Environmental Influences for the Tree Canopy Preference of *Culex pipiens* and *Culiseta melanura*

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ABSTRACT

In our study we found that when mosquito collections were made on the same night and location, with one trap placed in the tree canopy and the other trap at ground level, there were a significantly higher number of *Culex pipiens* and *Culiseta melanura* in the tree canopy traps. These two trap levels also exhibited no significant difference in temperature, although it was determined that there was a significantly higher relative humidity at the ground level than in the canopy. This difference in relative humidity was also found to not be significantly correlated with the collections. By learning more about the biology of *Culex pipiens*, as well as other mosquitoes, we will be able to devise more effective methods to hamper their negative effects on humans without impacting other parts of the ecosystem.

INTRODUCTION

Since the discovery of West Nile virus (WNV) in the United States in 1999, much emphasis has been placed on learning more about its transmission and characteristics of the specific mosquito species involved (Kulasekera, 2001; Nasci, 2001; Kilpatrick, 2005). The first known human case of WNV was reported in New York City, in August of 1999. After this first case there were an additional 61 humans positive for WNV in New York, from August to October of 1999, consisting mostly of elderly people (Enserink, 2000; Rappole, 2000). As of March 2005, WNV has infected over 17,000 and killed over 670 people in North America (Kilpatrick, 2005). From its initial discovery, WNV quickly spread across the U.S. and has made its way down into Mexico and Central America (Knight, 2003).

An important vector of WNV in the United States is the mosquito species *Culex pipiens* (Goddard, 2002; Anderson, 2004; Kilpatrick, 2005). It has been suggested that these mosquitoes act as hosts for overwintering flaviviruses such as WNV, until they reemerge in the spring (Goddard, 2002). Some studies suggest that *Culex pipiens*, along with *Culex restuans*, may in fact be responsible for up to 80% of human WNV infections in the northeast United States (Kilpatrick, 2005). Previously believed to feed mainly on birds, and therefore reducing their likelihood of infecting humans, *Culex pipiens* are now thought to more commonly feed on humans than previously thought (Kilpatrick, 2005). By learning more about the biology of *Culex pipiens*, as well as other mosquitoes, we will be able to devise more effective methods to hamper their negative effects on humans without impacting other parts of the ecosystem.

The vast majority of female adult mosquitoes require a blood meal to begin development of each clutch of eggs, and obtain this from a variety of sources (Bates, 1949; Knight, 2003). Most mosquito species will feed on warm-blooded animals after receiving cues to induce biting. These signals include carbon dioxide and ammonia, especially when coupled with a temperature and moisture level similar to breath (Bates, 1949). Respiration of animals, along with color, motion, and smell to a lesser degree attract the mosquitoes to feed upon various hosts (Bates, 1949). Some mosquitoes exhibit host preference while others do not. For example, past studies have the *Culex pipiens* species preferentially feeding on birds, but also feeding on assorted mammals (Nasci, 2001).

Once they have acquired their blood meal necessary for egg development, mosquitoes may use many different types of areas for breeding, including irrigated agricultural lands, shallow isolated pools, dumping areas, and wetlands (Knight, 2003). After obtaining a blood meal, the female mosquitoes will usually have a resting period before oviposition. It has been shown that mosquitoes don't lay eggs randomly but instead may lay eggs where there are fewer predators present (Kiflawi, 2003).

In many aspects of mosquito life history, temperature seems to play a very influential role. Low air temperatures in the winter lead many mosquito adults to enter a hibernation state and high temperatures in the summer can also lead to decreased adult mosquito activity (Knight, 2003). As noted before, temperature also plays an important role in the feeding habits of mosquitoes. *Culex pipiens* have been shown to prefer host temperatures between 32° C and 43°C, with temperatures above 49°C and below 30°C showing less attraction. Temperature also seems to have an effect on oviposition, with mosquitoes avoiding water temperatures outside the range of 20°C to 30°C (Bates, 1949).

There are several common trapping methods for adult mosquitoes. These include gravid traps that simulate oviposition habitat, light traps, and carbon dioxide traps with the latter two possibly being combined. With carbon dioxide being a major attractant for mosquitoes, yields from these traps are especially clean, containing almost no unwanted insects. The traps with light alone can produce many kinds of non-targeted insect species, which can slow research.

