Preferential mannerisms in invasive ant species in varied levels of urbanization at Texas A&M University

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Abstract: This study was intended to provide information on how environmental conditions affected food preferences in different environments. It was hypothesized that a difference between food preference would arise between the urban and rural environments. Eugenol based mouse traps were placed at three different locations around Texas A&M University. At each location protein, starch, and glucose were set in traps to determine preferences. The traps were placed during the month of November for four trials, which each lasted four days. Fire ants (*Solenopsis invicta*) were collected and identified at every location. Tawny crazy ants (*Nylanderia fulva*) were collected and identified during the second trial. It was indicated that there was a stronger correlation between urbanization of a location, than preference in food source. There was also evidence to show a potential correlation between *S. invicta* and a reliance on humans for a protein source. In the absence of students in the dorms during Thanksgiving break, there was an influx in specimens collected. Red Imported Fire Ants were collected more frequently than Tawny Crazy ants. The lack of native Texas species of ants indicated a dominance of invasive ant species at the Texas A&M University campus. In these invasive ant populations, it was determined that there were preferential differences in urbanization levels on the Texas A&M University campus. Preference between foods was not significant, and a preference for one location at Legett Hall was determined to be near-significant with a p-value of 0.06. Urbanization increased specimen collection, and a reliance on humans for protein in these areas is plausible.

Key words: *Ants, Environment, Food, Preference, Invasive, Urbanization*

Through the deployment of three different bait traps in three different environments, the question of whether different environmental conditions affected food preferences was tested. The trials performed in this experiment served to differentiate preferences in each environment, but differences between species were not relevant due to low specimen count. It was hypothesized that different environments would cause differences in nutritional needs and deficiencies amongst ants. Data was compiled on the Texas A&M campus in College Station, Texas. There was a high prevalence of red imported fire ants (*Solenopsis invicta*). The range for this species spans a large portion of the United States, and in particular in the Southern United States, has caused medical issues for children and adults in the area (Nguyen and Napoli 2005). In addition to *S. invicta*, the Tawny crazy ant (*Nylanderia fulva*). Both species are indigenous to South America, and are a prevalent invasive species found in Texas, specifically the Harris and Brazos county areas (J. C. 2014). The increased prevalence of these invasive species has decreased the native population of ants in Texas and has caused severe issues with natural ecosystems that rely on ants. This is often seen because invasive species do not have natural predators in their invaded ecosystems.

Humans experience negative effects from these invasive species in aspects of psychological and medical ailments. In severe cases, the sting of these ants can cause anaphylaxis, which is a severe allergic response that requires immediate medical attention (Rhoades et al. 1989). In addition ants can damage food stores. Some species such as *Camponotus sericeiventris,* also known as carpenter ants, are capable of chewing through wood for nutritive or nesting purposes (Yamamoto and Del-Claro 2008). This experiment was designed to investigate the more common problem of ant infestation in urban settings. Different possible attractants of different ant species on the Texas A&M campus in College Station, Texas were used to examine these factors. Results from this study can be used to educate college students and the general populace on how to understand the psychological effects of ant infestations, as well as the foraging behavior of *S. invicta* in differing levels of urbanization.

The composition of food sources does have an effect on ant preference because different species of ants require different dietary food sources, whether solid, liquid, protein based, or carbohydrate based (Tan et al. 2012). In several studies, it has been found that there is a strong correlation between amino acids and single fatty acids acting as chemical triggers in ants in relation to food transport to the colony (Reifenrath et al. 2012). These chemicals in high protein food sources are favorable for achieving the nutritional requirements of the ants and allow for the ant's best chance of survival. In sterile environments with different glucose tests, the effects of sugar content preference can also be seen (Nyamukondiwa and Addison 2014). Many species of ants will tend and feed on the honeydew of aphids (Silverman and Roulston 2001). This is an energy-rich source that provides many benefits for the ants. Ants can also feed on extrafloral nectaries from plants for their high sugar content (Del-Claro and Oliveira 1993). The worker ants prefer a food source that is easily obtained and that can be easily transported back to the colony. They are also looking for a food source that will quickly nurioush them in a time of need. The species *Iridomyrmex humilis* prefered a watered-down sugary substance when given the choice between different glucose options (Bakeret al. 1985). A watery sugar substance was made in return to validate this research and determine its effects on the native populations in Texas. A 5:1 ratio of sugar water was created to keep the sugar content high, while preserving the low viscosity of the bait.

