Effect of *Solenopsis invicta* presence on species diversity of ground-dwelling arthropods at Brackenridge Field Laboratory

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Abstract

The invasive ant species *Solenopsis invicta,* more commonly known as the Fire Ant, made its first appearance in the Southern United States in the 1930's. These ants adapted quickly to the landscape of Southern states and grew rapidly in population size. Soon, *S. invicta's* widespread and invasive presence was reflected in vertebrate and invertebrate populations alike, a trend which encouraged studies over the environmental impact of the ant. While earlier studies in areas with *S. invicta* concluded that the species diversity of ground-dwelling arthropod populations was significantly less, later studies in those same areas concluded that the species diversity of ground-dwelling arthropods had returned to pre-invasion levels. As a response to these former experiments, I conducted an experiment at Brackenridge Field Laboratory in Austin, Texas, which replicated previous testing methods for arthropod diversity. This experiment was conducted using several plots in which bait traps were first deployed to test for the presence of *S. invicta* in the plot. Once *S. invicta* presence was determined in a plot, pitfall traps were purposefully set in marked plots both with and without *S. invicta* in order to test for arthropod species richness. After counting and identifying the collected arthropods, one-tailed and two-tailed t-tests were run to determine the significance of the difference between the species richness of arthropod populations in plots with *S. invicta* presence and arthropod species richness in plots lacking *S. invicta.* I concluded that there was no significant difference in species diversity of arthropod populations between plots containing *S. invicta* and plots lacking *S. invicta.* Despite some external variables such as inconsistencies of weather and sampling procedures, the resulting conclusions corresponded with my null hypothesis and expectations from preliminary research on the subject.

Introduction

Solenopsis invicta, more commonly known as the red invasive fire ant, is notable for the negative effect it has on other species within its environment. The correlation between *S. invicta* and population shifts in arthropod and small vertebrate communities has been historically negative, indicating that the presence of *S. invicta* has had a consistent impact on its surrounding environment (Epperson and Allen 2010). This correlation has led several researchers (such as those in Morrison 2002; Morrison and Porter 2003) to look further into the phenomenon of fluctuation within the arthropod communities which are sharing habitats with *S. invicta*, and more recent studies have found a reversal in this trend. Experiments were conducted with the goal of understanding how this invasive species affects native species of plants and animals, and it was discovered on several occasions that the foraging patterns of *S. invicta* had caused a depletion in the nearby population of arthropods (Epperson and Allen 2010). In this paper, I will discuss my personal continuation of the research on the relationship between *S. invicta* and the arthropod community.

Soon after their arrival from South America in the late 1930's, *S. invicta* grew quickly accustomed to utilizing and altering the environment of the Southern United States (Davidson 2002). Statistically shown to be one of the most environmentally destructive invasive ants in North America, the impacts of this ant can be witnessed on multiple plains (Allen 2004). In the Southern United States, the major region the ant has invaded, several experiments have shown that the presence of *S. invicta* affects vertebrates such as small birds and reptiles (as studied in Allen 2004). Researchers determined that this resounding response within vertebrate populations is a clear reflection of the dietary and territorial needs of *S. invicta* (Wojcik 2001; Taber 2000). In other words, studies have consistently shown that *S. invicta* has depleted the populations of arthropod and plant life so greatly that it has traveled up the food chain, also affecting vertebrate populations (Allen 2004).

Originally, research was prompted by human encounters with the 'pest' *S. invicta* and the recognition of a new species within the American landscape (explained by Mann 1994; Schwartz 1991). This research quickly grew into a concerned documentation of *S. invicta*'s impact on several native species within the invaded environments. Initially, this research resulted in the expected feedback that *S. invicta* was depleting arthropod populations and heavily altering its surrounding landscape. However, over seventy years of study following their arrival in the U.S., the later results of studies claim that there is a non-existent, or even moderately positive correlation between arthropods and the presence of *S. invicta* (Morrison 2002; Morrison and Porter 2003). The previously mentioned studies (Morrison 2002; Morrison and Porter 2003) that claim this trend reversal were conducted in central Texas at Brackenridge Field Laboratory, the same site at which I am conducting my experiment.