Culex pipiens, as well as other mosquito species, has been discovered to prefer inhabiting tree canopies, or at least seem to frequent tree canopy height. The specific reasons for this behavior are not clear although it has been speculated that they may be influenced by temperature, humidity, light, as well as the potential feeding of nesting birds (Anderson, 2004). This project was geared toward gathering data on two of these possibilities, temperature and humidity. My hypothesis is that *Culex pipiens* and *Culiseta melanura* will both show a significant preference for the canopy level, but that this will not have a significant relationship with either temperature or humidity.

METHODS

Data collection for the project was started in late May 2005 and ended September 2, 2005. There were three different sites, two in Westborough, MA, and the other in neighboring Hopkinton. The two sites in Westborough were located off of Rogers St. (42°16.427'N, 071°36.033'W) and Hopkinton Rd. (42°15.709'N, 071°35.812'W), while the Hopkinton site was located off of Woods St. (42°15.354'N, 071°35.149'W).

Trapping involved using two CDC light/CO_2 mosquito traps (John W. Hock Co., model 512) with net collection bags, one placed approximately 6.5 meters into the air and the other about 1.5 meters high at the same site. Carbon dioxide was used as the only means of attractant, with the light feature of the traps being disabled to avoid non-target insects. The CO_2 tanks were adjusted with regulators to 15psi. On each trap there was a temperature/relative humidity data logger (Onset 64K HOBO Pro RH/Temp Logger) that logged each every 40 seconds while the trap was collecting.

The traps were set and collected overnight and retrieved approximately 24 hours later and set again usually at one of the other sites, with new collection bags, new batteries and new CO_2 tanks. The data logger information was downloaded and reset at each retrieval. The specimen collections were knocked down and stored in a refrigerator until identification. The specimens were identified as *Culex pipiens*, *Culiseta melanura*, or "other," by using the Darise mosquito index (1981) and a dissecting microscope.

The data collected from the data loggers and mosquito identification was then used in several ANOVAs to determine whether there were significantly different findings for the two trap levels, three trap sites, any interaction between those factors, and also for the temperature and relative humidity of the two trap levels. Significantly different mosquito numbers were then put through a test for normality and then a Spearman correlation test to determine if they were associated with any of the two possible environmental influences that were tested.

RESULTS

There were 42 viable collections made, which included both canopy and ground traps along with complete temperature and relative humidity data sets (Tables 1-4). An ANOVA for the number of *Culex pipiens* caught was performed against the two trap levels and the three sites. It was then determined that there was a significantly higher number of these mosquitoes caught in the canopy traps than in the ground traps (Figure 1), but no significant difference between any of the sites and any interactions within the trap levels and sites (Table 5).

Table 1: Descriptive Statistics for *Culex pipiens* Collections

Variable	N	Mean	Median	TrMean	StDev	SE Mean
CUL (Canopy)	42	5.98	3.00	5.03	7.65	1.18
CUL (Ground)	42	2.43	1.00	1.37	6.70	1.03
	Min.	Max.				
CUL (Canopy)	0.00	33.00				
CUL (Ground)	0.00	43.00				

Table 2: Descriptive Statistics for *Culiseta melanura* Collections

Variable	N	Mean	Median	TrMean	StDev	SE Mean
MEL (Canopy)	42	11.31	5.00	8.030	20.00	3.09
MEL (Ground)	42	2.00	1.00	1.579	2.98	0.46
	Min.	Max.				
MEL (Canopy)	0.00	123.00				
MEL (Ground)	0.00	15.00				

Table 3: Descriptive Statistics for Average Temperature (°C) Readings

Variable	N	Mean	Median	TrMean	StDev	SE Mean
Ave. Temp. (Canopy)	42	19.278	20.325	19.596	4.622	0.713
Ave. Temp. (Ground)	42	19.070	19.825	19.380	4.462	0.689
	Min.	Max.				
Ave. Temp. (Canopy)	6.700	25.980				
Ave. Temp. (Ground)	6.780	25.400				

