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Effects of Blackberry Leaf Infusion on Survival Rate and Outcomes of Intraspecific Competition of *Culex restuans* and *Aedes aegypti*

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ABSTRACT

The American Mosquito Control Association has estimated that mosquito-borne diseases kill more than one million people each year. Diseases such as West Nile Virus, Dengue fever, and various forms of encephalitis are present in countries across the globe. Many are spreading throughout the United States. Despite the importance of controlling mosquito populations, most widely used pesticides are inefficient and harmful to humans or the environment. Research has shown that natural larvicides may offer a valid alternative to traditional pesticides in targeting mosquitoes. This study focused on understanding the effects of a blackberry leaf infusion on *Culex restuans* and *Aedes aegypti* mosquitoes. While the survival rate of the *Aedes aegypti* mosquitoes was not lowered by the use of the infusion, the survival rate of the *Culex restuans* decreased significantly.

INTRODUCTION

Methods for controlling mosquito populations are a necessity due to the ability of mosquitoes to transmit several harmful diseases. Mosquito-borne diseases kill more than one million people each year. Diseases spread by mosquitoes are endemic to hundreds of countries worldwide, but they are also becoming disturbingly prevalent in many U.S. states (AMCA 2014). In 2002, Illinois was struck with a West Nile Virus (WNV) epidemic. There were 884 cases of the disease and nearly seventy deaths (IDPH 2014). Mosquitoes of the *Culex* species, such as *Culex restuans*, are frequent vectors of WNV as well as St. Louis Encephalitis and Equine Encephalitis. *Culex* species are widely spread and able to live in even the most polluted water in catch basins, storm drainage systems, and sewage systems (Crans 2013) Another species, *Aedes aegypti*, is able to live in similar conditions and is the primary vector of Dengue fever. Because these species are both well established in a variety of human-inhabited areas they pose a great threat to humans as disease transmitters (Crans 2013).

Pesticides have frequently been used as a method of eliminating mosquitoes, but the efficacy of these chemicals is very low. Only a tiny part of pesticides sprayed in the air actually reach mosquitoes; one study found that even in an optimal situation, only 10% of aerosol pesticides reach flying insects (Fitz 2014). Pesticides have also been linked to various human health issues such as asthma, frequent headaches, burning and itching skin, and even seizure and disruptions in the endocrine system (TAC 2014). Because adult mosquitoes are widely dispersed and difficult to target effectively, larvicides, or pesticides added to water to target the mosquitoes in their aquatic life stage, are another popular solution. Although they are generally not as harmful to humans as adulticides, larvicides pose many environmental threats as they may harm other species that are present in larvae-inhabited water. Because of concerns about the health effects, environmental dangers, and efficiency of chemical pesticides, research on natural methods of mosquito population control is becoming increasingly important. Two of the most harmful diseases spread by mosquitoes, WNV and Dengue fever, do not yet have a vaccination, making mosquito control one of the only ways to prevent them. If fewer larval mosquitoes are able to emerge as adults, the number of outbreaks and spread of these diseases as well as many others will be lessened.

One study focusing on similar topics such as the effects of specific detritus infusions on larval survival and competition found that a blackberry leaf infusion is especially attractive as an oviposition-site to female *Culex pipiens*, but that the effects of this infusion have a negative impact on the survival rates of the larval mosquitoes (Gardner *et al.* in review). Other studies have found that the use of various leaf infusions can alter or mitigate the expected outcome of larval competition at different densities (Murrell and Juliano 2008).

The objectives of this study are to observe the effects of a blackberry infusion on the survival and larval competition of *Culex restuans* and *Aedes aegypti* using methods similar to those used in a another study, which focused on the similar species *Culex pipiens* (Gardner *et al.* in review).

Based on the results of previous studies it is hypothesized that the blackberry leaf infusion will be harmful to the survival of the *Culex restuans* but will not change the effects of the intraspecific competition of each species.

LITERATURE REVIEW

A variety of studies in recent years have focused on various leaf infusions and how they affect larval mosquitoes. These studies have provided the basis for this research, which attempts to use similar methods to focus on blackberry leaf infusion and its effects on *Aedes aegypti* and *Culex restuans*.

In a study conducted by Ebony Murrell and Steven Juliano in 2007, results showed that the effects of interspecific competition can be altered or eliminated by detritus type. This research focused on *Aedes albopictus* mosquitoes, which have been shown to be stronger resource competitors than *Aedes aegypti*. It was hypothesized that different detritus types would reverse or alter the asymmetrical competition between these species. To test this hypothesis, forty treatments using either oak, pine, grass, or insect detritus were tested at varying larval densities. Ten different combinations of mosquitoes testing intraspefic and interspecific competition at high medium, and low densities were tested with each detritus type (Murrell and Juliano 2008).

