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The Role of the Arista on Lucilia sericata in Sensing Wind and Airflow, Relative Humidity and Volatile Compounds

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Honors Thesis

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Abstract

Lucilia sericata is a species of blow fly that has important applications in the fields of forensic entomology and medicine. *L. sericata* is one of the first organisms that arrives at decaying carrion in response to decay odors released by the carrion. The attraction stimuli are presumably the decay volatiles, but wind flow, and humidity may also influence the blow fly resource-oriented behavior. This fly species has feather-like structures known as arista that project from the antenna. The function of these structures is not completely understood, however they may play a role in sensing airflow and wind, humidity, and volatile organic compounds. The goal of this project is to gain a deeper understanding of the function of the arista so that it may provide a greater insight into the behavior of the organism. Flies were subjected to arista ablation and were exposed to of a series of choice experiments: air flow with versus without humidity, variable air flow, and with or without carrion odors. Flies were placed in a reaction chamber and allowed to choose between the wind, speed, humidity, and odor variables and their behavior was observed. Fly choice was recorded as landing/hovering in the area of the stimulus introduction port. Preliminary data indicate that non-ablated flies actively select humid airflow. The data showed the arista played a role in sensing wind and airflow.

Dedication

I would like dedicate this thesis to my parents, Robert and Elaine Jacob, for their constant support.

UNIVERSITY of DAYTON

Table of Contents

Introduction

Known for its medical and forensic importance, the green bottle fly *Lucilia sericata* has been the subject of much study. The species' most notable contribution to the field of forensic analysis results from this organism's role as a primary colonizer of carrion and human remains [1,2]. *Lucilia sericata* is attracted to a body immediately following death, often within minutes of occurrence [3]. Because this species is typically the first to colonize human remains, it is the most important and precise means for determining time of death and post mortem interval [2]. In fact, *L. sericata* allows one to determine time of death for two or more weeks. This is much more accurate than a medical examiner who is only able to determine time of death within the span of two days [3]. Thus, this species is one of the prime tools in the field of forensic entomology, which is defined as the use of insects and other arthropods in mediocriminal investigations [3]. The carrion is critical for the life cycle of *L. sericata* in that it provides a food source, mates, and a suitable oviposition site. However, the occurrence of carrion in an ecosystem is unpredictable. Thus, there is intense competition among species for these resources [4]. Additionally, in order for proper oviposition and development, the decomposition stage at which the fly arrives is crucial. Inaccurate arrival time could be detrimental to the offspring [4]. As a result, it is important for most insects, including *L. sericata*, to be able to rapidly sense their environment and modify their behavior accordingly.

An organism's fitness and success is often evaluated in terms of their ability to thrive and produce viable offspring that contribute to the next generation [5]. In order for an organism to produce offspring, it must avoid death and debilitation, as well as locate and utilize certain resources. As a result, an organism must respond to a multitude of stimuli in order to find these resources. The complexity of an organism's resource-oriented behavior varies with the number of resources needed and the spatial separation between them. Insects vary in the number of resources required according to their stage of development and sex. Specifically, a food source is required by all the larvae of all species, and by the adults of most species. Only adults, however, require resources related to reproduction. For example, adult males and non-parthenogenetic females need to locate a mate, and adult females need to find an appropriate location to deposit eggs or living offspring [5]. Further, relative humidity is important for arthropods to sense because it affects their own viability as well as the growth and development of their offspring. Specifically, it has been shown that low relative humidity increases mortality in many species of insects, especially during the egg stage [6]. Additionally, *L. sericata* locate carrion and host organisms by volatile organic compounds. It has been shown that these flies activate specific resource-oriented behavior, orient themselves upwind and land in response to sulfur-rich volatiles [7]. In all animals, including insects, behavior is a result of physiological processes. Thus, physiological processes are the underlying determinants of all resource-oriented behavior. The most critical sensory organ to flies is their antenna and antennal sensilla. These structures are the means through which *L. sericata* responds to environmental stimuli and modifies its behavior. As a result, the antennae and antennal sensilla are crucial to the organism's resource-oriented behavior. Specifically, these organs allow the organism to find food sources, mates, and acceptable oviposition sites [2]. The antenna of *L. sericata* consists of three segments: a proximal scape, a pedicel, and a distal flagellum which is comprised of an elongated funiculus and a feather-like projection known as the arista [2].

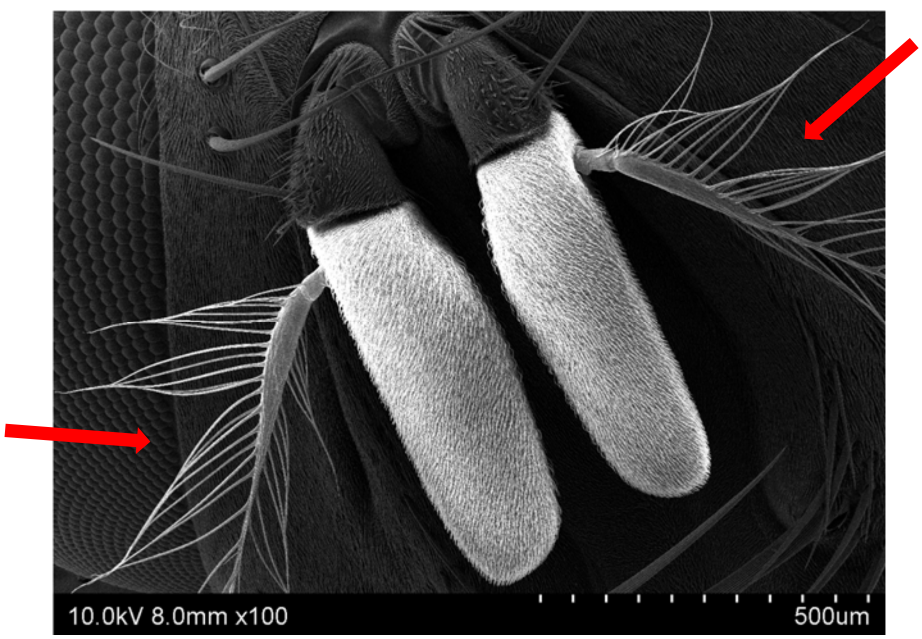

Figure 1: SEM picture showing the antennal sensilla of *L. sericata.* The arista are highlighted by the arrows. Photo courtesy of Allissa Blystone.

