5-2020

Exposure of Phlebotomus Argentipes to Alpha-Cypermethrin, Permethrin, and DDT Using CDC Bottle Bioassays to Assess Insecticide Susceptibility

Jacob Rex Andersen
Utah State University

EXPOSURE OF PHLEBOTOMUS ARGENTIPES TO ALPHA-CYPERMETHRIN, PERMETHRIN, AND DDT USING CDC BOTTLE BIOASSAYS TO ASSESS INSECTICIDE SUSCEPTIBILITY

by

Jacob Rex Andersen

Capstone submitted in partial fulfillment of the requirements for graduation with

University Honors

with a major in

Biology

in the Department of Biology

Approved:

Capstone Mentor
Dr. Scott Bernhardt

Departmental Honors Advisor
Dr. Brett Adams

N/A

Committee Member [optional: type name or N/A]
N/A

University Honors Program Director
Dr. Kristine Miller

UTAH STATE UNIVERSITY
Logan, UT

Spring 2020

Abstract

Background: Insecticide resistance for sand flies is a concern since sand flies are vectors for *Leishmania* spp. parasites which cause leishmaniasis affecting millions of people each year. The CDC bottle bioassay is used to assess resistance by comparing known insecticide diagnostic doses and diagnostic times from an insecticide-susceptible population. The objective of this study was to determine diagnostic doses and diagnostic times for α-cypermethrin and the lethal dose for 50% and 90% mortality for α-cypermethrin, permethrin, and DDT for *Phlebotomus argentipes*.

Methods: The CDC bottle bioassays were performed in 1,000 mL glass bottles with 15-25 sand flies from a laboratory strain of insecticide-susceptible *P. argentipes*. A range of concentrations of α-cypermethrin, permethrin, and DDT were evaluated. Approximately four replicates at each concentration were completed with a 24-hour recovery period after the exposure tests. 24-hour mortality dose-response survival curves were created. A time-to-knockdown test was conducted with α-cypermethrin to determine the diagnostic doses with diagnostic times.

Results: α-Cypermethrin had the lowest LD_{50} and LD_{90} followed by permethrin and then DDT with the highest values. Diagnostic doses with (diagnostic times) for α-cypermethrin were 7.5 µg/mL (30 minutes), 5.0 µg/mL (35 minutes), and 3.0 µg/mL (45 minutes).

Conclusions: The dose-response survival curves, diagnostic doses, and diagnostic times can be utilized by control programs in assessing insecticide resistance in field populations of *P. argentipes*. The control programs can apply the appropriate insecticide and dose to effectively manage the population. The data presented can also be used a starting point for determining diagnostic doses and diagnostic times for other sand fly species.

Acknowledgments

I would like to thank Dr. Scott Bernhardt for allowing me to do research in his lab. He has been an incredible mentor throughout my undergraduate and in this project. He was very encouraging during my whole experience despite multiple setbacks. Also, I would like to thank Shawna Hennings for her support in the lab and in performing some of the bottle bioassays. None of this project would be possible without the help from both Dr. Scott Bernhardt and Shawna Hennings in helping to maintain the sand fly colonies. In addition, I would like to thank Dr. Scott Bernhardt and Dr. Brett Adams for their feedback on the written portion of the project. Lastly, I would like to thank the Department of Biology and the Honors Program at Utah State University for opportunities to apply my knowledge and further my skills in research.

Exposure of *Phlebotomus argentipes* to α-cypermethrin, permethrin, and DDT using CDC bottle bioassays to assess insecticide susceptibility

Abstract

Background: Insecticide resistance for sand flies is a concern since sand flies are vectors for *Leishmania* spp. parasites which cause leishmaniasis affecting millions of people each year. The CDC bottle bioassay is used to assess resistance by comparing known insecticide diagnostic doses and diagnostic times from an insecticide-susceptible population. The objective of this study was to determine diagnostic doses and diagnostic times for α-cypermethrin and the lethal dose for 50% and 90% mortality for α-cypermethrin, permethrin, and DDT for *Phlebotomus argentipes*.