The survival of both species varied greatly among detritus types, and was lessened by a high conspecific density. *Aedes albopictus* density had a negative effect on *Aedes aegypti* survival in pine, oak, and insect detritus infusions but with the grass detritus *Aedes aegypti* survival was not affected by *Aedes albopictus* density. The grass infusion lessened the effects of interspecific competition, but intraspecific competition was still present (Murrell and Juliano 2008).

This work aims to apply similar methods to test the effects of a blackberry infusion on intraspecific competition of *Aedes aegypti* and *Culex restuans* mosquitoes.

In another study performed by Allison Gardner in 2014, infusions of exotic invasive plants were shown to affect larval development and adult emergence of *Culex pipiens*. Infusions of exotic Amur honeysuckle, autumn olive, and multiflora rose were used, as well as those made with native species: blackberry, elderberry, and serviceberry. To test the attractiveness of these various infusions to gravid females, six oviposition traps, each containing one of the six species, were placed in residential Champaign-Urbana, Illinois. These traps were surveyed daily and the number of egg rafts was recorded. The greatest number of egg rafts was collected from the blackberry leaf infusion, while the lowest number was collected from the serviceberry and autumn olive infusions. The different infusions also displayed different effects on adult emergence; the lowest rates of emergence were in blackberry and multiflora rose infusions, while honeysuckle and autumn olive infusions produced the lowest numbers of adults. Although the infusions were tested at varying larval densities, the honeysuckle mitigated the negative effects of the expected competition created by higher intraspecific densities (Gardner *et al.* in review).

While it is clear from this study that the blackberry infusion caused a negative effect on the survival of the mosquitoes, more research is necessary to discover if all species react similarly to this treatment and how it may effect larval competition. This study aims to use similar methods and a similar experimental design to test the effects of a blackberry infusion on the *Aedes aegypti* and *Culex restuans* species.

In a study performed by Loganathan Ponnusamy in 2008, bacterial and bacteria-associated chemical cues were determined to affect oviposition site preferences of *Aedes aegypti* (Ponnusamy *et al.* 2008). This study hopes to use the related knowledge that blackberry-leaf infusion is an especially attractive oviposition-site to discover further information on the possibilities of using blackberry leaf infusion as an effective natural larvicides.

There are some major commonalities between these studies, despite the fact that they focus on a variety of leaf infusions and mosquito species. From these studies it can be gathered that particular leaf infusions are preferred as oviposition sites and that particular leaf infusions can effect mosquito survival as well as the outcomes of larval competition.

METHODOLOGY

Origin of Mosquitoes

To obtain mosquitoes for this experiment *Culex restuans* were collected from grass infusion baited ovitraps (as described by Barbosa, Rosângela MR, *et al.*) placed in residential neighborhoods in Urbana, Illinois (Barbosa *et al*. 2007) The eggs were hatched overnight and applied to the experimental treatments as first instar larvae. *Aedes aegypti* were sourced from a laboratory colony maintained in the medical entomology lab at the University of Illinois. The colony originated in Florida and the mosquitoes used for this experiment were generation F21.

Preparation of Blackberry Leaf Infusion

The blackberry infusion used in this study was prepared by fermenting 80g of fresh leaves with stems removed in 4 gallons of tap water for seven days. The leaves were gathered on 7/3/14 from South Farm Plantation in Urbana, Illinois. Before adding larvae to the infusion, it was filtered through cheesecloth to remove particulates. After adding the larvae to the treatment, the cups were stored in a walk-in incubator at 25 degrees Celsius, 70% relative humidity, and 16:8 Light: Darkness photoperiod.

Bacterial Makeup of Blackberry Infusion

In previous experiments it has been found that the main types of bacteria in blackberry leaf infusion are from three phyla. 43% of the bacteria in blackberry infusions is from the phylum bacteroidetes, 25% from the phylum alpha-proteobacteria, and 16% from the phylum gamma-proteobacteria (Gardner *et al.*, in review).

Treatment Setup

For this study three treatments were tested on the two species, with five replicates for each treatment and species (15 containers per species). Each species was added to 360 mL of the blackberry infusion at a density of 10, 20, or 40 larvae. After five days the containers were checked for pupae, and any found were moved to separate vials until their emergence. Also on the fifth day 100 mL of the infusion was removed from each container and replaced with fresh infusion to maintain suitable bacterial resources for the larvae to feed on. This process was repeated once more on the 11th day of the experiment.

