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Harvester Ant *(Pogonomyrmex sp.)* Seed Preferences and Distribution in a Suburban Setting

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HARVESTER ANT (*POGONOMYRMEX SP.*) SEED PREFERENCES

AND DISTRIBUTION IN A SUBURBAN SETTING

A Thesis

by

LILLY V. ELLIOTT

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May 2022

ABSTRACT

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Harvester ants (Pogonomyrmex sp.) are omnivorous ants native to Texas and are the main food source for the threatened Texas Horned Lizard (*Phrynosoma cornutum*). Little research has been conducted on harvester ants in the Lower Rio Grande Valley (LRGV) and their interactions with their environment. For this purpose, a variety of experiments were conducted to better understand these interactions and preferences. In CHAPTER II, suburban harvester ants were exposed commonly used cover crop seeds in the LRGV and inoculated seeds via seed depots over the course of 24 hours. We found that harvester ants do have preferences for some seeds over others and are impartial to the addition of inoculum. In CHAPTER III, spatial analysis, elevation, impervious surfaces, and soil moisture were analyzed and compared between areas with and without ant colonies. We found that ant colonies were significantly clustered together within this landscape. Within the larger landscape, elevation but not impervious surface was a significant factor in colony placement. Within the subset area soil moisture was not an important predictor. Determining the specific conditions harvester ant colonies choose to reside in could help citizens take proper measures to reduce the likelihood of colony establishment on their property.

DEDICATION

For my mother, who has consistently strived for better since I can remember, and who has never let any opportunity for growth and prosperity slip away. Who has taught me that anything is possible by leading by example. For my mother, who showed me the greatest amount of unconditional love in the smallest of actions. I hope you find peace and know I will be okay. For I had a wonderful role model.

For the late and current matriarchs in my family and the women in my life who have believed in me from the beginning. Your strength, compassion, and unrelenting support is what has led me to be in the position I am today. I am forever grateful to have learned many lessons under your guidance and only hope every step I take past this point brings you pride, for I am a product of your guidance.

For my late grandfather who was the first of many examples that familial love can exist outside of blood. For he who taught me true patience and to never take things for granted. To appreciate life for every moment its worth, for it is constantly fleeting.

For teaching me to not fear death but accept it as the outcome of living.

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CHAPTER I

INTRODUCTION

The Lower Rio Grande Valley (LRGV) is a fast-growing region on the Southern border of Texas. Running along the US-MEX border, population and trade have been growing in numbers since the 1940s leading to mass urbanization in counties closest to the border (Bounoua, Nigro, Zhang, Thome, & Lachir, 2018; Huang & Fipps, 2006; Leslie, 2016; Lombardi, Perotto-Baldivieso, & Tewes, 2020). This growth in urbanization has been at the expense of agricultural plots and leftover natural areas in the region that house a large diversity in wildlife and contribute to large migration events of birds and insects (Huang & Fipps, 2006; Leslie Jr, 2016). Prior to this population growth in the second half of the 20^{th} century, the region was being converted from natural areas to agriculture, displacing much of the native wildlife (Leslie Jr, 2016). Outside of the mammals and reptiles, insects, the typical foundation to some areas, have had to adapt to the change. One native species that's been regularly displaced is the Red Harvester ant (*Pogonomyrmex barbatus* F. Smith).

Overall, harvester ants *(Pogonomyrmex sp.)* measure up to a centimeter in length and are common inhabitants of arid to semi-arid regions of the United States (Luna, García-Chávez, & Dáttilo, 2018; MacMahon, Mull, & Crist, 2000a; Tizon, Peláez, & Elía, 2010; Vieira-Neto, Vasconcelos, & Bruna, 2016). In Texas, there are 12 harvester ant species distributed throughout the state (*P. anergismus, P. apache, P. bigbendensis, P. californicus, P. comanche, P. desertorum, P. maricopa, P. occidentalis, P. rugosus, P. texanus and the most widely distributed P. barbatus*) (Davis, 2016; Taber, 1998). Though widely spread, harvester ant colonies in Texas are constructed similarly to one another, situating themselves on flat ground with a bare disk devoid of vegetation surrounding the colony. Spotted in a variety of environments, harvester ants are able to withstand semi-extreme conditions and have an affinity for frequently disturbed areas such as roadsides, trails, etc. (Davis, 2016; Shaw, 2011; Taber, 1998).

Of the twelve species, South Texas would likely appeal to *Po. rugosus* and the more common *Po. barbatus*, these two species have been known to establish themselves in soils of higher clay contents, something commonly found in the region (R. A. J. E. E. Johnson, 2000; R. A. J. O. Johnson, 1992; MacMahon, Mull, Crist, & Systematics, 2000). With clay's ability to absorb higher amounts of moisture and retain it for a longer period, it could be presumed that colonies in semi-arid to arid areas would try to keep their colony from drying out as much as possible (R. A. J. E. E. Johnson, 2000; R. A. J. O. Johnson, 1992; MacMahon, Mull, Crist, et al., 2000). Retaining moisture in the colony is pivotal for central colony functions such as colony foundation and nuptial flights (R. A. Johnson, 2001; Taber, 1998). Though what comes as a requirement also can be detrimental if too much. Excessive moisture in the colony caused by flooding or rain can cause ants to drown. This scenario is what may serve as a deterrent for colonies to establish themselves in some areas, for example those that are regularly watered or those at a low elevation. Frequently used sprinkler systems insuburbanareas might be a reason as to why harvester ants have been observed to avoid areas of UTRGV even with the amount of space and vegetation available. Colony distributions avoid areas with a high density of sprinklers in coordination with large amounts of tree cover (which could further preserve soil moisture due to prevention of evaporation) and instead concentrate in open grassy areas that are not prone to having a large irrigation system in place (Figure 1.2). In addition to soil moisture, in hurricane-prone areas such as south Texas, residing on a higher elevated surface would be imperative in the event of a flood.

Outside of living arrangements, harvester ants are seed feeders, scavengers, and occasional predators. In lower densities these scavenger/hunting tendencies can be a beneficial force within agriculture; UTRGV students in an organic corn field (26°19'28.1"N 98°07'50.5"W/26.324477, -98.130699) have witnessed and photographed these beneficial interactions first hand (Figure 1.1) (Baraibar, Ledesma, Royo-Esnal, & Westerman, 2011). Harvester ants partake in group foraging and together form trails extending from their colony up to 60m away in high colony dense areas to food sources to sustain their underground granaries (Reed & Landolt, 2019). This movement causes displacement of seeds and loss of potential vegetation growth, actively changing the surrounding fields' species composition and density (Gentry & Stiritz, 1972; MacMahon, Mull, Crist, et al., 2000).

Harvester ants pick off their most preferred seeds first and fill their granaries with as much as they can before moving on to less preferred seed varieties, tend to highly prefer density seed patches (MacMahon, Mull, Crist, et al., 2000). In times of low diversity and their preferred seeds are unavailable, they collect these leftover varieties. However, as soon as other more desirable seed varieties begin to emerge, harvester ants will empty their seed depots and quickly begin to replace their old seeds with these newer options (MacMahon, Mull, Crist, et al., 2000). Though have little effect (around 10%) on the total amount of seeds taken from around the area annually they can take up to 100% of a preferred seed in an area (Thomas O Crist & MacMahon, 1991). As a result, they will reduce seed heterogeneity in those areas (MacMahon, Mull, Crist, et al., 2000).

As for all the material collected, it is transported to the colony for consumption and is returned to the soil via nutrient-rich fecal matter. They increase total nitrogen, increase soil pH over time, and increase aeration via tunnels allowing for better root penetration from surrounding plants. Roots of vegetation outside the disk can absorb the available nutrients in the soil and enhance plant growth. Frequent movement, breakdown, and deposition of material, allows an increase of microbial diversity due to the relocation of settled microorganisms and making space for higher diversity of microbes (Gentry & Stiritz, 1972; Kubicek & Druzhinina, 2007; MacMahon, Mull, & Crist, 2000

Harvester ants in Agriculture

With the urbanization increase in the LRGV, harvester ants have become prevalent insuburbanareas as well as agricultural plots. Given their granivorous nature, this can be especially damaging if their preferred seed of choice happens to be that belonging to an agricultural crop rather than a neighboring weed. Given their specificity for seeds, mass foraging would occur during periods of sowing and the seed will not have the opportunity to germinate before being taken off to the nearest colony. This means that area will be left bare for a whole season. Bare soil is more susceptible to soil erosion from wind, water, ice, etc. (Labrière, et al. 2015). Since bare soil is highly susceptible to erosion in comparison to other land use alternatives, preventing harvester ant interference would help preserve. Efforts to combat soil erosion during non-growing periods include the use of cover cropping. Cover cropping typically consists of a low-effort crop that can provide enough above ground biomass and below ground support via root systems during fallow periods (Meerkerk, 2008). These dual forms of support come in handy during intense rain or wind events that could displace soil and serve as a protective armor to soil from the elements.

Without cover crops, nutritious topsoil would be relocated to other areas and leave the field with less and less nutrients over time. Once it is time to re-sow for cash crops, fertilizers will need to be added to the soils can support growth, costing the farmer more money.

If harvester ants were to grow fond of a cover crop seed, they would remove as much as they can from the field before germination can secure the seed to the ground, leaving bare spots areas closest to the colony. As time goes on and the rest of the field grows, these barren areas will experience higher impact by erosion and feel more nutrition loss than their covered counterparts. Using a seed that harvesters are prone to ignore could help reduce field nutrient loss and save the farmer time and resources.

Just like cover crops help cash crops in the long run, using alternative methods of reducing harvester ant predation would be useful. Some potential preventatives used in this study is seed inoculation and seed vinegar soaks. Perks of inoculating cover crop seeds, bacteria aids with the growth of the plant. If the bacteria repel the ants, inoculation of seeds could be a potential alternative for reducing harvester ant predation while helping with crop productivity. As for vinegar, we’re anticipating its strong scent will deter harvester ants who rely on pheromone trails to travel to and from the colony.

Hardships the Colony Faces

Other than urbanization, other natural enemies of harvester ants consist of their predator: the threatened Texas Horned Lizard, *Phrynosoma cornutum*. Of insects and spiders, 65% of the threatened Texas horned lizards’ diet consists of Pogonomyrmex ants. The main reason behind Texas Horned Lizards’ decline in population is due to food and habitat loss (Davis & Parks, 2012).

Protection of this lizard requires protection of its food source as well, and with pesticides, urbanization, and competition with invasive species. Harvester ants' value is inherently higher than their peers due to the support they offer to their threatened predators. The importance of preserving harvester ants derives from their value within the native ecosystem. They provide several services to the ecosystem, allowing different plant species compositions to fluctuate in an area, creating more biodiversity (Thomas O Crist & MacMahon, 1991). They work as decomposers and help redistribute nutrients within a system (Thomas O Crist & MacMahon, 1991).

Harvester ants aren't only targeted in agricultural settings, but in suburban areas as well. Insecticides that suburban residents use also kill off harvester ants whether they are specifically targeted or not. In the fifties, insecticides used against harvester ants were in the forms of dusts and fumigants (Barnes & Nerney, 1953). Dusts like Dieldrin were banned in 1974 by the EPA due to the harm it may cause to human lives and dusts like Chlordane were also banned in 1983 except for use of removing termites until 1988 where all uses were banned. (ToxFAQs: Hazardous Substance Fact Sheets, 2015). Fumigants like Carbon Disulfide and Methyl Bromide are still in use to this day and are used for a variety of reasons in an agricultural setting. These fumigants were recommended for dealing with pesky harvester ant colonies. Another article recommended Sodium Cyanide, Paris Green, Carbon Bisulphide, and Paradichlorobenzene + Calcium Cyanide as effective insecticides against Harvester Ants (Nichol, 1931).

Decline in harvester ants in bothsuburbanand agricultural settings can directly affect the population of Texas Horned Lizards through urbanization and lack of prey due to harvesters' ongoing competition with the invasive Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren (Davis & Parks, 2012; Henke & Fair, 2019). RIFA colonies inhabiting the region thin out resources for harvester ants. The relationship between harvester ants and the RIFA in Texas would be described as a competitive one (Davis & Parks, 2012). These fire ants are opportunistic omnivores, like harvester ants, and are known to consume seeds and affect local seed assemblages through seed transport. They consume germinating seeds, fruits, other insects, and roots (Davis & Parks, 2012). RIFA are very aggressive and attack any intruders in comparison to the slower moving and larger, tamer harvester ant (Reed & Landolt, 2019). In fact, according to a study conducted by Hook and Porter, of 5 red harvester ant colonies targeted by invasive fire ants only 1 survived (Hook & Porter, 1990).

