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Research Article

Insect Succession on Pig Carrion in North-Central Mississippi

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Abstract: A freshly-euthanized 90 kg Yucatan mini pig, *Sus scrofa domesticus,* was placed outdoors on 21March 2012, at the Mississippi State University South Farm and two teams of students from the Forensic Entomology class were assigned to take daily (weekends excluded) environmental measurements and insect collections at each stage of decomposition until the end of the semester (42 days). Assessment of data from the pig revealed a successional pattern similar to that previously published – fresh, bloat, active decay, and advanced decay stages (the pig specimen never fully entered a dry stage before the semester ended). Over 2,300 immature and adult insects were collected from the pig or its immediate surroundings during the observation period, representing 34 distinct taxa. Their appearance at the carcass clearly varied by stage of decomposition. Blow flies and fire ants were among the first insects to colonize the carcass, followed by beetles. The very first responding insect group was the blow flies (Calliphoridae), specifically *Phormia regina,* appearing within minutes of pig placement. A sub-sample of 756 larval specimens collected from the pig during the study revealed that over 90% of specimens were *Phormia regina*. Three hundred twenty-five beetles were collected during the active and advanced decay stages, mostly in the insect families Staphylinidae, Histeridae, Cleridae, Nitidulidae, Dermestidae, and Trogidae. One of the most commonly collected beetle species was the red-legged ham beetle, *Necrobia rufipes.* Interestingly, burying beetles (Silphidae), which are extremely common on carrion in Mississippi, were never seen or collected throughout this study.

Keywords: Forensic entomology, insect succession, blow flies, decomposition, carrion

Introduction

In the broadest sense of the term, forensic entomology means using entomological evidence in courts of law. This may include entomological evidence in court cases about termite damage to homes and other structures, insect pests in food, cases of neglect in nursing homes and other facilities, or similar situations. However, for most people, the term forensic entomology brings to mind using insects during investigations of murders to help establish a post-mortem interval (PMI) (Gennard 2007). In order to accurately estimate PMI, key indicator species (mostly flies and certain beetles) must be known for each particular area. Knowledge about species involved in corpse or carrion decomposition in one region may be of little value in cases in another region since diversity of species and relative abundance varies with geography and physiographic regions. There have been almost no systematic surveys of arthropod fauna attracted to carrion in Mississippi. One study evaluated blow flies attracted to various carrion types near Oxford, MS (Goddard and Lago 1985), and an annotated list of blow flies of Mississippi was generated from that same study (Goddard and Lago 1983). Nearby, in Tennessee, a thorough study of insects colonizing dog carcasses was conducted in the 1950's (Reed 1958), and others have been conducted with other animal hosts, such as those in Texas and Virginia (Tabor et al. 2004, Bucheli et al. 2009). In an effort to understand the succession of insects at carrion for our physiographic region and in particular for our location (Mississippi), we observed and collected insects from a freshly-euthanized dead pig for 42 days as it progressed through successive stages of decomposition.

Site description

The pig used in this study was placed outdoors on the Mississippi State University South Farm, also known as the Leveck Animal Research Center, an agricultural setting with limited public access. South Farm is a 1000 plus acre research facility used primarily for agricultural projects. The facility is located within the black prairie physiographic region of Mississippi (Lowe 1919). The enclosure used for our pig study was located at N33°25'21"W88°46'57", near an area used for burning vegetation and debris (but not garbage) from the campus (Figure 1).

Figure 1. Cage and surroundings where pig was placed for decompositional study.

Pastures surrounding most of the study site are actively grazed by cattle, although there is a small wooded ephemeral creek/drainage ditch on the northeast side. The site has also been bulldozed multiple times, leaving the ground cover at approximately 20-30% (primarily grasses). Overall, the site is very disturbed, and this disturbance is noteworthy as it may impact the diversity and abundance of insects available to utilize the pig carcass as a resource (Bestelmeyer and Wiens 1996).

