Cover Crop Seed preferences among harvester ants in the Lower Rio Grande Valley

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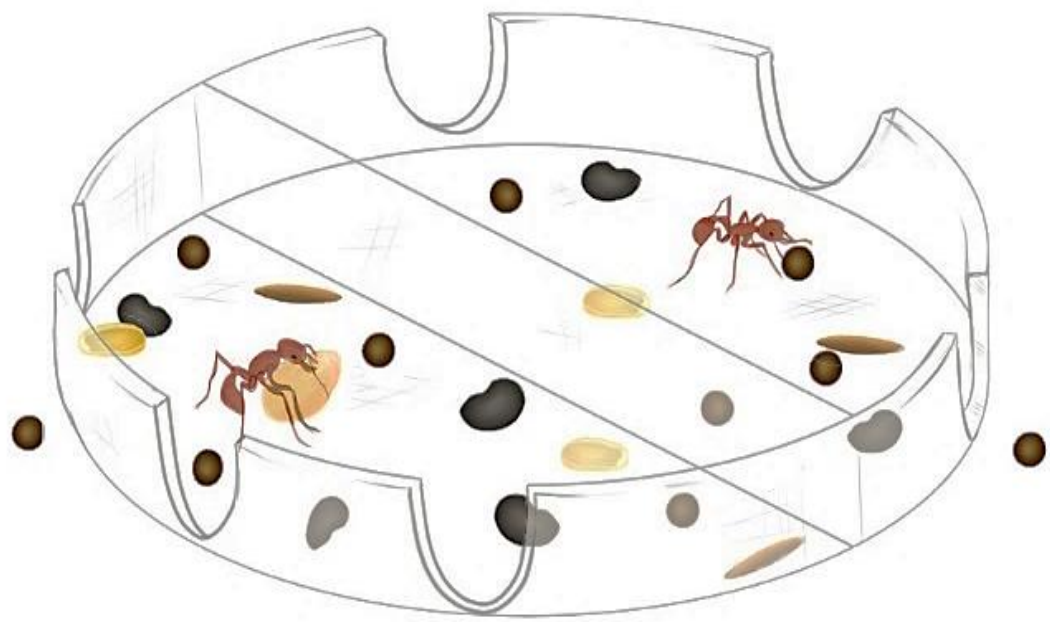

1. Abstract

Harvester ants (Genus Pogonomyrmex) are known to forage seeds up to 25m away from the colony to store in their underground granaries. Though quick, harvester ants can be fastidious in their seed selection for consumption – and will collect as many favored seeds possible. This study aims to determine if local red harvester ants prefer specific varieties of cover crop seeds commonly used in the Lower Rio Grande Valley. If there is a strong preference by the Harvester Ants for a commonly used cover crop seed, it could prove detrimental to farmers that are trying to prevent topsoil erosion. To determine this, red harvester ant colonies around UTRGV with no prior exposure to cover crop seeds were chosen as test subjects. Trials were run with groups of 9 colonies (minimum of 10m apart) for a cafeteria study where several seed varieties were tested, wheatgrass and radish being preferred among the rest. To collect data on which seeds they were taking, we categorized 10 seeds of each variety into Petri dishes placed 2m from the entrance of the colony along a foraging trail. All Petri dishes were placed within a wire cage to protect the seeds from vertebrates that could interfere with data collection. 2 more seed studies were conducted with amendments to see if alteration can change survivability of the seeds in the petri dish.

Introduction/Background

Pogonomyrmex ants, measuring up to a centimeter in length and part of the New World genus, are common inhibitors of the arid to semiarid regions in the United States. Seed feeders and occasional corpse scavengers, these ants are most notably known for the bare disks surrounding the entrance to their colony of ~10,000 individuals, a relatively small number in comparison to other ant species. They remove vegetation from the entrance to warm their colony. In comparison with grass, bare soil absorbs much more heat. With hairs protruding on the underside of their head, these ants use these “beards” to excavate tunnels from 1 to 10 meters in diameter with a depth of as much as 5m into the dry soil (Reed & Landolt, 2019). Harvester ant wise, south Texas would likely attract harvester ant species of *Po. rugosus* and *Po. barbatus*, who are attracted to soils of higher clay contents likely due to a longer-lasting moisture availability after rains. Moisture is very important to ants and aids in anywhere from colony foundation to nuptial flights (Johnson, 2001). What comes as a requirement also can be detrimental if too much. Though moist soil is a necessity, like all things, too much of a good thing is bad. Harvester ants can drown if the soil moisture content is high especially since they’re prone to arid/semi-arid regions. This might be a reason as to why they have been observed to avoid areas of UTRGV with a high density of sprinklers and instead concentrate in open grass areas that aren’t prone to having a large sprinkler system in place.

In Texas, there is one major predator of harvester ants: the Texas Horned Lizard, *Phrynosoma cornutum*. Of insects and spiders, 65% of the threatened Texas horned lizards’ diet consists of Pogonomyrmex ants. Their numbers are declining due to food and habitat loss (Davis & Parks, 2012). To protect themselves from predation the ants cease foraging activities and hide in the colony until the lizard moves on, sometimes even plugging up the entrance to the colony (Johnson, 2001). Protection of this lizard requires protection of its food source as well, and with pesticides eradicating harvester populations in agricultural areas, as well as invasive species there’s no surprise of the population’s decline. Harvester ants aren’t only targeted in agricultural settings, but in urban areas as well. The bare disks that result from their presence interfere with the popular ‘clean lawn’ homeowners prefer, causing them to specifically be targeted. If not specifically targeted, generic insecticides that suburban residents use against fire ants also kill off harvester ant. This population decline in harvester ants in both urban and agricultural settings can directly affect the population of Texas Horned Lizards through urbanization and lack of prey due to harvesters’ ongoing competition with invasive fire ants (Henke & Fair, 2019).