Methods: The CDC bottle bioassays were performed in 1,000 mL glass bottles with 15-25 sand flies from a laboratory strain of insecticide-susceptible *P. argentipes*. A range of concentrations of α-cypermethrin, permethrin, and DDT were evaluated. Approximately four replicates at each concentration were completed with a 24-hour recovery period after the exposure tests. 24-hour mortality dose-response survival curves were created. A time-to-knockdown test was conducted with α-cypermethrin to determine the diagnostic doses with diagnostic times.

Results: α-Cypermethrin had the lowest LD_{50} and LD_{90} followed by permethrin and then DDT with the highest values. Diagnostic doses with (diagnostic times) for α-cypermethrin were 7.5 µg/mL (30 minutes), 5.0 µg/mL (35 minutes), and 3.0 µg/mL (45 minutes).

Conclusions: The dose-response survival curves, diagnostic doses, and diagnostic times can be utilized by control programs in assessing insecticide resistance in field populations of *P. argentipes*. The control programs can apply the appropriate insecticide and dose to effectively manage the population. The data presented can also be used a starting point for determining diagnostic doses and diagnostic times for other sand fly species.

Keywords: *Phlebotomus argentipes*, CDC bottle bioassay, insecticide susceptibility, alpha-cypermethrin, permethrin, DDT

Word Count: 2530

Background

There are over 90 proven or suspected species of sand flies, which includes *Phlebotomus argentipes*, that are vectors for *Leishmania* spp. parasites which cause leishmaniasis and affects millions of people worldwide each year [1–2]. Approximately 70,000 deaths occur each year due to leishmaniasis [3]. Sand flies have been a target for insecticides to control populations, but there is a growing concern throughout the world for insecticide resistance of these sand fly populations [4,5]. Being able to assess insecticide resistance can be useful in developing new or utilizing current insecticide control strategies more effectively for controlling the sand fly populations [5,6].

There are two common techniques used to quantify a species' susceptibility to an insecticide: The World Health Organization (WHO) susceptibility test and the Centers for Disease Control and Prevention (CDC) bottle bioassay [7–8]. The WHO susceptibility test kit has been used to measure insecticide susceptibility on many different insects [9–11]. However, the WHO susceptibility test kit is simplistic and is unable to do range analysis since the insecticides for purchase for the kit are limited in different concentrations and different insecticides [11]. An alternative to the WHO susceptibility test is the CDC bottle bioassay. Both the WHO susceptibility test and CDC bottle bioassay have been shown to give similar results of susceptibility to an insecticide [11,12]. However, the CDC bottle bioassay uses fewer insects, and the concentration of whichever insecticide needed can be controlled with dilution of the insecticide in a solvent which will allow for a range analysis of insecticides [8,11]. In addition, the CDC bottle bioassay's portability and the availability of the materials can be potentially used in remote locations for monitoring insecticide resistance in field populations [12].

When dichlorodiphenyltrichloroethane (DDT) and pyrethroids are used in the CDC bottle bioassay, a 24-hour recovery period must be allowed after the exposure [13]. The knockdown point and 24-hour mortality point represent different resistance mechanisms (knockdown resistance by target site insensitivity or metabolic detoxification) for insects [14]. This raises the question of whether to assess resistance by time-to-knockdown which is faster or by waiting for the 24-hour mortality [8,14]. Without the 24-hour recovery period, a metabolic detoxification resistance could be missed when assessing insecticide resistance [14]. Using both the 24-hour mortality point and the time-to-knockdown point will quantify the knockdown resistance via target site insensitivity and metabolic detoxification resistance by using the CDC bottle bioassay for determining the diagnostic doses (lowest dose of insecticide that causes 100% mortality) and diagnostic times (between 30-60 minutes) for an insecticide [8,14]. The diagnostic doses and diagnostic times of a susceptible population to an insecticide can be used to assess and monitor over time the insecticide resistance in field populations by comparing the diagnostic doses and diagnostic times [15,16]. There have been limited sand fly studies that have determined diagnostic doses and diagnostic times for sand flies which makes a comparison between species difficult without more data [17–20]. Dose-response survival curves using CDC bottle bioassays for *Phlebotomus papatasi* and *Lutzomyia longipalpis* for pyrethroids and DDT can be used as starting points for *P. argentipes* [17].

The objective of this study is to determine diagnostic doses and diagnostic times for α-cypermethrin and the lethal dose for 50% and 90% mortality for α-cypermethrin, permethrin, and DDT by using the CDC bottle bioassay for *P. argentipes*. The diagnostic doses and diagnostic times determine in this study can be utilized by future comparative studies and studies in assessing insecticide resistance in field populations of sand flies.

