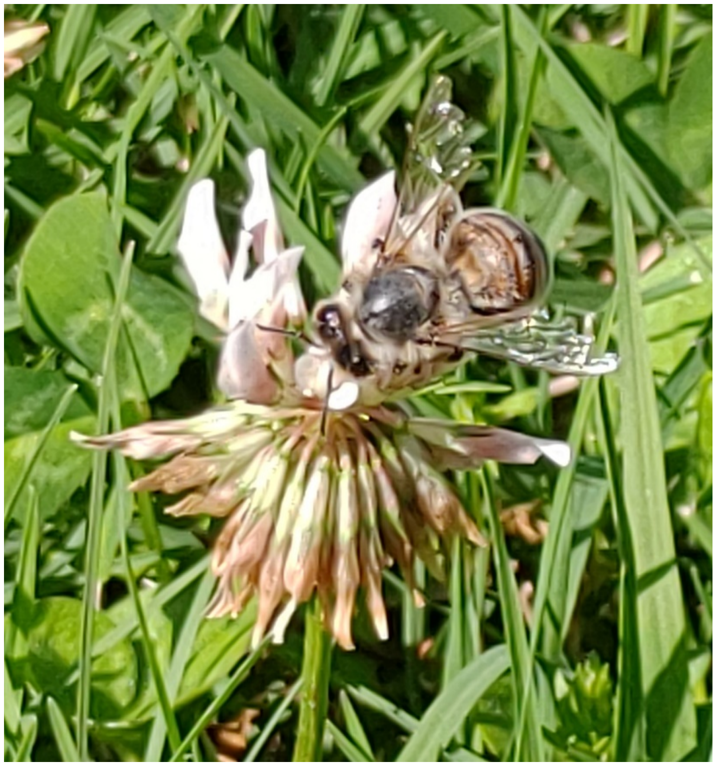

Out with the New, In with the Old: How the Use of a Decoy Asian Citrus Psyllid Mating Call Impacts Honeybee Population Density

By Eleanor Shore

Department of Integrative Biology, Oklahoma State University

Stillwater, OK 74078

ABSTRACT

Asian citrus psyllids carry a bacterium that causes citrus greening disease. In order to combat this disease, researchers developed a machine that broadcasts a decoy mating call to disrupt mating of Asian citrus psyllids. The noise this machine makes in order to disrupt the psyllids has not been tested on an important pollinator, the honeybee. The goal of this study was to test how a decoy mating call of the Asian citrus psyllid affected the population density of honeybees (*Apis mellifera*) in the area immediately around where the call was being broadcasted. There was no statistically significant difference in the population density of bees when the decoy mating call was playing versus the population density of bees when there was no call playing. The data support the hypothesis that the decoy mating call noise does not have a significant impact on honeybees (does not drive away honeybees nor lure honeybees). Further testing is required to see if this finding holds true at different volumes and other settings.

KEYWORDS

Honeybee, Asian Citrus Psyllid, Sound Insecticide, Apis mellifera, Acoustics

INTRODUCTION

You probably have some form of citrus in your house right now. Whether it's in your juice, your fruit bowl, your cleaning products, or even your favorite fragrance, citrus is all around us. It's not surprising to learn that the United States citrus industry makes billions every year (USDA 2019). However, the U.S. citrus industry could be making even more if it weren't for a small bug called an Asian citrus psyllid (*Diaphorina citri*) (Entomology Today 2014).

These pests may be only three to four millimeters long, but they are devastating to citrus trees (Grafton-Cardwell *et al*. 2006). While the Asian citrus psyllid is, as the name implies, originally from Asia, they made their way over to the United States in 1998. At first, they were just in Florida, but in 2001 they spread to Texas and in 2008 they arrived in California (Grafton-Cardwell *et al*. 2006, Grafton-Cardwell 2020). Now in these states, the Asian citrus psyllids damage local citrus trees by feeding on their sap, making honeydew that encourages mold growth, and injecting malformity-causing toxins into the leaves and shoots just to name a few things (Grafton-Cardwell *et al*. 2006). However, these direct attacks on citrus trees are not what earned Asian citrus psyllids their place on the United States Department of Agriculture's (USDA) list of top invasive pest threats (USDA 2020). Asian citrus psyllids are a vector for a bacterium called *Candidatus* Liberibacter asiaticus or LAS (Hall *et al*. 2012). This bacterium causes Huanglongbing or citrus greening disease, considered by many to be the world's most serious citrus disease.

Citrus greening disease spread much like the Asian citrus psyllid; starting in Asia and coming into the United States through Florida (Grafton-Cardwell *et al*. 2006). The disease came to the U.S. in 2005 and has been difficult to combat ever since. The disease is fatal to trees, killing them in only a few years (Kaplan 2018). In those few years, an infected tree will turn mottled and yellow and its growth will be stunted. Additionally, and perhaps most noticeably, the fruits of the infected tree will stay green and small while also becoming lopsided, hard, bitter, and full of dark, aborted seeds. There is no cure for citrus greening disease. Infected trees are removed in hopes that the disease will not spread from tree to tree.

