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Article

Do attractants bias the results of malaise trap research?

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SUMMARY

The study of biodiversity is crucial to the stability of the planet as it assists scientists with the knowledge and tools necessary to maintain a functional and sustainable environment. Previous research has utilized malaise traps to collect insects in order to study trends in biodiversity. However, malaise traps may have a potential for bias, depending on the type of attractant used, given that flies are attracted to rotting, fermented fruit. This study aims to test whether inadequate sampling occurs during the collection of flying insects. We hypothesized that attractants do bias the results of malaise trap research. The project was designed to test this hypothesis. We placed two identical traps in similar areas of the Southwestern Riverside County Multi-Species Reserve near Western Center Academy. All variables were maintained across these traps, except for the type of attractant used (independent variable). Traps were placed three feet apart, both parallel to the prevailing wind in a homogeneously vegetated field. After one week, we counted and identified the insects down to order manually under a microscope and their genomes were sequenced. This process was repeated the following week. The data from the two traps were compared to each other and to a concurrent Mount San Jacinto Junior College study to test our hypothesis. Based on analysis of our data, we found our hypothesis to be supported by the data and there was indeed a bias when using denatured alcohol.

INTRODUCTION

As the most abundant taxon in the animal kingdom, insects play a critical role in their communities, while both positively and negatively impacting our ecosystem. To illustrate, insects are pollinators, decomposers of organic matter, and significant sources of energy in the food chain. However, insects can also carry disease, harm crops and livestock, and damage landscapes (1). To investigate these impacts, researchers commonly use malaise traps to collect flying insects. Swedish entomologist, Rene Malaise, discovered in the 1930s that more flying insects were captured by using his tent than by using traditional netting. Malaise traps, resembling tents, are now the most effective and ubiquitous flying insect trap in the world (2). However, bias resulting from attractants used in malaise traps could create inadequate sampling in studies, ultimately leading to a misunderstanding of the relative diversity and ecology of insects and perhaps cause scientists to overlook potential patterns and inaccurate trends.

The state of California contains over 14,000 protected areas, administered by various public and non-profit organizations, in addition to various private conservation areas and easements. Although it is difficult to estimate the exact area of all protected land in California, the California Protected Area Database estimates protected land to comprise almost 47% of the state's total area (3). State law protects these lands in order to preserve the biodiversity and maintain the landscape ecology of flora and fauna populations. As a result, these protected lands are ideal for conducting various types of research. In our study, we used malaise traps just inside and just outside one of these protected lands to collect flying insects that we then sorted, identified by order, and counted. Collaborators at Mount San Jacinto College (MSJC) used the same type of traps to collect and identify insects from a location approximately 11 miles southwest of our study location. In total, we identified nearly a thousand insects and to verify our identifications, we extracted, purified, and amplified the DNA of 150 insect specimens. We sent all specimens for this portion of the study to the University of Guelph to be barcoded for inclusion into the International Barcode of Life Database, as part of the 2016 international School Malaise Trap project. This database, maintained by the University of Guelph, is used worldwide to study the biodiversity genomics and identification of insects (10).

Informed by previous literature, we hypothesized that the townes-style malaise trap is preferred for unbiased insect collection, as this trap involves a passive form of collection with minimal required maintenance (4, 5). Scientists commonly use townes-style malaise traps to assess the relative abundance and diversity of flying insects that are active in shrublands where there is a reasonable amount of shelter. This was most consistent with the specific protected reserve area chosen. Some research, however, suggests the shape, model, and size of the trap may contribute to varying data sets (6). Still, others question the very materials the trap is made of (7). We utilized the townes-style malaise traps to test our hypothesis that attractants bias the results of malaise trap research. Denatured ethanol was used as one of the independent variables, not only because ethanol anesthetizes and preserves the insects over the course of a week, but it minimizes the Lepidopteran from damage and sticking to

other insects. At the same time, the Vapona insecticide strips were used as the other independent variable in the dry trap. We decided to check on the traps daily since dry attractants do not preserve the specimens as well as the ethanol.