Invasive species, pesticides, predation, and urbanization all play a role in the survival of the harvester ant and in turn the ecological services they provide to the diversity in the LRGV. In this study methods of finding the foundation for alternative methods to reduce harvester ant disturbance in human spaces by passive forms that could push harvester ants into safer areas that prevent disruption of daily anthropogenic functions. First question is determining whether harvester ant have preferences within a variety of cover crop seeds and whether alteration of the seed can reduce its likelihood to be foraged by said colonies. The second is learning what characteristics are present in suburban areas where harvester ant colonies are able to withstand establishment long term by using data taken from the Texas Natural Resource and Information System (TNRIS), field sampling, and ArcMap.

Table and Graphs

Figure 1.1 Harvester ant's consuming a Fall Armyworm (Spodoptera sp.) found on an organic corn crop in Edinburg, TX.

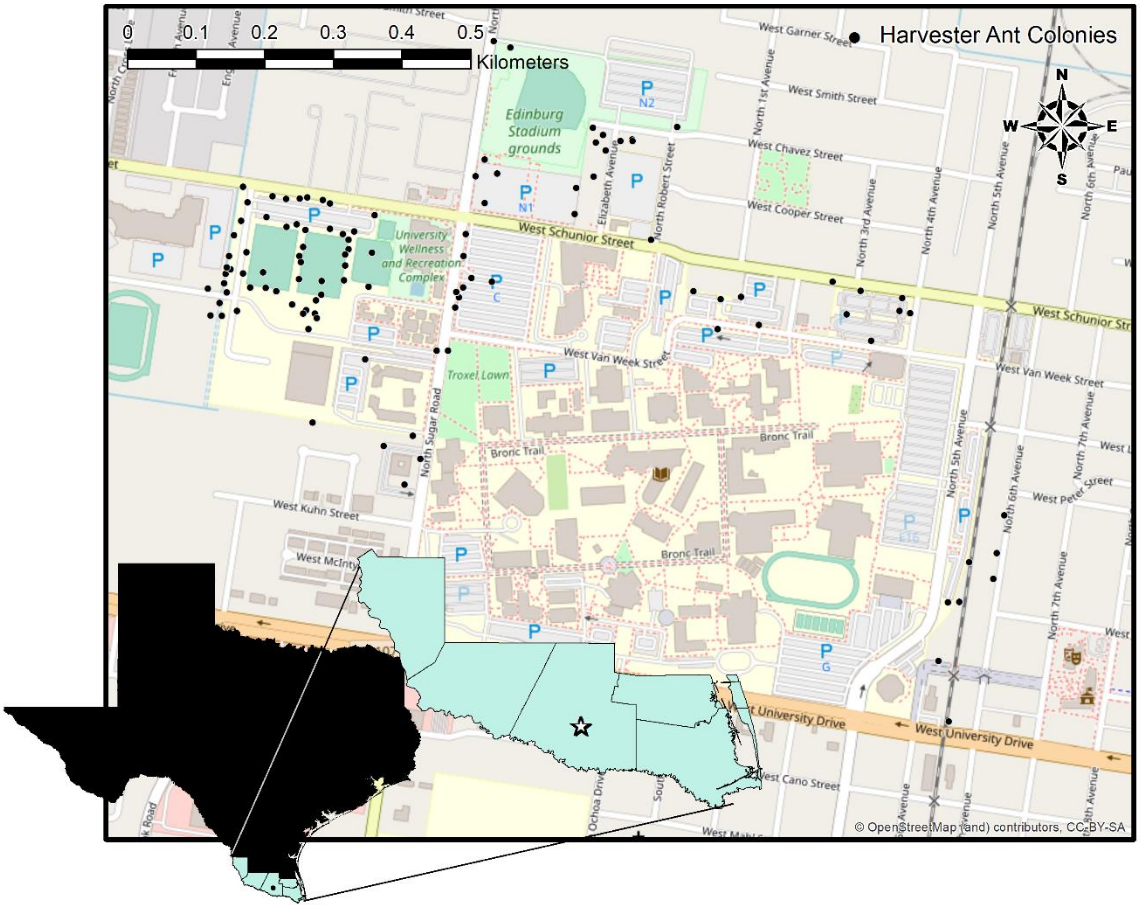

Figure 1.2 Map of known Harvester ant colonies on and surrounding the University of Texas Rio Grande Valley Edinburg Campus.

CHAPTER II

HARVESTER ANT SEED PREFERENCES

Study Objectives

Harvester ants exhibit seed preferences based on a combination of relative seed abundance, size/shape, and nutritional content of the seeds (MacMahon, Mull, et al., 2000; Penn & Crist, 2018; Traniello, 1989). For instance, *P. occidentalis* Cresson prefers to forage with high species fidelity in seed-dense patches, which can reduce local seed bank heterogeneity (Thomas O. Crist & MacMahon, 1992; Luna et al., 2018; MacMahon, Mull, et al., 2000; MacMahon, Mull, & Crist, 2000b). When the seed bank has low seed diversity, ants will collect less preferred seed varieties until more desirable seeds are available (MacMahon, Mull, et al., 2000, 2000b). When more preferred seeds return, ants will empty colony seed stores of the less desired seeds to replace them with preferred options (MacMahon, Mull, et al., 2000). Harvester ant seed foraging is not limited to natural areas and may occur in agricultural matrices where seed preferences may benefit or harm crop production. Although harvester ants are known to consume weed seeds, their seed preferences may also include consumption of crop seeds (Baraibar et al., 2011; Barbercheck, Wallace, & Reddy, 2021; Taber, 1998). Ant removal of crop seeds and vegetation causes economic loss, especially if the crop is situated within areas of high colony density (Borth, 1982; Reed & Landolt, 2019). Red harvester ants in particular are found in agricultural areas in the LRGV (personal observations) and may have a large impact on the plant community through removal of vegetation surrounding their nest entrance (1-5 m in diameter) or through seed collection (MacMahon, Mull, et al., 2000b; Reed & Landolt, 2019).

In addition to cash crops, harvester ants in agricultural fields may forage on cover crop seeds but have not been well documented. In the LRGV, cover crops are used during fallow periods to prevent soil erosion from wind or water (Bodner, Himmelbauer, Loiskandl, & Kaul, 2010; Labrière, Locatelli, Laumonier, Freycon, & Bernoux, 2015; Martinez, 2020; Pushpa Soti & Racelis, 2020). Presumably, if an ant-preferred seed is sown within the foraging range of a colony, it will not have time to germinate before being taken by a forager to the colony granary. The lack of a root system and above-ground vegetation in the foraged field area can then potentially increase economic loss for the farmer (Bodner et al., 2010; Martinez, 2020; Pushpa Soti & Racelis, 2020). So, preventing harvester ant interference with cover crops could potentially reduce soil exposure to erosion as well as save the cost of having to re-seed foraged areas.

The primary objective of the study was to determine red harvester ant preferences for commonly used cover crop seeds in the LRGV. We chose members of the families Fabaceae, Poaceae, and Brassicaceae that are currently being evaluated by farmers in the LRGV - hairy vetch (*Vicia villosa*), oat (*Avena sativa*), sunn hemp (*Crotalaria juncea*), radish (*Raphanus sativus*), and fescue (*Festuca arundinacea*). Wheatgrass (Poaceae: *Triticum aestivum*) was also included as a known preferred food for harvester ants and served as a control for the experiments (Brito-Bersi, Dawes, Martinez, & McDonald, 2018; Ryti & Case, 1988). Based on known baseline preferences that harvester ants exhibit towards grasses, we anticipated that oat, fescue, and wheatgrass would be most preferred as they are sugar-rich grasses from the family Poaceae. (MacMahon, Mull, et al., 2000b; Taber, 1998). In addition to the use of cover crops, LRGV farmers may inoculate cover crop seeds with nitrogen-fixing bacteria to facilitate root nodulation to further benefit the soil (S. Kasper, Christoffersen, Soti, & Racelis, 2019; S. L. Kasper, 2019). As such treatments may influence ant foraging decisions, the second objective was to determine if seed inoculation treatments used for increased germination rates would alter the previously established cover crop seed preferences.

Methodology

Site Description

The study site was located within the Lower Rio Grande Valley in South Texas. This area is considered a local steppe climate that is subtropical subhumid marine with an average annual temperature of 24 °C (16.3-30.2 °C) and 572 mm of precipitation. Soils in these regions of the Rio Grande Plain are considered deep loamy soils with moderately sloped planes and an average altitude of 34 m (USDA 2008). Specifically, all trials were conducted at the University of Texas at Rio Grande Valley (UTRGV) campus (~ 1.5 km^2) in Edinburg, Hidalgo County, TX, USA (26°18'24.0"N, 98°10'15.4"W). This site was selected as the ant’s present would have no prior exposure to the species of seeds presented during the study, but would also still experience disturbance pressures such as irrigation and routine mowing (a proxy for agricultural practices relative to natural settings).

On the campus, most vegetation included grasses used for lawns intermixed with weeds (primarily grass burr/sticker burr) and punctuated by standard suburban ornamental plants (such as *Asclepias curassavica* and *Lantana sp.*). As of publication of the 2020 Tree Campus USA Report, there are 53 different species of trees present with live oak (*Quercus virginiana*), Texas ebony (*Ebenopsis ebano*), and honey mesquite (*Prosopis glandulosa*) being most common (Sustainability, 2020). The immediate land use surrounding the study site is considered a combination of suburban and peri-urban with intermixed sorghum fields, pasture, and citrus groves. Land use within the LRGV more generally also includes mixed fruit and vegetable crops as well as sugarcane production. Active *Pogonomyrmex barbatus* colonies (n = 37) with no prior exposure to cover crop seeds near were mapped throughout the site using an eXplorist 610 GPS unit (Magellan, San Dimas, CA, USA). Colony activity was determined by whether there were foraging trails present with active bidirectional ant traffic.

Seed Preference Trials

To determine whether size differences among seeds could impact ant preference, 50 seeds of each type were weighed with seed texture noted. For the preference trial, the cover crop seeds - hairy vetch (Johnny's Selected Seeds, Winslow, ME), oat, sunn hemp (Johnny's Selected Seeds, Winslow, ME), wheatgrass (Todd's Seeds, Livonia, MI), radish (Johnny's Selected Seeds, Winslow, ME), and fescue (GreenCover, Bladen, NE) - were pre-counted in groups of 10 seeds per cover crop and stored in microcentrifuge tubes at room temperature before transport to the field. Seed depots were constructed out of I-plate Petri dishes (100 mm × 15 mm) (Figure 2.1). Petri dishes were sanded to produce a rough surface to increase traction, and 3 U-shaped entrances were created with a soldering iron at 45° and 90° angles on each half of the Petri dish to allow for easy ant entry to the dish.

The seed depot was placed 2 m from the nest entrance along the primary foraging trail with seed depot entrances facing the foraging trail (Figure 2.2). Upon initiation of each trial, the seeds were placed into a depot, with even numbers per side and a total of 10 seeds per cover crop available per colony. After the addition of the seeds, cages (1 cm × 1 cm hardware cloth [Everbilt, The Home Depot, Atlanta, GA] shaped into a 23 cm × 23 cm square) were placed on top of the depots and secured into the ground with 3 cm fence staples to prevent vertebrate removal of the seeds and indicate human interference (Campagnoli & Christianini, 2021; Hughes & Westoby, 1990; Thomson, Auld, Ramp, & Kingsford, 2016). Seed removal was documented at intervals of 1, 2, 4, and 24 h. During each inspection, temperature, wind speed, and cloud cover percentage were measured and the seeds within and outside of the depots were counted. Seed preference trials were conducted from February to June 2020 in groups of 8-10 colonies per observation period. The tested colonies ($n = 37$) were a minimum of 10 m apart to prevent overlap of colony foraging. All trials were conducted within a temperature range of 20.5-36.6 °C and wind speeds ≤ 32 km/h to optimize ant foraging time but minimize the risk of wind overturning the seed depots.

Due to a delay in shipping, the colonies observed in the first two days of trials ($n = 12$) were not immediately exposed to fescue seeds. These colonies were re-tested later with a depot mix including fescue seeds. They were compared to colonies that were exposed to fescue from the start and they did not demonstrate any difference in preference. Because of this lack of difference, we decided to use the full data set from the second round of trials from the initial twelve colonies for data analyses.

Seed Inoculation Trials

The experimental design for the seed inoculation trials was conducted in a similar manner to the seed preference trials. The same colonies (n = 34) and number of colonies per observation period (n = 8-10) were used. To differentiate which side held inoculated seeds and which held non-inoculated, the undersides of depots were marked with a small section of tape. Two preferred seed types from the seed preference trials belonging to different plant families (wheatgrass and radish) were used to ensure that any inoculation effects would not be confused with lack of preference. Seeds were inoculated in the laboratory with the Guard-N Omri Seed Inoculant (Johnny's Selected Seeds, Winslow, ME) via slurry method. For every 90 g of seeds, 0.7 g of inoculant was added to the container and shaken. Seeds were then stored at room temperature in a marked microcentrifuge tube until use in the field. Trials were completed between July and August 2020 according to the previously used seed preference methods.