There have been many attempts to get rid of U.S. Asian citrus psyllids and the disease they carry. Chemical insecticides have been implemented, but this strategy has been shown to be ineffective as well as costly (Hall *et al*. 2012). Additionally, there is evidence that Asian citrus psyllids are growing increasingly more resistant to a variety of insecticides (Tiwari *et al*. 2011).

However, chemical insecticides aren't the only type of insecticide scientists have been looking at.

Recent research suggests that sound may be a way to combat the Asian citrus psyllid problem (Mankin *et al*. 2015). Male Asian citrus psyllids call for mates; female Asian citrus psyllids call back. Researchers discovered that this mating call could be disrupted by a decoy female mating call. The researchers created a machine that could detect the male Asian citrus psyllid mating call and reply back with the decoy Asian citrus psyllid mating call faster than an actual female Asian citrus psyllid. The researchers discovered that this machine significantly disrupted the mating of Asian citrus psyllids. The researchers even put a fly-paper like material around the machine to tempt male Asian citrus psyllids to fly over and stick to it while following the decoy call (Gammon 2015).

This machine is not in widespread use, in part, because the ecological impacts of the machine outside of the influence on Asian citrus psyllids remain unknown. Ecological impacts on pollinators, for instance, might present a problem. Honeybees are an important pollinator of citrus trees (Sanford 1992). When thinking of using Mankin and his colleagues' machine, it would be of the upmost importance to find out if the sound of the machine has a significant impact on the population density of honeybees within hearing distance of the machine. If the machine drives the bees away, then that leaves the citrus trees with fewer pollinators. If the machine attracts the bees, then the bees are in danger of getting caught in the adhesive surface covering the machine.

In this study, I tested if the population density of honeybees, *Apis mellifera,* differed in the presence or absence of the decoy call of the Asian citrus psyllid. If honeybees were attracted to the decoy call, their population density would be higher compared with that of the control, or if honeybees were driven away by the call, their population density would be lower compared with that of the control. The control for this study is the population density of honeybees around a speaker while there is no sound playing. The treatment for this study is the population density of honeybees around a speaker while the decoy Asian citrus psyllid mating call is playing. It was predicted that the population density of honeybees around the speaker when the sound was playing would not be significantly different from the population density of honeybees around the speaker when there was no sound playing from the speaker.

METHODS

All trials were conducted in spring 2020 in Oklahoma in the Stillwater and Glencoe area. The specific coordinates of the trials were (36.12º, -96.87º) for the trials done in Glencoe and (36.13º, -97.03º) for trials done in Stillwater. Only two decimals were used due to privacy precautions. All trials were done with wild members of the species *Apis mellifera* due to their abundance in the field and their status as an important pollinator to citrus fruits (Sanford 1992).

For each trial, a small (6.35cm by 13.5cm), black Bluetooth® speaker was used. The same volume was used for all sound trials. All trials were done between 1300 and 1900 hours CDST, with 3% or less cloud cover, 7 mph wind, and a temperature between 19℃ and 32℃. Before each trial, the weather conditions including temperature, cloud cover, wind speed, wind gust speed, humidity, pressure, visibility, and ceiling were recorded. After each trial, the coordinates of the speaker were recorded.

The audio file used during the trials was a modified version of the audio file used for the Asian citrus psyllid catching machine (Intagliata 2015). The portion of the podcast containing the decoy female Asian citrus psyllid mating call was isolated using MAGIX audio cleaning lab. A video of Asian citrus psyllids calling was then taken and the pattern of calls identified using MAGIX music maker (USDA 2016). The audio for the decoy female Asian citrus psyllid mating call was then made to mimic the same pattern of calls, both in duration and spacing, of the video of Asian citrus psyllids calling.

The speaker was placed in an area with visible foraging bees and connected to a phone via Bluetooth®. The speaker was always placed on the ground. When the speaker ran out of battery, it was hooked up to a small black battery pack that was then placed beneath the speaker. A white connection cable was used for this. When the battery pack ran out of energy, the speaker was hooked up to a white charger which was then hooked up to a 30.5-meter orange extension cable.

During the entirety of each trial, the observer sat one meter away from the speaker and wore a white beekeeping hat with black mesh. All data were recorded in a field journal.

Each trial consisted of thirty minutes broken into three ten-minute sections. The time at which each section started and ended was recorded. The first section, herein referred to as part A, consisted of a ten-minute trial in which the mating call was played or nothing was played. Whether the speaker was playing the call or not was determined by a coin flip. This trial consisted of recording the number of bees within half a meter of the speaker every fifteen seconds for ten minutes straight. The time was observed via phone. At the end of the ten minutes, the second section of the trial would begin. In this section of the trial, herein referred to as the intermission, the speaker would play no sound and no data would be taken for ten minutes. A ten-minute timer was used during this section. At the end of these ten minutes, the third and last section of the trial, herein referred to as part B, would begin. In this section, the mating call would either be played or not based on the opposite of what occurred in part A. Data collection then proceeded as in part A. This process was repeated nine times for a total of ten trials.