While the function of the proximal scape, pedicel, and funiculus are somewhat understood and described in the literature, the function of the arista remains unclear. As a result, further genomic, developmental, and physiological studies are necessary to better understand the function of the arista and the behavior they elicit in response to environmental stimuli [2]. Thus, this study sought to determine how the arista affects the organism's resource-oriented behavior and the role this structure plays in sensing wind and airflow, relative humidity, and volatile organic compounds.

Materials and Methods

Colony Maintenance

Lucilia sericata were reared in a fly enclosure that was kept between 83 and 86 degrees Fahrenheit, 30 ± 4% humidity, and with a 12 hour light/dark cycle. Small sub-populations used for experimentation were kept in Bug Dorms. The Bug Dorms were mesh cubes measuring 30 x 30 x 30 cm. Flies were fed a diet of organic beef liver and honey-water, they were also given access to a water source *ad libitum.* The honey-water solution was applied to one third of a paper towel and placed on half a cell-culture dish. The honey-water solution was prepared with a ratio of one part honey and one part water and stored in the refrigerator to prevent bacterial contamination.

Survivability Study

30 flies were captured from a small sub-population using small vials. These flies were placed in an ice bath for 10 minutes for anesthetization. Once the flies were anesthetized, the aristae were ablated using needle-nose tweezers. In order to ensure the arista were removed properly and completely, the organism was checked under a microscope. Once the arista were removed from this group, these flies were placed in a Bug Dorm and fed beef liver and honey water and given access to a water source *ad libitum*. Each day, the number of surviving flies was counted.

Another 30 flies were captured from a small sub-population using small vials. The aristae were not removed from this set of flies in order to serve as a control condition. These flies were placed in a Bug Dorm and fed a diet of organic beef liver, honey water, and given access to a water source *ad libitum*. The number of surviving flies was counted each day.

Experimental Chamber

The experimental chamber was built from a round plastic container measuring 11 inches across. A vertical line was drawn on the outside bottom of the container 5.5 inches across to indicate a difference between the two sides. Two Y-tube ports penetrated opposite ends of the chamber. One branch of the Y-tube port was connected to a different condition with flexible plastic tubing. The other branch of the Y-tube was covered with Parafilm. Around each Y-tube port, a 3 by 5 inch glue trap was placed. The area covered by the glue trap was used to indicate if the organisms exhibited a strong preference toward the particular condition. See Appendix A for a picture of the chamber.

Wind and Airflow Experiment

In this experiment, an air pump was connected to a flexible drying tube containing silica beads. The silica beads served to dry the air and eliminate any humidity present in the ambient air. This tube was then connected to a 1000mL glass media bottle. Once the air was pumped into the media bottle, it was picked up and taken to the chamber by another length of flexible tubing. This tubing was connected to one branch of the Y-tube. The stalk of the Y-tube penetrated into the chamber and was surrounded by a 3 by 5 inch glue trap. Thus, a small stream of dry air entered this side of the experimental chamber. The other branch of the Y-tube was covered with Parafilm so as to prevent inflow of ambient air. Both branches of the Y-tube on the opposite side of the experimental chamber were covered with Parafilm. This side also had a 3 by 5 inch glue trap placed around it.

In the control experiment, 3 sets of 10 flies were placed in the experimental chamber. The flies were given 15 minutes in the chamber and their behavior was observed and recorded.

In the experimental condition, 3 sets of 10 flies were placed in an ice bath for 10 minutes in order to be anesthetized. Once they were anesthetized, the aristae were removed using needle-nose tweezers. To ensure the aristae were removed completely and properly, the flies were checked under a microscope. Each set of 10 flies was placed in the experimental chamber for 15 minutes and their behavior was observed and recorded.

Relative Humidity Experiment

Prior to this experiment, flies were deprived of water for 15 hours in hopes that it would increase the response of flies to humid conditions. For this experiment, the air pump was split into two outputs using a Y-tube. One side of the Y-tube was connected to the same drying apparatus as described above. The other side of the Y-tube was used to bubble air through water to create humidified air. From the Y-tube on the air pump, a length of flexible tubing was attached to a 10mL pipet. This pipet was submerged in a 1000mL glass media bottle filled halfway with distilled water. Another length of tubing was placed inside the media bottle in the airspace above the water. This length of tubing connected to another Y-tube that penetrated the experimental chamber. The stalk of this Y-tube penetrated the chamber with a 3 by 5 inch glue trap placed around it. This set up produced an air stream at approximately 40% relative humidity.

In the control experiment, 3 sets of 10 flies were placed in the experimental chamber. The flies were given 15 minutes in the chamber and their behavior was observed and recorded.

In the experimental condition, the aristae were ablated from 3 sets of 10 flies according to the procedure outlined above. Each set of ten flies was placed in the experimental chamber for 15 minutes and their behavior was observed and recorded.

Volatile Organic Compound Experiment

This experiment began by performing serial dilutions of a stock solution of dimethyl disulfide (DMDS). DMDS is a sulfur-rich hydrophobic compound, and as such was diluted using hexane. A dilution of 1×10^{-3} was prepared. The experimental chamber was set up such that a length of clear tubing was run from the air pump into an empty 1000mL media bottle. A second length of tubing was run from the 1000mL media bottle to an empty 500mL media bottle. A third length of tubing was run from the 500mL media bottle and attached at one branch of a Y-tube that penetrated the chamber. The other branch of the Y-tube was covered with Parafilm. The other side of the experimental chamber was set up in a similar manner. One length of tubing ran from the air pump to an empty 1000mL media bottle. A second length of tubing was run from this bottle to a 500mL media bottle. In the bottom of this 500mL bottle, two rounds of filter paper with a 5.5cm diameter were placed. Each round of filter paper were saturated with 0.5mL of the of 1×10^{-3} dilution of DMDS for a total of 1mL. A third length of tubing ran from this bottle, and connected to the second Y-tube penetrating the chamber. The other branch of this Y-tube was covered with Parafilm.