In shrubland, forest, and grassland areas, there has not been a correlation between species richness (Retna and Cerda 2000). The abundance of dominant groups are typically in comparable proportions, but the competitive nature of different species for a food source can be a hindrance for learning how different ants choose which food they are going to eat. Further hindrance comes from the presence of invasive species that outcompete the native populations of ants. When ants outcompeted other species for a territory, they allowed for the food source provided to be dominated by that species. Competition between trials corresponding to food sources was completed during the preliminary trial completed for each location of the experiment and the different collective responses were consistent with previous experiments (Nicolis and Deneubourg 1999). The inferior native species was not accurately represented in the collections data and caused variances from the intended results. The current dominant species is *S. invicta*, which are an invasive species that typically outcompete the native species in Texas and the rest of the southern United States (LeBrun, Abbott, and Gilbert 2013). *S. invicta* can take over other species territories by dominating the resources currently available to the native ant species. They have very few predators in the United States, but some common competitors and other ant species in the area that were likely to be encountered included: *Dorymyrmex spp., Pheidole spp., Monomorium pharaonis, Camponotus sp., Nylanderia fulva,* and *Paratrechina longicornis* (Lennon).

Based on this research, the food preferences of different species of ants and the effect of environment on those preferences was analyzed. Traps were chosen to avoid preferential species selection. Baited ant traps tend to incorporate pheromones that attract only certain species, so a captivation trap with a eugenol base was instead used. Eugenol only attracts various species of bees and has a negligible effect on the olfactory system of ants in their ability to detect the different baits placed on each of the traps. (Tan and Nishida 2012).

Materials and Methods

Traps were designed to collect ants that came to each of the three food sources provided. The food sources used were protein based (hot dog), sugar based (sugar water), and starch based (potato). The traps used to capture the ants were mouse sized glue traps with eugenol for enhanced stickiness by Tomcat® Glue Traps (Marysville, Ohio). Although these traps emphasize a use for mice, they are also sticky enough to trap cockroaches, spiders, scorpions, and ants. These traps were also pesticide free, which allowed for the reduction of confounding from the ants being attracted to a different bait than the ones intended for this experiment. Specifically, ant traps were avoided for the reason that they contained premade baits designed to lure ants into them. In addition, ant baits are often species specific, and could not prove the validity of the food sources administered in this experiment. The potato bait used was Russet potatoes, and it was a H-E-B (San Antonio, Texas) brand. One potato was purchased and the same potato was used for all the traps. Hill Country Fare (San Antonio, Texas) sugar was used to create the sugar water mixture and it was in a 5:1 ratio of sugar to water. Bar S® Signature Smokehouse Franks made with pork and chicken were used for the protein source (Phoenix, Arizona). These traps were placed in three different areas on the campus of Texas A&M University. Traps with each of these food sources were placed by Legett Hall, in between Appelt Hall and the Commons, and in Research Park near white creek on west campus. When measuring out the baits, 10.23 grams (+/- 0.1 grams) of each food source were measured out. A kitchen gram scale provided by the Department of Entomology was used to obtain these weights. These baits were refrigerated until use and administered on to the traps directly before deployment. Traps were placed in each of these locations completely exposed to the environment to allow for the greatest travel of pheromones from the baits. Figure 1 below shows the set up for the ant traps. All traps were placed within a 15 cm distance from one another.

Figure 1:
These traps were designed identically for each of the locations and this figure shows a guideline for how each of the traps were constructed. The leftmost trap contained the sugar water bait, the top right trap contained the potato bait, and the bottom trap contained the hot dog bait.

A preliminary trial was run in each of these locations and the baits were monitored daily for the span of four days. By the fourth day, most of the bait had been eaten or had become spoiled, so a deployment length of 4 days for the traps was chosen. Quantitative data from the preliminary trial run was not collected. Traps were then deployed during the month of November in four-day increments for a total of 4 trials. During each of these trials, the traps were placed in each of the locations between the hours of 10:00 and 11:00 am by all parties administering the traps. These traps were placed within one meter of one another. The traps were placed with signs declaring the progress of this experiment to avoid vandalization attempts and the destruction of the trap sites. The traps were monitored daily and on the fourth day, were collected at the same time the new traps were deployed, between the hours of 10:00 and 11:00 am. With these preventative measures in place, the vandalization of traps at the Legett Hall location still occurred during one of the trials. The traps at this location had to be moved during the second trial to a location further away from the foot path of residents of Legett Hall. New traps were placed before the new deployment and retrieval period had arisen. New traps and all further traps in this location were moved fifty meters away towards the Sbisa dining hall to prevent further tampering. No further vandalization occured after the relocation of these traps for the remainder of the experiment.