My experiment, a collaboration with Arielle Lutfak, is a contemporary response to the series of previous studies done on *S. invicta* and the way this invasive ant has affected its surroundings. This paper discusses my own experiment, in which I am drawing on and replicating previous experiments (conducted by Morrison 2002 and Timothy 2002) in order to answer the question: What is the effect of *S. invicta* on biodiversity (species richness, species evenness, and abundance) of non-ant, ground-dwelling arthropods at Brackenridge Field Laboratory? Through continuing this decades old research topic, this new research of the *S. invicta* impact contributes to the general knowledge of how *S. invicta* is active in current environments, and how it affects an environment after several decades of exposure. The new research will aid in the understanding of environmental patterns surrounding *S. invicta,* and the prediction of environmental trends caused by this ant. The research will also investigate the latter discovered (by Morrison in 2002) reversal of the trend of the *S. invicta*'s impact and question whether this trend reversal is still apparent or consistent throughout the BFL site. Lastly, my current research can point to potential discoveries concerning why the *S. invicta* impact may have decreased or reversed within BFL.

Methods and Materials

For this experiment, data indicating (1.) the presence or absence of *S. invicta* in 10 separate plots of land and (2.) the species abundance, species richness, and species evenness of ground-dwelling arthropods in these same plots was collected through the use of bait traps and pitfall traps, respectively (as in Morrison 2002). This population data was then analyzed to determine what effect, if any, the presence of *S. invicta* had on the abundance, richness and evenness of arthropod populations. In total, this study requires an account of three separate populations: *Solenopsis invicta,* ground-dwelling arthropods sharing a habitat with *S. invicta,* and ground-dwelling arthropods in habitats lacking *S. invicta.* The first step in this process was to collect the data for the presence of *S. invicta* using bait traps. After the presence or absence of this first population was confirmed, the next step was to collect samples of ground-dwelling arthropods via pitfall traps. The correlation between *S. invicta* and arthropod species abundance, richness, and evenness was then determined through methods of analysis.

During the experiment, *S. invicta* was the first species to be located and trapped. Ten total sites, varying in size from 10m X 15m to 10m X 10m were sought out amongst several different ecosystems at BFL. The minor size variance between plots can be attributed to limited accessible ground space in certain plots. These sites were marked with flags placed at the corners and along the edges. Additionally, these sites were assigned numbers and named according to site-specific geographical features and map zones within BFL. For sampling purposes, it is ideal that 5 of 10 sites contain *S. invicta*, and the other 5 of 10 sites do not contain *S. invicta*. In order to influence the chance of finding *S. invicta* in a given plot of land, the plots were placed in habitats where the presence of *S. invicta* could be predicted. When the goal was to set a plot which had a higher likelihood of housing populations of *S. invicta,* the ideal place for a plot was by a trail, a garden or a meadow- ultimately places with disturbed soil. During this process, it was also applicable to seek out potential active nests as an indicator of *S. invicta's* presence. When a plot was placed with the aim of avoiding *S. invicta,* the location was in proximity to a body of water or in a forested area with a higher amount of canopy cover and leaf litter.

Bait traps were used in the plots to indicate the presence of *S. invicta*. Each site was tested for the specific ant with seven bait traps. These bait traps were laid out evenly throughout the site (1.) for purposes of randomness in results and (2.) to determine if a given area of the plot contained a higher concentration of ants. In total, there were 70 bait traps constructed, 10 for each plot, each using 50 mL falcon tubes, granulated sugar, and hotdogs. For each trap, ¼ oz. of a hot dog was finely diced and placed in a falcon tube. The diced hot dog was coated with a teaspoon of sugar as an added measure to attract the *S. invicta*. The tubes were then randomly dispersed five meters apart throughout the sectioned off plots. Their location was marked with a flag and recorded on a handheld GPS so that the collection of the traps would be timely, allowing for more consistent results between plots. The bait traps were then left out for approximately 5 hours before collection. All of the bait traps were set out and collected on the same day to avoid weather becoming a potential confounding variable.