Methods

Sand flies

Laboratory strains of insecticide-susceptible *P. argentipes* were maintained at Utah State University [21–22]. The laboratory strain comes from a >30-year-old established colony at the Walter Reed Army Institute of Research which had not been exposed to any insecticides.

Insecticides

Insecticides used for this study were α-cypermethrin (100 mg/vial), permethrin (250 mg/vial), and DDT (350 mg/vial). A working solution for each insecticide was made by diluting the active ingredient with acetone in glass bottles. The bottles were wrapped in aluminum foil to prevent photodegradation and stored at 4°C unless in use [8]. Table 1 lists the concentrations used of each insecticide in the study.

Insecticide	Concentrations (µg/mL)
α-cypermethrin	0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 7.5
Permethrin	1, 3, 5, 6.5, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 65, 80, 100
DDT	1, 3, 5, 10, 15, 20, 30, 40, 75, 100, 175

Table 1. Insecticide concentrations diluted in acetone used to expose *Phlebotomus argentipes* to during the experiment.

Preparation of exposure bottles

CDC bottle bioassay procedures have used 1 mL of working solution of insecticide to coat a 250 mL glass bottle [8]. 1,000 mL glass bottles were used to scale up the procedure. To maintain the concentration of insecticide (µg/250 mL bottle), 4 mL of the insecticide solution were used instead of 1 mL to compensate for the increased bottle size [17]. The 1,000 mL bottles were coated inside with 4 mL of the insecticide solution via rotation to coat the bottom, sides, and lid. The bottles were then put on a mechanical roller for 45-60 minutes, and roughly every 10-15 minutes each bottle was rotated again to coat the bottom, sides, and lid, then placed back on the mechanical roller. The lids were loosened slowly over the 45-60 minutes on the mechanical roller to allow the acetone to evaporate. Then the lids were completely removed, and the bottles continued to roll until all the acetone evaporated. Bottles were left overnight to dry. For every test replicate, a control 1,000 mL glass bottle was prepared with 4 mL of acetone without any insecticide to coat the inside of the bottle and lid then was allowed to evaporate following the same procedure using the mechanical roller [8]. Bottles coated in the acetone or the insecticide solution were used within 5 days of preparing the exposure bottles. The bottles and lids were reused throughout the experiment which were cleaned with a triple-rinse of acetone, placed in soapy water, rinsed in cold water, then autoclaved and allowed to dry before reuse [17].

Insecticide exposure tests

For the CDC bottle bioassays, 15-25 sand flies (roughly equal numbers of male and female sand flies) were aspirated into each bottle [8]. Sand flies were first aspirated into the control bottle then into increasing insecticide concentration bottles. Once the sand flies were aspirated into a bottle, the timer began for 60 minutes. After the 60-minute exposure, the sand flies from one bottle were aspirated into a cardboard container with a mesh cover and were kept in the same conditions as the main colony for approximately 24 hours to recover. Approximately four replicates were performed for each insecticide concentration. After the recovery period, the number of alive and dead sand flies were recorded for the 24-hour mortality survival curve. Sand flies were considered dead if they had difficulty flying or righting themselves up [8]. If the control group for the trial had a mortality of 20% or more, then the whole test replicate was not used [23]. For α-cypermethrin, a time-to-knockdown exposure test was also performed which follows the same (24-hour mortality) procedure described above. For the time-to-knockdown exposure test, the number of sand flies that were dead ("knocked down") were recorded at 0, 15, 30, 35, 40, 45, and 60 minutes [24].

Survival curves

The percent of mortality after the 24-hour recovery period from the exposure test was determined for each replicate and insecticide concentration. Survival curves were made using QCal Dose Response software which utilized a logistic regression model and was used to find the LD_{50} and LD_{90} for each insecticide [25]. In the time-to-knockdown exposure test for α-cypermethrin, the average percent of dead ("knocked down") sand flies from the four replicates were calculated at each time point. The diagnostic doses were determined by a dose that caused a 100% mortality rate with a diagnostic time between 30 and 60 minutes [8].