In the control experiment, 3 sets of 10 flies were placed in the experimental chamber. The flies were given 15 minutes in the chamber and their behavior was observed and recorded.

In the experimental condition, aristae were removed from the flies. 3 sets of 10 flies were placed in the experimental chamber. The flies were given 15 minutes in the chamber and the behavior was observed and recorded.

Results

Survivability Study

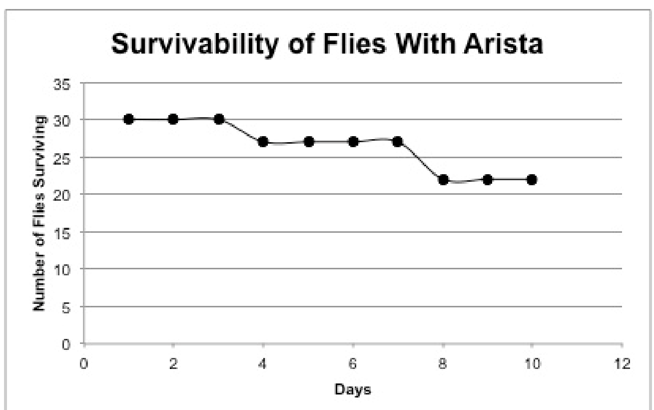

Figure 2: Survivorship of *L. sericata* over a 10 day period with arista intact.

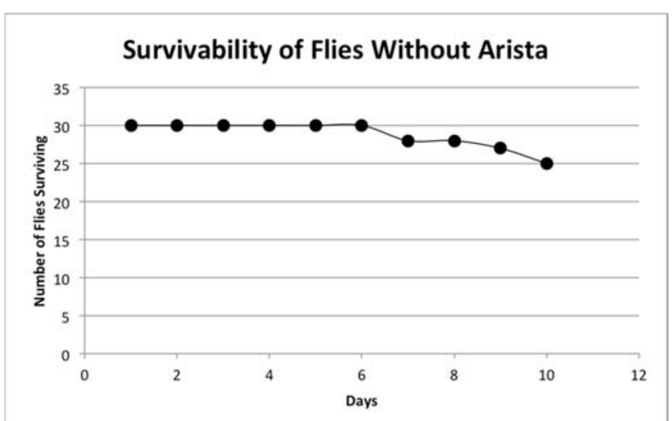

Figure 3: Survivorship of *L. sericata* over a 10 day period with arista ablated.

Wind and Airflow Experiment

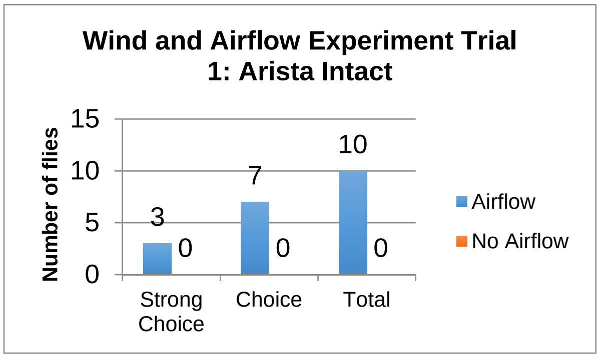

Figure 4: Number of flies with arista displaying a preference for the airflow or no airflow condition in the first trial.

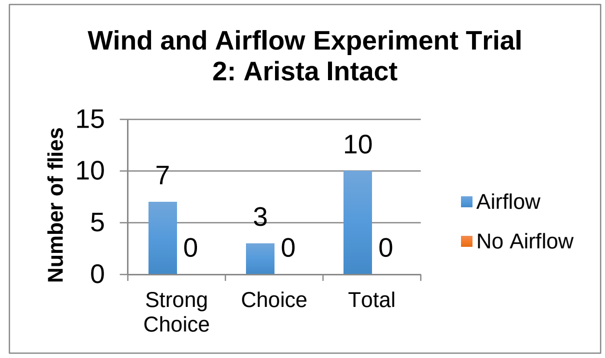

Figure 5: Number of flies with arista displaying a preference for the airflow or no airflow condition in the second trial.

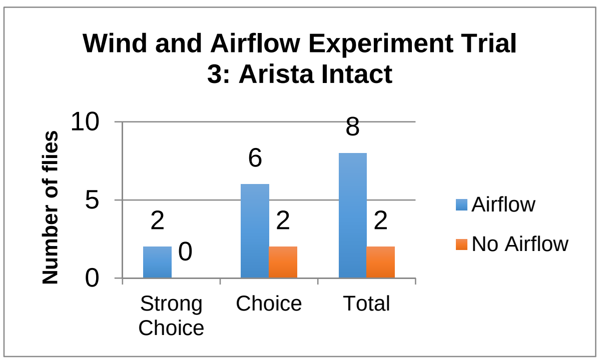

Figure 6: Number of flies with arista displaying a preference for the airflow or no airflow condition in the third trial.

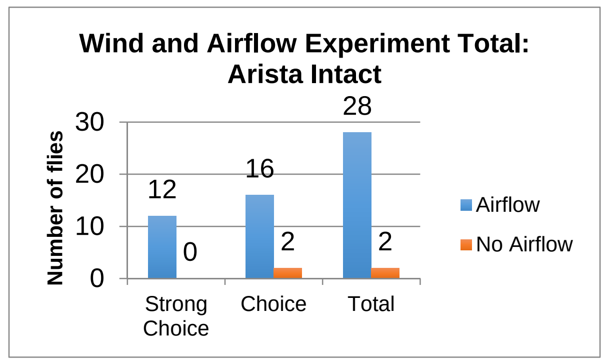

Figure 7: Total number of flies with arista displaying a preference for the airflow or no airflow condition.