RESULTS

In order to test our hypothesis that attractants bias the results of malaise trap research we collected insects in two identical malaise traps at a predetermined location inside the nature reserve, near the Western Center Academy (WCA) using a wet and dry attractant (**Figure 1A**). A similar collection was made during the same time period near the MSJC campus approximately 11 miles southwest of our location by an MSJC Honors Biology students research team (**Figure 1B**). The collections made at the Western Center Academy were specifically designed to test the hypothesis and to simultaneously participate in the School Malaise Trap program. The insects collected at MSJC verified that our traps worked properly and the expected number and variety of insects we anticipated were caught. We caught and counted mostly Diptera, which we believed would be the most abundant. We also caught Hymenoptera, Lepidoptera, Hemiptera, Coleoptera, Orthoptera, and Trichoptera The DNA of 150 insects collected from the WCA site were sequenced the first week. The results of the DNA sequences helped us to realize that an insect of the order Lepidoptera was misidentified as belonging to the order Trichoptera; we went back and corrected the data to reflect this. Our findings concluded a much greater population of insects (625%) had been caught with the wet denatured ethanol traps when compared to the dry Vapona traps. The greatest increase of insects, however, were those in the order Diptera. When compared to a dry anesthetic trap, insects of the order Diptera were caught an average of 1,024% more in both weeks one and two (**Figure 2 & 3**). The results appeared to show a clear bias in the population of insects caught when using either the denatured ethanol or Vapona. This data supports that there may be a bias based on the type of attractant used. Specifically, denatured ethanol traps may enrich for Diptera, as these insects normally feed on rotting fruits which produce alcohol. After a quantitative comparison of the findings, we concluded that our malaise traps research revealed that attractants do bias the results of malaise trap research.

DISCUSSION

We aimed to investigate whether attractants may bias the results of malaise trap research. Because no previous work in the field has addressed our hypothesis, we decided to make this question the focus of our research. We set up two malaise traps, with all variables identical, except for the type of attractant used (denatured ethanol or Vapona). We quantitatively measured and identified all the insects by morphology in order to place each of the insects into the appropriate Order. We verified our insect identifications via DNA barcodes. When we initially submitted our data, the barcode data revealed that we had misidentified one species of insects by hand, so we went back and corrected our mistake in order to ensure the data was correct. We clearly found a much larger number of Diptera caught in the denatured alcohol trap than in the Vapona trap, as well as many more insects overall. We compared our research data with that performed by Dr. Reeves and his MSJC team. In their project, they studied how human impact affects the genetics of specimens in high traffic areas as evidenced by gene flow, genetic drift, and other biodiversity trends. However, their study only used traps with denatured ethanol so it is conceivable that bias may have occurred in their collection. We used their data as additional verification to confirm that we collected very similar insects when using the same attractant.

Figure 1: Experimental set up and location. (A) Photo of townes-style malaise trap used and its location near Western Center Academy in the northeastern part of the Southwestern Riverside County Multi-Species (SWRCMS) Reserve located west of Diamond Valley Lake. (**B**). Aerial map of northern portion of Southwest Riverside County Multi-Species Preserve located in Southern California. The locations are 11 miles from where the MSJC and WCA research teams placed their malaise traps. WCA set up their traps in an uninterrupted area of the reserve, while MSJC set their traps in a high-traffic area of the campus.

The most difficult decision we made when setting up the malaise traps was finding their best placement for collection (8). We considered a diverse range of variables, including the insects' flight line, outside day and night temperatures, wind direction and speed, and hours of direct sunlight (9). We also had to consider that the reserve management board did not want the trap visible from any roads. We were able to set up both our wet and dry traps in the identical spot for two weeks in September 2016 during a time and at a location where we felt there would be minimal negative environmental influences and we would get the best, most consistent collection.

In addition, insects we collected had their DNA extracted, sequenced, and barcoded for possible inclusion in the International Barcode of Life Project database maintained