Methods

The carcass used in this study was a 90 kg Yucatan mini pig, *Sus scrofa domesticus*, provided by the Mississippi State University College of Veterinary Medicine. The pig was previously used in veterinary medicine research at the college, and already had been scheduled for euthanization. The pig carcass was placed in a 3m-x 3m chain link fence enclosure, with strings running across the top of the enclosure to prevent vultures from disturbing it. Further, two stab wounds and a gunshot wound were inflicted upon the dead pig to mimic wounds that potentially could be found on a human corpse and to provide information about insect activity upon wounds. Once the pig was placed, two class teams (A and B) were assigned to take daily temperature and humidity readings (weekends excluded) and insect collections at each stage of decomposition until the end of the semester. All data from both teams were combined for this paper.

At each visit, insects were collected with an aerial sweep net over the pig prior to any other disturbance activity. This was to ensure that "shy" species were gathered prior to the carcass being disturbed. After the initial sweeping, collecting was done by hand to gather beetles and fly larvae. Additional sweeping with a net was performed to collect as many representatives as possible of the Diptera species at the carcass. Six pitfall traps, consisting of small plastic jars buried such that their openings were level with the ground surface, were also placed near the carcass primarily to collect beetles. Placement of these traps was random, with two traps placed at soil level within the cage, approximately 10 cm from the head and 10 cm from the back, and four traps placed within 3 m of the enclosure. Insects collected with sweep nets were killed using both ethyl acetate jars and potassium cyanide jars (by team leaders only). Hand-collected insects were either placed directly into vials of 70% ethanol or into empty specimen jars for rearing. Insects from pit fall traps were rinsed in 70% ethanol and either pinned or stored in vials of 70% ethanol.

Representatives of all insects were labeled and either pinned or stored in 70% ethanol as appropriate. Specimens examined for this report were identified to family level and, where possible, to genus or species. Identifications were made using standard keys (Hall 1948, Hall and Townsend 1977, Whitworth 2006) and museum reference material. In addition, representatives of all taxa identified were re-examined by various experts in the entomology department at Mississippi State University. Voucher specimens were deposited in the Mississippi Entomological Museum, Mississippi State University.

Upon each visit, ambient temperatures were recorded above the pig carcass at 0.3 and 1.2 m. Recordings were also taken from the body surface of the pig, the interface of the pig and soil, and direct soil temperature at an approximate depth of 5 cm. When present, maggot mass temperatures were recorded for each location on the pig (head, rear, or stomach).

Results

Temperature observations. Ambient temperatures recorded above the pig (1.2 m) ranged from 7.7^0 C to 38.8^0 C, while temperatures directly on the body surface were much warmer, 16.1^0 C to 50.5^0 C (note: temperatures were taken during the day when the pig was in full sun). Under-the-body temperature readings ranged from 17.7^0 C to 34.4^0 C. During the active decay stage there was development of a maggot mass (Figure 2), with recorded temperatures ranging from 36.1^0 C to 38.3^0 C. On average, the maggot mass temperature was approximately 8.0^0 C warmer than the 1.2 m ambient temperatures.

Figure 2. Measuring maggot mass temperature.

Stages of Decomposition and Succession. Assessment of the data from the pig appeared to follow the expected successional pattern (Catts and Goff 1992) – fresh, bloat, active decay, and advanced decay stages (Figure 3) (the pig specimen never fully entered a “dry” stage before the semester ended). Over 2,300 immature and adult insects were collected from the pig or immediate surroundings during the 42-day observation period, representing 34 distinct taxa (Table 1), and their appearance varied by stage of decomposition (Table 2). Blow flies and fire ants, *Solenopsis invicta x richteri,* were among the first insects to colonize the carcass, followed by beetles. The very first responding insects were blow flies (Calliphoridae), specifically *Phormia regina,* appearing within minutes of pig placement. A sub-sample of 756 larval fly specimens collected from the pig during the study and identified by the first author revealed that over 90% of specimens were *Phormia regina* (Figure 4). After 4 weeks, a drop-off of fly activity occurred with the continued presence of beetles. The following stage assessments are based on our observations and sampling data.

Figure 3. Stages of decomposition studied.

Table 1. Summary of insects collected from pig carcass on Mississippi State University South Farm, 21 March - 2 May, 2012.

