Filth Fly Activity Associated with Composted and non-composted Beef Cadavers and Laboratory Studies on Volatile Organic Compounds

Justin L. Talley, Ph.D., Professor

Department of Entomology and Plant Pathology, Oklahoma State University, justin.talley@okstate.edu

Trisha Dubie, Ph.D., Graduate Research Assistant

Department of Entomology and Plant Pathology, Oklahoma State University, trishadubie@gmail.com

Josh Payne, Ph.D., Technical Services Manager

Jones-Hamilton Co., Agricultural Division, jpayne@jones-hamilton.com

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Abstract. *Commercial livestock facilities are faced with the challenge of managing large amounts of waste including manure and animal mortalities. One method of disposing of dead animals is composting. The cadavers are enveloped in carbon material that creates a barrier between the dead tissue and the surrounding environment. Dead tissue can release materials that not only contaminate the soil but also the groundwater and nearby surface water. Animal cadaver composting is designed to facilitate decomposition without the aid of carrion feeding insects and reduce the presence of common pathogens associated with animal waste and dead tissue. The aim of this study was to evaluate insect activity associated with composted and exposed beef cadavers, specifically filth flies that can serve as mechanical vectors of important human pathogens such as E. coli 0157:H7. Greater numbers of all types of arthropods were trapped overall at the exposed animal site than the composted animal site. Most importantly, the number of filth flies was significantly lower at the composted site (P=0.0009). Laboratory analysis of volatile organic compounds from composted and non-composted rats indicated that known fly attractants such as dimethyl disulfide may be inhibited by the composting process. Implementing composting programs at livestock facilities could reduce the risk of flies spreading harmful pathogens to surrounding areas.*

Keywords. bovine cadaver, decomposition, carcass disposal, filth flies

Introduction

Catastrophic events such as disease outbreaks and natural disasters can dramatically increase the number of livestock mortalities, and these circumstances require a safe and effective means of carcass disposal. Exposed livestock cadavers can contain harmful zoonotic pathogens that can be transmitted to other livestock animals (Lloyd-Smith et al. 2009), and many filth flies are competent mechanical vectors of pathogenic microorganisms that can be acquired from animal manure and animal carcasses (Greenberg 1973, Graczyk 2001). Filth flies have also been implicated in the transmission of antibiotic resistant bacteria (Zurek and Ghosh 2014). Filth flies are potential vectors for contaminating produce on-farm, and therefore their control is an essential component of food safety (Talley et al. 2009, Wasala et al. 2010). Conditions within a compost pile reach temperature that theoretically should be too high for filth fly development, and the envelope of composting materials should also prevent flies from accessing the carcass. Composting cadavers could reduce filth fly activity overall and lessen the public health risks associated with the disposal of livestock mortalities. The aim of this study was to evaluate the activity of carrion feeding insects, specifically filth flies, associated with composted and exposed bovine cadavers, and to determine what effect, in any, the composting process has on the emission of volatile organic compounds that play in important role in filth fly olfaction and carrion detection.

Materials and Methods

Carcass sites: Bovine carcasses, Bos taurus, were acquired from the Oklahoma State University's Willard Sparks Beef Research Center and North Lake Carl Blackwell Beef Research Range in Stillwater, OK. Each site was in a partially wooded habitat with similar surrounding vegetation. Both field sites were equipped with a 3m x 3m fence, approximately 1.5 m high, to keep large scavenger animals from disturbing the carcasses and compost. All animals were obtained within 12 hours of death, and each set of animals, one composted and one exposed for each replicate, was observed simultaneously throughout the entire period of decomposition.

Temperatures inside of the compost piles and under the exposed cadavers were monitored throughout the period of decomposition. A HOBO U23 Pro v2 External temperature/relative humidity data logger with a sensor on the end of a 2 m long cable was placed directly under the posterior end of each carcass (U23-002, Onset Computer Corp. Bourne, MA). A mixture of different types of hard and soft wood chips, associated leaf litter, and sawdust obtained from the OSU Botanical Garden was used for the composting media, and the material was moistened with water per the standard recommendations for mortality compost which is approximately 50% moisture (Payne and Pugh 2010). Following standard recommendations for C:N ratio of 25:1, composted animal sites were constructed by establishing a pad of composting media that was approximately 0.5 m thick with an envelope of approximately 0.5 m surrounding the carcass to ensure adequate insulation (Rynk 1992, Payne and Pugh 2010). Before burial in composting media, the rumen of each animal was punctured in order to avoid excessive bloating. The exposed and composted cadavers remained undisturbed throughout the entire period of decomposition.

