in Melbourne, Fl. The first set of candles was received in November of 2010 and was kept in a climate controlled area until testing started; a second set was received on April 28<sup>th</sup>, 2011 and was used immediately, as the first set had been depleted. To improve attraction to the traps, TrapTech® lures³, a combination of R-Octenol and Ammonium Bicarbonate, were used as added attractants for repetitions two through six. Low numbers in the first repetition led to the decision to add attractant lures to both traps.

<u>Methods.</u> Traps were positioned on opposite ends, diagonally, of a large residential backyard separated by an approximately 40' x 40' screened pool area. Candles were randomly placed, coin flip application, at Site 1, which was located on the eastern side of the yard. Candles were set up 11"-12" from each corner of the trap on 8" cans, they were spaced 34"-36" apart, Figure 1. A repetition consisted of two trap nights (4.5 hours), candles rotated and tested at each site. Traps remained stationary. Candles were lit at either 5 pm or 6 pm and allowed to form a wax pool for approximately 40 minutes before traps were turned on. Traps have a warm up period of 10 – 20 minutes, so nets were checked and traps were started 40 minutes after candles were lit. Twenty minutes later, traps were checked to verify operation and candles were checked to verify that they remained lit. Four and one-half hours after candles were

<sup>&</sup>lt;sup>1</sup> Cloak & Scatter® Trademarks are registered to Bedoukian Research, Inc. and are marketed by Bite-Lite® LLC.

<sup>&</sup>lt;sup>2</sup> Mosquito Magnet Liberty® Trademarks are registered to Woodstream Corporation.

<sup>&</sup>lt;sup>3</sup> TrapTech® Trademark is registered to Bedoukian Research, Inc.

lit, candles were covered with the mason jar lids, nets collected and traps shut down. Collected nets were placed in the freezer overnight before being emptied for evaluation. All mosquitoes were identified to species and other non-target specimens were noted. This was repeated each evening until five full repetitions were completed. Note that morning repetitions were also attempted; the same method was used starting at 5 am, however after four morning attempts with only one morning in which mosquitoes were collected in the control trap, the morning testing was cancelled.



Figure 1: Mosquito Magnet Liberty® Trap Surrounded by Four Bite-Lite® Repellent Candles, 7 Spring 2011.

<u>Statistical Evaluation.</u> A Shapiro – Wilks test was used to determine if Trap site and Treatment versus Control data were normally distributed. Based on the results of those tests the Mann-Whitney U test was used to determine statistical differences.

#### **Results & Discussion**

Testing was conducted in late February, Mid March and finally from Mid April through the beginning of May. The repetitions were spaced out due to weather issues as well as travel schedules. The first repetition was conducted early in the season (the evenings of 2/24/11 and 3/18/11) and collected low numbers. At this point it was decided to add TrapTech mosquito lures to both traps to help boost the numbers attracted to the traps. Testing from April 16<sup>th</sup> – May 1<sup>st</sup>, 2011 only showed a large increase in numbers attracted for the first night, after that it was a little higher on some nights but not by much. Although, the average number of mosquitoes collected in the control trap was low, it was high enough for statistical analysis. There were some trap nights that had to be repeated due to no activity in the control trap, this lack of mosquito activity could have been due to higher than normal winds, cooler nights or a combination of these factors. April was an extremely windy month with temperatures varying from a high of 90° to a low of 48°. Regardless of weather conditions, six repetitions were attempted on 14 different days, from February 27<sup>th</sup> – May 1<sup>st</sup>, 2011, with five evening repetitions succesfully completed.

A total of 212 mosquitoes were collected in 18 identifiable species, there were two additional specimens that were not identifiable, to species, due to damage, Table 1, Figure 2.

Table 1: Number of Adult Female Mosquitoes Collected by Species, Florida; Spring 2011.

	Number	% of Total Number
Ochlerotatus infirmatus	66	31.13%
Ochlerotatus taeniorhynchus	46	21.70%
Culex erraticus	20	9.43%
Mansonia dyari	17	8.02%
Coquillitidia perturbans	15	7.08%
Psorophora columbiae	12	5.66%
Aedes vexans	9	4.25%
Ochlerotatus atlanticus	7	3.30%
Culex nigripalpus	4	1.89%
Anopheles barberi	2	0.94%
Anopheles crucians	2	0.94%
Anopheles pseudopunctipenis	2	0.94%
Culex restuans	2	0.94%
Culex salinarius	2	0.94%
Culex iolambdis	1	0.47%
Culex quinquefasciatus	1	0.47%
Ochlerotatus excrucians	1	0.47%
Psorophora ciliata	1	0.47%
Psorophora sp.	1	0.47%
Unidentifiable	1	0.47%
Total	212	100%

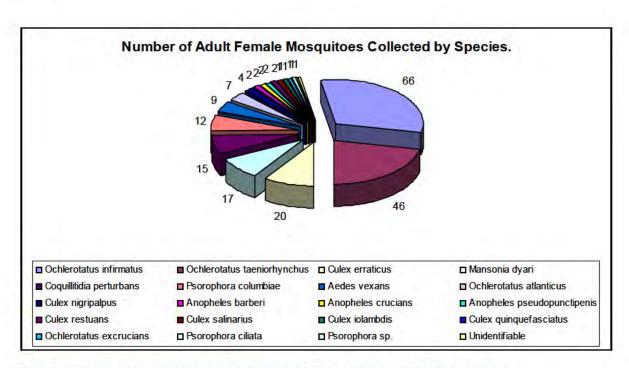


Figure 2: Species Diversity and Number of Adult Female Mosquitoes Collected, Florida; Spring 2011.