Order	Family	Genus and species
Coleoptera	Carabidae	
	Cicindellidae	
	Chrysomelidae	
	Cleridae**	*Necrobia rufipes***
	Dermestidae**	
	Histeridae**	
	Nitidulidae**	
	Scarabaeidae	
	Staphylinidae**	*Creophilus maxillosis***
		Other staphylinid spp.
	Trogidae**	
Diptera	Calliphoridae**	*Phormia regina***
		*Lucilia coeruleiviridis***
		*Cochliomyia macellaria***
		*Lucilia cuprina***
	Dolichopodidae	
	Fannidae	*Fannia scalaris*
	Muscidae**	*Ophyra leucostoma***
		Other *Orphyra* spp.**
	Piophilidae**	*Piophilia casei***
		Prochyliza brevicornis
		Prochyliza xanthostoma
		Stearibia nigreceps
	Sarcophagidae**	
	Scathophagidae	
	Sepsidae	
	Ulidiidae (formerly Otitidae)	*Euxesta* spp.
Hemiptera	Geocoridae	*Geocoris uliginosis*
Hymenoptera	Formicidae	*Solenopsis invicta x richteri*
		Camponodis subbarbatus
		Monomorium minimum
		Hypoponera opacior
Orthoptera	Tetrigidae	*Tettigidea lateralis*
	Acrididae	*Cortaphagae viridifaceatae*

**Insects primarily associated with the decomposition process.

Table 2. Duration of stages and dominant insect taxa for pig carcass on Mississippi State University South Farm, 21 March – 2 May, 2012. AD = adults; LV = larvae.

Stage	**Duration**	**Dominant Taxa and Stage**
Fresh	Days 1-4 March 21-23	Diptera Calliphoridae *Phormia regina* AD, LV *Lucilia coeruleiviridis* AD, LV *Cochliomyia macellaria* AD, LV Sarcophagidae *Sarcophaga* spp. AD Unidentified sarcophagids AD Hymenoptera Formicidae *Solenopsis invicta* AD
Bloat	Days 5-9 March 23-27	Diptera Calliphoridae *Phormia regina* AD, LV *Lucilia coeruleiviridis* AD, LV *Cochliomyia macellaria,* AD, LV Sarcophagidae *Sarcophaga* spp. AD Unidentified sarcophagids AD Ulidiidae (formerly Otitidae) AD *Euxesta* spp. Unidentified Diptera Coleoptera Staphylinidae AD *Creophilus maxillosus* AD Unidentified staphylinids AD Histeridae AD Unidentified Coleoptera Hymenoptera Formicidae *Solenopsis invicta* AD
Active decay	Days 10-20 March 27- April 14	Diptera Calliphoridae *Phormia regina* AD, LV *Lucilia coeruleiviridis* AD, LV *Cochliomyia macellaria* AD, LV *Lucilia cuprina* AD Sarcophagidae *Sarcophaga* spp. AD Unidentified sarcophagids AD Muscidae *Ophyra* spp. AD Unidentified Muscidae AD Piophilidae *Piophilia casei* AD *Prochyliza brevicornis* AD *Prochyliza xanthostoma* AD *Stearibia nigreceps* AD Ulidiidae (formerly Otitidae) AD *Euxesta* spp. Unidentified Diptera AD, LV Coleoptera Staphylinidae AD *Creophilus maxillosus* AD Unidentified staphylinids AD Histeridae AD Dermestidae AD Cleridae *Necrobia rufipes* AD Unidentified Coleoptera AD, LV Hymenoptera Formicidae *Solenopsis invicta* AD

Advanced decay	Days 21- 42 April 14- May 2	Diptera Calliphoridae *Phormia regina* AD, LV *Lucilia coeruleiviridis* AD, LV *Cochliomyia macellaria* AD, LV Sarcophagidae *Sarcophaga* spp. AD Unidentified sarcophagids AD Muscidae *Ophyra* spp. AD Unidentified Muscidae AD, LV Piophilidae *Piophilia casei* AD *Prochyliza brevicornis* AD *Prochyliza xanthostoma* AD *Stearibia nigreceps* AD Fannidae *Fannia scalaris* AD, LV Unidentified Diptera Ad, LV Coleoptera Staphylinidae AD *Creophilus maxillosus* AD Unidentified staphylinids AD Histeridae AD Dermestidae AD, LV Trogidae AD Unidentified Coleoptera AD, LV Hymenoptera Formicidae *Solenopsis invicta* AD
Dry	Days 42 onward	Not done