Results

The 24-hour mortality dose-response survival curves for each insecticide are shown in Figure 1. From the survival curves in Figure 1, the LD_{50} and LD_{90} for each insecticide were calculated and summarized in Table 2.

Insecticide	LD_{50}	LD_{90}
α-Cypermethrin	1.16 µg/mL	3.07 µg/mL
Permethrin	10.1 µg/mL	39.3 µg/mL
DDT	34.6 µg/mL	120.1 µg/mL

Table 2. LD_{50} and LD_{90} for *Phlebotomus argentipes* for each insecticide.

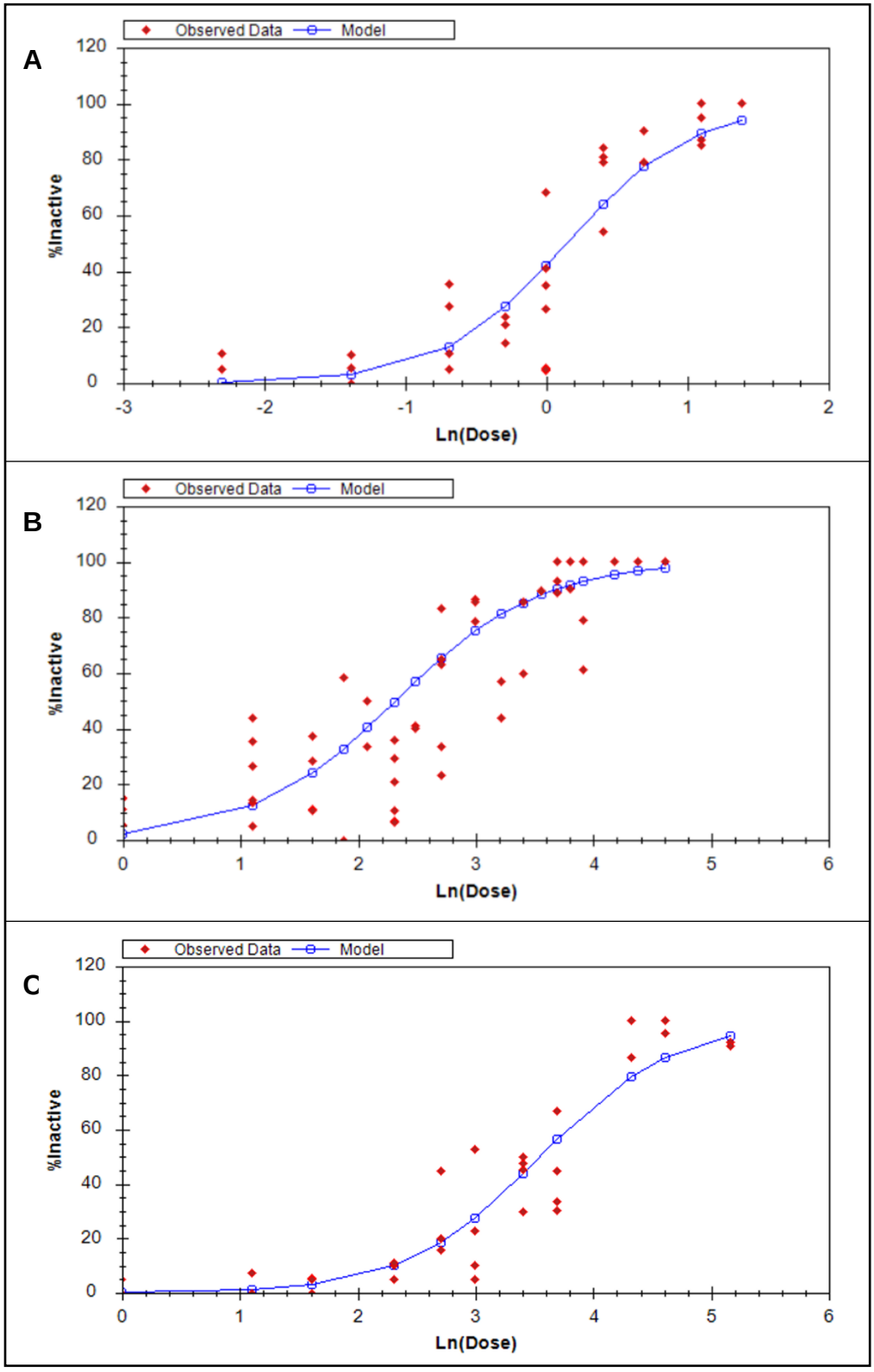

Figure 1. 24-hour mortality dose-response survival curves for *Phlebotomus argentipes* with each insecticide shown in a natural logarithm (µg/mL) using QCal software. **A.** α-cypermethrin. **B.** permethrin. **C.** DDT.