Collecting Wing Measurement Data (Not included in results)

After the pupae emerged the adult mosquito wings were dissected from the bodies in a standard wing dissection procedure. These wings were placed on labeled slides and measured using an Olympus IX51 Stereomicroscope and the computer program CellSens Entry.

Statistical Analysis

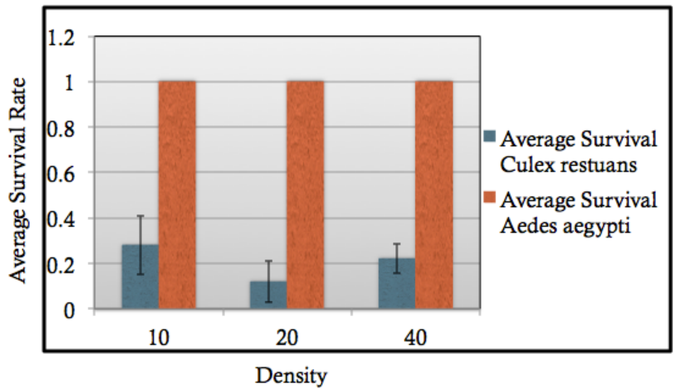
The data collected was not analyzed statistically, but the standard deviation was calculated for the values used to create the graphs of this study's results.

FINDINGS

The results of this study support the hypothesis that the use of the blackberry infusion would negatively impact the survival rate of the *Culex restuans.* For this study, survival was defined as reaching the pupal stage. In the density=10 treatments, only 28% ±13% of mosquitoes survived. In the density=20 treatments, 12% ± 9% survived and in the density=40 treatments 22% ±6% of the *Culex restuans* survived. In all treatments 100% of *Aedes aegypti* mosquitoes survived.

Figure 1

Indicates the average survival rates of both mosquito species as a percentage of the total number of mosquitoes in each density treatment.



The results of this study support the hypothesis that the blackberry infusion would negatively impact the survival rates of the *Culex restuans* mosquitoes. The findings of this research are similar to those discussed in the work of Allison Gardner, whose research focused on the effects of a blackberry infusion on *Culex pipiens* mosquitoes. In her study the majority of the *Culex pipiens* raised in the blackberry infusion did not survive. The results of this experiment, as well as results from previous studies may indicate a trend in the ability of blackberry infusion to lower the survival rate of mosquitoes in the *Culex* genus (Gardner *et al*. in review).

It is less clear as to whether or not the second hypothesis, that the use of a blackberry infusion would have no effect on the outcomes of the outcomes of intraspecific competition, is supported by the results of this study. Previous studies have found that mosquitoes in environments with a higher larval density are less likely to survive than those in environments with a lower larval density and thus, less competition for limited food resources (Reiskind and Lounibos 2009) However, in a study conducted by Allison Gardner, it was found that a honeysuckle infusion mitigated the effects of competition at varying larval densities (Gardner *et al*. in review).

In this study the *Culex restuans* mosquitoes seem to have benefited from a low-density, low-competition environment. The greatest survival rate for the *Culex restuans* mosquitoes was found in the density=10 treatments. However, the lowest survival rate was found not in the density=40 treatments, but in the density=20 treatments. The *Aedes aegypti* mosquitoes had a 100% survival rate at all tested densities, which may signal that the blackberry infusion was able to mitigate or even fully negate the negative effects of a high-competition environment for this species.

Some limitations have impacted the depth of this study. The largest of these was a seven-week time constraint, which forced the project to be shortened or simplified in multiple ways. Although this study originally aimed to also test the same hypotheses on *Aedes albopictus* mosquitoes, data was not able to be collected or analyzed from this part of the experiment because of the time constraints. Wing dissections were performed on all adult mosquitoes from the first experiment, but due to time constraints and limited number of some groups (e.g. female *Culex restuans* from density=40 treatments) this data was also unable to be included in the results. Preliminary results from the wing dissections showed lower average wing lengths in higher-density treatments.

Future research on the effects of blackberry leaf infusion may aim to understand whether the bacteria formed as the infusion is created or the chemicals that leech into the water from the blackberry leaves themselves are harmful to the mosquitoes. Another goal for future research based on the findings of this study might be to understand why the *Aedes aegypti* species is unresponsive to the blackberry leaf infusion treatment.

CONCLUSIONS

The results of this study clearly show that a blackberry infusion negatively impacts the survivorship of *Culex restuans* mosquitoes. Further research into the usage of this infusion as well as others will aid in the development of safer and more effective natural larvicides. As new methods of controlling mosquito populations are devised, fewer numbers of adult mosquitoes will survive and the spread of mosquito-vectored diseases will be slowed.

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