Wind and Airflow Experiment Continued

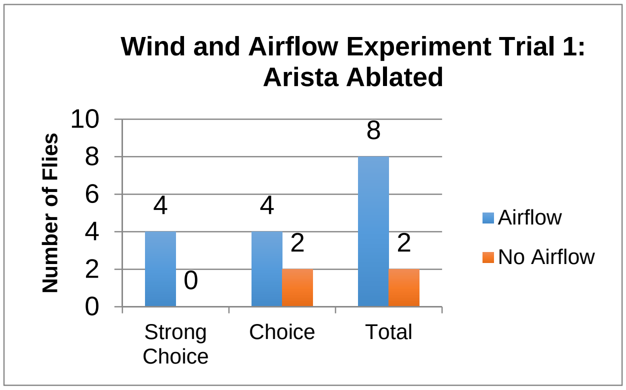

Figure 8: Number of flies without arista displaying a preference for the airflow or no airflow condition in the first trial.

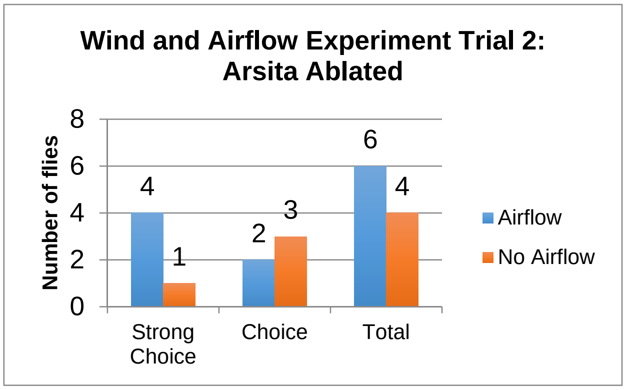

Figure 9: Number of flies without arista displaying a preference for the airflow or no airflow condition in the second trial.

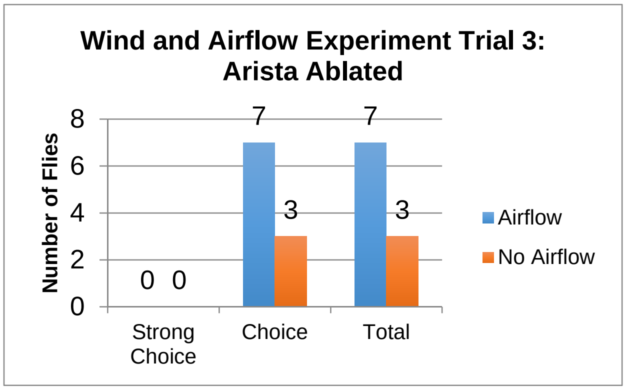

Figure 10: Number of flies without arista displaying a preference for the airflow or no airflow condition in the third trial.

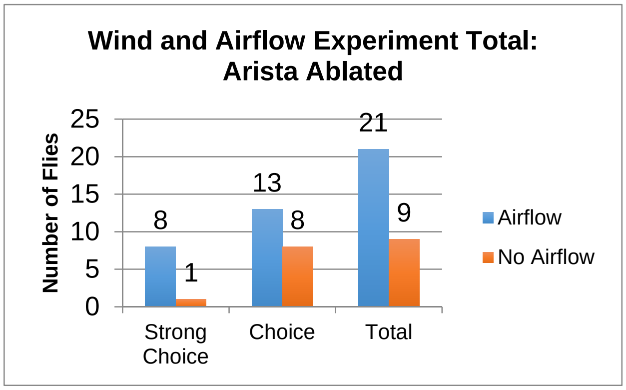

Figure 11: Total number of flies without arista displaying a preference for the airflow or no airflow condition.

Relative Humidity Experiment

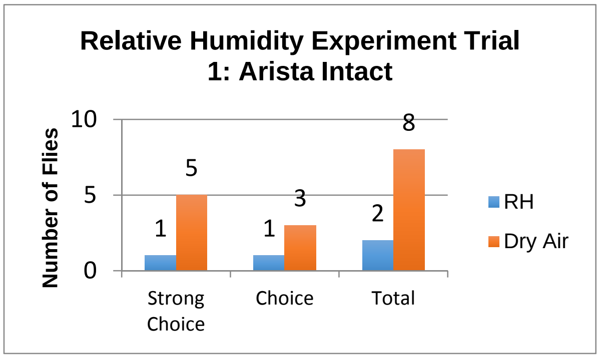

Figure 12: Number of flies with arista displaying a preference for the humid or non-humid condition in the first trial.

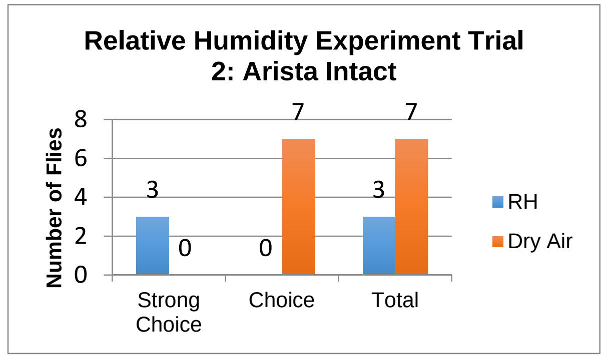

Figure 13: Number of flies with arista displaying a preference for the humid or non-humid condition in the second trial.

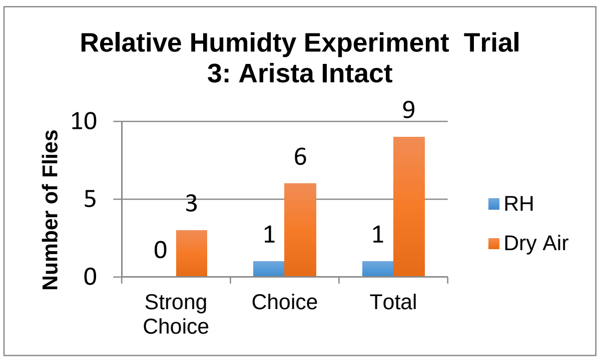

Figure 14: Number of flies with arista displaying a preference for the humid or non-humid condition in the third trial.