α-Cypermethrin had the smallest concentration of insecticide for both LD_{50} and LD_{90} followed by permethrin then by DDT. For the 24-hour-mortality diagnostic doses, α-cypermethrin had a diagnostic dose of 4 µg/mL, and permethrin had a diagnostic dose of 65 µg/mL. From the time-to-knockdown exposure tests which are shown in Figure 2, three diagnostic doses for α-cypermethrin were determined 7.5 µg/mL, 5.0 µg/mL, and 3.0 µg/mL which had diagnostic times of 30 minutes, 35 minutes, and 45 minutes respectively. 1.0 µg/mL and 2.0 µg/mL doses of α-cypermethrin did not reach a 100% mortality within the 30-60 minutes diagnostic time range.

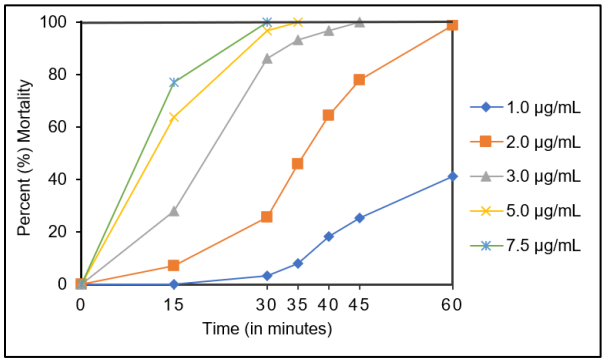

Figure 2. Time-to-knockdown survival curve for *Phlebotomus argentipes* with α-cypermethrin.

Discussion

The objective of this study was to quantify insecticide susceptibility with the CDC bottle bioassay of a laboratory strain of *P. argentipes* to α-cypermethrin, permethrin, and DDT. This study added to the collection of diagnostic doses and diagnostic times of insecticides that used the CDC bottle bioassay across different sand fly species that are vectors of *Leishmania* spp. parasites [18–20,24]. LD_{50}, LD_{90}, and diagnostic doses for some insecticides can now be compared between species in the *Phlebotomus* genus.

When converted to µg permethrin/bottle for comparison, *P. argentipes,* from our colony, had a LD_{50} of 40.4 µg permethrin/bottle and LD_{90} of 157.2 µg permethrin/bottle which were close to the concentrations that *Phlebotomus papatasi* had of a LD_{50} of 41.3 µg permethrin/bottle and LD_{90} of 188.6 µg permethrin/bottle [17]. Likewise, *P. argentipes* has a diagnostic dose of 65.0 µg/mL of permethrin after the 24-hour recovery which was a similar concentration to *P. papatasi* with a diagnostic dose of 55.0 µg/mL [24]. As for DDT, *P. papatasi* had a LD_{50} of 15.0 µg DDT/bottle and LD_{90} of 296.0 µg DDT/bottle while *P. argentipes* from our colony, when converted to µg DDT/bottle for comparison, had a much higher concentration for a LD_{50} of 138.4 µg DDT/bottle and LD_{90} of 480.4 µg DDT/bottle [17]. This shows a wide range of DDT susceptibility between *Phlebotomus* spp. Unfortunately, the 24-hour diagnostic dose of DDT for *P. argentipes* could not be determine due to insufficient data at higher concentrations. Additional data points at higher concentrations of DDT will be needed in order to compare the concentration to a diagnostic dose of 470.0 µg/mL for *P. papatasi* [24].

Both the 24-hour mortality dose-response survival curves for permethrin and DDT from this study can be used as starting points for determining diagnostic doses with diagnostic times in the time-to-knockdown test. The diagnostic doses with diagnostic times for α-cypermethrin can be used in future comparative studies of insecticide susceptibility. There is a potential diagnostic dose of 2.5 µg/mL α-cypermethrin with a diagnostic time of 60 minutes that was unable to be tried due to a limited number of sand flies which were needed to maintain the colony population.