Statistical Analysis

R version 3.6.2 (RStudio Team, 2020) was used to conduct all statistical analyses. Within each dataset, each seeds' time to removal was categorized individually with censoring due to external events (e.g., flipped depots due to high wind speeds, removal of the cage prior to the 24 hours period, etc.) denoted. The survdiff function from the survival package was used to determine if there was a significant difference in ant cover crop preference (T. Therneau, 2015; T. M. Therneau & Grambsch, 2000).The Kaplan-Meier survival estimator, which estimates the likelihood of an event occurring at a point in time, was used to calculate seed removal event likelihood over time (L. L. Johnson, 2018). The log-rank test using the lifelines package (Rickert, 2017), a hypothesis test that compares the survival distribution between two samples, was used to compare the survival distribution of cover crop seeds to the wheatgrass and non-inoculated controls. To further investigate these differences while incorporating other variables such as observation month, we used Cox proportional hazard models and preferences compared against the wheatgrass standard using the ggforest function from the survival package (T. Therneau, 2015; T. M. Therneau & Grambsch, 2000).

Results

Seed Preference Trials

Kaplan Meier survival curves were used to visually compare removal rates of the different cover crop seed types ($p < 0.0001$; Figure 2.3; Table 2.1). The Cox proportional hazards model determined the only significant differences in removal were between wheatgrass and vetch (*P* < 0.001; Figure 2.4; Table 2.1), wheatgrass and sunn hemp (*P* < 0.001; Figure 2.4; Table 2.1), and wheatgrass and fescue (*P* = 0.023; Figure 2.4; Table 2.1). During the trials, ants exhibited a preference for wheatgrass and oat seeds, often removing all the seeds before 24 h (Figure 2.4; Table 2.2). For differences between seed types outside of wheatgrass, a pairwise log rank test was used.

The pairwise log rank test provided differences in survival between the seeds amongst themselves (Table 2.2). Vetch and Sunnhemp, though not significantly different from one another, were the types that were significantly less harvested in comparison to other seed types outside of wheatgrass. Overall, vetch was found to be significantly less harvested when compared to oat, wheatgrass, or radish (Fig. 2.3; Table 2.2). Sunn hemp was found to be significantly less harvested when compared to oat, wheatgrass, or radish (Fig. 2.3; Table 2.2). Like the Cox proportional hazards model, Fescue, while not being significantly different from vetch or sunn hemp, was significantly less harvested than wheatgrass (Fig. 2.3; Table 2.2), another member of the Poaceae family. Other than seed types, seed collection differed among months. Over time, seed collection significantly decreased from February to June.

The physical characteristics of the seeds in the depot did not appear to affect preference as the preferred seeds in the study did not consistently share characteristics. Non-preferred seeds also did not share seed shape or texture, only color and nitrogen-fixing abilities. Weight differed significantly between preferred seeds, a linear regression plot demonstrated a lack of correlation between seed weight and survivability (Figure 2.5; Figure 2.6). Vetch and radish shared physical characteristics - both were round and uneven in texture, but they were treated differently by the ants. Sunn hemp was smooth, and bean shaped, while oat and fescue appeared fibrous towards the ends with a thin and elongated shape. Wheatgrass was oblong in shape and relatively smooth.

Seed Inoculum Trial

Unlike the seed preference trials, inoculum trials did not indicate significant differences in preference. The Kaplan Meier curve created from the collected data further demonstrated the visual lack of preference between inoculated versus non-inoculated seed between the same seed type (Figure 2.7). Additionally, the Cox proportional hazards data demonstrated that the difference in preference between the inoculated and non-inoculated seeds was not significant (Figure 2.8; Table 2.3). This lack of overall preference also meant that there was no preference between one another ($P = 0.87$). Surprisingly, the only significance found within the trial was a change in seed removal. Depot harvesting was significantly higher in July in comparison to June or August.

Discussion

The goal of the study was to determine if red harvester ants exhibit preferences among different cover crop seeds used in the Lower Rio Grande Valley and whether inoculating preferred seeds with nitrogen-fixing bacteria would inhibit the desirability of the seed. We introduced naïve harvester ants to selected cover crop seeds via seed depots deployed over 24 h. We found that harvester ants had a significant preference for grass seeds such as wheat and radish seeds compared to nitrogen-fixing sunn hemp and vetch seeds. However, we did not observe any difference in preference between inoculated and non-inoculated seeds of either wheatgrass or radish.

We had assumed harvester ants would prefer to forage on certain seeds based on physical characteristics and family (Poaceae) (MacMahon, Mull, et al., 2000b; Penn & Crist, 2018; Taber, 1998). As anticipated due to prior work on seed preferences in natural areas, all grass seeds were similarly preferred within this study. However, the attributes of radish overlapped with the less preferred seeds of sunn hemp and vetch in terms of shape, color, and/or weight, indicating these physical trails were not the only driver of preference within this context (MacMahon, Mull, et al., 2000b; Taber, 1998).

Alternatively, seed preferences could have been based on seed availability in the surrounding habitat, which likely changed from February to August. Throughout the study, we observed native seed burrs (Genus *Cenchrus* L.) being taken into the colony often as well as smaller grass seeds. Prior documentation of burrs in and around Hidalgo County indicates that burrs are annual grasses with an affinity for frequently disturbed sites such as roadsides, similar to the study sites (Goel, Singh, & Raina, 2011; Shaw, 2011). *Cenchrus echinatus* L. is known to germinate in the late spring, continuing through the fall (Cope & Gray, 2009; Smith, Ferrell, & Sellers, 2012), corresponding to the timing of the summer seed depot trials. Given these observations, the interactions of cover crop seeds with weed banks within agricultural settings needs to be evaluated further, particularly in regard to sowing timing.

Outside of seed preference changes due to the surrounding seed pool, *P. barbatus* activity is closely related to rainfall, peaking in the summer months and correlated with overall seed availability. With additional rainfall, more grasses outside of drought resistant varieties such as *Cenchrus sp*. potentially germinated, allowing for more diversity in the seed pool (Cope & Gray, 2009; MacMahon, Mull, et al., 2000b; Smith et al., 2012). The additional surrounding native seeds could have been another cause for the reduction in depot harvesting over time from February to June. Alternatively, during the sudden increase in depot harvesting from June to July could be in preparation for August, which is usually known for its higher temperatures. In August, activity significantly decreased in comparison to both June and July, implying that high amounts of collection in June could have been done to avoid excess water loss for the colonies in August.

Another interesting, isolated event was recorded on July 23rd, 2020, two days prior to the touch down of Hurricane Hanna in the LRGV. Within one hour, 8 of 9 colonies had completely emptied the depots. The impacts of such weather events are known to affect insect behavior in response to changes in barometric pressure; many insects exhibit sudden insatiable appetites likely preparing for weather events that follow (Fernando R. Sujimoto, 2019; Flitters, 1963). Leaf-cutter ants have been observed to significantly increase foraging during periods of low barometric pressure, and harvester ants may do the same (Fernando R. Sujimoto, 2019). Future studies regarding the correlation between harvester ant foraging intensity and barometric pressure could help determine risk during certain planting dates in regions along the gulf coast that have the potential to experience tropical cyclones annually.

Harvester ants have been previously observed to have contradictory behavior regarding the same seed species based on other aspects such as seed germination or fungal infection (Thomas O Crist & Friese, 1993; MacMahon, Mull, et al., 2000b; Taber, 1998). However, in our trials, inoculated and non-inoculated seeds were not treated differently, indicating that the presence of nitrogen-fixing bacteria did not inhibit or encourage harvester ant predation. Regardless, there is conflicting data regarding the amount of microbial diversity/biomass within the soil around ant colonies (Boulton, Jaffee, & Scow, 2003; Ginzburg, Whitford, & Steinberger, 2008; Wagner, Brown, & Gordon, 1997). *Pogonomyrmex barbatus* in the study showed no preference towards or against inoculated seeds, hinting that their granaries could be potentially rich in microbial activity. Alternatively, harvester ants do partake in seed cleaning behavior that could occur at any point prior to introduction to the granary.

In subtropical areas such as the LRGV, prior studies recommend the use of warm season cover crops due to subtropical climate and promotion of native mycorrhizal fungi (Rugg, 2016; PG Soti, Rugg, & Racelis, 2016). Based on the data collected in this study, harvester ants were exhibited lower levels of preference towards certain seed types such as sunn hemp. The benefits that these nitrogen fixing types, such as sunn hemp, hold towards the soil can be extremely beneficial. Sunn hemp, for example, conserves phosphorus in the soil, increases nitrates, and has the potential to improve soil health in subtropical agroecosystems such as the LRGV (Mansoer, Reeves, & Wood, 1997; Rugg, 2016; PG Soti et al., 2016; Treadwell & Alligood, 2008; Uhey, Cummins, Rotter, Lassiter, & Whitham, 2021). Not only does sunn hemp have the potential to be an excellent South Texas cover crop, but it is also increasing in popularity in other southern areas of the U.S. like Florida and Louisiana. Similarly, hairy vetch also has potential to be a great cover crop due to the low ant preference and its weed suppression and nitrogen-fixing abilities (Moran & Greenberg, 2008). Given these cover crops are not preferred over grasses in the seed depot study, which are common in the non-crop habitats surrounding LRGV crop fields, harvester ants would likely predate on surrounding weeds and grasses instead of the chosen cover crop.

Harvester ants can be a substantial disturbance agent in arid to semi-arid regions of the United States and Mexico. *Pogonomyrmex sp.* have a pest status for seed collection and plant removal in agricultural areas and can remove all of the preferred seeds within their foraging range (Thomas O Crist & Friese, 1993; Taber, 1998). Our data suggests we can recommend nitrogen-fixing cover crops like sunn hemp and vetch to farmers as a potential cover crop during fallow periods and could be paired with the fact that seed inoculation is neither preferred nor rejected by harvester ants. Inoculating these nitrogen-fixing seeds could help with nodulation, nitrogen-fixing processes, and benefit the soil health below ground while protecting topsoil from erosion. Not only that, using the pair for a cover crop trial, could in turn encourage harvester ant predation on weed species or surrounding native plants that could limit crop yields (Baraibar et al., 2011). Additional research should be conducted regarding harvester ant preferences. For example, conducting preference studies with rural harvester ants that have more exposure to different agricultural seed types and in turn, potential differences in preferences. A better understanding of harvester ant seed preferences can be used to encourage predation on native or weed seeds while reducing the need to eradicate native harvester ant colonies.

Table and Graphs

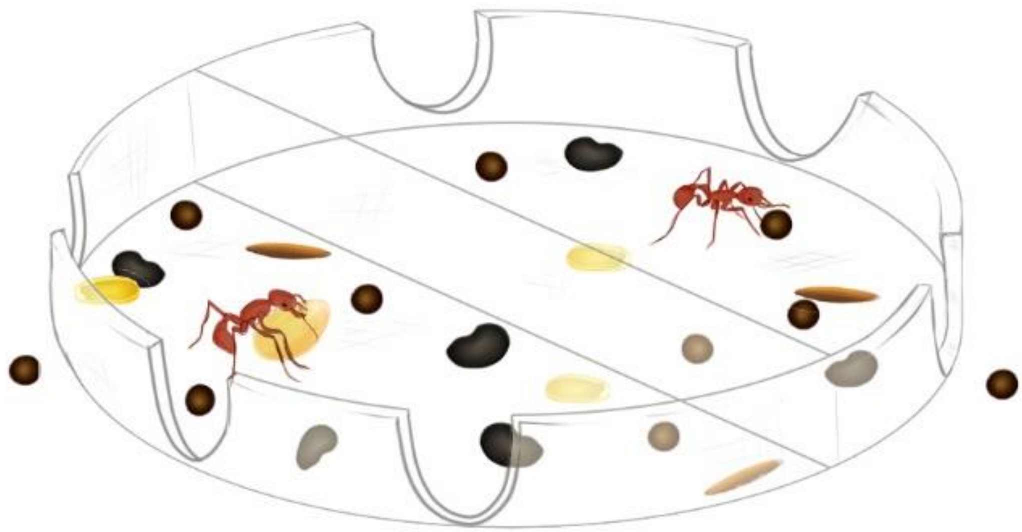

Figure 2.1: Petri-Dish modifications to allow ants to have easy access to seeds.