Trapped ants were collected and identified by the primary author using the appropriate dichotomous keys from the Department of Entomology at Texas A&M University (Cook, O'Keefe, and Drees 2014). Further steps to prove identification of specimens were taken by contacting members of the pesticide and pest control unit through Facilities Services. Specimen identification was validated by these employees. Each sticky trap was kept separate and labelled with the trial information of trap site, date, and food source. The location to which they were obtained was also labeled and the traps were frozen to prevent desiccation of the specimen until identification. In the events that the traps were moved, ants from a two-meter radius of the trap were collected to compile data on the specimen present at each trapping site. The two species of ants collected and identified were Red imported fire ants (*Solenopsis invicta*) and Tawny crazy ants (*Nylanderia fulva*). The majority of the specimens collected were *S. invincta.*

Data was compiled using Excel sheets to categorize which site each specimen came from and which bait it had been captured on. These spreadsheets served to compare the bait preferences between protein, starch, and sugar and how it differed in each environment. The data was also used to determine if there was a correlation between the species of ant and bait preference. Further analysis of this data and the data compiled is presented in the results and discussion section. A one variable ANOVA test was performed, as well as regression analysis to analyze p-values of urbanization and specimens collected for *S. invicta* using an α-value of 0.05.

Results

Location:	Legett Hall			Research Park			Between Appelt and The Commons		
Bait Type:	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato
Trial 1:	17	3	6	4	39	4	1	7	1
Trial 2:	23	4	4	0	0	3	0	11	0
Trial 3:	15	0	8	8	15	5	1	3	0
Trial 4:	100	2	9	4	19	4	1	5	2
Total:	155	9	27	16	73	16	3	26	3

Table 1. Raw Data counts of collected species in all locations spanning four trials.

Figure 2, also shown below, depicts a sample of *Solenopsis invicta* that were collected. Figure 3 shows the differences between *Solenopsis invicta* and *Nylanderia fulva.* The morphological differences between these species of ant consist of color differences, with *Solenopsis invicta* having a larger amount of red present cranially. Structurally, the two can be differentiated by petiole number, as *N.* fulva has one petiole and *S. invicta* has two, the presence of a stinger in *S.* invicta, and size differences, with *Solenopsis invicta* being larger in size. Identification for these specimens was achieved by using the Texas Agrilife Extension Texas Pest Ant Identification Key (Cook, O'Keefe, and Drees 2014).

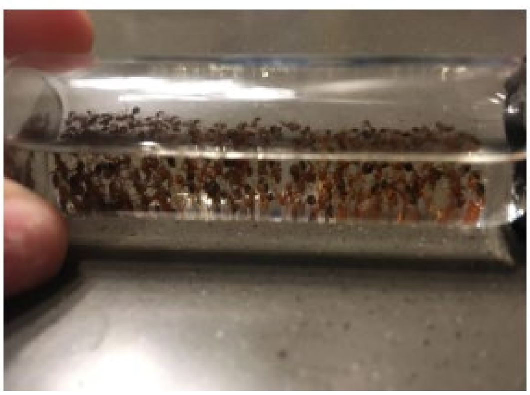


Figure 2:
A vial sample of Red imported fire ants (*Solenopsis invicta*) collected from the Legett Hall location.

Figure 3: Red imported fire ants (*Solenopsis invicta*) Is pictured on the right and Tawny crazy ants (*Nylanderia fulva*) is pictured on the left.