Disturbances, such as gusts of wind or other organisms, were noted in the notes section of the field journal. Whether the speaker and bees were in the shade or not was not recorded as this remained constant throughout all three sections of a given trial.

The data were then entered into an Excel spread sheet. The trials with the call not playing (sound off) were placed together in a table while the trials with the call playing (sound on) were placed in another table. The average population density of bees in each part of each trial was then determined. Using these averages, a t-test was run to compare the average population density of honeybees when the call was playing to the population density of honeybees when the call was not playing. The two-tailed t-test was used since this study is exploring if there were any significant changes in honeybee density in either direction (a higher density or a lower density).

RESULTS

During each trial, the population density of honeybees seemed to come and go in gradual waves rather than erratically jump up and down. In the context of fifteen second intervals, the honeybees seemed slow to enter and leave the half meter area around the speaker. This trend seemed to hold true across all trials.

The mean of each group was compared to each other (Table 1). The mean of the treatment group was found to be 11.67% less than the mean of the control group. There was no significant difference in the averages of the control (sound off) compared to the averages of the treatment (sound on) ($t=0.47$, $df=9$, p-value=0.649; Table 1). Since the two-tailed p-value was far more than the alpha value of 0.05, the two-tailed p-value was not statistically significant in either direction, positive or negative.

The variance of the control group and the variance of the treatment group were compared to each other and their difference found to be negligible (Table 1). This indicates that the p-value given by the t-test is likely to be accurate since there was little to no interference from the difference in variances.

The sample standard deviation of the control group and the treatment group was calculated. Standard deviation =0.542344068 for the control group and standard deviation = 0.451852628 for the treatment group. From this, the standard error for each group was calculated. Standard error = 0.171504253 for the control group and standard error = 0.142888347 for the treatment group. These standard errors coupled with the means from each group (Table 1) were then used to create a 95% confidence interval. The 95% confidence interval for the control was (2.828495747, 3.171504253) and the 95% confidence interval for the treatment was (0.857111653, 1.142888347).

DISCUSSION

The null hypothesis that the mean population densities of honeybees between the two groups (sound on and sound off) are not significantly different from each other is supported. Therefore, we found no effect of the decoy Asian citrus psyllid mating call on the behavior of *A. mellifera*. This means the use of a decoy Asian citrus psyllid mating call to disrupt the mating behavior of Asian citrus psyllids in and around citrus tree farms is not expected to negatively impact the population density of honeybees in and around said farms.

The mean honeybee density when the decoy Asian citrus psyllid sound was playing was slightly less than the honeybee density when there was no sound, but this was not to a significant degree. However, a larger number of trials might yield a larger, more significant difference in means in the same direction as the difference found here. Such results would then be more likely to find a significantly lower density of honeybees when the decoy call was playing compared to when there was no sound.

The confidence intervals for each group are rather large due to the small number of trials. The possible means that lie within these confidence intervals could potentially create a statistically significant difference in population density if found to be the true means. A larger number of trials would combat this by creating a smaller confidence interval, allowing us to be more sure that the honeybee population density was or was not significantly different between the control group and the treatment group.

Originally, the trials were going to be conducted indoors in a temperature-controlled room. There, the bees would have been closely observed and the time spent at certain distances away from the speaker recorded. However, with the coronavirus pandemic reaching the United States and soon Oklahoma, these trials had to be cancelled. I would recommend that further studies take place in controlled lab environments rather than in the field as there were many problems encountered with field trials. These problems were both environmental and technological in nature. Environmental difficulties included wind interference making bees move away from where they had been and other organisms making noises that interfered with the honeybee's behavior. Technical difficulties included the black speaker slowly heating up over the course of the trials, the speaker making a loud noise when it powered off, and the speaker dying in the middle of a trial, leading to the speaker having to be removed, plugged into a power source which made a slight noise, and replaced. However, all research encounters some difficulties and I am confident in the data gathered and the conclusions drawn from said data. I do not think any of the problems encountered to be great or numerous enough to significantly alter the data that was collected.

Further studies should test different volumes of the decoy Asian citrus psyllid mating call. It would be beneficial to discover if there is a volume which has a statistically significant impact on honeybee population density. Further studies should also do a larger number of trials in order to create more robust means for the treatment and control groups. Trials should also be conducted in other locations, especially citrus groves.

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TABLES

t-Test: Paired Two Sample for Means		
Trial Type	*OFF*	*ON*
Mean	1.0243902	0.904878
Variance	0.2941371	0.2041708
Observations	10	10
Pearson Correlation	-0.299393	
Hypothesized Mean Difference	0	
df	9	
t Stat	0.4705621	
P(T<=t) two-tail	0.6491487	
t Critical two-tail	2.2621572	

Table 1: Two-tailed t-test for two paired variables comparing the population density of honeybees in the control group (no sound) to the treatment group (decoy sound playing). Off denotes the group with no sound or the control group and on denotes the group with the decoy sound playing or the treatment group.