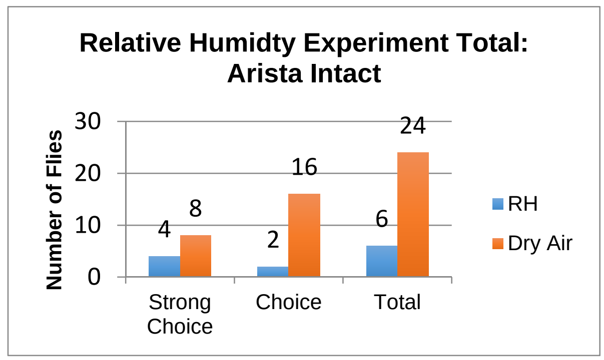

Figure 15: Total number of flies with arista displaying a preference for the humid or non-humid condition.

Relative Humidity Experiment Continued

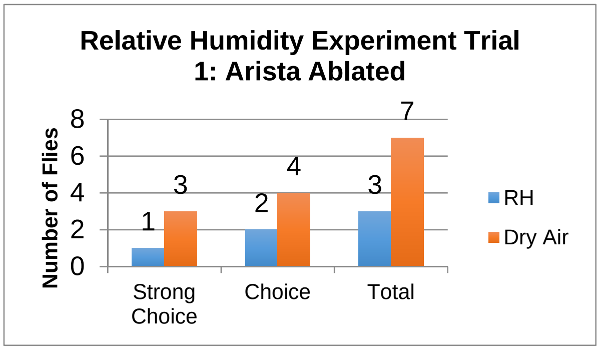

Figure 16: Number of flies without arista displaying a preference for the humid or non-humid condition in the first trial.

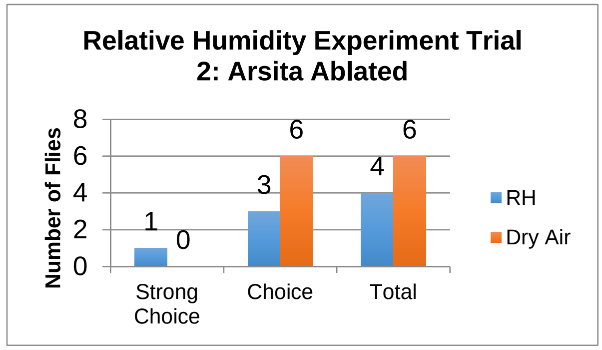

Figure 17: Number of flies without arista displaying a preference for the humid or non-humid condition in the second trial.

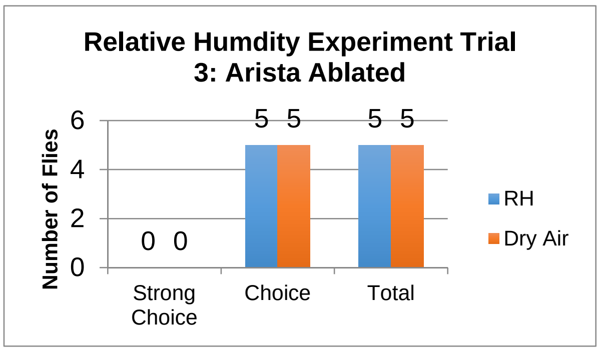

Figure 18: Number of flies without arista displaying a preference for the humid or non-humid condition in the third trial.

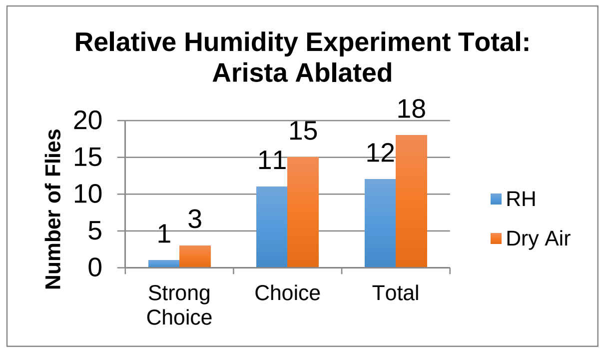

Figure 19: Total number of flies without arista displaying a preference for the humid or non-humid condition.

Volatile Organic Compound Experiment

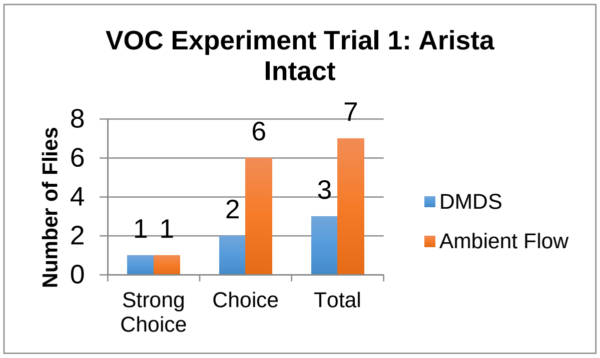

Figure 20: Number of flies with arista displaying a preference for the VOC or ambient flow condition in the first trial.

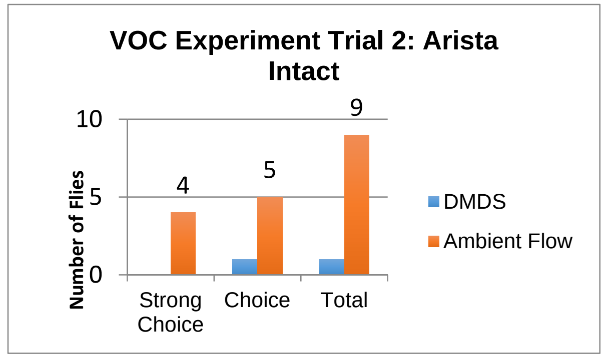

Figure 21: Number of flies with arista displaying a preference for the VOC or ambient flow condition in the second trial.

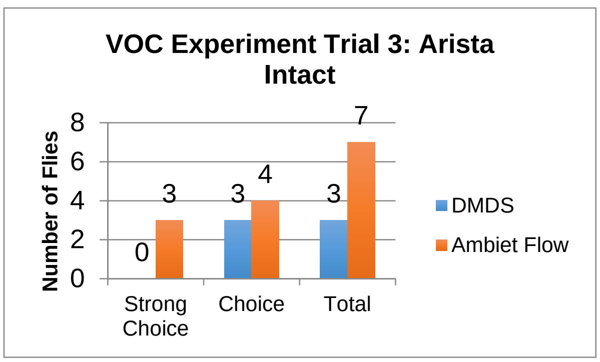

Figure 22: Number of flies with arista displaying a preference for the VOC or ambient flow condition in the third trial.

