Effects of Quicklime on Flesh Fly (Diptera:Sarcophagidae) Colonization

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Edited by Carla M. A. de Loera

Abstract: Entomological evidence in the form of immature blowflies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) are often used in forensic investigations to estimate the minimum postmortem interval (mPMI). This is done by using species-specific growth charts established under ideal conditions. Clandestine burials often do not fall within these ideal conditions, and insect colonization can be influenced by the presence of chemicals meant to enhance the rate of decay, such as quicklime. While quicklime in fact slows decomposition, the direct effect of the presence of quicklime on insect colonization is poorly represented in literature. Two lamb livers were used as analogues for human remains in a field experiment. They were left open to colonization both treated with quicklime and untreated, while being monitored for insect activity. The results suggest a significant increase in attraction to the quicklime-treated liver, 70.52%, implying that quicklime might have acted as an olfactory attractant rather than an olfactory deterrent to gravid Sarcophagidae females.

Keywords: postmortem, lime, colonization, liver, attraction

Exploring insect behavior in the context of forensic entomology often concerns insect responses to decomposing humans remains. These responses act as ephemeral resource pulses and elicit specific behavioral responses from insects in the surrounding ecosystem (Yang et al. 2008). Entomological evidence on decomposing human remains can come in multiple forms but are often collected as the larvae of blowflies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae). There are several factors that influence insect detection of carrion and subsequent oviposition including internal (i.e., age, physiology, nutrition [Haskell 2008]) and external (i.e., environment, physical access to the resource, odor cues [Bell 1990]) factors, which converge for an insect to discover and colonize a body. Forensic entomologists often exploit these predictable insect behaviors to estimate the minimum postmortem interval (mPMI). These mPMI calculations, however, are often based on data and species-specific growth charts established under ideal conditions at known temperatures. Clandestine burials often do not fall within these ideal conditions. Insect colonization in these cases can be affected by several factors, including manner of death, physical access to remains, and the presence of chemicals, such as quicklime.

A common misconception both in the criminal eye is that quicklime hastens the decay of remains or destroys evidence. In reality, quicklime serves to preserve soft tissue and slows the rate of decomposition (Thew 2000, Schotsmans et al. 2012). However, the effects of quicklime on insect colonization is poorly represented in literature. Because quicklime can affect some of those external detection factors (i.e. pH of the environment, skewed or masked odor cues), its presence could influence how and when insects colonize the remains. This could directly impact the mPMI estimation. Using lamb livers as a carcass model, the flesh flies will be introduced to quicklime. This study will serve to explore the difference in attraction of flesh flies to the presence of quicklime on remains. The attraction of flesh flies to quicklime can help understand their behavior towards decomposing human remains.

Materials and Methods

Experimental Setup. The experiment took place on an agriculture research farm approximately 14 miles southwest of College Station, Texas in Caldwell county. To prevent scavenging, two metal kennels measuring 30 in. x 21 in. x 24 in. (Midwest Life Stages, Irvine, California) were staked down five feet apart on a corner of a livestock grazing field. The bottoms of each kennel were then filled with approximately one inch of soil collected from the surrounding area to allow for pupation of the larvae. Two lamb livers weighing approximately 1,500 grams each were used as analogues for human remains. Using latex gloves (Curad, Medline Inc., Katy, Texas), each liver was placed in a separate kennel. One liver was left untreated, while the other was coated in one cup of quicklime (Mississippi Lime Company, St. Louis, Missouri) . Once both livers were placed in the kennels and the experiment setup was completed, the doors of the kennels were sealed with zip ties (Commercial Electric Products, Cleveland, Ohio).

Experimental Procedure. Data was collected every twelve hours every day for one week (03/23/16-03/29/16) and ambient temperature was recorded (Table 1). After seven days, the livers and all insect specimens were collected from the kennels and sealed inside separate Tupperware containers (Tupperware, Orlando, Florida). The specimens were then brought back to College Station, Texas for blanching at 100°C and preservation in 80% ethyl alcohol (Fisher BioReagents, Pittsburg, PA) solution. All specimens were identified to family and measured to the nearest half millimeter.