Nine of the species were collected in amounts greater than 1%, with the most abundant species collected being *Ochlerotatus infirmatus*. More diversity was observed at Site 2 which is most likely in direct relation to the increased numbers collected at Site 2, Table 2.

Table 2: Number of Adult Female Mosquitoes Collected by Site, 7; Spring 2011.

Rep*	Site 1	Site 2
1	3	5
	9	5 5
2	4	77
	20	77 4
4	9	3
	2	13
5	32	4
	32 2	4 5
6	5	1
	1	8
Total	87	125

<sup>\*</sup> Repetition 3 was discarded due to an inability to attract mosquitoes to the control trap at site 1 at dawn.

A Shapiro-Wilks test was utilized to determine if the data were normally distributed. It was determined that neither site had data that were normally distributed and therefore a non-parametric test was used to evaluate the statistical relationship. A Mann-Whitney U test was conducted and although there were 43.7% more mosquitoes collected at Site 2 than at Site 1, there was no significant difference (P > 0.05) between the two sites, Table 3.

Table 3: Mann-Whitney U Test Results Comparing the Total Number of Mosquitoes Collected by Site, 7 ; Spring 2011.

n <sub>1</sub>	n <sub>2</sub>	U	P (two- tailed)	P (one- tailed)
10	10	53.5	0.795936	0.397968
normal approx		pprox	0.791336*	0.395668*
z = 0.264575		4575		

The average number of mosquitoes collected in the control trap was 18.3 with the highest collection being 77 in one night and the lowest being 5. The average number of mosquitoes collected in the treated trap was 2.9 with the highest collection being 5 in one night and the lowest being 1, Table 4, Figure 3.

Table 4: Bite-Lite® Candle Repellency Evaluation: Number of Adult Female Mosquitoes Collected in Trap and Attractant Only Control versus Trap and Attractant with 4 Repellent Candles, 7; Spring 2011.

Rep*	Control	Treatment
1	5	3
	9	5
2	77	4
	20	4
4	9	3 2
	13	2
5	32	4
	5	4 2
6	5	1
	8	1
Average	18.3	2.9

<sup>\*</sup> Repetition 3 was discarded due to an inability to attract mosquitoes to the control trap at site 1 at dawn.

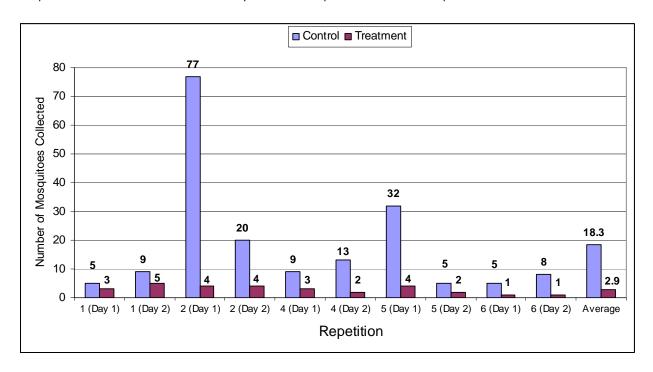


Figure 3: Total Number of Adult Female Mosquitoes Collected by Mosquito Magnet Traps Plus Attractant either with Bite-Lite® Candles or without (Control), 7 ; Spring 2011.

Again a Shapiro-Wilks test was conducted to determine if the data were normally distributed. The Control data was not found to be normally distributed, however the Treatment data was normally distributed. In general, trap data is not normally distributed, due in part to the natural variation of biological systems and the impact they have on mosquito populations. This generalization is supported by this Control data; however the normal distribution expressed by the Treatment data could possibly be considered evidence that the repellent being used is consistent in its rate of repellency and therefore not as effected by the daily biological variations. Data were again analyzed using a Mann-Whitney U test

to determine statistical relationship. It was found that there was a significant difference (P < 0.05) between the Control trap data and the Treatment trap data, Table 5.

Table 5: Mann-Whitney U Test Results Comparing the Total Number of Mosquitoes Collected by Control versus Treatment, 7 Spring 2011.

n <sub>1</sub>	n <sub>2</sub>	U	P (two- tailed)	P (one- tailed)
10	10	98.5	2.17E-05	1.08E-05
normal approx		pprox		
z =	3.666	326	0.000246128*	0.000123064*

### Conclusion

Therefore, in conclusion, it can be stated that the addition of the four Bite-Lite® candles with Cloak & Scatter® technology significantly decreased the attraction of the Mosquito Magnet Liberty® trap plus TrapTech® lure. Overall, there was an 84.1% reduction in attraction.

## **Future Testing**

As the addition of four Bite-Lite® candles with Cloak & Scatter® technology was successful in reducing the attraction of the Mosquito Magnet traps, follow-up testing could look at the percent reduction of attraction seen when using less candles.

#### References

Muller, GC, Junnila, A., Kravchenko, VD., Revay, EE., Butler, J., Orlova, OB., Weiss, RW., and Schlein, Y. 2008. Ability of Essential Oil Candles to Repel Biting Insects in High and Low Biting Pressure Environments. Journal of the American Mosquito Control Association, 24(1): 154-160.

Statistical Evaluation webpages

Shapiro-Wilk Normality test: <a href="http://dittami.gmxhome.de/shapiro/">http://dittami.gmxhome.de/shapiro/</a>

Mann-Whitney U test: <a href="http://elegans.swmed.edu/~leon/stats/utest.html">http://elegans.swmed.edu/~leon/stats/utest.html</a>