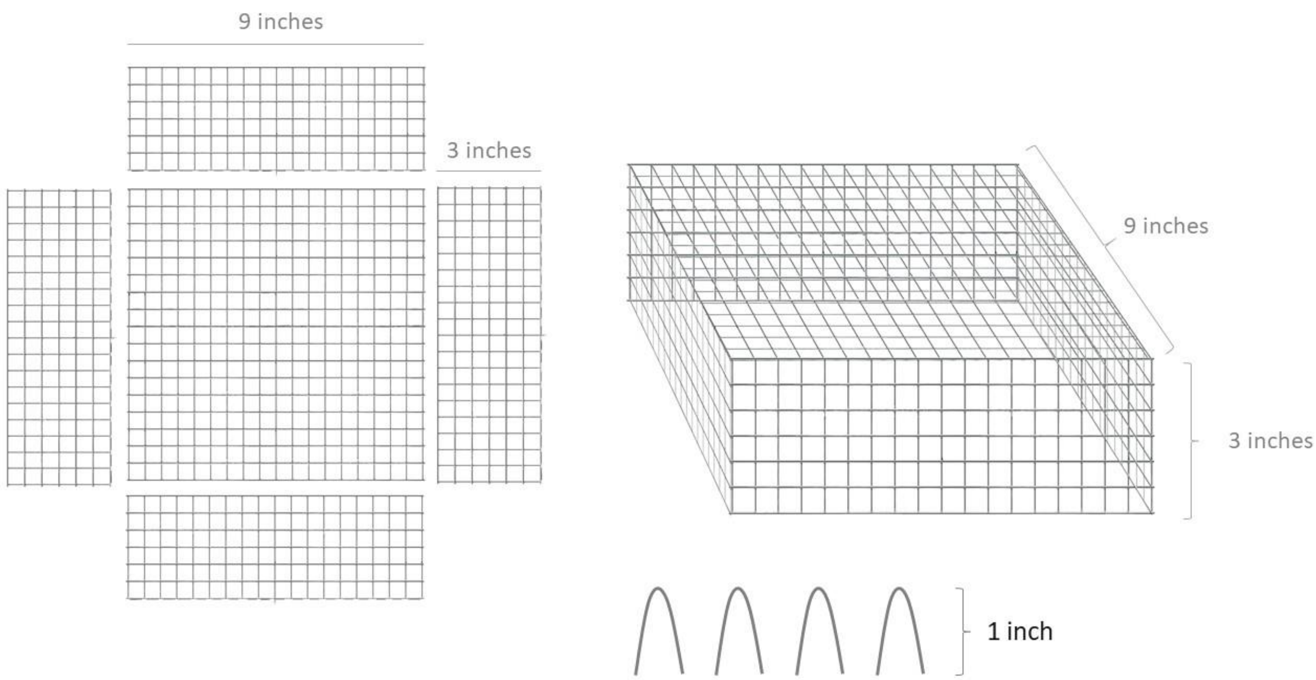

Figure 2.2: Cage constructed to protect seeds from herbivorous outsiders.

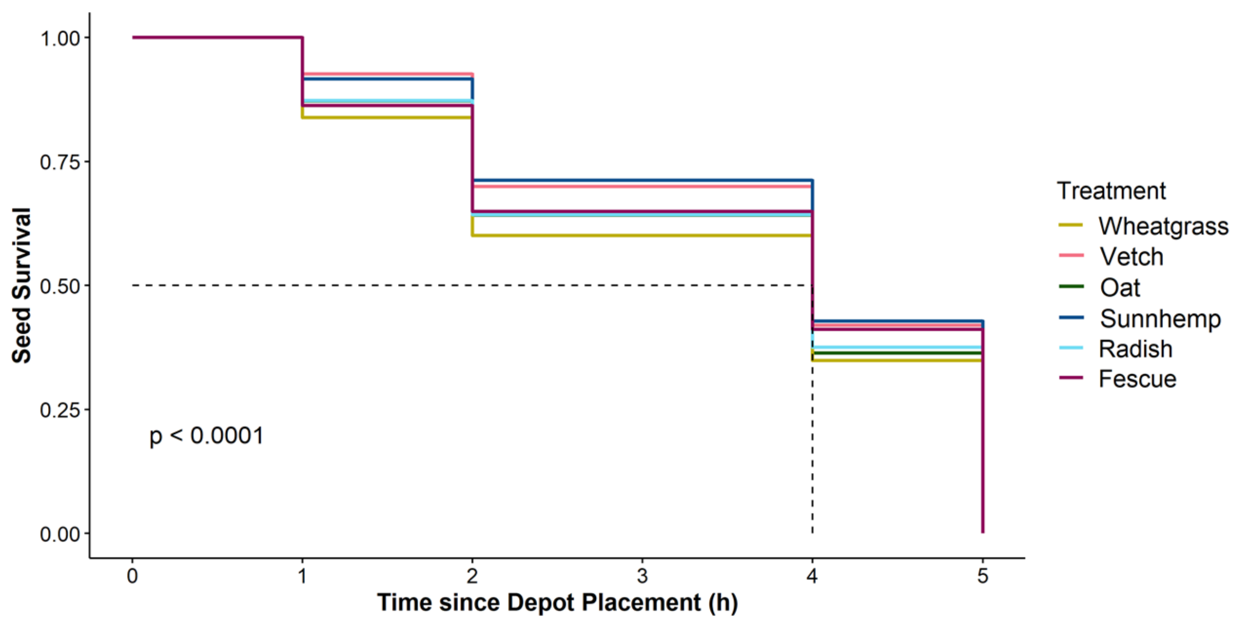

Figure 2.3 Kaplan Meier Survival curves of the six seed varieties introduced to the harvester ant colonies.

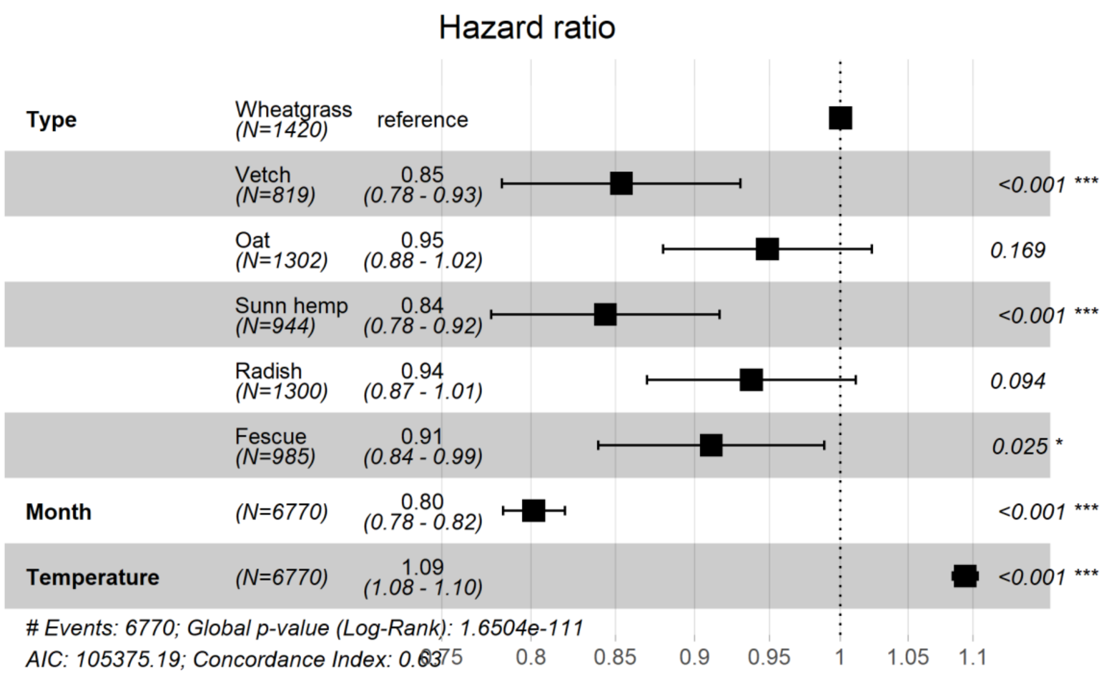

Figure 2.4 Hazard Ratio Analysis conducted between the five seed varieties to the control.

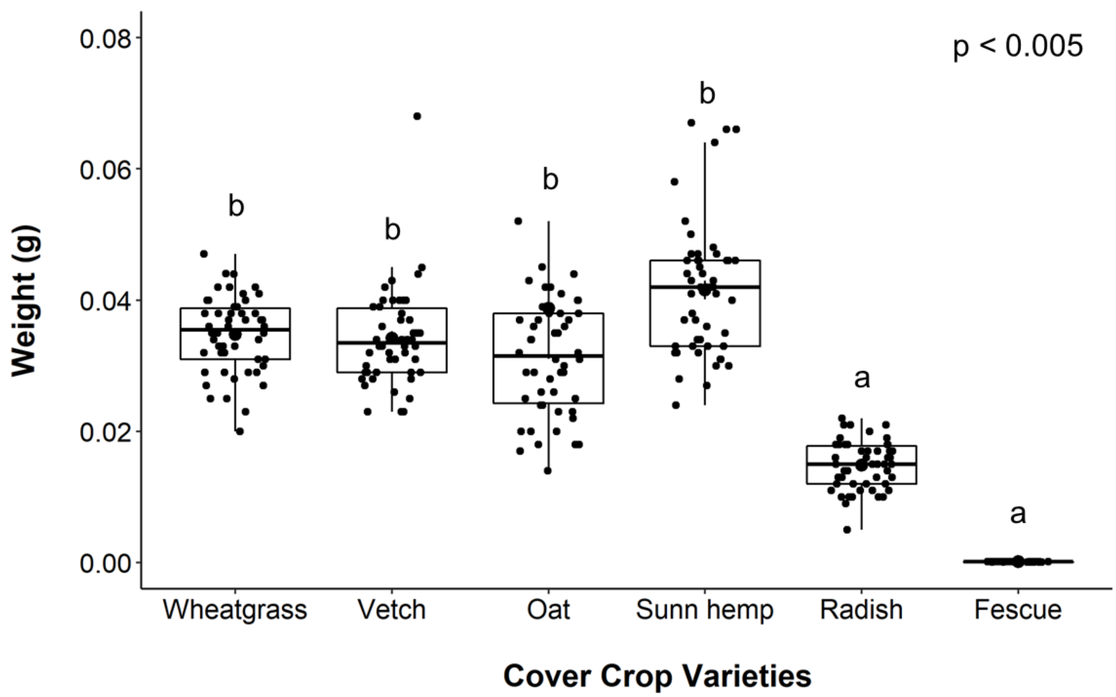

Figure 2.5 Boxplot comparing the weight of the six seed varieties. Weights were compared to one another via ANOVA and Post hoc test. Radish and fescue were significantly lighter in comparison to wheatgrass, vetch, oat, or sunn hemp ($p < 0.005$).

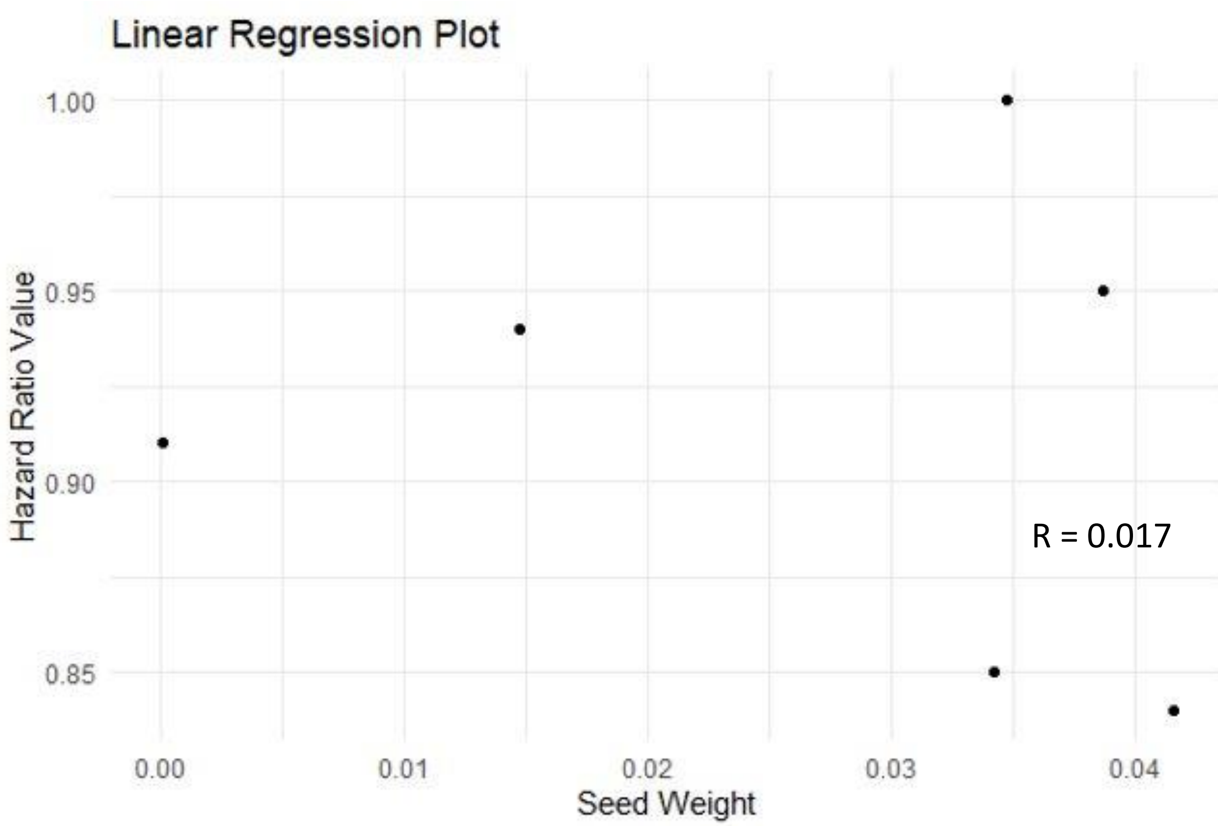

Figure 2.6 Linear regression demonstrating a lack of correlation between seed weight and survivability via hazard ratio value.