The use of 1,000 mL glass bottles instead of the standard 250 mL glass bottles may have some impact on the diagnostic doses and times [8]. However, there have been other studies that have assessed insecticide susceptibility using 1,000 mL glass bottles for the CDC bottle bioassay [17,24]. It was suggested that the diagnostic doses and diagnostic times would be similar even with the difference in the bottle volume [24].

The CDC criteria for resistance is if the mortality is less than 100% then there is resistance [8]. In order to test for the intensity of the insecticide resistance, the CDC bottle bioassay intensity rapid diagnostic tests (I-RDT's) were created to quantify the intensity of resistance based on known diagnostic doses [26]. The diagnostic doses and diagnostic times provided from this study can be used as a baseline for CDC bottle bioassay I-RDT's for *P. argentipes* in assessing resistance in field populations and resistant laboratory strains. The dose-response survival curves from this study can be used in an integrated method of control by comparing the results of the exposure test from field collected *P. argentipes* to the survival curves of the insecticide-susceptible *P. argentipes*. From comparing the field collected to the insecticide-susceptible population, control programs can apply

the appropriate insecticides and concentrations in an effective manner to control sand fly field populations and prevent exacerbating insecticide resistance [27].

Conclusions

Insecticide resistance has been a threat in managing sand fly populations with insecticides meant to reduce the spread of *Leishmania* spp. parasites in causing leishmaniasis. Having diagnostic doses, diagnostic times, and dose-response survival curves for an insecticide-susceptible sand fly population is crucial for comparison to field populations in order to have an effective control program. The data presented for an insecticide-susceptible *P. argentipes* in this study can be used as a starting point for determining diagnostic doses and diagnostic times for other sand fly species.

Ethical considerations

The maintenance of SKH1 hairless mice and experimental animal use protocol was approved by Utah State University's Institutional Animal-Care and Use Committee.