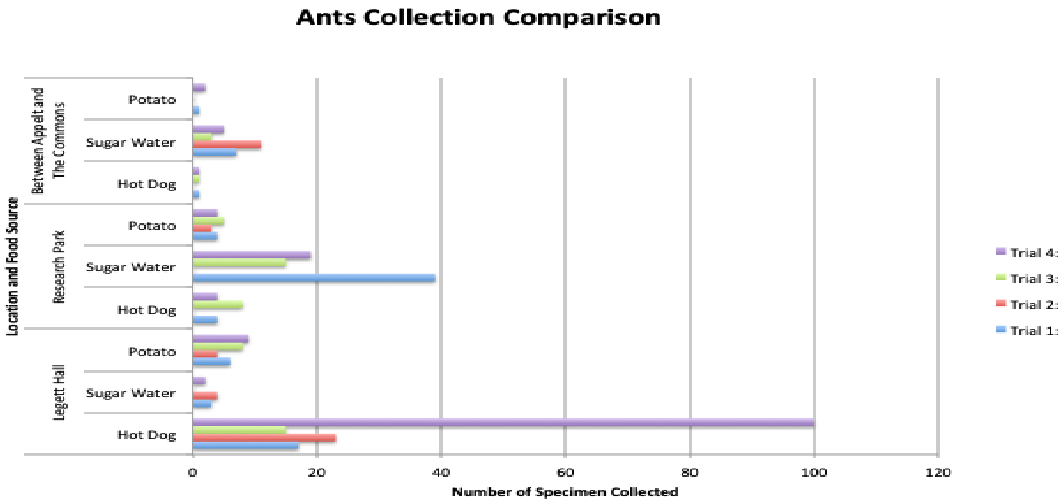


Fig. 4. The ratio of ants collected in comparison to the different environments and the correspondence to each of the food sources.

The location between Appelt and The Commons (AC) and the Research Park (RP) location yielded results that showed an increased number of specimens with a preference in the sugar water (S) source, while the Legett Hall (LH) location yielded results that indicated a higher count number of specimens toward the hot dog (H) as the favored food source. The potato (P) source did not appear to be preferential based on counts data alone. Figure 5 further shows this correlation between food sources.