Insect Activity: Malaise traps were installed to trap flying insects visiting each carcass site. The traps were fabricated specifically to be suspended above each carcass or compost pile without disturbing the sites with poles or stakes. Each cadaver was also examined for eggs and larvae

upon arrival, and additional activity outside of malaise traps, if any, was noted. Samples were collected every 2 to 4 days and frozen for subsequent identification. They were collected from both sites on the same days, and sampling continued throughout decomposition until visual inspection of the exposed carcass determined that it has progressed to the skeletal stage where only bone and hair remained (Lord and Goff, 2003).

Analysis of Volatile Organic Compounds: Volatile organic compounds (VOCs) were sampled using 75 µm carboxen®/polydimethylsiloxane solid-phase microextraction (SPME) fibers (Supelco #57344-U, Sigma-Aldrich Co. LLC, Bellfonte, PA). Individual compost containers were fitted with a large plastic jar to catch any VOCs emitted over a period of 48 hours, and Parafilm® (Bemis Company Inc., Neenah, WI) was used to completely seal the edges around each lid. SPME sampling was done on days 1, 5, 6, 12, 41, and 48 for all rats, and these dates were chosen based on the stage of decomposition observed in the exposed carcasses. The headspace in each container was homogenized by mild agitation, and individual fibers were exposed in the headspace for a period of 20 minutes (Hoffman et al., 2009). The plastic jars and lids were removed after each sampling event in order to release remaining odorant compounds and purify the headspace between samples. The samples were immediately analyzed using gas chromatography/mass spectrometry (GC/MS).

The instrument used was an HP6890 gas chromatograph (GC) with a 5973 mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA). The instrument was equipped with a DB5-MS capillary column 30 meters long with an internal diameter of 0.25 mm, a film thickness of 0.25 µm, and a SPME injection port liner operated at 250°C. The carrier gas used was helium and the flow rate was set at 1.5mL/minute. The oven temperature was set at 40°C to begin, held for 1 minute, ramped to 80°C at 3°C/minute, then up to 120°C at 10°C/minute, and lastly raised to 260°C at 40°C/minute. The total run time for the program was 21.83 minutes. The MSD was scanned 10 to 700 amu at a rate of 2.94 scans per second. Protocols for this study were adapted and modified from Hoffman et al. (2009). Data was collected using ChemStation and the spectra deconvoluted using AMDIS32 software. Compounds were identified using the NIST mass spectral library (National Institute of Standards and Technology, Gaithersburg, MD).

Statistical Analysis: Data from malaise trap samples collected throughout the entire period were pooled for each treatment. A two-sample t-test was conducted to determine any difference between the mean total abundance of arthropods and the mean total abundance of filth flies (SAS. Version 9.3; SAS Institute, 2013).

Results and Discussion

The mean total abundance of all insects collected from malaise traps throughout both replicates was numerically higher at the exposed carcass site, not statistically significant from the composted site ($F=1.05$, $df=1$, $p=0.2730$). The mean number of filth flies trapped at the exposed cadaver site was significantly higher than those trapped at the composted site ($F=1.96$, $df=1$, $p=0.0009$) (Fig. 1). Temperatures within the compost pile were consistently much higher than both the ambient temperature and temperature under the exposed carcass for both replicates reaching their peak at 48°C and 59°C for the first and second round, respectively. During the

initial heat cycle, which corresponded with the active decay stage in the exposed carcasses, odor was detectable only in close proximity to the compost pile.