References

1. Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. Med Vet Entomol. 2013;27:123-147.
2. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;7:e35671.
3. Reithinger R, Dujardin J, Louzir H, et al. Cutaneous leishmaniasis. Lancet Infect Dis. 2007;7(9):581–96.
4. Rivero A, Vézilier J, Weill M, Read AF, Gandon S. Insecticide control of vector-borne diseases: when is insecticide resistance a problem? PLoS Pathog. 2010;6:e1001000.
5. Amóra SSA, Bevilaqua CML, Feijó FMC, Alves ND, Maciel MDV. Control of phlebotomine (Diptera: Psychodidae) leishmaniasis vectors. Neotrop Entomol. 2009;38:303–310.
6. Surendran S, Karunaratne SHPP, Adams Z, Hemingway J, Hawkes NJ. Molecular and biochemical characterization of a sand fly population from Sri Lanka: evidence for insecticide resistance due to altered esterases and insensitive acetylcholinesterase. Bull Entomol Rev. 2005;95:371–380.
7. W.H.O. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: W.H.O.; 2013.
8. Brogdon WG, Chan A. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay/ Methods in *Anopheles* research. Second edition. CDC Atlanta, USA: CDC technical report; 2010. p. 343.
9. Braverman Y, Chizov-Ginzburg A, Pener H, Wilamowski A. Susceptibility and repellency of *Culicoides imicola* and *Culex pipiens* to lambda-cyhalothrin. Vet. Ital. 2004;40:336–339.
10. Faraj C, Ouahabi S, Adlaoui EB, Elkohli ME, Lakraa L, El Rhazi M, Ameur B. Insecticide susceptibility of *Phlebotomus (Paraphlebotomus) sergenti* and *Phlebotomus (Phlebotomus) papatasi* in endemic foci of cutaneous leishmaniasis in Morocco. Parasite Vectors 2012;5:51.
11. Aïzoun N, Ossè R, Azondekon R, Alia R, Oussou O, Gnanguenon V, et al. Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. Parasite Vectors. 2013;6:147.
12. Perea EZ, Leo´n RB, Salcedo MP, Brogdon WG, Devine GJ. Adaptation and evaluation of the bottle assay for monitoring insecticide resistance in disease vector mosquitoes in the Peruvian Amazon. Marlar J 8;208.
13. Davies TGE, Field LM, Usherwood PNR, Williamson MS. DDT, pyrethrin, pyrethroids, and insect sodium channels. IUBMB Life. 2007;59:151–162.
14. Owusu HF, Jančáryová D, Malone D, Müller P. Comparability between insecticide resistance bioassays for mosquito vectors: time to review current methodology? Parasite Vectors. 2015;8:357.
15. da-Cunha MP, Lima JB, Brogdon WG, Moya GE, Valle D. Monitoring of resistance to the pyrethroid cypermethrin in Brazilian *Aedes aegypti* (Diptera: Culicidae) populations collected between 2001 and 2003. Mem Inst Oswaldo Cruz. 2005;100:441–444.
16. Ochomo E, Bayoh MN, Brogdon WG, Gimnig JE, Ouma C, Vulule JM, et al. Pyrethroid resistance in *Anopheles gambiae s.s.* and *Anopheles arabiensis* in western Kenya: phenotypic, metabolic and target site characterizations of three populations. Med Vet Entomol. 2013;27:156–164.
17. Denlinger DS, Lozano-Fuentes S, Lawyer PG, Black WC, IV, Bernhardt SA. Assessing insecticide susceptibility of laboratory *Lutzomyia longipalpis* and *Phlebotomus papatasi* sand flies (Diptera: Psychodidae: Phlebotominae) J Med Entomol. 2015;52:1003–1012.
18. Santamaría E, Munstermann LE, Ferro C. Aproximación al método CDC para determinar susceptibilidad a insecticidas en vectores de leishmaniasis. Biomédica 2003;23:115–121.
19. Marceló C, Cabrera OL, Santamaría E. Indicadores de sensibilidad de una cepa experimental de *Lutzomyia longipalpis* (Diptera: Psychodidae) a tres insecticidas de uso en salud pública en Colombia. Biomedica. 2014;34:624–630.
20. Henriquez C, Pereira Y, Cochero S, Bejarano EE. Dosis diagnóstica y umbral de resistencia de *Lutzomyia evansi* (Diptera: Psychodidae), a dos insecticidas utilizados en salud pública en Colombia: deltametrina y lambdacihalotrina. Rev Entomol Soc Argent Mendoza. 2009;68:287–294.
21. Lawyer PG, Rowton ED, Perkins PV, Johnson RN, Young DG. Recent advances in laboratory mass rearing of phlebotomine sand flies. Parassitologia. 1991;33:361-364.
22. Young DG, Perkins PV, Endris RG. A larval diet for rearing phlebotomine sand flies (Diptera: Psychodidae) J Med Entomol. 1981;18:446.

23. Saeidi Z, Vatandoost H, Akhavan AA, Yaghoobi-Ershadi MR, Rassi Y, Sheikh M, et al. Baseline susceptibility of a wild strain of *Phlebotomus papatasi* (Diptera: Psychodidae) to DDT and pyrethroids in an endemic focus of zoonotic cutaneous leishmaniasis in Iran. Pest Manag Sci. 2012;68:669–675.
24. Denlinger DS, Creswell JA, Anderson JL, Reese CK, Bernhardt SA. Diagnostic doses and times for *Phlebotomus papatasi* and *Lutzomyia longipalpis* sand flies (Diptera: Psychodidae: Phlebotominae) using the CDC bottle bioassay to assess insecticide resistance. Parasites & Vectors. 2016;9:212.
25. Lozano-Fuentes S, Saavedra-Rodriguez K, Black WC, IV, Eisen L. QCal: A software application for the calculation of dose-response curves in insecticide resistance bioassays. J. Am. Mosq. Control Assoc. 2012;28:59–61.
26. Bagi J, Grisales N, Corkill R, Morgan JC, N'Falé S, Brogdon WG, et al. When a discriminating dose assay is not enough: measuring the intensity of insecticide resistance in malaria vectors. Malar J. 2015;14:210.
27. Maharaj R. Global trends in insecticide resistance and impact on disease vector control measures. OAIP. 2011;3:27-33.