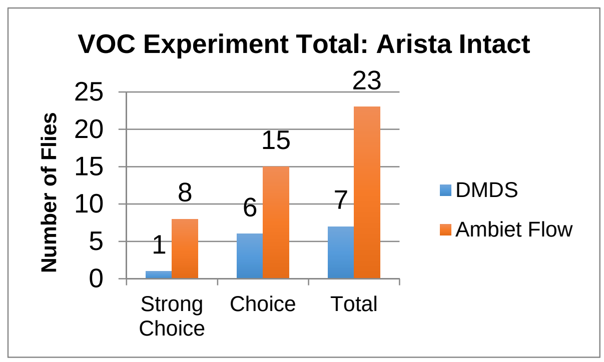

Figure 23: Total number of flies with arista displaying a preference for the VOC or ambient flow condition.

Volatile Organic Compound Experiment Continued

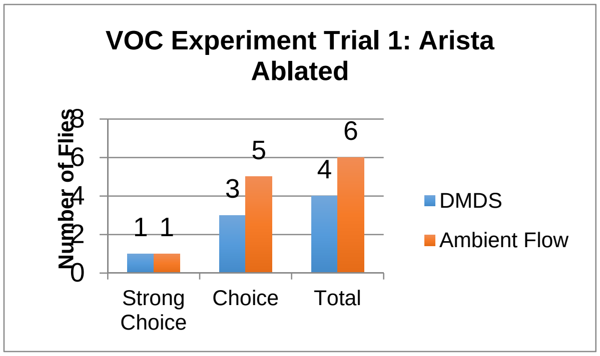

Figure 24: Number of flies without arista displaying a preference for the VOC or ambient flow condition in the first trial.

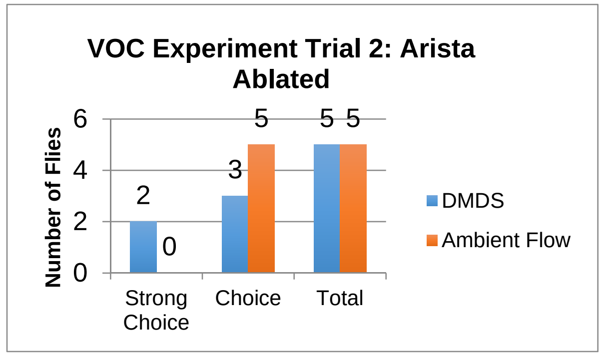

Figure 25: Number of flies without arista displaying a preference for the VOC or ambient flow condition in the second trial.

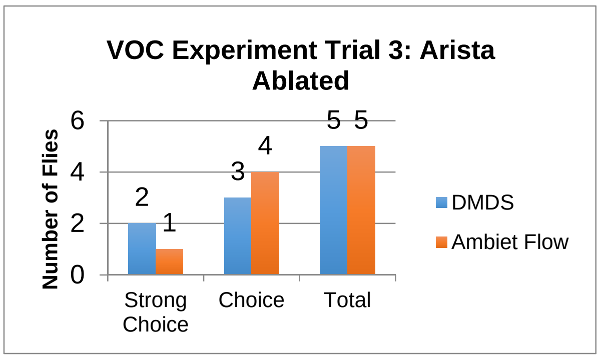

Figure 26: Number of flies without arista displaying a preference for the VOC or ambient flow condition in the third trial.

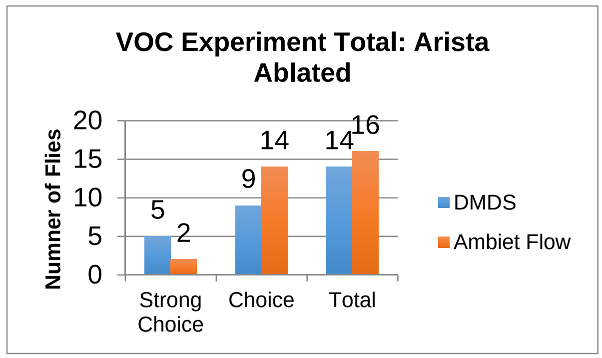

Figure 27: Total number of flies without arista displaying a preference for the VOC or ambient flow condition.

Discussion

The results from the Survivability study indicated that removal of the arista did not affect the overall survivability of the organism. This was crucial in determining whether or not to continue with the rest of the experiments as planned. If removal of the arista had affected the survivability of the organism it would have been impossible to determine if behavioral changes occurring after the arista were ablated were caused by sensing or overall lack of viability.

The second study examined the role of the arista in sensing wind and airflow. The results of this study revealed that when the aristae were intact, 28 out of 30 flies exhibited a preference toward the side of the chamber with dry airflow. When flies with ablated arista were tested in the chamber, less chose the side with airflow. This could suggest that the arista play a role in sensing the physical environment of the organism. Specifically, they assist the fly in orienting itself in the environment.

The third study examined the role of the arista in sensing relative humidity. Water was removed from the Bug Dorm containing the flies to be experimented on in an effort to exaggerate the flies' response to the humid conditions in the chamber. Originally, it was believed that the flies with intact arista would chose the side of the chamber with the stream of humid air, because humidity indicates the presence of water, an important resource. However, the opposite was true. The files with intact arista overwhelmingly chose the dry air condition. There are several possible reasons this was observed. One explanation for this behavior could be attributed to the fact that the experiments were run in a room with 33% relative humidity. The humid environment coupled with the fact that air bubbled through water only produces 40% relative humidity could have diminished the flies' attraction to the humid air stream. The approximate 7% difference might have been too small to elicit a different physiological response. However, this response might also indicate a hierarchy of resources that dictates a fly's resource-oriented behavior. Since the flies in the chamber were already sensing humidity, it may have been more important for them to sense air flow because airflow could bring VOCs, which indicate the presence of carrion. Even though the flies with arista did not act as predicted, when these results are compared to the results of the flies without arista, there is a clear difference in behavior. Once the arista were ablated and the flies were placed in chamber, 12 chose the relative humidity condition, while 18 chose the dry air condition. This data compared to the 6 flies that chose the humid condition and the 24 flies that chose the dry air condition indicates a strong behavioral change. Without the arista, the flies showed much less preference to the dry air. This data suggests that without arista, the flies are less able to discern a difference between the two conditions. Thus, the flies with ablated arista did not exhibit a preference.