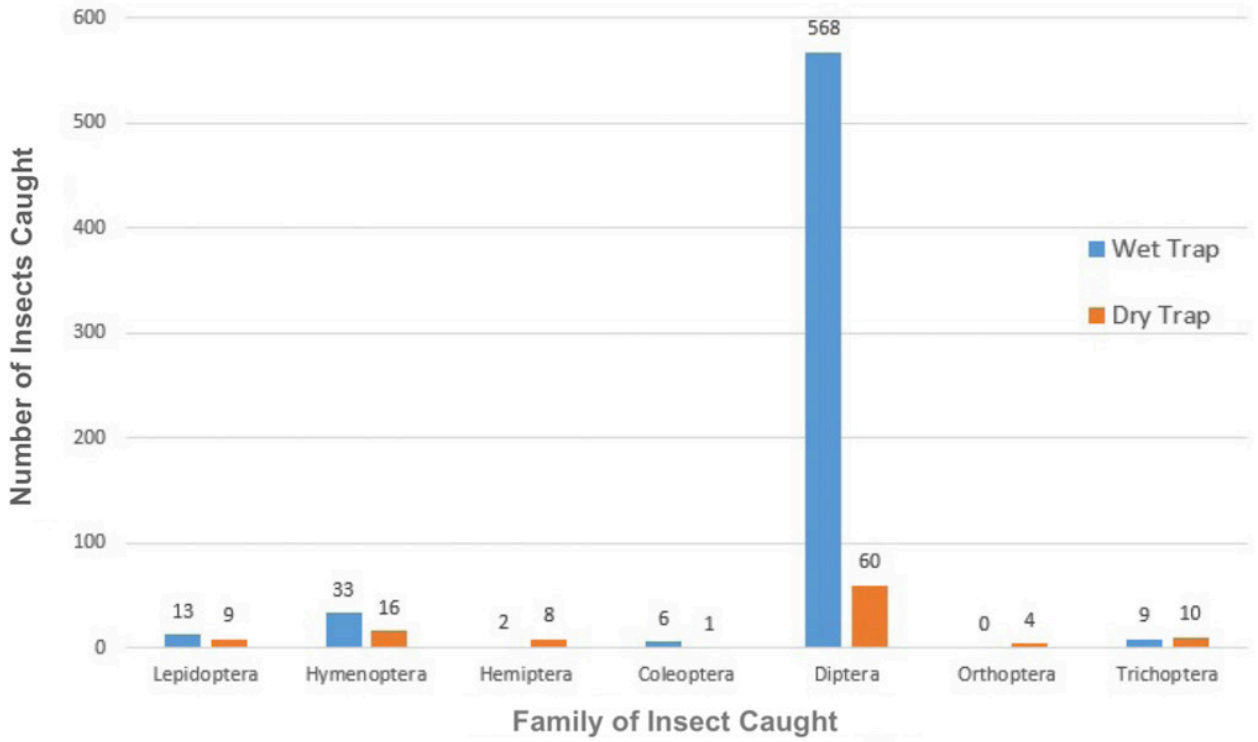

Figure 2: Distribution of insects caught week 1. Bars indicate type and number of insects caught in traps during week 1 of collections. Blue bars indicate insects caught by wet traps while orange bars indicate dry traps.

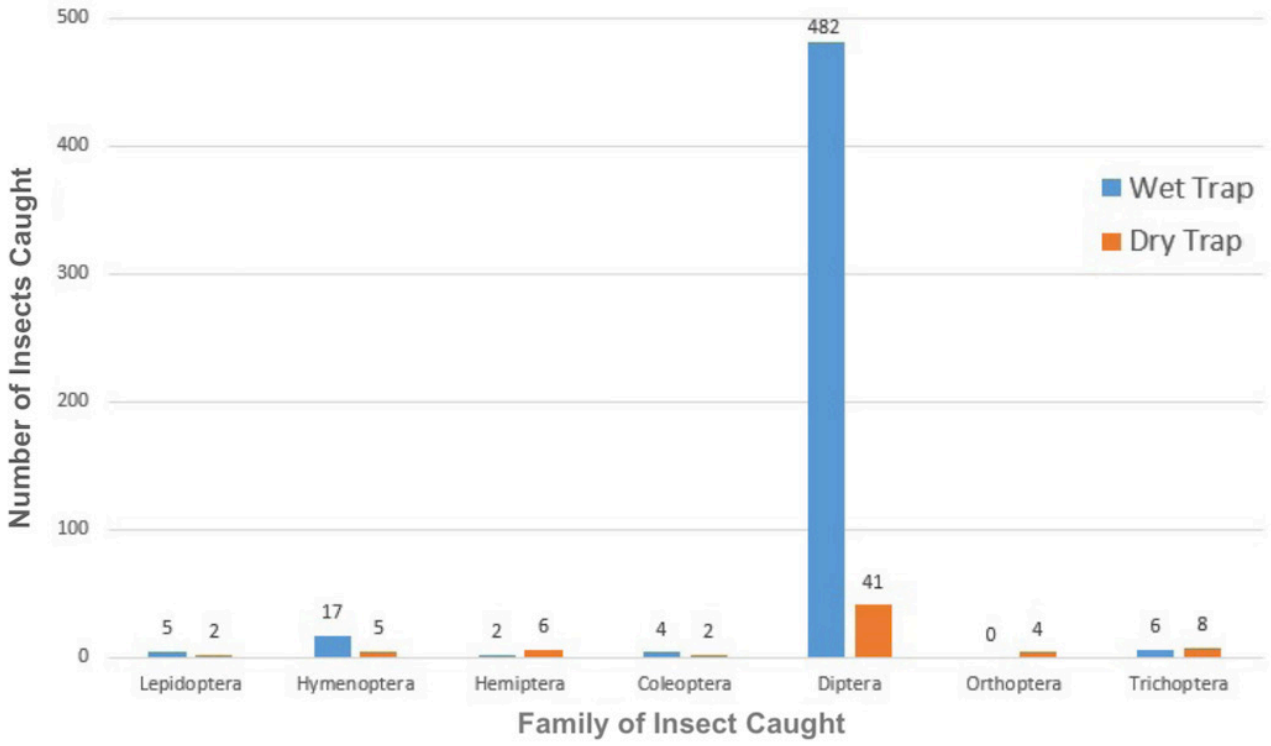

Figure 3: Distribution of insects caught week 2. Bars indicate type and number of insects caught in traps during week 2 of collections. Blue bars indicate insects caught with wet traps while orange bars indicate dry traps.