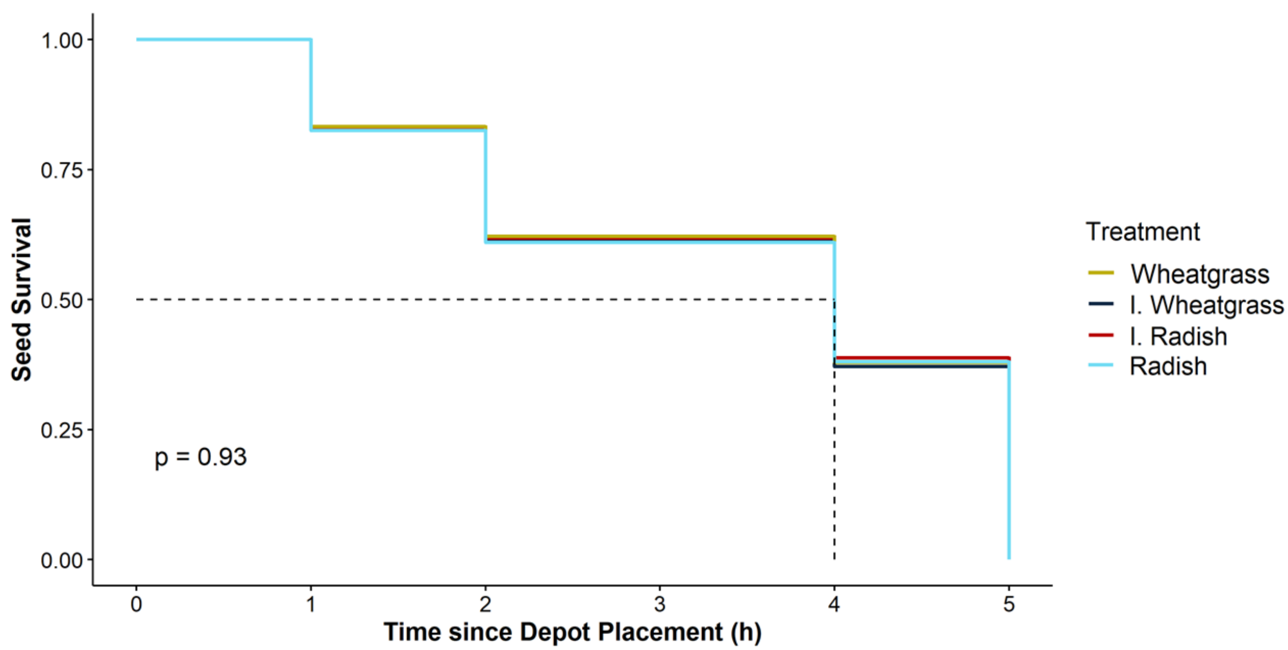

Figure 2.7 Kaplan Meier Survival curves of the six seed varieties introduced to the harvester ant colonies.

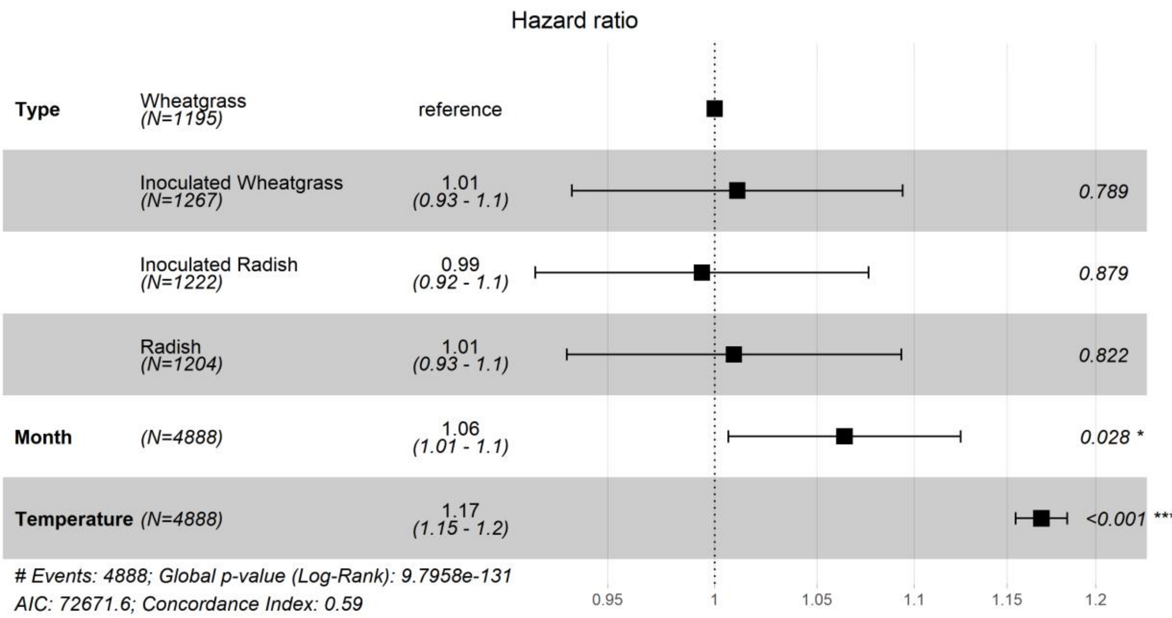

Figure 2.8 Hazard Ratio Analysis conducted between the inoculated seeds and uninoculated radish to the control.

Table 2.1 Summary of the fitted cox model for cover crop seed preferences.

Cover Crop	coef	exp(coef)	se(coef)	z	***P*-value**
Vetch	0.000	1.000	0.040	-0.040	0.970
Oat	0.000	1.000	0.040	-0.080	0.930
Sunn hemp	0.000	1.000	0.040	-0.110	0.910
Radish	0.010	1.010	0.040	0.150	0.880
Fescue	-0.170	0.840	0.040	-4.130	0.000

Table 2.2 P-values for the pairwise comparisons using Log-Rank Test between seed types for the seed preference study (n = 6770 total seeds).

	Vetch	**Oat**	**Sunn hemp**	**Wheatgrass**	**Radish**
Oat	0.003				
Sunn hemp	0.733	<0.001			
Wheatgrass	<0.001	0.149	<0.001		
Radish	0.011	0.664	0.003	0.068	
Fescue	0.190	0.121	0.115	0.003	0.230

Table 2.3 Summary of the fitted cox model for inoculated seed preferences.

Cover Crop	coef	exp(coef)	se(coef)	z	Pr(>|z|)
Inoculated Wheatgrass	0.010	1.010	0.040	0.260	0.750
Inoculated Radish	-0.010	0.990	0.040	0.680	0.820
Radish	0.000	1.010	0.040	0.410	0.930

CHAPTER III

HARVESTER ANT SUBURBAN DISTRIBUTION

Introduction

Harvester ants are native inhabitants of arid to semi-arid regions of the United States and commonly reside in rural to peri-urban environments with significant effects on their surroundings in the form of soil aeration, plant species distribution, and nutrient accumulation within and around their colony (MacMahon et al. 2000; Tizon et al. 2010; Vieira-Neto et al. 2016; Luna et al. 2018). Their body size ranges up to a centimeter in length and come in a variety of warm tones ranging from orange to red; they also have large mandibles and are known for their useful "beards" (Davis, 2016; Taber, 2998). These beards consist of hair-like structures that protrude on the underside of their head, the beard projects outwards in a curved fashion. The beards are used to facilitate excavation and further expand the colony into neighboring territory.

These colonies can grow widely in size and in depth, with tunnels that can range up to 10m in diameter and 5m deep into dry soil (Reed & Landolt, 2019; Taber, 1998). This large amount of available space allows for potential mobility within the colony, through the soil column in times of varying climates. Extreme weather events are commonplace in the range of red harvester ants; regardless of disaster type, whether it be drought zones or flood plains, each colony has a way to overcome their surrounding environment (Friedman et al., 2019; Sundaram et al. 2021). Hurricanes are frequent in south Texas given its proximity to the Gulf of Mexico. Though rainfall is highly beneficial and vital to ants (especially aiding the colony during events such as initial foundation and nuptial flights and providing water to fuel localized seed production) excessive localized rainfall can cause flooding events (Johnson, 2001). To avoid drowning from flood events, establishment in highly elevated areas would prevent the colony becoming waterlogged. Prevention in establishment in consistently moist areas would also be recommended to further prevent risk of waterlog.

These environmental requirements for the optimal nesting location can make it difficult for ants to initially excavate a new nest. Though harvester ant queens can lose their wings during nuptial flights, these ants can travel a decent average distance of around 65 to 85m away from their original colony to avoid intraspecies competition (Taber, 1998; Suni & Gorden, 2010). The subsequent limit on movement after nuptial flights can make it even more difficult for new queens and moving colonies to find an ideal location for establishment. The amount of suitable habitat within dispersal distance may differ between land uses, subjecting the ants to different levels of competition between colonies. Space is not an issue for harvester ants in grasslands (unless the area already has high nest-density area) and have no problem surviving in their native habitat. However, in urbanized areas open space is more limited, particularly areas providing enough food resources.

Urbanization has been a fast-growing process in the United States with the state of Texas having some of the of the fastest recent growth of impervious surface areas in the country (Bounoua et al., 2018; Lombardi et al., 2020). This increase in urbanized areas in Texas has led to habitat loss for a variety of native plants and animals, prompting relocation or population decline

(Leslie Jr, 2016). Due to its proximity to the US-MEXICO border, population growth has caused urbanization to rise in the Lower Rio Grande Valley (LRGV) since the 70s, with a recent increase in population of over 55% between 1990 and 2021 (Leslie, 2016). In response to the increase in human population, land use has changed drastically over the years as well. The amount of land in three counties of the LRGV categorized as “urban” has increased over 46% over the span of 10 years (Huang & Fipps, 2006; Lombardi et al., 2020). This increase in one land type has caused the surrounding types to shift in response. Among the different land cover types, rangeland-herbaceous (includes grasslands), is one of the categories that’s being affected the most by urbanization and has been on the decline (Lombardi et al., 2020). Given the amount of discreet diversity already present in urbanized locations from insects who were able to adapt to a unique environment, one new insect entering might not seem as harmful. One native species still present in suburban areas is *Pogonomyrmex barbatus*, the red harvester ant. However, red harvester ant colonies are known to remove the vegetation surrounding their colonies, potentially causing conflict with land managers who desire a manicured lawn (Davis, 2016; Taber, 1998). The bare disks surrounding the nest supply many benefits and are routinely maintained by younger members of the colony; however, the removal of surrounding vegetation can be a major disturbance in urban and suburban settings especially in case of infestations.

In suburban and urban areas, ants may nest in areas as such as roads, firewalls, trails, sidewalks, etc. (Stiles & Jones, 1998; Tizon et al., 2010; Uhey et al., 2021). Establishment of ant colonies in [human] population-heavy areas allow for more interaction between human beings and harvester ants. Red harvester ant stings are among the most painful of all insects in North America and can have detrimental effects for people who have a strong allergic response to their sting; sting symptoms can range from a minor reaction to life-threatening anaphylaxis (Schmidt, 2016; Klotz

& Rust, 2007; Klotz et al., 2005; Taber 1998). Given harvester ants' affinity for large open spaces like playgrounds and athletic fields, the likelihood of a human interactions with harvester ants increases.