The final study examined the role of the arista in sensing volatile organic compounds. DMDS was selected for this study because it is both sulfur-rich and a known fly attractant [4]. Because DMDS is a fly attractant it was believed that the flies with arista would exhibit a preference toward the side of the chamber emitting DMDS. When flies with intact arista were placed in the chamber, 23 chose the side with ambient flow and 7 chose the side with DMDS flow. When flies with ablated arista were placed in the chamber 14 chose the DMDS flow condition, while 16 chose the ambient flow. While the flies with intact arista did not behave as expected, there was a once again a drastic difference in behavior. This data suggests that the flies with the arista could discriminate the VOC and show a preference toward the side without the VOC. On the other hand, flies without arista showed no preference. Moreover, this could indicate that the aristae do play a role in sensing VOCs in some capacity. While it is unclear while the normal flies did not choose the VOC condition, it could be attributed to an unfavorable concentration of the VOC or the age of the flies.

In addition to this data, another interesting effect was observed. In almost every trial, a number of flies with arista exhibited a "clustering" effect in which the flies oriented themselves in close proximity to one another.

Figure 28: Clustering effect observed in flies with arista intact.

In contrast, flies without arista did not exhibit this "clustering" effect. In every trial, these flies were spread out in the chamber. This suggests that the removal of the arista played a role in this effect. While this observation was not directly examined in this study, it does point to evidence of other functions of the arista, and thereby offers a starting point for future research.

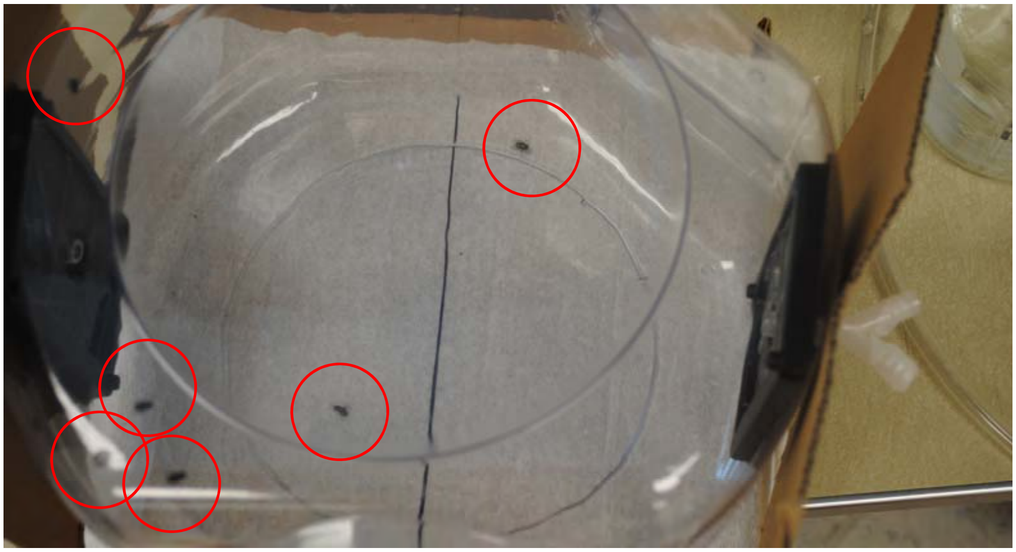

Figure 29: Clustering effect was not observed in flies without arista. These flies were spread out in chamber.

As a result of these experiments, it appears that the aristae play a role in sensing airflow. As stated previously, upwind orientation is important for flies in locating host organisms. Perhaps flies in the chamber were attempting to orient themselves in upwind in order to locate a food source or oviposition site. The data also suggests that the arista play a role in sensing the environment, and their removal affects the flies' ability to sense their surroundings. Flies without arista did not exhibit a preference that mirrored that of the anatomically normal flies. Thus, removal of the arista affected the behavior of the organism.

Ultimately, a greater understanding of the antennal sensilla of *L. sericata*, and how these structures allow the organism to sense its environment will allow for an even more accurate estimation of time of death and post-mortem interval in medical and criminal cases. This alone, has many practical applications to the field of forensic science. Moreover, deeper knowledge of the physiology of this fly's antennal sensing could allow for the development of a biosensor. A biosensor modeled after the incredible sensing capabilities of these flies would allow humans to detect small concentrations of VOCs, and thereby accomplish tasks otherwise impossible to us, such as locating lost human remains. Consequently, this field of research has great potential and there is still much to be learned about these organisms.

Acknowledgements

I would like to thank my advisor Dr. Karolyn Hansen, for her guidance and support. I would also like to thank TJ Lee, Casey Walk, and Erin Filbrandt for their help. Finally, I would like to thank the University of Dayton Biology Department and the Honors Department for both this opportunity and their contributions to this project.