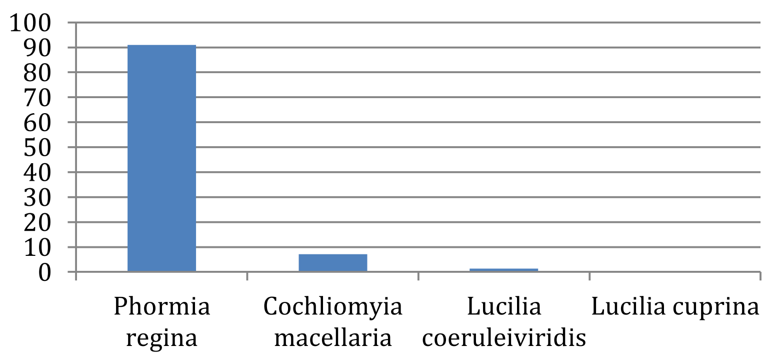

Figure 4. Proportion of blow flies collected at the pig carcass.

Fresh stage, 21-23 March. The fresh stage is defined from the time of death until first signs of bloating. In this experiment, this stage lasted approximately three days. On the third day there was obvious bloating of the carcass. During the fresh stage the predominant insects collected were the red imported fire ant hybrid, *Solenopsis invicta* x *richteri*, and calliphorid flies such as

Phormia regina, Cochliomyia macellaria, and *Lucilia coeruleiviridis.* No beetles were collected during this stage. However, at the end of the fresh stage, one ground beetle (Carabidae) was collected. By the second day, fly eggs and 1st instar larvae were present on the eyes and mouth of the pig carcass (Figure 5). However, fly activity at the wound sites on the abdomen was prohibited and exploited by fire ants (Figure 6). Fire ants were observed actively feeding on and in the open wounds, removing fly eggs almost as fast as they were deposited.

Figure 5. Blow fly eggs laid in/around eye during fresh stage.

Figure 6. Fire ants foraging in wounds of dead pig, primarily collecting fly eggs and larvae.

Bloat Stage, 23-27 March. The bloat stage begins with buildup of gasses inside the carcass as it goes through decomposition and ends with release of these gasses, evidenced by decrease in size of the carcass. The bloat stage was evident by 23 March, with high levels of blow fly activity, both adults and larvae (Figure 7-8), sarcophagid flies, and the advent of several beetle groups including Histeridae, Staphylinidae, and Cleridae. The end of the bloat stage became evident on 27 March when skin slippage, a sign of active decay, occurred. Further support that the carcass was entering the active decay stage was a peak in insect activity which also occurred around 27 March.

Figure 7. Blow fly species appearing at pig carcass.