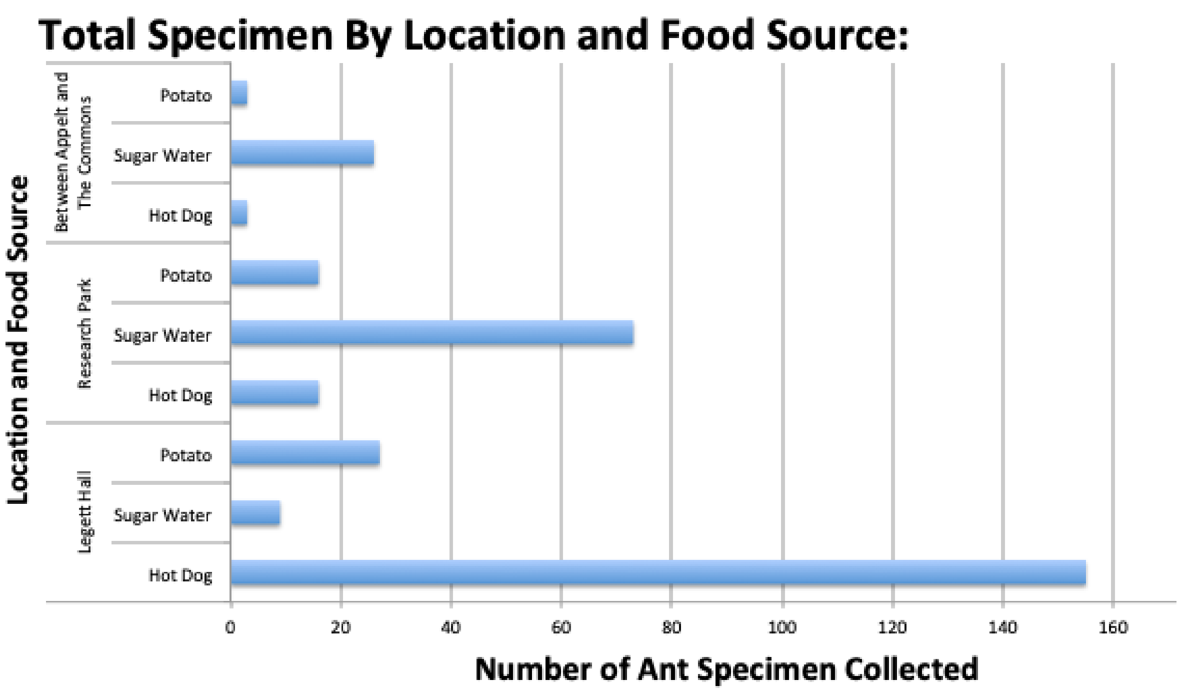
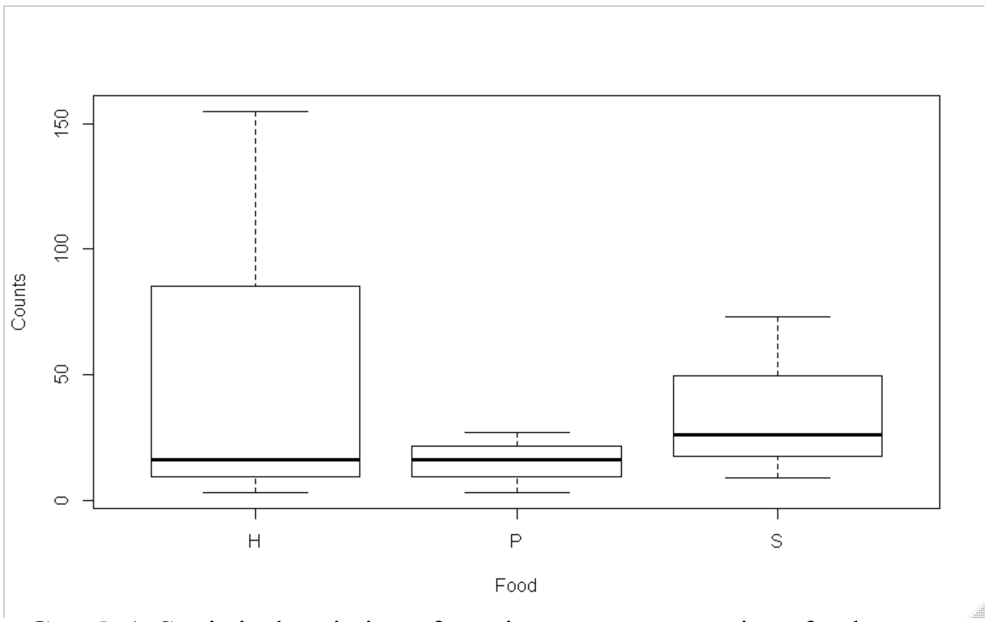
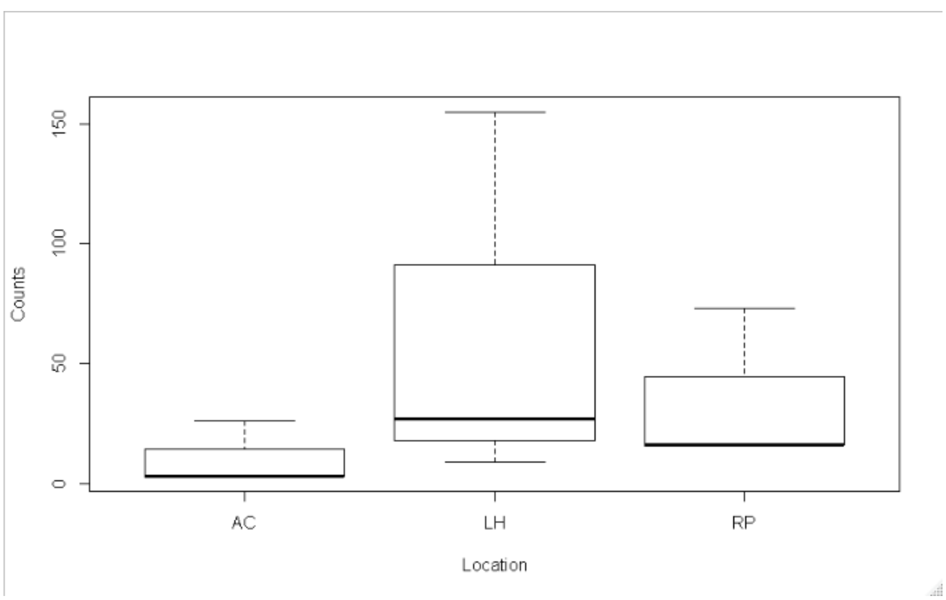


Fig. 5. Specimen count in each location with regards to food sources.