By determining which characteristics of a landscape in a suburban area that promote the establishment of harvester ant colonies, land managers can make decisions about how to prevent establishment of these ants without the use of pesticides. Further, by reducing pesticide use on ant colonies and using passive changes to discourage ants from nesting in certain locations allows for the ants' beneficial contributions as ecosystem engineers and food resources for the threatened Texas horned lizards to remain. The objective of this study is to determine established colony distribution in suburban settings and how they may correlate to environmental factors such as elevation, canopy cover, soil moisture, surrounding pervious/impervious material as well as compare colony density changes from 2020 to 2021. With this information, general recommendations can be made to those interested in passively lessening the likelihood of long-term harvester ant colony establishment.

Methodology

Site Description

The area of interest (~1.5km^2) was located within the boundaries of UTRGV Edinburg campus (26°18'33.1"N 98°10'26.8"W). The UTRGV Edinburg campus lies in the northern section of Hidalgo County, a rapidly growing sub-tropical/semi-arid region of Texas (Davis 2016, Martinez 2020). Hidalgo county is one of five counties encompassing the LRGV. The LRGV is situated on the Rio Grande Plain, with deep loamy soils, moderately sloped planes, and an average altitude of

34m (NRCS, 2008). This region has a climate that is humid with a temperature range of 8°C to 35°C, with an average annual precipitation of 609mm (Nielson-Gammon, 2011; Jacobs, 1981).

Colony Localization and Documentation

In August 2020 and August 2021 established harvester ant colonies with a visible foraging trail and active foragers were documented using an eXplorist 610 GPS unit (Magellan, San Dimas, CA, USA). The coordinates collected using the GPS unit were converted from Latitude/Longitude to the Universal Transverse Mercator Coordinate System (UTM) for importation to the software ArcMap 10.2.2. (ESRI, 2011). Within ArcMap a point shapefile of colony locations for the two years was created to determine ArcMap/ArcGIS. Shapefiles of colony locations were used to determine colony density and distribution in a suburban environment.

Colony Density and Spatial Autocorrelation

The geographic locations tied to the shapefiles allowed for a Spatial Autocorrelation Analysis (Moran's Index) to determine whether colonies were dispersed from one another, randomly distributed, or clustered together. Nearest Neighbor Distance (NND) analysis was also conducted to measure disbursement at multiple scares (1^{st}, 2^{nd}, …10^{th}), these were compared with the same number of points as colonies that were randomly distributed in the same area. Using the date, a ratio of observed versus expected values was created and compared.

Foraging area

Foraging trails may extend from 1m away to 25m away from the colony on average (Detrain et al., 1999; Harrison and Gentry, 1981). In cases of high-density foraging trails have been reported to extend up to 60m away from the colony (Reed and Landolt 2019). Foraging distance can also be a direct impact of impervious surfaces; with less available food sources due to sidewalks or parking lots, foragers may have to move farther to get sustenance. Harvester ant colonies documented on the shapefile were input into RStudio where Voronoi tessellation was used to determine the average amount of available foraging area available for use between colonies within the intramural fields of UTRGV and overall campus. The intramural fields (26°18'33.9"N, 98°10'47.8"W) are within campus premises and were used for the Voronoi analysis due to its uniformity.

Given harvester ant affinity for disturbed areas, we also measured the average distance to roads from colonies using ArcMap (Davis, 2016; Shaw, 2011; Taber, 1998). Elevation was also taken into consideration as a potential reason for establishment along certain boundaries. Elevation data was extracted from DEMs provided by TNRIS. The data provided from the file was used to compare areas with and without harvester ant colonies.

Percent Impervious

All pervious/impervious analyses were conducted in the software ArcMap 10.2.2. And ArcGIS Pro (ESRI, 2011). To confirm these data, we created a training sample where classes could be specified. These classes are categorized by different pixel values and are created via supervised classification method. Rooftops, roads, parking lots, bodies of water, and sidewalks were some of the items highlighted and identified as impervious surfaces. The pervious classes created consisted of grassy areas and bare soil. The accuracy and detail in the training sample trains the model to be able to identify and categorize the rest of the pixels on the raster map as pervious or impervious material. To measure accuracy of the model, a confusion matrix was created from 100 random points distributed within the area of interest. A column was made with the values of the models' prediction and another for the ground truth. The columns were compared via confusion matrix to gauge the margin of error of the model. This layer was then saved as a raster and the shapefile containing the colony data was imported into the map.

Tree Canopies

Using NAIP imagery, a Normalized Difference Vegetation Index (NDVI) analysis was conducted on the area to determine the amount of available vegetation in the region. Differences in reflectance from various vegetation types can be differentiated within the range of the analysis (Gitelson, 2004; Buddheswar et al., 2015). This range was narrowed to represent tree canopies within the vicinity of area surveyed for colonies. Areas composed of grass can range in value from 0.18 to 0.4, while the NDVI value for forest can range from 0.4 to 0.74 (Buddheswar et al., 2015; Akbar et al., 2019). For our analysis, our NDVI value was reduced from -1-1 to 0.4-0.7. The NDVI of the area was averaged and split by those with an absence or presence of colonies prior to being compared to one another.

Soil Moisture

Soil moisture samples were taken in the Intramural fields at the University of Texas Rio Grande Valley (UTRGV) (0.02 km^2) using Field Scout TDR 350 Soil Moisture Sensor (Geneq, Vaughan, Ontario, Canada). Samples were taken uniformly over the soccer field where 41 harvester ant colonies are present. For an even distribution of data for an interpolation map, sample sites were approximately 50m by 50m of one another. Over the area, 27 sample locations were chosen on the site, 3 sub-samples were taken in each sample location to average. The instrument used for measurements was the Field Scout TDR 350 Soil Moisture Sensor. The sensor takes water moisture data in the form of Volumetric Water Content (VWC%), electric conductivity (EC), temperature of the soil in both Celsius and Fahrenheit. The probe used was 8cm in length.

Statistical Analysis

A Binary Logistic analysis was run to determine significance in colony establishment between different variables (i.e., elevation) of areas with a presence or absence of harvester ant colonies. To summarize the areas with and without colonies, a fishnet of 20 x 20 cells was created in ArcMap over UTRGV grounds. Areas that were not university property or surveyed for colonies were removed, leaving 252 cells. Cells with colonies present were tagged as 1 or 0 if there were none. The zonal statistics tool was used to classify the average elevation for each cell within the fishnet file. The output table was then used to further determine the cells with a presence/absence of harvester ant colonies.

Data used

Raster and vector data were collected to run the proper analysis. Satellite data consisted of National Agricultural Imagery Program's (NAIP) Imagery from 2020 of the Edinburg Southeastern Quad (USDA, 2021). Raster data consisted of Digital Elevation Models (DEM) extracted from the Texas Natural Information System (TNRIS) data hub. Vector data were created using colony coordinates that were converted into shapefiles.

Results

Spatial Autocorrelation

In the observed area, colonies can be seen within areas like large athletic fields, front lawns, playgrounds, along sidewalks, and in more deserted locations such as railroad tracks (Figure 1.2). Colony density decreased from around 84 colonies per hectare to 74 colonies per hectare from 2020 to 2021. Nearest Neighbor Distance (NND) of harvester ant colonies on ArcMap was determined to be on average 31m in 2020 and 30m in 2021. For both years, colonies were significantly clustered together ($p < 0.0005$).

Binary Logistic Regression

Using this analysis, we could analyze our environmental data discreetly between sites with and without ant colonies. In 2020, areas with a presence of harvester ant colonies were significantly higher elevated in comparison to areas without colonies present (Figure 3.3A, $W = 4698$, $P < 0.001$). This significance only increased in 2021 (Figure 3.3B, $W = 3392$, $P < 0.0001$).

The pervious/impervious model was created with an error of 94%, demonstrating high accuracy in representing the two surfaces over the area (Table. 3.1; Figure 3.4). However, there was no significant difference for pervious material in areas with a presence or absence of harvester ant colonies for either year (Figure 3.5A, $P < 0.492$; Figure 3.5B, $P < 0.942$ in 2021). In the second year, the percentage of pervious material surrounding harvester ants decreased but not significantly. Finally, there were less NDVI value associated with tree canopies in areas with colonies present versus absent in 2020 (Figure 3.6; Figure 3.7A, $W = 6993$, p-value = 0.060) and significantly less in 2021 (Figure 3.7B, $W = 7459$, p-value = 0.022).

Intramural fields

Within the Intramural fields, soil moisture was lower but not significantly in areas with ant colonies for the first year, this difference in both areas decreased in 2021 ($W = 8329$, $P < 0.213$; $W = 8199$, $P < 0.428$). Voronoi tessellation within the intramural fields averaged in 913m^2 (30.215m x 30.215m) in 2020 and 860m^2 (29.312m x 29.312m) in 2021, a loss of 53m^2 (Figure 3.1; Figure 3.2). The same analysis conducted on the entirety of campus also saw a decline in average tessellation area over time (loss of 135m^2) as well as a decline in established colonies.

Discussion

Harvester ant colonies were distributed in areas alongside roads/disturbed areas, in significantly higher elevated areas, and were clustered together in groups. In 2020 to 2021 there was a decline in colonies, due to either death, dormancy, or relocation within or outside of the survey parameters. Decline in colonies is not unusual and is common as younger colonies as they tend to have a higher mortality rate, which lowers over time as the colony matures or crowding decreases (Tschinkel, 2017; Sundaram et al., 2021). Interestingly, the Voronoi tessellation also determined a decline in foraging areas surrounding the colonies, something unexpected if crowding were to be reduced in the area (Sundaram et al., 2021). Within the intramural fields, four overall colonies in the northern and eastern sections of the intramural fields were observed in 2020 that did not return in 2021. In the second year however, three additional colonies were documented on the western strip of the fields. The western fields are slightly higher elevated, watered at the same rate, and were less tampered with in comparison to the soccer fields outside of mowing. Unfortunately, this section was outside of the parameters of our soil moisture survey, so the soil moisture is unknown. Future studies what characteristics in a different location within the LRGV make it desirable for relocation within the harvester ants on campus over time.

There was a lack of significant difference seen in the ratio of impervious to pervious surfaces from areas with and without a presence of harvester ant colonies. We believe colonies found on the northern side of the surveyed region were the reason for the shift in mean (Map 1.2). These colonies are found on small "islands" of vegetation in the middle of parking lots, thus surrounded by a large amount of impervious area. With a limited amount of food sources available in their immediate surroundings, this brings up more questions as to how they sustain the colony within areas with little to no nearby food sources. Ants may have been traveling across asphalt with higher risks of dehydration or at the cost of limiting the amount of time they forage outside to avoid unnecessary moisture loss. The minimum distance between the ~4.5m x ~1.5m islands within the parking lot and neighboring vegetation along the border of the parking lot was around 15m, well within the length of a foraging trail (Reed & Landolt, 2019; Detrain et al., 1999; Harrison and Gentry, 1981). Harvester ant foragers often travelled across the asphalt to neighboring, uninhabited islands for seeds (personal observation).

Though there was a lack of significance in soil moisture in areas with and without harvester ant colonies, we did see that areas with colonies were significantly elevated than those without. In periods of intense rainfall, the water that cannot percolate into the soil fast enough will runoff into lower elevated surfaces. This can cause flooding to occur in these lower elevated areas and pose more risk to harvester ant colonies. In the future, more investigations can be done to see why there is a lack of harvester ants specifically within the University grounds. The area receives the same amount of irrigation as the intramural fields but has significantly more trees. UTRGV was classified as a Tree Campus by the Arbor Day foundation, with over 2,000 trees on the Edinburg campus with large tree canopy's stretching over large areas (The University of Texas Rio Grande Valley, 2020). The canopy serves as protection against sun, storms, and ground erosion. They also cast lots of shade, further preserving soil moisture for longer periods of time in between irrigation, allowing for the soil to remain moist for longer periods of time. Though there is a significant difference in NDVI from tree canopies between areas with and without colonies, we cannot say that this is the reason for a void of colonies in the area. That being said, other studies have found harvester ant species that rejected areas below tree canopies to nest (Azcárate & Peco, 2003). This is due to the thermal service the sun provides; during shaded periods or in shady areas, productivity decreases within the colony (Bucy & Breed, 2006). Harvester ants are not shade-tolerant and in shaded areas have a later start time, leading to reduced foraging activity during the day and potentially getting outcompeted by unshaded neighbors for resources (Bucy & Breed 2006; Reyes-López, Ruiz, & Fernández-Haeger, 2003). Aside from canopies, areas with higher tree counts also typically had increased proximity to more heavily [human] populated areas of our study. As a result, the likelihood of conventional preventative methods of pests via insecticides grows. Other species of ant colonies are visible on these inter-most sections of the campus grounds. Leaf cutter ants and red imported fire ant colonies (Hymenoptera: Formicidae) have both been observed in the same area's harvester ants are absent (personal observations).