After the collection of bait traps, and the determination of the presence or absence of *S. invicta* in each plot, the next step was to collect data on the biodiversity of ground-dwelling arthropods within said plots via pitfall trapping. The pitfall traps were constructed with red, plastic 9 oz. disposable cups and propylene glycol. Each trap consisted of one plastic cup containing 2 oz. of propylene glycol added to the bottom of the cup to serve as a preservative. Bait traps results showed that 3 plots did not have *S. invicta* and 7 plots did have *S. invicta,* and these results were accounted for in the pitfall trapping process. There were a total of 70 pitfall traps, with 7 traps set out at each of the 10 plots. Similar to the bait traps, the pitfall traps were also evenly dispersed throughout the plot and set up five meters apart. To place the pitfall traps in the plot, they had to be placed in a hole close to the size of the cup, where the top of the cup was flush with the surface of the dirt. These traps were also marked with flags and GPS via Google Earth for location purposes. Once distributed throughout the plots, the pitfall traps were left out for 1-2 days before collection.

After the collection and identification of arthropods from the pitfall traps, each arthropod was identified to order. Then species richness, abundance, and evenness from the collected samples was compared between plots to calculate the relative species diversity between plots containing *S. invicta* and plots lacking *S. invicta*.

Results

A sample of the arthropod population, demonstrating species abundance and richness in each plot, was collected from the pitfall traps. The results below show that the means concerning arthropod species diversity, as calculated from species richness, evenness, and abundance, of the samples (using the Shannon-Weaver diversity index) in plots containing *S. invicta* and plots lacking *S. invicta* are 0.532294905 and 0.512216632, respectively (table 1). The difference between these means is approximately 0.02, which is greater than 0, the hypothesized mean difference. In the table below, the means for *S. invicta* and *no S. invicta* reflect species diversity of the arthropods collected in the presence of *S. invicta* and of absence of *S. invicta.* I used t-tests to determine whether the P value was greater or lesser than alpha. In the one-tailed and two-tailed t tests calculated from the collected samples, P = 0.476187354 and P = 0.952374708, respectively. The results for both the one-tail and two-tail tests show that P > .05, which is alpha for the relevant value of critical t.

Table 1: Chart containing the statistical results of the pitfall trap findings. These statistics measure the current effect of *S. invicta* on its surrounding arthropod community. In this case the means of the two scenarios, *S. invicta* and *no S. invicta,* are compared to derive the results.

t-Test: Two-Sample Assuming Unequal Variances		
	S. invicta	*No S. invicta*
Mean	0.532294905	0.512216632
Variance	0.035725741	0.239206208
Observations	4	3
Hypothesized Mean Difference	0	
df	2	
t Stat	0.067428847	
P(T<=t) one-tail	0.476187354	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.952374708	
t Critical two-tail	4.30265273	

The correlation between *S. invicta* and arthropod diversity is also modeled by the graph shown below in fig. 1. This graph is a visual representation of the difference between the means of arthropod diversity in plots containing and lacking *S. invicta*. The graph also contains standard error for the diversity findings, which for plots containing *S. invicta* is approximately 0.095, and about 0.283 for plots without *S. invicta*.