A one variable ANOVA test was used to assess if there was a statistically significant difference in counts and preference in location and food types. Graph 1 below shows that the means between food preference and count were all similarly driven and that there was not likely a preference in the food source. Graph 2 showed that the distribution of means between locations shows that it is more likely that there was a correlation between urbanization and counts rather than the bait provided. The Legett Hall location counts data yielded a p-value of 0.0664, which was near significant to show preference in that location versus the others. The other p-values gathered were not significant or near significant to show a relationship between food preference of any of the food sources.

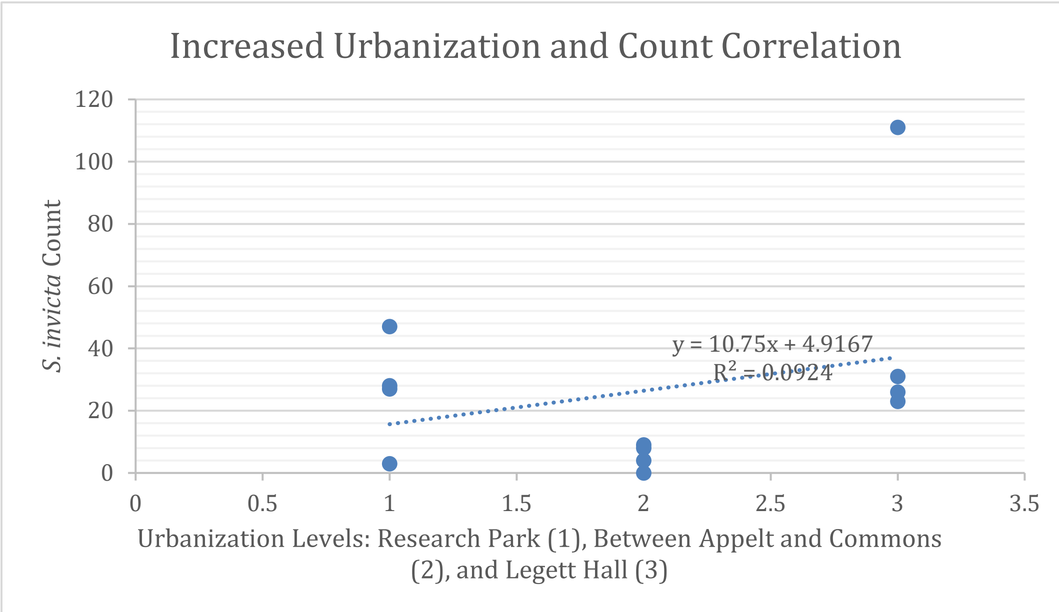


Graph 1. Statistical variation of specimen counts at various food sources.



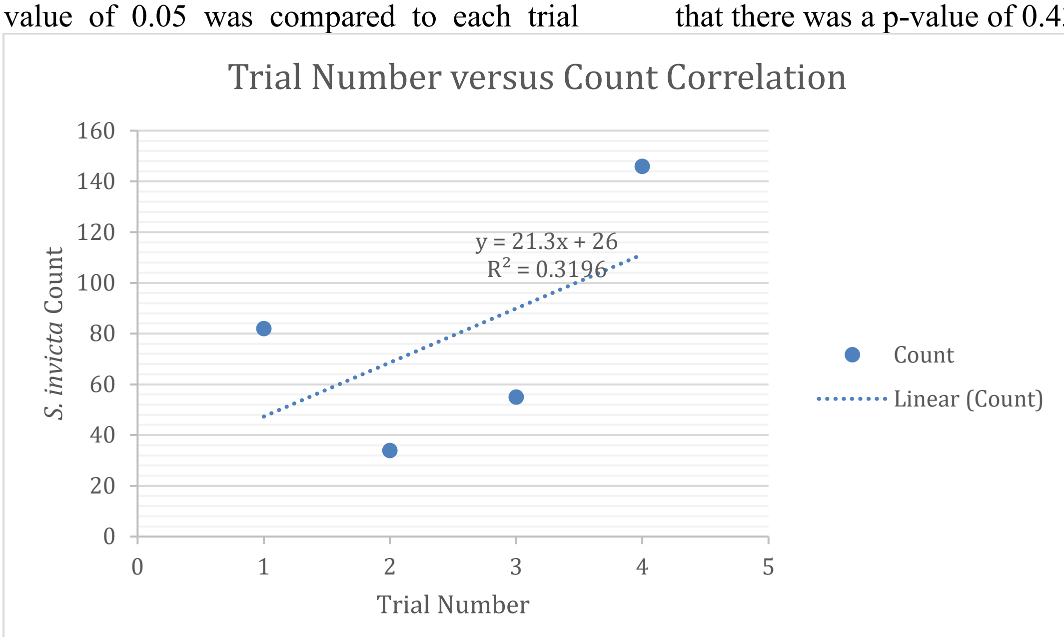
Graph 2. Statistical variation of collected specimens at selected locations.