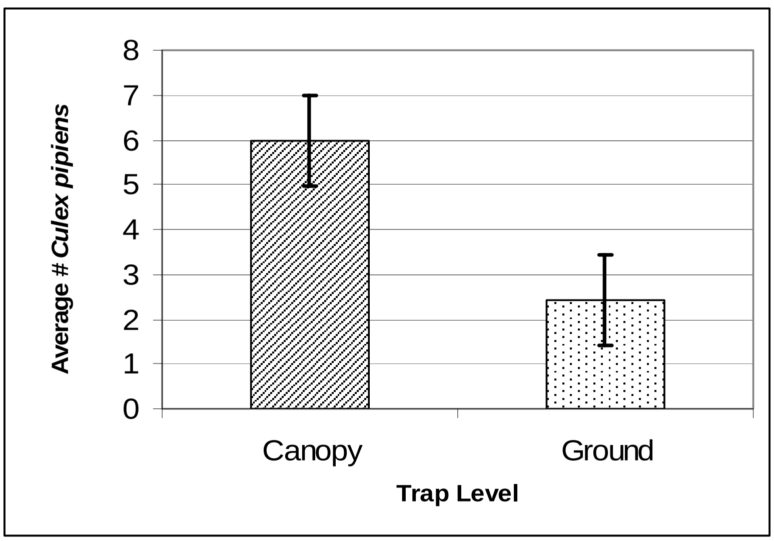
Table 4: Descriptive Statistics for Average Relative Humidity (%H) Readings

Variable	N	Mean	Median	TrMean	StDev	SE Mean
Ave. RH (Canopy)	42	84.57	84.94	84.98	8.04	1.24
Ave. RH (Ground)	42	88.21	87.84	88.56	6.82	1.05
	Min.	Max.				
Ave. RH (Canopy)	58.20	98.41				
Ave. RH (Ground)	67.14	99.45				

Table 5: Analysis of Variance for *Culex pipiens*

Source	DF	SS	MS	F	P
Canopy/Ground	1	264.3	264.3	5.30	0.024
Site	2	145.2	72.6	1.46	0.240
Interaction	2	200.2	100.1	2.01	0.141
Error	78	3889.9	49.9		
Total	83	4499.6			

Figure 1: Average # *Culex pipiens* at Canopy and Ground Trap Levels

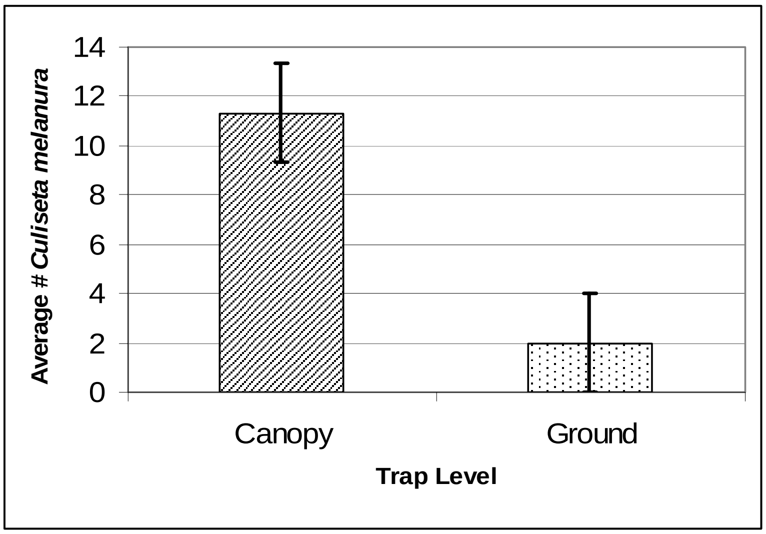


Similar results were found when an ANOVA was performed for the number of *Culiseta melanura* caught against the two trap levels and the three sites. There was a significantly higher amount of *Culiseta melanura* mosquitoes found in the canopy traps as opposed to the ground traps (Figure 2). Again, there was no significant difference between the number caught from the three sites or any interactions between the levels and sites (Table 6).