Results

Table 1. Temperature Data collected onsite

Date	3/23/16	3/24/16	3/25/16	3/26/16	3/27/16	3/28/16	3/29/16
Morning Temperature (˚F)	65°	48°	44°	40°	53°	45°	60°
Evening Temperature (°F)	73°	62°	63°	68°	63°	71°	70°

Count Difference Between Treated vs Control. After a week of observation, a total of 821 Sarcophagidae larvae were collected. Of these larvae, 242 (29.48%) were gathered from the control liver; 579 (70.52%) were gathered from the quicklime treated liver (Fig. 1). Using the Fisher's exact test, a *p*-value of 0.3561 from the two-sided probability was calculated. A *p*-value of greater than 0.05 normally indicates weak evidence against our hypothesis (Fig. 2). Of all specimens collected, 94% were third instar larvae; only 6% of all larvae were first or second instars (Fig. 3).

Size Difference Between Treated vs Control. Larvae collected from the quicklime treated liver were significantly larger than those collected from the control (Table 2). However, since the total number of the larvae between the groups are skewed in the direction of the treated liver, there is not a normal distribution or equal variance. To address this, the Wilcoxon/Kruskal-Wallis Test and Welch's Test (Fig. 4) determined that there was significant variance between the two groups in size caused by the manipulated variable.

Discussion

Based on the analysis of the quantities of larvae present, as well as their average lengths, the data collected provides statistical evidence that the quicklime treated liver showed a greater attraction than the control liver for colonization. Larvae collected from the quicklime treated liver were significantly larger and more numerous than those which

Fig. 1. Mosaic plot of larvae count from control and treatment

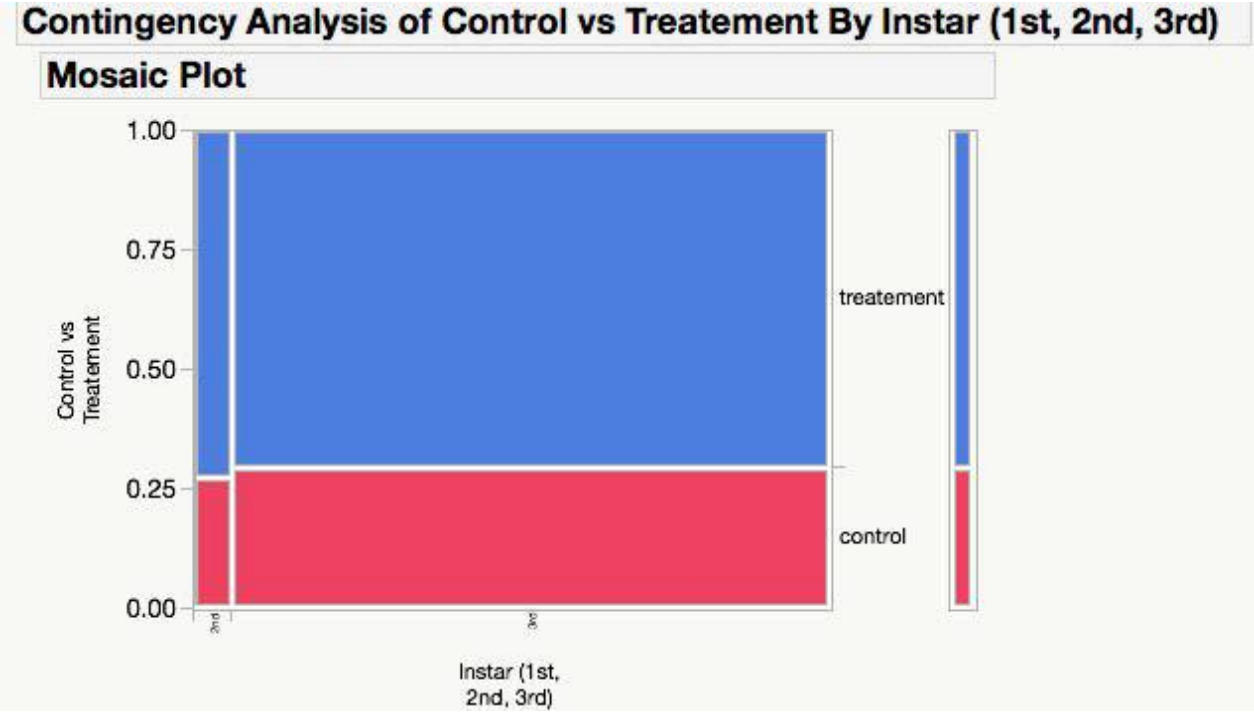


Fig. 2. Pearson's chi squared test and *p*-value from Fisher's exact test.