Linearization of the data and further analysis showed that there was a moderate correlation between urbanization and the number of specimens collected at each location. This can also be seen in Graph 3 below.



Graph 3. Linear correlation between urbanization and specimen count

Only specimens of the species *S. invicta* were used using an α-value of 0.05. The p-value for the above data was 0.3367. After looking at this data, total specimen count of specimens collected for *S. invicta* using an α-value of 0.05 was compared to each trial rather than location. This linear regression showed a slightly moderate correlation between trial number and the total number of *S. invicta* collected. Graph 4 displays a summary of this data, and it was determined that there was a p-value of 0.437.



Graph 4. Summary of trials and count correlation

Figure 5 depicts the deployment site of the traps and Table 2 depicts the data collected from this site. There were no *Nylanderia fulva* collected from this site, and the traps were specifically placed near *Solenopsis invicta* mounds. During the fourth trial, the ants in the closest mount actually carried the trap baited with hot dogs to their hill and by the fourth day, had begun to bring the trap into their mound. The trap was covered in pebbles and dirt from the ants moving and bringing the trap into their hill, so a specific quantity to how many ants were attracted to the hot dog bait could not be accurately obtained. The largest attraction to the hot dog food source, based on counts data, was found at this location.

Fig. 6. Legett Hall Location

Location:	**Legett Hall**					
	Solenopsis invicta			***Nylanderia fulva***		
Bait Type:	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato
Trial 1:	17	3	6	0	0	0
Trial 2:	23	4	4	0	0	0
Trial 3:	15	0	8	0	0	0
Trial 4:	100+	2	9	0	0	0

Table 2. Specimen counts of food preference during each trial at Legett Hall

Figure 7 depicts the location where the traps were deployed for the Research Park. There were also no *Nylanderia fulva* collected from this location.

Fig. 7. Research Park Location

Location:	Research Park					
	Solenopsis invicta			***Nylanderia fulva***		
Bait Type:	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato
Trial 1:	4	39	4	0	0	0
Trial 2:	indeterminat	indeterminat	3	0	0	0
Trial 3:	8	15	5	0	0	0
Trial 4:	4	19	4	0	0	0

Table 3. Specimen counts of food preference during each trial at Research Park

The location between Appelt and The Commons collected the least amount of ants, but the preferred food source was congruent to that of Research Park, being that the sugar water solution was the preferred food source. This was the only site where *Nylanderia fulva* was collected. This location collected 11 specimens of *Nylanderia fulva*, as seen in Table 4. The traps in this location retained the least amount of consistency and the location for the traps was altered a total of 3 times. The trap location was altered due to the low collection of ant specimens and was enacted in an attempt to gain more statistically significant data for the location. Figure 7 shows the areas where the traps were set and relocated and the location for each trial is indicated below. The change in location was a variable that did not hinder the collection of data in this area because there was a low collection of ant specimen in the area to begin. Regular pesticide and insecticide spraying in this area may be an indicator to the low collection from this location.

Trials 1 and 4 are indicated by the blue circle.
Trial 2 is indicated by the blue square.
Trial 3 is indicated by the blue triangle.