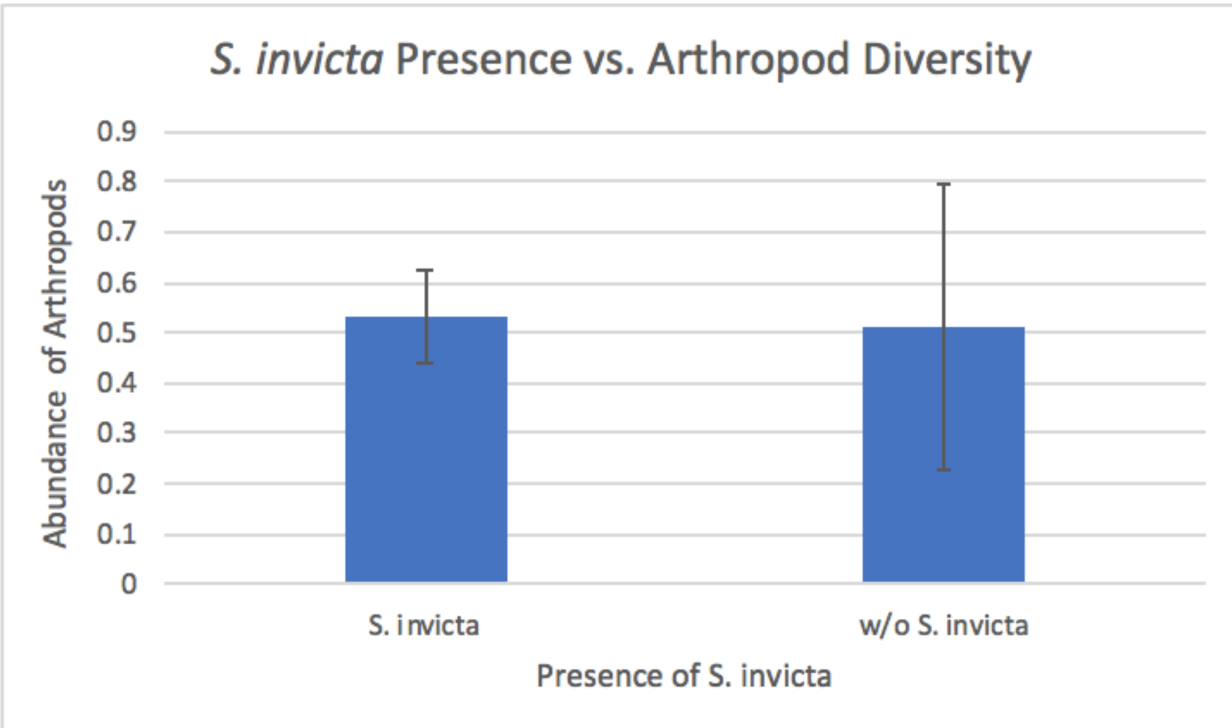

Fig. 1: In this graph, the mean of arthropod diversity in plots with *S. invicta* is compared to the mean for plots without *S. invicta.* The grey line in the center of each bar shows the standard error calculated for the results found in the two types of plots.

Amongst the arthropods gathered in the pitfall traps, the most commonly found species was the *Coleoptera* species, returning at approximately 90% (26 counted) in the *S. invicta* plots and 81% (21 counted) in the *no S. invicta* plots (table 2). *Coleoptera,* the most popularly trapped arthropod in the experiment, are small, glossy insects with black bodies and red-orange legs. These insects averaged about ¾" in length. Three traps containing flies (about 1 cm) represented the *Diptera* species. Three crickets (*Orthoptera*) about 2" in length were found in plots with and without *S. invicta,* one Pholcidae (*Opiliones*) totaling 3" in length, and one moth (*Lepidoptera*) about 2" in length were found in the traps. The species *Opiliones* and *Lepidoptera* did not appear at all in plots containing *S. invicta.*

Table 2: This table shows the percentage of the different arthropod species collected in the plots in comparison to the total number of arthropods (*S. invicta* and *W/O S. invicta* plots have separate totals). *Hypothesis and findings only account for non-ant, ground-dwelling species of arthropods.