Tests

N	DF	-LogLike	RSquare (U)
821	2	1.2894050	0.0026

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	2.579	0.2754
Pearson	2.526	0.2828

Warning: 20% of cells have expected count less than 5, ChiSquare suspect.

Fisher's Exact Test	Table Probability (P)	Two-sided Prob ≤ P
	0.036525	0.3561

Fig. 3. Count of specimens at different larval stages collected in both setups.

Contingency Table

Control vs Treatement

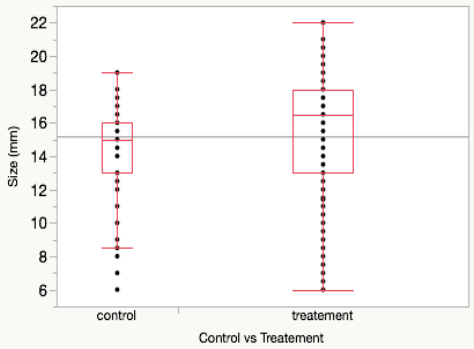
Instar (1st, 2nd, 3rd)	control	treatment	Total
Count Total % Col % Row % Expected			
1st	1 0.12 0.41 100.00 0.29476	0 0.00 0.00 0.00 0.70524	1 0.12
2nd	13 1.58 5.37 27.08 14.1486	35 4.26 6.04 72.92 33.8514	48 5.85
3rd	228 27.77 94.21 29.53 227.557	544 66.26 93.96 70.47 544.443	772 94.03
Total	242 29.48	579 70.52	821

developed on the control liver. This could have been caused by several factors.

Rather than the presence of quicklime deterring insect colonization with additional olfactory cues, it is possible that the

Fig. 4. Graphs and tables showing the non-normal distribution and unequal variances as well as the results of the statistics tests.

Oneway Analysis of Size (mm) By Control vs Treatement



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
control	242	78944.0	99462.0	326.215	-6.643
treatement	579	258487	237969	446.437	6.643

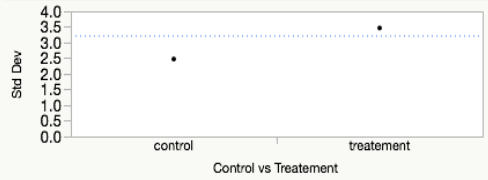
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
78944	-6.64310	<.0001*

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
44.1329	1	<.0001*

Tests that the Variances are Equal



Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
control	242	2.474818	1.908698	1.811983
treatement	579	3.466790	2.879672	2.798100

Test	F Ratio	DFNum	DFDen	p-Value
O'Brien[.5]	33.2236	1	819	<.0001*
Brown-Forsythe	35.8209	1	819	<.0001*
Levene	48.0979	1	819	<.0001*
Bartlett	34.7759	1	.	<.0001*
F Test 2-sided	1.9623	578	241	<.0001*

Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

F Ratio	DFNum	DFDen	Prob > F
33.4821	1	623.55	<.0001*

t Test
5.7864

quicklime served as a direct attractant to gravid Sarcophagidae females. Additionally, several ant species (Hymenoptera: Formicidae) were found on the control liver

Table 2. Counts and average lengths of the groups of larvae.

	Control	Treated
Total Larvae	242	579
First Instar	1	0
Second Instar	13	35
Third Instar	228	544
Avg Length - First Instar	6 mm	N/A
Avg Length - Second Instar	8.461538 mm	8.6714286 mm
Avg Length - Third Instar	14.63377 mm	15.948346 mm
Avg Length - All Specimens	14.26653 mm	15.508463 mm

that were not present on the quicklime treated liver. These ant species were notably preying on smaller larval instars. This predation could have contributed to the marked difference in total larvae on the control, with the quicklime acting as a deterrent to ant species and thus protecting the developing larvae.

Future iterations of this experiment should include a more analogous carcass model (i.e. cattle liver, hog carcass). The larger carcass model might allow for colonization by more than one species. Additionally, adjustment in the experimental design to prevent predation on either set of specimens and an increase in experimental replicates would provide cleaner data. Further exploration should be done into the olfactory attraction of quicklime to gravid Sarcophagidae females.

Acknowledgments
The authors thank Dr. Leslie Frenzel, Mark Frenzel, Ray Riley, and the rest of the team at the Rosenthal Meat Science and Technology Center for the donation of the lamb livers used in the experiment; Shane Halfmann for the use of the experiment site; and Taylor Donaldson for his assistance, support, and guidance throughout the process.

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