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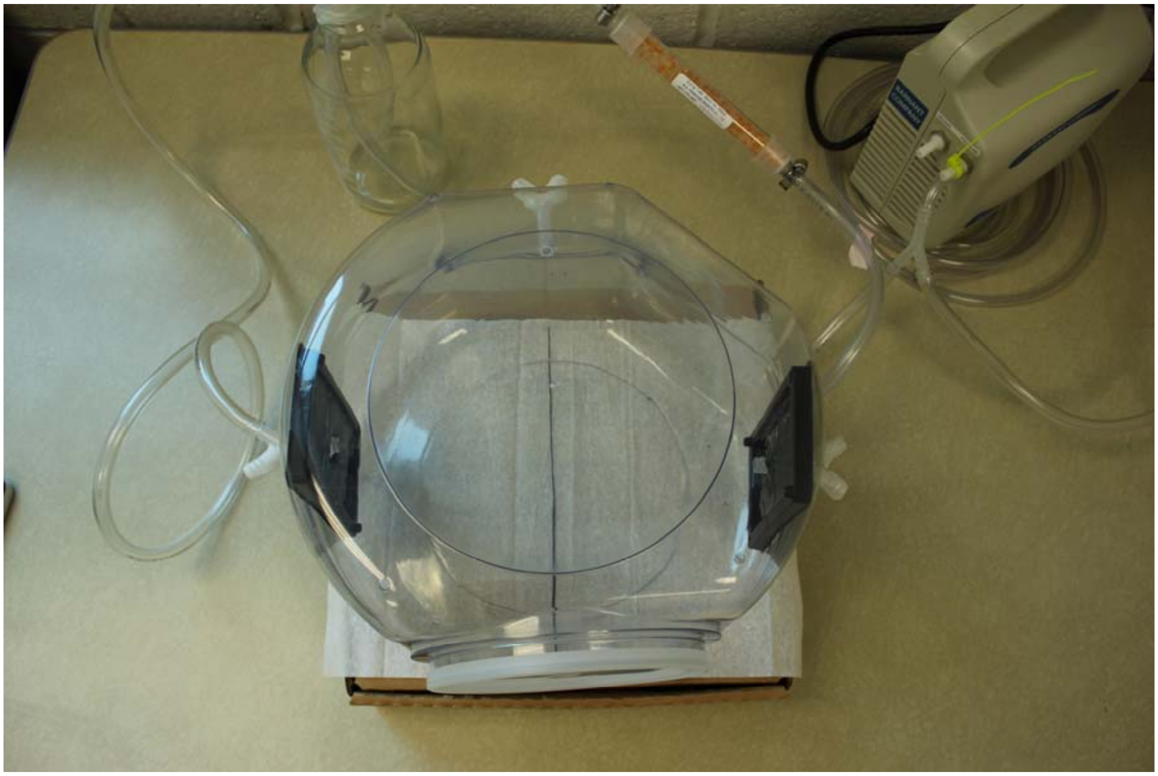
Appendix A



Experimental chamber used in the experiments. It was altered according to procedures outlined in Materials and Methods section.

Appendix B: Raw Data

Survivability Study Raw Data:

Survivability of Flies with Intact Arista	
Day	Number of Flies
1	30
2	30
3	30
4	27
5	27
6	27
7	27
8	22
9	22
10	22

Table B.1: Survivability of flies with arista.

Survivability of Flies With Ablated Arista	
Day	Number of Flies
1	30
2	30
3	30
4	30
5	30
6	30
7	28
8	28
9	27
10	25

Table B.2: Survivability of flies without arista.

Wind and Airflow Study Raw Data:

Arista Intact:

Trial 1			
	Strong Choice	Choice	Total
Airflow	3	7	10
No Airflow	0	0	0

Table B.3: Strong choice and choice of Trial 1 arista intact.

Trial 2			
	Strong Choice	Choice	Total
Airflow	7	3	10
No Airflow	0	0	0

Table B.4: Strong choice and choice of Trial 2 arista intact.

Trial 3			
	Strong Choice	Choice	Total
Airflow	2	6	8
No Airflow	0	2	2

Table B.5: Strong choice and choice of Trial 3 arista intact.

Arista Ablated:

Trial 1			
	Strong Choice	Choice	Total
Airflow	4	4	8
No Airflow	0	2	2

Table B.6: Strong choice and choice of Trial 1 arista ablated.

Trial 2			
	Strong Choice	Choice	Total
Airflow	4	2	6
No Airflow	1	3	4

Table B.7: Strong choice and choice of Trial 2 arista ablated.

Trial 3			
	Strong Choice	Choice	Total
Airflow	0	7	7
No Airflow	0	3	3

Table B.8: Strong choice and choice of Trial 2 arista ablated.

Relative Humidity Study Raw Data:

Arista Intact:

Trial 1			
	Strong Choice	Choice	Total
RH	1	1	2
Dry Air	5	3	8

Table B.9: Strong choice and choice of Trial 1 arista intact.

Trial 2			
	Strong Choice	Choice	Total
RH	3	0	3
Dry Air	0	7	7

Table B.10: Strong choice and choice of Trial 2 arista intact.

Trial 3			
	Strong Choice	Choice	Total
RH	0	1	1
Dry Air	3	6	9

Table B.11: Strong choice and choice of Trial 3 arista intact.

Arista Abated:

Trial 1			
	Strong Choice	Choice	Total
RH	1	2	3
Dry Air	3	4	7

Table B.12: Strong choice and choice of Trial 1 arista ablated.

Trial 2			
	Strong Choice	Choice	Total
RH	1	3	4
Dry Air	0	6	6

Table B.13: Strong choice and choice of Trial 2 arista ablated.

Trial 3			
	Strong Choice	Choice	Total
RH	0	5	5
Dry Air	0	5	5

Table B.14: Strong choice and choice of Trial 3 arista ablated.

Volatile Organic Compound Study Raw Data:

Arista Intact:

Trial 1			
	Strong Choice	Choice	Total
DMDS	1	2	3
Ambient Flow	1	6	7

Table B.15: Strong choice and choice of Trial 1 arista intact.

Trial 2			
	Strong Choice	Choice	Total
DMDS	0	1	1
Ambient Flow	4	5	9

Table B.16: Strong choice and choice of Trial 2 arista intact.

Trial 3			
	Strong Choice	Choice	Total
DMDS	0	3	3
Ambiet Flow	3	4	7

Table B.17: Strong choice and choice of Trial 3 arista intact.

Arista Ablated:

Trial 1			
	Strong Choice	Choice	Total
DMDS	1	3	4
Ambient Flow	1	5	6

Table B.18: Strong choice and choice of Trial 1 arista ablated.

Trial 2			
	Strong Choice	Choice	Total
DMDS	2	3	5
Ambient Flow	0	5	5

Table B.19: Strong choice and choice of Trial 2 arista ablated.

Trial 3			
	Strong Choice	Choice	Total
DMDS	2	3	5
Ambiet Flow	1	4	5

Table B.20: Strong choice and choice of Trial 3 arista ablated.