Arthropod Species	*S. invicta*	w/o *S. invicta*
Coleoptera	0.8965517	0.807692308
Diptera	0.0344828	0.038461538
Orthoptera	0.0344828	0.076923077
Opiliones	0	0.038461538
Lepidoptera	0	0.038461538

Discussion

Since the value of P was statistically insignificant, the experimental results fail to reject the null hypothesis, which predicted that there would be no significant difference in the species diversity of ground-dwelling, non-ant arthropod populations between sites containing and lacking the invasive species *Solenopsis invicta*. Between the three plots lacking the specific ant and the four plots containing this ant, there were similar sample sizes of ground-dwelling arthropod found, and nearly similar levels of species abundance reported. These findings, supported by the results of one-tailed and two-tailed t-tests, conclude that the nature of arthropod species diversity currently experiences very few, if any, effects of the invasive *S. invicta.*

Despite these experimental results and their correspondence with my expectations based on former topical research by other scientists, there are unintended and external factors which could have affected the outcome of my results (Morrison 2002). This experiment was conducted in the field and therefore affected by some variables outside of my control. The temperature during the latter half of the bait trapping and pitfall trapping process was often in the range of 30 to 50 degrees Fahrenheit, as a week-long cold front had begun. Additionally, this period of weather also harbored several rain showers. This cold, wet, and overcast weather (different from usual Texas weather) may have affected both arthropod and ant activity, causing the traps to collect unusual, likely lower, sample sizes (Davidson 2002). A second factor which had the potential to affect the collected sample sizes was the varying times for which the bait and pitfall traps were left out. The usual protocol for bait and pitfall traps is to set them out as the same time, or under similar conditions, and to leave each of them out for similar amounts of time (Work *et al.* 2002). Whereas some bait traps in this experiment were left out for 4 hours, others were left out for up to 7 hours. Similarly, some pitfall traps were left out for 38 hours, while others were left out for up to 72 hours. This variation may have compromised the collected sample sizes in both cases, affecting the results.

Due to the limited time frame of this experiment, the sample size was also limited to 7 total plots (for arthropods) and 7 traps per plot. In addition, only one round of the experiment was conducted and there were no repetitions. With a larger sample size and more repetitions, the samples may have led to different or potentially more accurate results. Lastly, a variable that may have affected the outcome of both methods of sampling was the habitats in which the plots were placed. I took into consideration the preferred habitat of *S. invicta* when searching for places to lay plots, and this resulted in a bias of the placement of plots, as some were meant to contain the ants while others were not (Taber 2000). This means that many plots containing *S. invicta* were in open spaces with disturbed ground, while plots without *S. invicta* were in denser areas with more canopy cover. While open plots attract the ants, they may deter arthropods, and vice versa, giving plot location the potential to affect the experimental results.

At the beginning of the experiment, I had predicted the accuracy of the null hypothesis due to research I had done on previous findings in the area (by Morrison 2002 and Morrison and Porter 2003) which concluded that arthropod species diversity in areas containing *S. invicta* had rebounded to pre-invasion levels. The information from Morrison and Porter's research, as well as my recent research, is telling of an important trend concerning areas which have come into contact with this invasive species. Whereas early experimental results from 1990 at Brackenridge Field Laboratory showed as much as a 40% decline in species richness of arthropods in response to *S. invicta* presence, later research in 2002 showed a reversal in this trend. As a continuation, my research, 29 years after the former experiment and 17 years after the latter, supports Morrison's conclusions of a trend reversal and the rebound of the arthropod species diversity despite on-going *S. invicta* presence in the area. This progression of repeated experiments and their successive conclusions could lead to broader understandings and implications about the process of native arthropod species' recovery in the face of *S. invicta* impact.

Similar trends of arthropod diversity rebound have been found at other affected locations in the United States as well, indicating that this trend of native species' recovery from *S. invicta* may be widespread (Epperson and Allen 2010). However, due to the unique conditions (in terms of different flora and fauna, weather and climate, etc.) of the different sites across the Southern United States impacted by this invasive ant, it should not be assumed that other cases of arthropod rebound will happen at the same pace, if at all (Allen and Epperson 2004). This is where further research on this same topic could be applied in other areas affected by *S. invicta* invasion.

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