Table 6: Analysis of Variance for *Culiseta melanura*

Source	DF	SS	MS	F	P
Canopy/Ground	1	1820	1820	8.72	0.004
Site	2	329	164	0.79	0.458
Interaction	2	150	75	0.36	0.700
Error	78	16278	209		
Total	83	18577			

Figure 2: Average # *Culiseta melanura* at Canopy and Ground Trap Levels



When two-way ANOVAs were used with average temperature and average relative humidity against the trap levels and different sites, it was determined that there was a significant difference in the relative humidity readings of the canopy and ground level traps, but not in those of the average temperature of the two levels. There was no significant difference in the environmental factors between each site and also no significant difference in any interaction between trap level and site (Tables 7, 8).

Table 7: Analysis of Variance for Average Temperature

Source	DF	SS	MS	F	P
Canopy/Ground	1	0.9	0.9	0.04	0.835
Site	2	49.4	24.7	1.17	0.315
Interaction	2	0.2	0.1	0.00	0.995
Error	78	1642.8	21.1		
Total	83	1693.3			

Table 8: Analysis of Variance for Relative Humidity

Source	DF	SS	MS	F	P
Canopy/Ground	1	277.6	277.6	5.09	0.027
Site	2	270.7	135.3	2.48	0.090
Interaction	2	27.5	13.7	0.25	0.778
Error	78	4253.9	54.5		
Total	83	4829.6			

A test for normality showed that the mosquito collection data was not normal, and so the resulting Spearman correlation test showed that there was not a significant positive correlation between the *Culex pipiens* and *Culiseta melanura* canopy preference and the significantly different relative humidity of the two levels (Table 9; Figures 3, 4).

Table 9: Spearman Correlation Test for Relative Humidity and Mosquito Collections (Canopy:Ground)

RH Difference: CUL Difference	P-value = .280
RH Difference: MEL Difference	P-value = .943

Figure 3: Scatter Plot of Relative Humidity vs. # *Culex* Differences (Canopy-Ground)

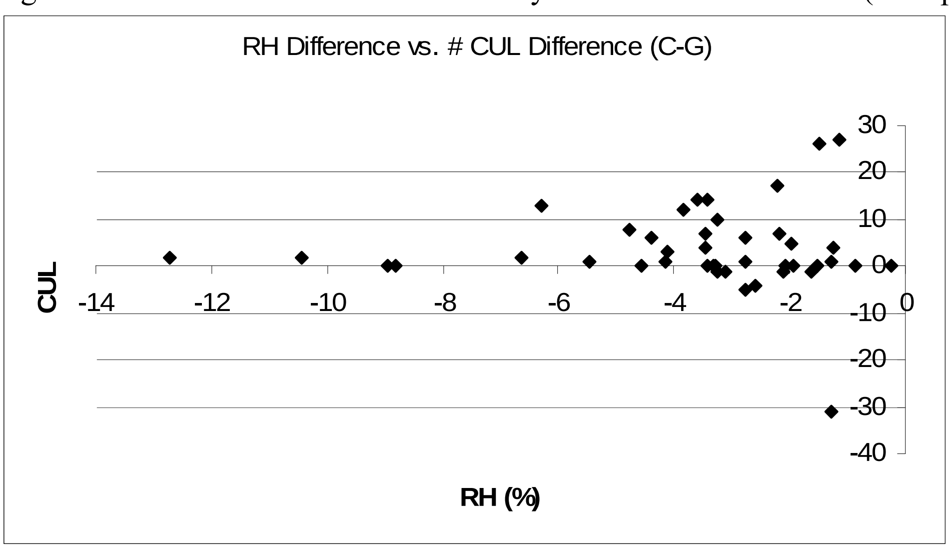
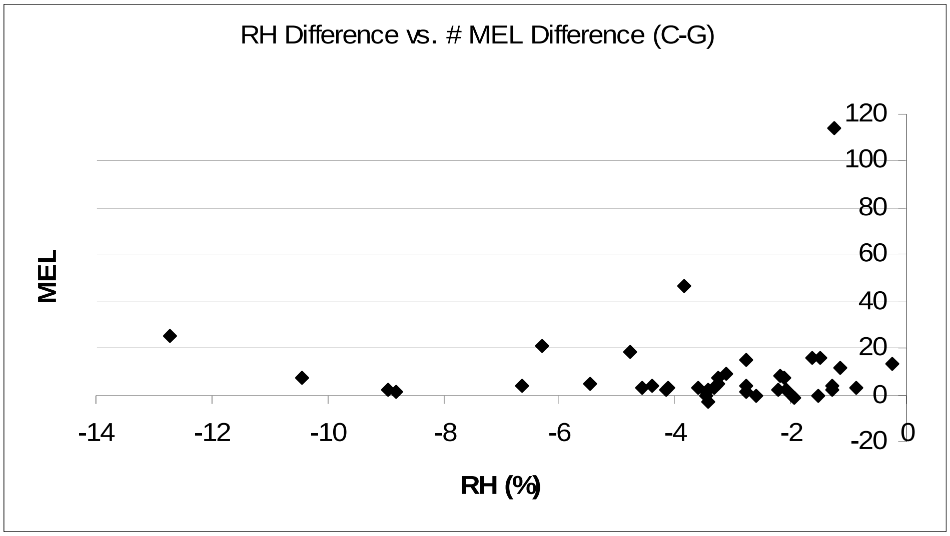