by the University of Guelph in Ontario, Canada. This DNA barcode-based reference library is used worldwide by scientists to study the diversity and identification of insects internationally (10). This research made an important contribution to the International Barcode of Life project, as new and rare species were added to their DNA barcode library database. We sent 820 specimens to the University of Guelph. A summary of our data was compiled, and our sample ranked 35th in biodiversity out of 67 other research groups who contributed to the program (11).

The purpose of this study was to determine if attractants influence and bias collected data when looking at malaise traps and the type of attractant used. Based on our data analysis, we concluded that the denatured alcohol trap proved to entice far more insects, particularly those in the order Diptera. Thus, Diptera may be especially attracted to the trap for they are naturally attracted to fermented fruits. Our data suggests that the denatured alcohol trap tends to bias the results of malaise trap diversity research. This could call into question any diversity studies done with malaise traps. Our data supports our hypothesis that there is a bias when using denatured ethanol in malaise traps.

METHODS

Malaise traps are made to resemble tents using the polyester fiber Terylene. This design allows insects to fly into the tent and get funneled into a collection jar which is located at the highest point. The collection jar uses a killing agent. We used both a wet (ethanol) and dry killing agent (Vapona) as the independent variables to test our hypothesis. We found that the agent ethanol tends to damage Lepidopteran but at the same time preserves them long enough for the purpose of our study. It is used during research primarily for the collection and preservation of flies (Diptera) and wasps (Hymenoptera). However, they can also be used to catch many other flying insects. Such traps are normally placed in predetermined locations for long periods of time, but they need to be checked daily or weekly depending on the type of killing agent used. Our malaise traps had two short walls, one middle wall, and a roof peaked on one end. The walls can vary in color and the roof is usually white. Poles are used to support the trap at each corner like a tent, and at the peak in front. Poles can be adjustable so that the sample jar may be raised or lowered to fit the specific circumference (8).

We set up two identical townes-style malaise traps for our research. Both traps were 5-feet high at the highest peak in the front, 4-feet high in the back, 6-feet in length, and 3-feet in width. Our traps were placed three feet apart from each other to ensure that variables such as outside temperature, wind direction and speed, hours of direct sunlight, and path of insect flight line remained constant. The traps were placed parallel to the wind direction to make certain that one trap was not up-wind from the other. The capture principle of the wet and dry traps given the natural features of the environment were based on positioning the traps for maximum interception of the insects' flight by means of a fabric barrier and subsequent positive phototropism by the flying insects. Since the intercepted insects are attracted by the sunlight at the top of the trap, they would subsequently hit the fabric barrier, be funneled upwards, and fall inside a collection jar with either attractant. The jar was removed while the malaise trap was left in place after the first week. During the first week, we checked the traps every day to confirm that the traps were all intact and not damaged or altered in any way by wind, birds, or animals that inhabit the preserve. We put new collection bottles for the second week, at which time we switched the independent variables just in case there was a difference in location. We noted that collections were similar for both weeks, so the external variable which could affect the results remained constant.

We found that denatured ethanol works best as it kills and preserves insects caught during the week. In addition, it keeps Lepidopteran scales that cover the wings, head, and parts of the abdomen from getting damaged or clinging to other insects in the collection bottle. Alternatively, a dry killing agent Vapona, was used in the collection jar of the second trap.

We hand sorted the collected insects in each jar using fine point precision forceps (Fisherbrand) and identified each one down to their order by using a dichotomous key provided by Dr. Reeves. Samples of each order of insects were mounted and labelled for display. This method was conducted by inserting the pin into a top hole on the 3-stair block making sure the tear drop paper is aligned with the hole. After this, a smudge of silicone gel (shellac) is pasted on the tear drop paper. Finally, a pair of tweezers was used to place the insect on its left side with its wings and antennae facing up. The pin was stabbed through the small blank paper and the identity was labeled.

In the first week, we collected 484 insects in total. We counted the number of insects belonging to each order. The following week, we returned to the same spot, inserted a new collection bottle, and collected an additional 336 insects.

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