Fig. 8. Appelt and The Commons Locations

Discussion

From the three locations, most ants were trapped at Legett Hall, which is located near Texas A&M University's Sbisa food court, and was also determined to be the most urbanized area. The Legett Hall location was unique in that the ants were most attracted to the hot dog protein source during the last trial. Because Legett Hall is in a more urban area than Research Park, Appelt and The Commons, the ants may have preferred the protein source, because protein isn't as readily available in a more urbanized setting as it would be in a more rural area where dead animals and bugs are more prevalent. The difference in this data was hypothesized to be due to Thanksgiving break on campus at Texas A&M University during the final trial. The Legett Hall location was the most urbanized of these locations, and the first three trials yielded an average of 27 specimens collected, whereas in the last trial, there were 111 specimens collected, with 100 of those being collected on the trap with the protein source. The conclusion drawn from this was that invasive species rely on human provided protein sources for survival in an urbanized setting. The influx in numbers during this campus break was not seen at the other locations, and the absence of students in the dorms near this trap site may be responsible for this deviation in data collected. *Solenopsis invicta* at Research Park preferred the sugar water solution over the protein and starch sources according to the counts data. As stated above, this may be due to the fact that ants at Research Park have an increased supply of protein available, such as dead insects and mice. This location had the highest number of ants attracted to the sugar source as seen in Table 3. For the second trial, the number of ants trapped for the protein and sugar sources were indeterminate due to storms that passed during the time. The traps were covered with grass and leaves, allowing ants to escape without coming into direct contact with the sticky traps. This also made it impossible to properly identify any ant specimens on the traps due to the high vegetation content that was stuck to the traps.

Location:	Between Appelt and The Commons					
	Solenopsis invicta			***Nylanderia fulva***		
Bait Type:	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato
Trial 1:	1	7	1	0	0	0
Trial 2:	0	0	0	0	11	0
Trial 3:	1	3	0	0	0	0
Trial 4:	1	5	2	0	0	0

Table 4. Specimen counts of food preference during each trial at Appelt and The Commons

Based on the data collected, it was determined that there were environmental differences in the attraction of either the Red imported fire ants (*Solenopsis invicta*) or the Tawny crazy ant (*Nylanderia fulva*) in regards to the protein and glucose baits. When placed in Research Park, a rural area, ants preferred the sugar source as seen in Table 5. There was likely an abundance of protein in this environment due to the lack of daily cleaning and sanitizing of this location, and thus the sugar source was favored. When the environment was switched to the urban area, close to Sbisa, the ants preferred the protein source. Protein sources, like dead animals, aren't as readily available in more urban locations due to sanitation laws and the daily cleanup on campus at Texas A&M. The location on the southside of The Commons was partially rural and partially urbanized.

Location:	Legett Hall			Research Park			Between Appelt and The Commons		
Bait Type:	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato	Hot Dog	Sugar Water	Potato
Total:	155	9	27	16	73	16	3	26	3

Table 5. Total specimen count for food preferences at each location

Ants are most active in the summer and months with warmer weather (Stinger 2017). This experiment was conducted in November, and with cold weather during trapping, there was a smaller quantity of ants collected than desired. As depicted in Table 6, only 328 ant specimens were collected during the duration of this experiment. Ants tend to disappear in the months of winter because their metabolism is slowed from the decrease in temperature and they live off the stored fat in their bodies and the food stored in their hills during cold weather (Stinger 2017). Ants burrow deep into the ground in the cold weather, and this hindered the collection of other specimens of ants during this experiment.

Total Species Collected:	
Solenopsis invicta	***Nylanderia fulva***
317	11

Table 6. Total species collected during all trials and at all locations

The complication of human interference arose in this experiment. The traps being left for four days of observation were tampered with by walking pedestrians and forging animals such as squirrels and beetles, this was especially seen in the second and third trials in the Legett Hall location. In addition, environmental hinderance also occurred, leaves falling into the traps and the wind or rain moving traps from the original location was a recurring problem. Leaves falling onto the traps either covered the ants and due to the stickiness of the trap they were unable to be uncovered or they provided the ants something to walk on and hindered the trapping of specimen. This problem was seen in the second trial in the Research Park location and in the last trial of the Legett Hall location where the fire ants had brought the baited hot dog trap into their ant hill. Table 7 shows the total number of species collected in this experiment and the plus (+) sign is indicative of these environmental hindrances.

Location:	Legett Hall	Research Park	Between Appelt and The Commons
Total Species Collected:			
Trial 1:	26	47	9
Trial 2:	31	3+	11
Trial 3:	23	28	4
Trial 4	111+	27	8

Table 7. The total number of species collected in this experiment; plus (+) sign is indicative of environmental hindrances.

There were no Texas native species of ants collected in this experiment and the large prevalence of fire ants can be indicative of an infestation and take over of these invasive species on the Texas A&M College Station campus. There is an exponential trend to the increase of fire ants in Texas, and this data further supports those findings (Mokkarala 2002). Environmental constraints to nutrient sources were also discovered with the variances for preferred food sources in the rural versus urban settings where traps were placed, but there was overall no desire for starch as a nutrient source in these species of ants.

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