Figure 4: Scatter Plot of Relative Humidity vs. # *Culiseta melanura* Differences (Canopy-Ground)



DISCUSSION

Our collections exhibited the canopy preference shown by *Culex pipiens* and other mosquitoes in previous studies (Table 5, 6; Figure 1, 2; Anderson, 2004). Our study also showed that there was not a significant difference in the average temperatures of the two traps level (Table 3). However, the relative humidity of the two levels did prove to be significantly difference (Table 4), leading us to perform a correlation, which showed that there was not a significant relationship between relative humidity and collections (Table 9).

Through the lack of a correlation, I believe our results seem to support the idea that the canopy preference is due more to the feeding habitat of these mosquitoes on roosting birds than abiotic environmental influences. The preference for obtaining blood meals through birds by *Culex pipiens* seems to be more behavioral than being influenced by certain environmental factors, temperature and relative humidity in this case. Our results support the possibility that these target mosquitoes are present in the canopy not because of the proposed abiotic factors but more likely because the dominant feeding patterns and the location of these organisms.

Because of the susceptibility of *Culex pipiens* and *Culiseta melanura* to acquire and transmit West Nile virus and also other diseases including Eastern Equine Encephalitis, it is important to know where they are predominantly located and also the reasons why. Previous studies along with this one seem to indicate that these mosquito species do prefer canopy level, which could be very influential in the control aspect of mosquito (Anderson, 2004). With the right thermal currents, a mosquito control application could be administered so that it would rise through the canopy, eliminating those targets before any virus is allowed to transfer and build in bird hosts. Lessening the amount of virus that bird host populations are exposed to could significantly decrease the chances of a mosquito with bird and mammal feeding preference to obtain virus and transmit it to humans.

Similar research of canopy preference of *Culex pipiens*, *Culiseta melanura* and other mosquito species, may lead mosquito surveillance projects to change their trapping protocol. By shifting the focus of surveillance techniques to the canopies as opposed to the standard ground level, there could be an increased chance of finding infected mosquitoes before they have a chance to infect birds, which would begin to build up the virus in themselves. Finding these infected mosquitoes before they have a chance to infect birds would give mosquito control projects a head start on signaling potentially high risk areas, and taking any proper actions.

These ideas were relevant during this project as one of the collections from a canopy trap was found to have West Nile virus. Signs were posted and a press release was announced, allowing local residents to take their own precautions to avoid contracting WNV. In response to these findings more traps were established in the local area, which later in the season resulted in a positive Eastern Equine Encephalitis pool of mosquitoes. These traps were located in an area that was frequented by children and senior citizens, emphasizing the importance of identifying it for infectious mosquitoes early.

In conclusion this study reinforces the canopy preference for *Culex pipiens* and *Culiseta melanura*. It was also found that there was no correlation between the canopy preference and canopy temperature and relative humidity. This finding leads one to believe that the canopy preference exhibited by these mosquitoes is influenced by something else, host availability being among the possibilities.

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