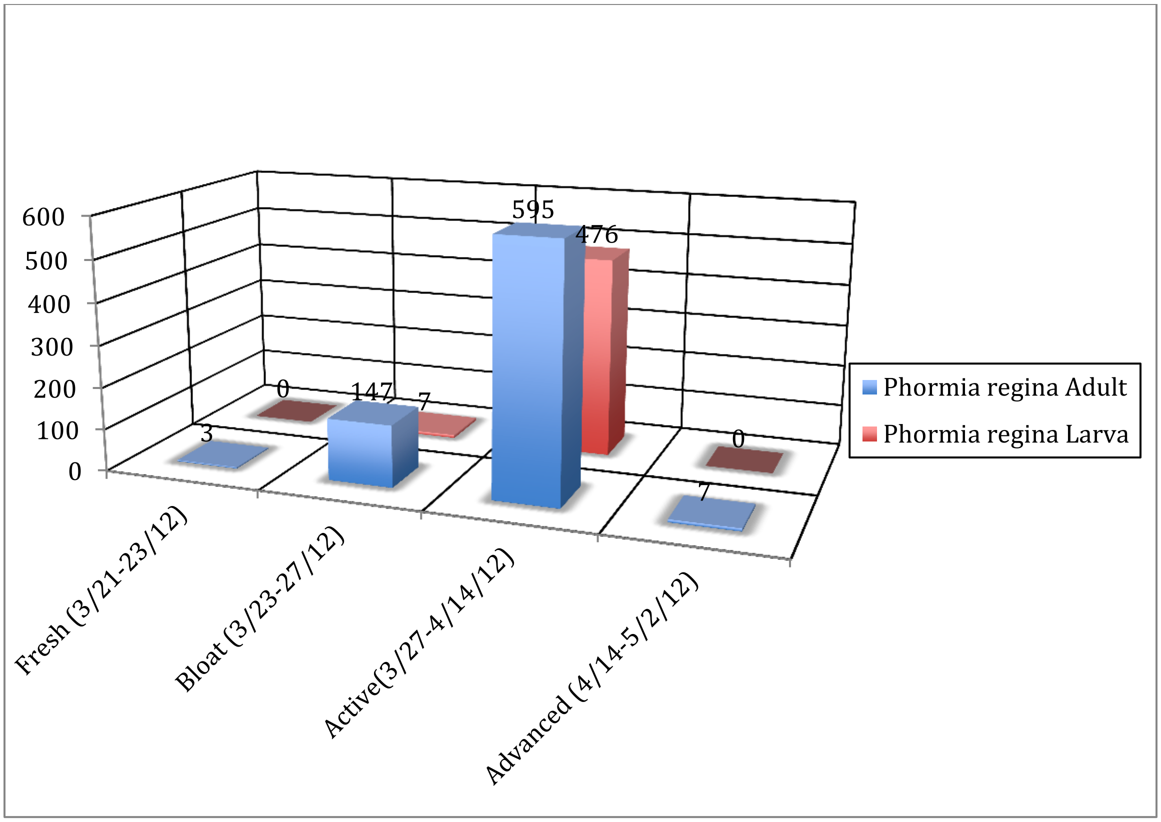

Figure 8. Comparison of blow fly, *Phormia regina,* activity pattern at each decompostion stage of pig carcass.

Active Decay Stage, 27 March - 14 April. Generally during the active decay stage, bloating ceases, and the decaying carcass begins to "lose shape". In this study, during active decay, insect activity was high for all insect groups. However, the composition of flies shifted from Calliphoridae and Sarcophagidae to predominantly smaller Muscidae and Piophilidae species. Three specimens of the blow fly *Lucilia cuprina* were collected, but this species is not usually an important insect in carrion decomposition. Beetle activity increased and continued to remain high from this point to the end of the study (Figure 9). One hundred seventy-three beetles were collected during the active stage, mostly members of the Histeridae and Staphylinidae families. Fire ants were also observed actively carrying maggots and eggs away from the carcass, even the largest 3rd instar Calliphoridae (Figure 10).