Finally, the intramural field is 75 times smaller than the area used for campus-wide comparisons. The difference in sampling amounts and in size could be a reason for a lack of significance to arise, perhaps with a higher sample size preference in establishment toward soil moisture may become clearer. Soil moisture varies with depth the farther away it is from thermal radiation; sometimes little difference has been found in areas with or without colonies due to this lack of depth. Other studies conducted in the past have determined significant differences in the soil column below the colony in comparison to surrounding control areas (Laundré, 1990; McGinley et al., 1994). Although, this significance is not always present in rural environments (Wagner, Brown, & Gordon, 1997). Further studies can be conducted to see if this variation in results also applies or will be different in urban to suburban environments.

With the results from this study, we can conclude that harvester ants have an affinity for significantly clustering together in open spaces, higher elevated spaces, with an average of 30.5m separating one another and a foraging area of an average of 886.5m^2. With this information land managers can passively reduce the likelihood of harvester ant colony establishment (i.e., planting more trees, diversifying the landscape with other non-grass native plants) without turning to conventional methods and in turn preserving the main food source of the threatened Texas Horned Lizard.

Tables and graphs

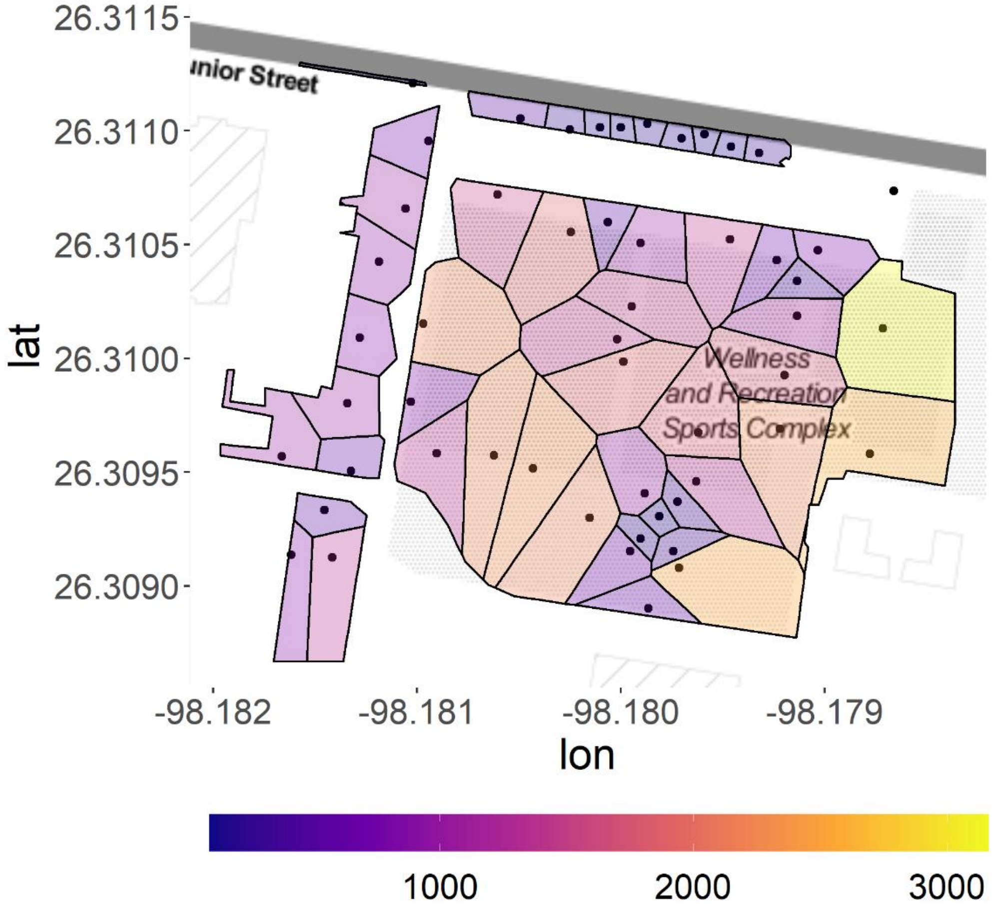

Figure 3.1 Voronoi tessellation conducted on 2020 harvester ant colonies on the intramural fields in Edinburg, TX. The median area of the Voronoi area is 913m^2.

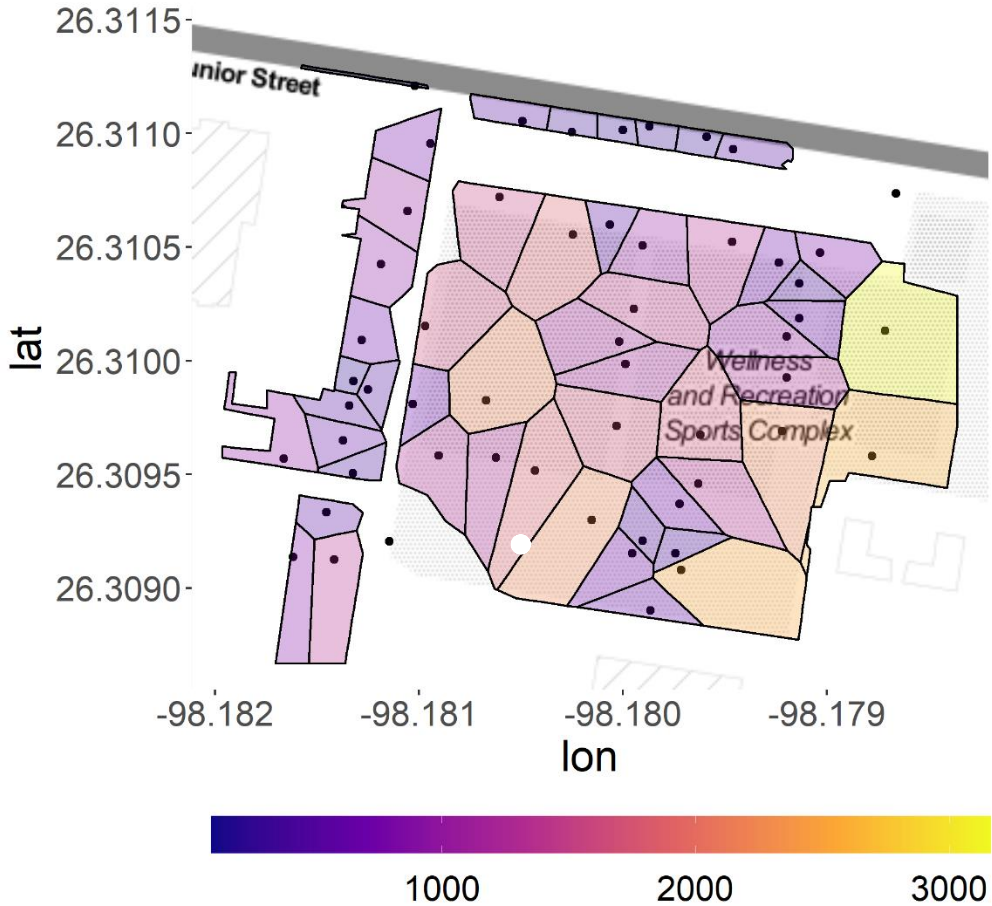

Figure 3.2 Voronoi tessellation conducted on 2021 harvester ant colonies on the intramural fields in Edinburg, TX. The median area of the Voronoi area is 860m^2.

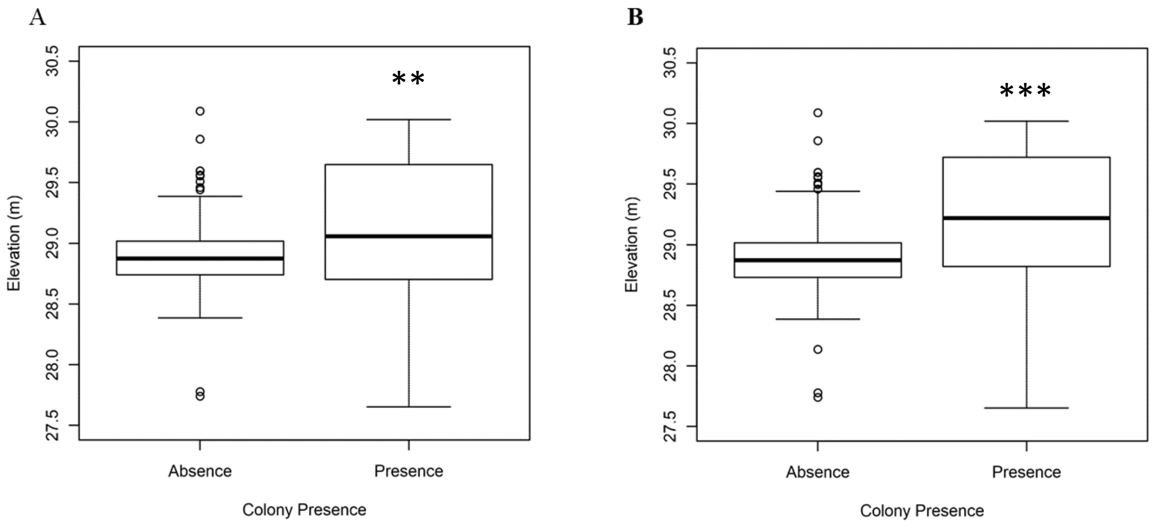

Figure 3.3 A) Box plot comparing the elevation of areas with and without harvester ant colonies in 2020 (W = 4698, p-value = 0.001) and B) 2021(W = 3392, p-value = 3.2e-06). The difference in elevation between the two areas increased from 2020 to 2021

Table 3.1 Classification matrix created from the 100 accuracy assessment points randomly dispersed on the .tif file.

Class Value	Impervious	Pervious	Total	U_Accuracy	Kappa
Impervious	47	3	50	.94	0
Pervious	3	47	50	.94	0
Total	50	50	100	0	0
P_Accuracy	.94	.94	0	.94	0
Kappa	0	0	0	0	.88

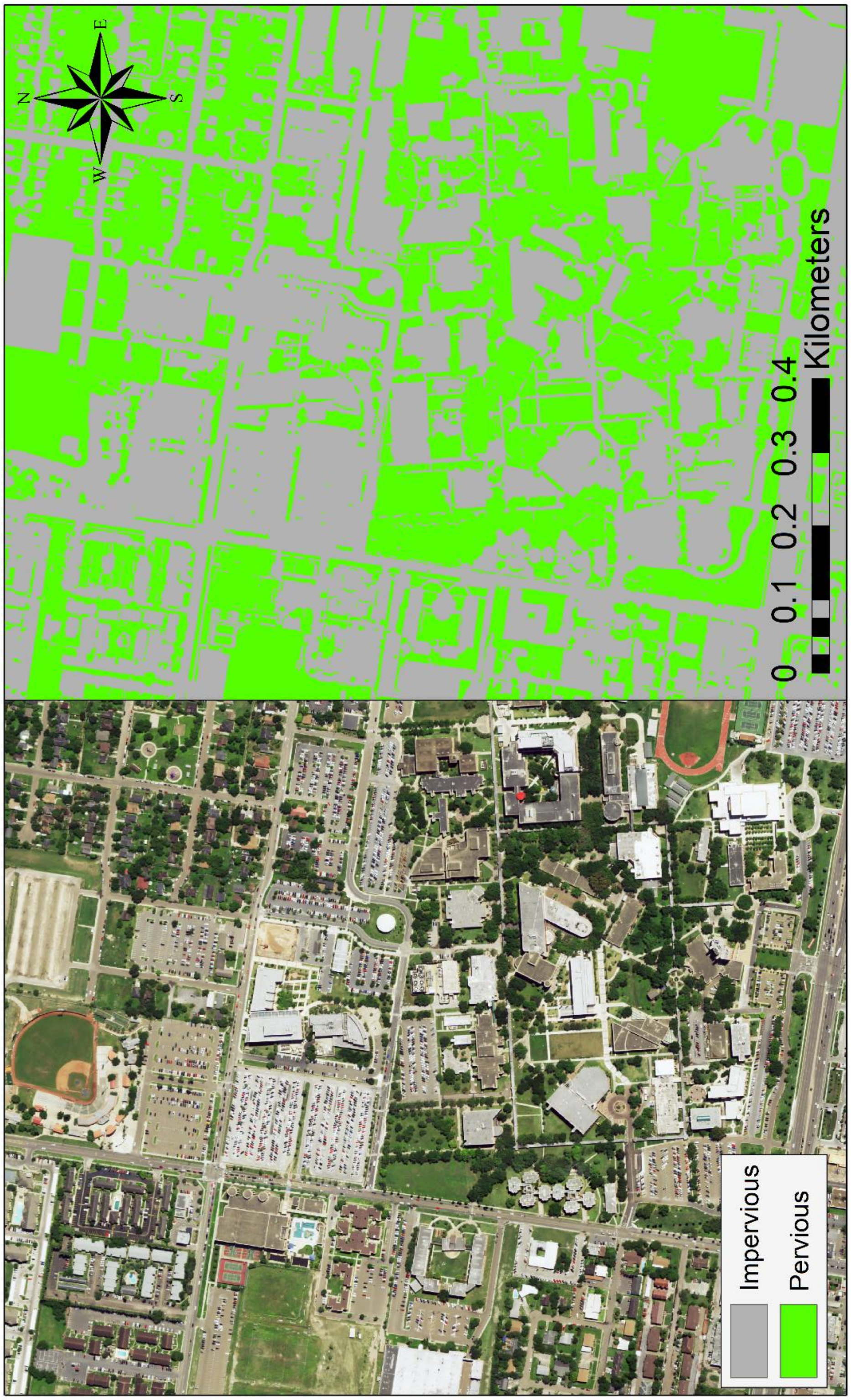

Figure 3.4 Predictive image of pervious and impervious surfaces generated by the model on ArcMap 10.2.2. (above) compared to satellite imagery (below). Pervious surfaces represented by green and impervious surfaces represented by gray.