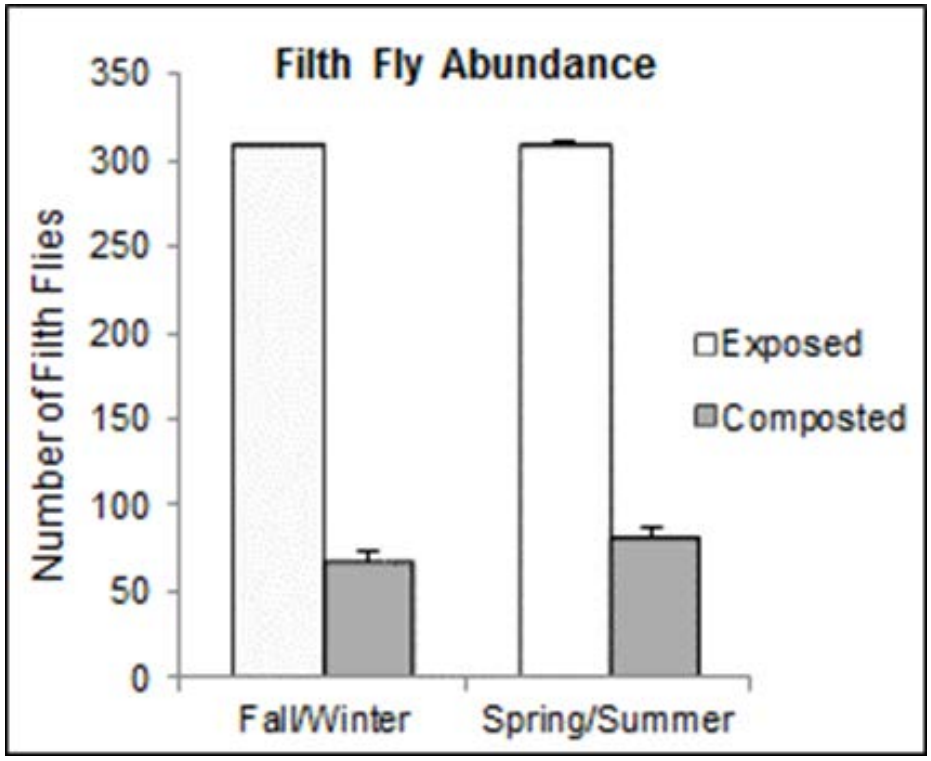

Figure 1 Total abundance of filth flies typically associated with carrion trapped near composted and non-composted beef cadavers throughout the entire period of decomposition (n=4). The number of filth flies at the exposed animal site was significantly higher than the composted site (p=0.0009).

There was a qualitative difference in the VOCs emitted from composted vs. exposed large rats. The only compound that was isolated from the composted rats was dimethyl disulfide, and it was not present in samples taken after day 6 (Table 1). The two compounds that were isolated from the exposed rats were dimethyl disulfide and dimethyl trisulfide, which were present on days 5, 6, 12, 41 and days 6, 12, 41, respectively (Table 1).

Table 1 Volatile organic compounds present in samples taken from exposed and composted rat cadavers.

	Exposed Animals						Composted Animals					
	Day 1	Day 5	Day 6	Day 12	Day 41	Day48	Day 1	Day 5	Day 6	Day 12	Day 41	Day48
	Fresh	*Active decay*			*Post decay*		*Fresh*	*Active decay*			*Post decay*	
Dimethyl sulfide	-	-	-	-	-	-	-	-	-	-	-	-
Dimethyl disulfide	-	✱	✱	✱	✱	-	-	✱	✱	-	-	-
Dimethyl trisulfide	-	-	✱	✱	✱	-	-	-	-	-	-	-

The relationship between livestock production and filth flies is inevitable, and the considerable amount of waste, including animal mortalities, generated annually by these facilities can have a profound effect on filth fly populations. This study has shown that composting livestock cadavers greatly reduces the abundance of adult filth flies. While similar groups of flies commonly associated with carrion were recovered at both sites, the abundance was significantly reduced

at the composted carcass site. This study establishes that composting bovine mortalities greatly reduces filth fly abundance.

The composting process also decreases emission of VOCs produced by animal decomposition. Early in the active decay stages, unpleasant odor is mild, detectable only by persons in close proximity, and quickly diminishes. After observing very few flies in association with the composted carcasses, laboratory studies were initiated to examine fly olfactory cues emitted from composted carcasses using a smaller animal model. Specific VOCs emitted from animal cadaver compost have been studied for use as indicators of composting efficiency, and dimethyl disulfide and dimethyl trisulfide are also known to be olfactory cues for blows flies (Zito et al., 2014). Odors of decomposition that have been specifically tested using electroantennogram (EAG) techniques and elicited a strong response in blow flies include dimethyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide (LeBlanc 2008), and more recent work has included indole, isobutylamine and phenylacetic acid (Liu et al., 2016). Results from this study indicate that emission of select fly olfactory cues from a rat carcass is decreased when it is composted.

Conclusions

Regular and catastrophic animal losses create additional challenges for animal waste management. The methods of disposal that have historically been popular and environmentally sound, such as rendering and incineration, may not be cost efficient or as locally available, and alternative means such as composting are being explored. Composting is an economical, environmentally sustainable means of disposing of dead animal carcasses, and this study examined the associated insect activity as well as volatile organic compounds emitted from the compost pile. Composting livestock mortalities greatly reduces filth fly activity and inhibits propagation. Results from laboratory studies with volatile organic compounds released from exposed and composted animals indicate that the composting process may degrade or otherwise inhibit the release of important olfactory cues that typically attract flies to carrion. The breakdown or suppression of chemical cues may contribute to the overall decrease in filth fly activity associated with composted beef carcasses. Reduction in filth fly activity within the grounds of livestock facilities by composting mortality waste could also reduce the risk of flies contaminating the surrounding area by spreading pathogenic microorganisms acquired from the dead animals.

*All of this work should be cited from the following publication:

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