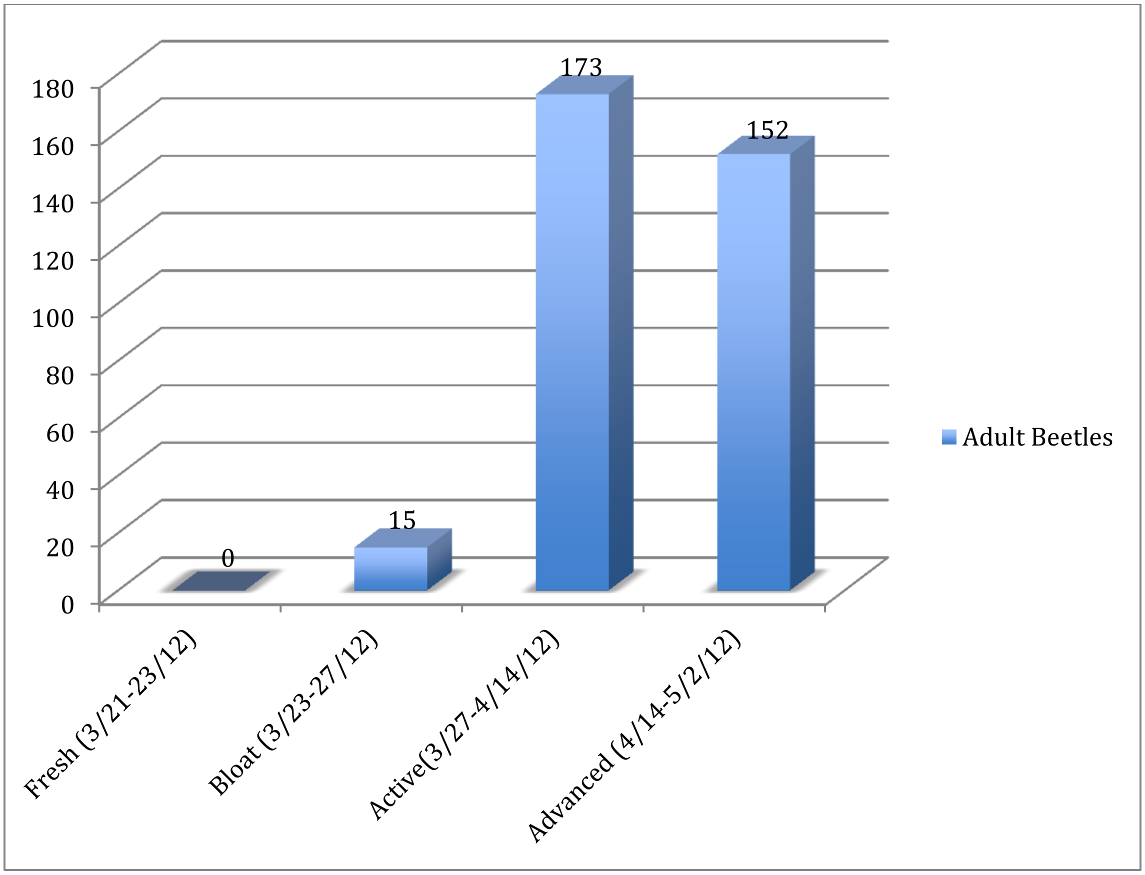

Figure 9. Beetle succession patterns at the pig, all families combined.

Figure 10. Fire ant attacking a 3rd instar blow fly maggot (Photo copyright 2012 by Jennifer Seltzer and Charlotte Chesnut).

Advanced Decay Stage, 14 April 14 - 2 May. During the advanced decay stage, there was a definite drop in the Diptera species attracted to the pig, but still considerable beetle activity. Teneral Diptera (mostly blow flies emerging from pupae) were collected from vegetation around the cage during this time period. In addition, members of the fly family, Piophilidae or "cheese skippers", were observed actively skipping on the body surface of the carcass. One hundred fifty-two beetles were collected during the advanced decay stage, mostly in the families Dermestidae, Trogidae, Nitidulidae, and Cleridae. Beetles dominating this stage included Dermestidae, Cleridae (primarily the red-legged ham beetle, *Necrobia rufipes*), and Trogidae (*Trox* spp.).

Conclusions

Our data show that blow flies, especially *Phormia regina,* are the primary indicator species in this region of Mississippi in forensic entomology investigations to estimate PMI. These blow flies arrive almost instantly and comprise over 90% of the blow flies collected at the carcass. This finding is consistent with that of Goddard and Lago which found that *P. regina* was the most common blow fly in Mississippi (Goddard and Lago 1983). Certainly, many other blow fly species occur in Mississippi and may have been present, but were not collected during our study.

Several different species of Piophilidae consistently appeared during the active and advanced decay stages of carrion decomposition (see Tables 1-2). Research is currently underway in our lab to study the Piophilidae in relation to forensic entomology.

Lastly, beetles dominate the active and advanced stages of decomposition, especially members of Histeridae, Staphylinidae, and Cleridae. Members of Trogidae, especially *Trox* species, and Dermestidae are good indicators of advanced decay. Oddly, in our study, no Silphidae (the carrion or burying beetles) were collected, even though these beetles are common on carrion in Mississippi. There have been some studies demonstrating negative effects of fire ants on Silphidae (burying beetles) (Scott et al. 1987, FWS 2008). Also, burying beetles may abandon carcasses that become heavily infested with fly larvae (Wilson 1983). In our study, there was an abundance of both fire ants and fly larvae, possibly prohibiting colonization of the carcass by Silphidae.

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