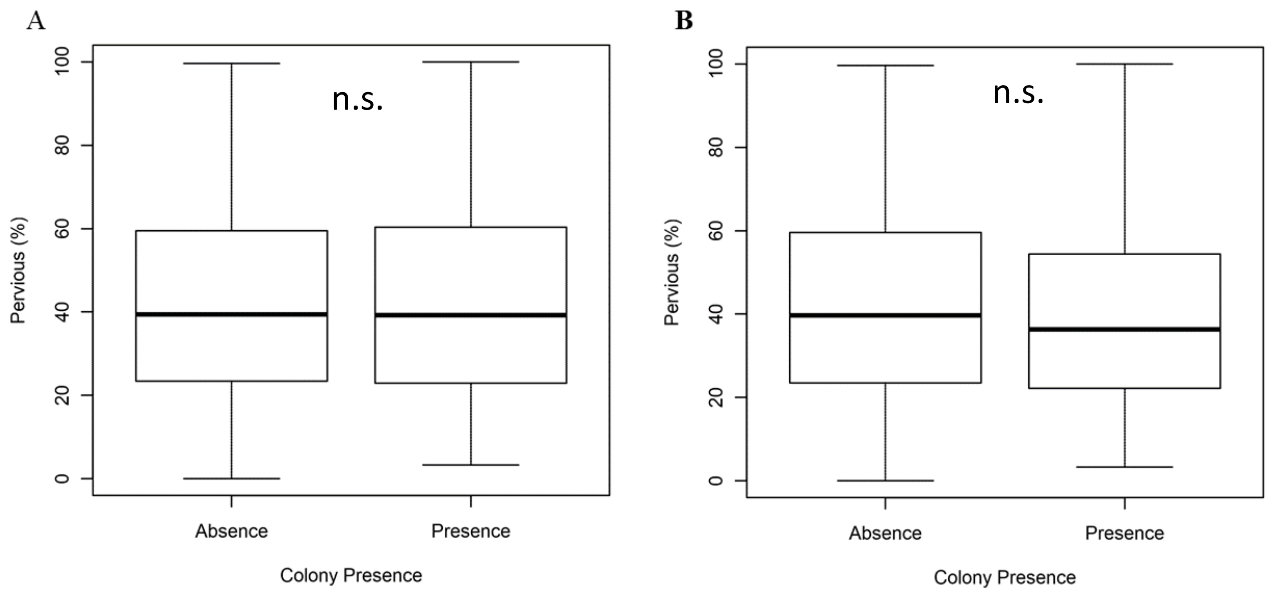

Figure 3.5 Box plot comparing the percentage of pervious surface of areas with and without harvester ant colonies in A) 2020 (W = 5698, p-value = 0.492) and B) 2021 (W = 6241, p-value = 0.9418).

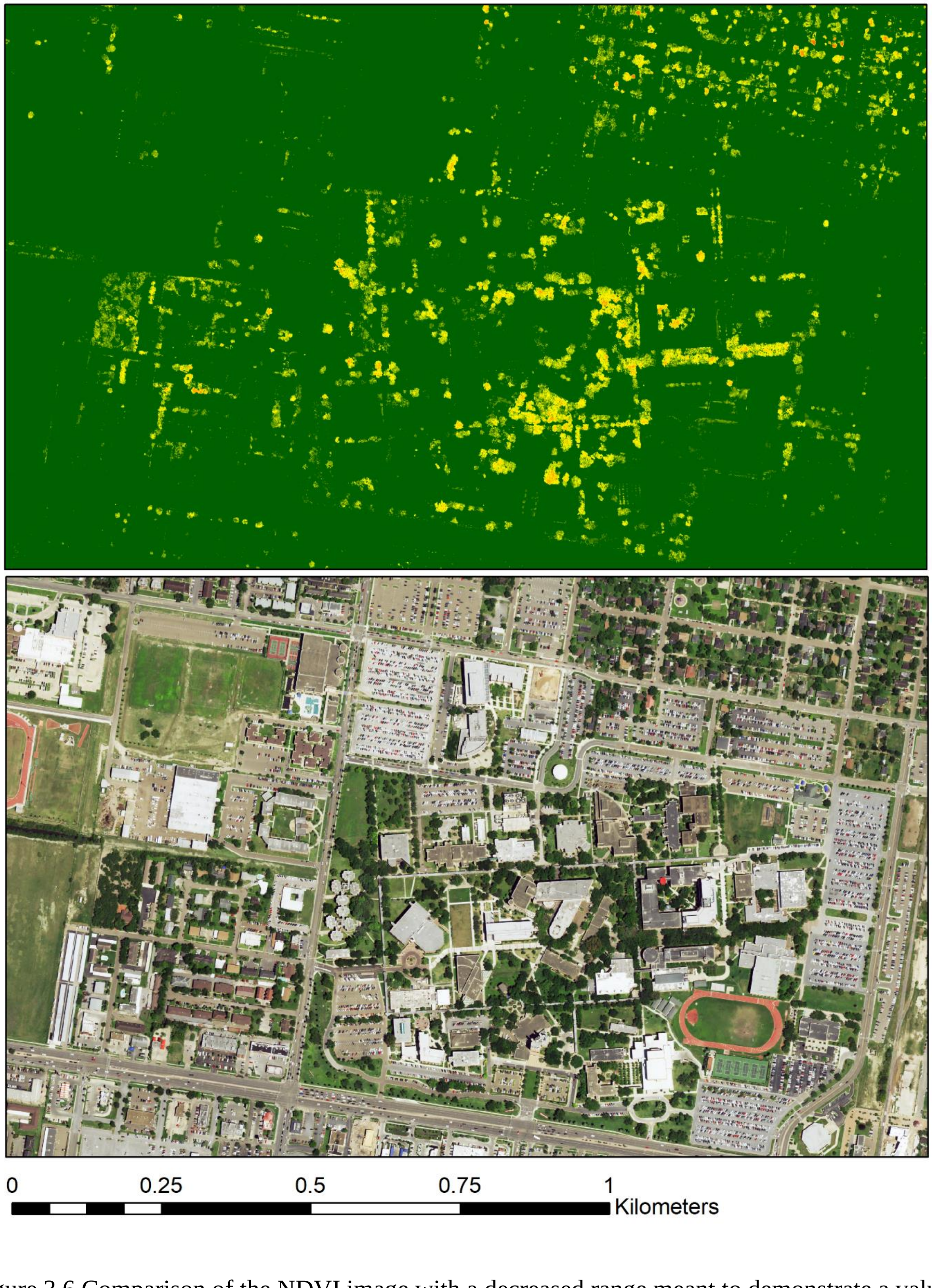

Figure 3.6 Comparison of the NDVI image with a decreased range meant to demonstrate a value where tree canopy cover is most visible (above) and the satellite image used to create it (below).

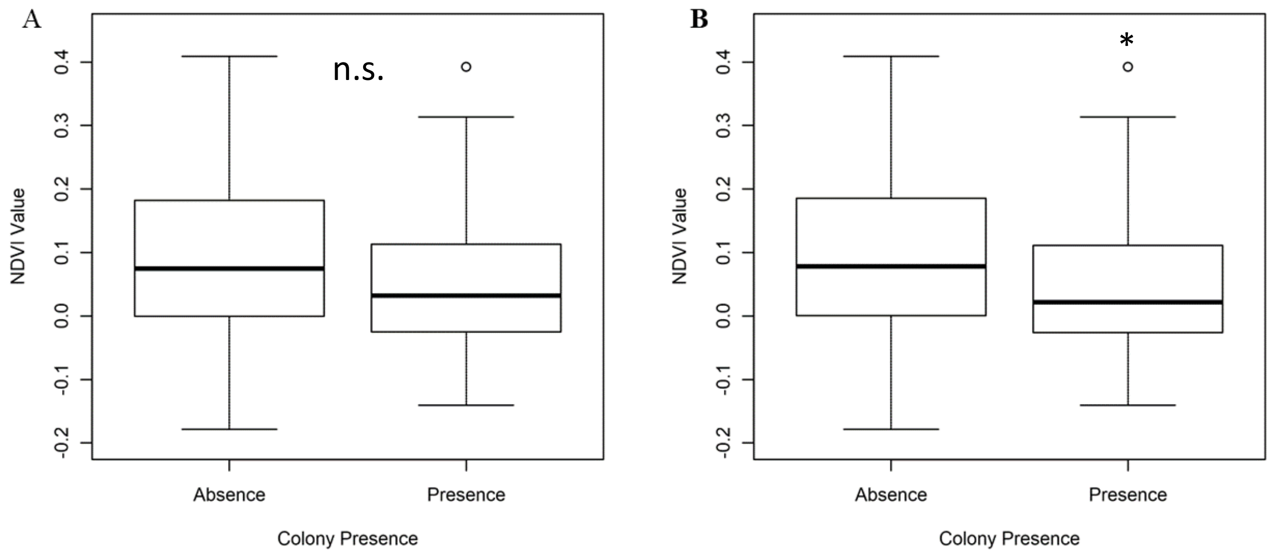

Figure 3.7 Box plot comparing the NDVI value average of areas with and without harvester ant colonies in A) 2020 and B) 2021. There were no significant differences in 2020 (W = 6993, p-value = 0.060) but there was a significant difference seen in 2021 (W = 7459, p-value = 0.02163).

CHAPTER IV

CONCLUSION

This research was conducted in part to learn more about conclusions we can draw from preferences harvester ants have within commonly used cover crop seed varieties in the LRGV and if amendments to the seeds can alter those predetermined preferences. Another objective of the study was to determine some characteristics associated with harvester ant colony establishment in order to provide recommendations to land managers on how to reduce the likelihood of colony establishment.

In CHAPTER II, Harvester ants demonstrated significant preferences to wheatgrass, oat, and radish. Nitrogen fixing varieties like sunn hemp and vetch were typically the last to be removed from the seed depot, if at all. Not only that, but we also found that inoculating preferred seed varieties like wheatgrass and radish with *Bradyrhizobia sp.* did not deter or encourage harvester ants to consume the seeds. With this information, recommending less preferred nitrogen fixing varieties, where inoculation can help with nitrogen sequestering, could be a beneficial option for farmers interested in cover cropping in between seasons without varied topsoil preservation due to harvester ant interference. Sunn hemp in particular is already popular in states like Florida and has the potential to be a great cover crop in the LRGV. The fact that it is not preferred by harvester ants makes it more valuable than before.

In CHAPTER III, established harvester ant colonies in suburban areas tended to cluster on significantly elevated area than their surroundings with significantly less tree cover. These highly elevated, sunny areas in a suburban setting make the likelihood of surviving a flood much higher than they would in a lower elevated, covered area. Encouraging land managers to plant native trees in suburban areas can help conserve the soil moisture in their land for a longer period of time, also requiring less water to maintain a lawn. Alternatively, planting other native, drought tolerant bushes and/or pollinator friendly plants such as milkweed can take up space that otherwise would have been inhabited by grasses, while promoting other beneficial insects like Monarch butterflies.

The beneficial services harvester ants provide have been overlooked in favor of focusing on their behavior. Information from the study brought valuable questions that can be further pursued by others who are interested in learning more about these interactions under different environments.

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