Reflection

I am extremely grateful for the opportunity to perform research during my undergraduate. It has helped me become a better candidate for medical school while exposing me to a glimpse of what research is like. From this experience, I have gained impactful relationships with my mentor, graduate students, and other fellow undergraduates. The capstone project was an excellent way for me to review everything I have learned throughout the past four years and apply it. The capstone project felt like a huge project up front; however, when it was broken down into parts, it seemed more manageable to complete in a timely manner. The actual project itself took a longer time than the written portion of my capstone project. There was a balancing problem during my project. I could only use so many sand flies at a time or else there would be a risk of losing the sand fly colony. Several times during the project, there had been too many sand flies used which almost lead to a loss of the colony. It takes roughly five weeks for these sand flies to go from an egg to an adult. When there were low numbers of sand flies, only a small number of eggs would be laid which would result in a decline in adult sand flies weeks after using too many. This put the project on standby until the colony stabilized again. It was important before starting the research to plan for some leeway in case this occurred.

One challenge that I thought would not have had a major impact on my project was that I had to share the same sand fly colony with a graduate student, who was performing her own research on the sand flies. I was very wrong in the sense of how many sand flies she would need. Fortunately, I had developed a working relationship with her, so there were never any hard feelings when the project was put on standby due to low numbers in the colony. It was a great learning experience that I would not have had if it were not for this project. I would highly recommend and encourage a good working relationship with anyone working in the same lab along with other labs because it could have easily turned into arguments over resources.

When the research was put on standby, this gave me the perfect opportunity to start working on the written portion of the project, so the whole project was not completely put on standby. In all honesty, I did not utilize the time effectively to write the research paper. I had put a majority of it off until the last few weeks of school. The Honors Programs always tells you to start early, so you do not scramble at the end to graduate. I have learned that firsthand. I would recommend to future students if there is a set back where they cannot perform any research in the lab that they start working on the written portion of the project instead of waiting until the end to do it. Even if it is just the background information of the paper, that is one less section to write after you complete the nonwritten portion of the project. With that being said, this whole process is definitely worth it in the end, even though you may not see it in the beginning. I have known many people who have started in the Honors Program but drop out before doing an Honors Capstone Project. I had thought about dropping out of the program as well,

but after reading other students' Honors Capstone Project reflections, it encouraged me to complete my own project since there are valuable lessons to be learned that you didn't think of before. I am positive that I have learned lessons from this experience that I do not fully realize how meaningful they are yet. I would encourage all students in the Honors Program to complete an Honors Capstone Project despite how difficult it may be on top of one's already large course workload since it will have many benefits down the road.

Doing an Honors Capstone Project has allowed me to gain a deeper understanding of why the research I was doing was important and the applications it had. When I first started in the lab almost three years ago, I had little understanding of what the big picture of the research with sand flies was. I hardly engaged myself in the lab for the first few months, and I only made simple connections between public health and biology with the sand flies. I was what people think of for what a typical undergraduate looks like in a research lab. However, I wanted a more meaningful experience, so I slowly began learning more about the potential applications of the research and making deeper connections. My capstone project pushed me even further with this by having me write a research paper. It has also allowed me to apply knowledge that I had gained from my classroom and lab experiences during my project to troubleshoot various problems that arose. In addition, I have been able to carry on meaningful conversations about research with other students in other disciplines which would not have happened without a deeper understanding of the research I was doing.

The whole experience has been amazing for me. I learned that it was okay to ask questions even if it may seem simple and is something you think you should already know. There are many resources available for you to succeed that I did not realize until later in my undergraduate career. Having a supporting mentor and graduate students in the lab made the goals of the project seem more achievable and added to the great experience. Honors students should not pass up this opportunity to connect with a mentor and graduate students, especially if one is planning on continuing their education in graduate school. The Honors Capstone Project also helps prepare you for what some of the research process looks like in graduate school, and it will help ease the transition from being an undergraduate to graduate student. The Honors Program has presented a great opportunity that will leave a lasting impact on one's life.

Word count: 1041

Author Biography

Jacob Andersen is a senior at Utah State University that is graduating in spring 2020 with a major in biology with an emphasis in cellular/molecule biology and a minor in chemistry. He did insecticide research for sand flies with Dr. Scott Bernhardt for almost three years, and he was an Undergraduate Teaching Fellow for two semesters for Dr. Shawn Miller's chemistry courses. During his undergraduate, he received multiple scholarships from the College of Science and the Biology Department. After graduating, he plans to apply to medical schools in aspirations of becoming a physician.