The relationship between Harvester ants and Invasive Fire Ants in Texas would be competitive (Davis & Parks, 2012). 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, and roots. This overlap in consumption preferences makes them deadly competition for Harvester Ants. They’re very aggressive and attack any intruders in comparison to the slower moving, 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).

Harvester ants can also do good for their surrounding ecosystem. They 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 a lot of displacement of seeds and loss of potential vegetation growth, in turn bringing the seeds to the colony for consumption and returning to the soil via feces. They also increase soil health by aeration via tunnels, increase total N, and decrease pH in their colony over time. Roots of vegetation outside the disk can absorb the available nutrients in the soil and enhance plant growth. These disturbances within the soil also increase microbial diversity due to the relocation of settled microorganisms and making space for new ones. Though they play a small impact in their surrounding environment, they're still a valid food source for a Texas species and should be studied more to ethically push them out of agricultural areas and towards native brushlands they can thrive in without disturbance.

Harvester ants can be heavily driven by preference if given a choice. They do not hesitate to empty already full seed depots if there is a sudden abundance of a preferred seed. In this experiment, we are looking at preventing harvester ant predation in agricultural areas. Knowing preference types within a pool of cover crop seeds. Removal of these cover crop seeds removes the protective barrier grown in the form of above ground biomass and below ground root systems. These are to protect nutritious topsoil from external forces like erosion in the forms of wind and water.

Do harvester ants have significant preferences between different seed types? How will a survival analysis properly compare preferences between different types of seeds if there is any? A survival analysis demonstrates length until 'death', though mostly used in life or death scenarios including ill patients and their mortality rate, we can apply this same principle to cover crop seeds and count their 'death' as an ant taking it into the colony for consumption. In figure 1, the graph demonstrates a visually similar curve depicting average mortality of vetch seeds over time. The final data will include other seed varieties, error bars, and comparisons of significances of differences over time.

If these preferences trials prove successful, more studies can be conducted by comparing 'disliked' seeds with common native seeds to see where the preferences lie in that instance. If native seeds prove to be preferred, this disliked seed could be the standard recommendation to Valley farmers who are looking to turn to alternative methods of avoiding harvester ant predation (this will serve well for organic farms who cannot use pesticides against pests).

Methodology

Following the methodology from https://pub.towardsai.net/survival-analysis-with-python-tutorial-how-what-when-and-why-19a5cfb3c312

The lifelines package will allow us to A Kaplan-Meier plot and a Log Rank test are going to be made to see the effect of seed type on survival. Some cages/dishes were compromised in the process of the study during certain trials. Under these circumstances, since the 'event' had not yet occurred we will censor that colony within that trial.

Once the excel sheet was converted from.xlsx to .csv (comma-deliminated), we upload the sheet to Jupyter notebook where we will be able to manipulate the data using Python. Before adding the .csv into the sheet, we imported pandas, numpy, matplotlib.pyplot. and the KaplanMeierFitter from the lifelines package we installed. After removing all the empty variables from the dataset (.dropna()), we were able to proceed with the next step.

New columns were created in the sheet named ‘dead’ where values within our data column that equate to 0 (or no seeds in dish of that variety) will be valued as ‘1’ in the ‘dead’ column while any other value that is not zero will equate to ‘0’. Below is a portion of the code used for radish, to see how this relabeling of the new column proceeded.

```
ant.loc[radish == 10, 'dead'] = 1
ant.loc[radish != 10, 'dead'] = 0
```

We created an object for the Kaplan-Meier-Fitter for each sample we were going to include in the estimate. For the first sample the following was typed out to determine the duration of the experiment and the event observed at the point in time. Our “Time point” column, depicting the time in which counts of the seeds were conducted, was renamed to ‘durations’.

```
kmf_r.fit (durations = ant['Time point'], event_observed = ant['dead'])
```

With this we were able to run a line of code to determine the likelihood of survivability for the sample (seed) at the point in time. In the code the likelihood of survival is determined by at risk of the event (determined by the data supplied) minus what is observed divided by the risk of the event. With this data you can see the probability of survival of the seed type over time.

```
event_0 = kmf_r.event_table.iloc[0,:]

surv_for_0 = (event_0.at_risk - event_0.observed)/event_0.at_risk
```

This methodology was repeated for all the samples we wanted to plot alongside one another and compare in a plot. Extra information was included including graph size, x and y axis labels, and legend axis labels.

```
f = plt.figure()
f.set_figwidth(14)
f.set_figheight(10)

kmf_v.plot(ci_show = False, label = 'Vetch')
kmf_s.plot(ci_show = False, label = 'Sunnhemp')
kmf_r.plot(ci_show = False, label = 'Radish')
kmf_f.plot(ci_show = False, label = 'Fescue')
kmf_o.plot(ci_show = False, label = 'Oat')
kmf_w.plot(ci_show = False, label = 'Wheatgrass')

plt.title("Seed Preference Trials")
plt.xlabel('Time (hours)')
plt.ylabel('Seeds taken')

plt.legend()
```

These lines of code gave us figure 1 below.

Results

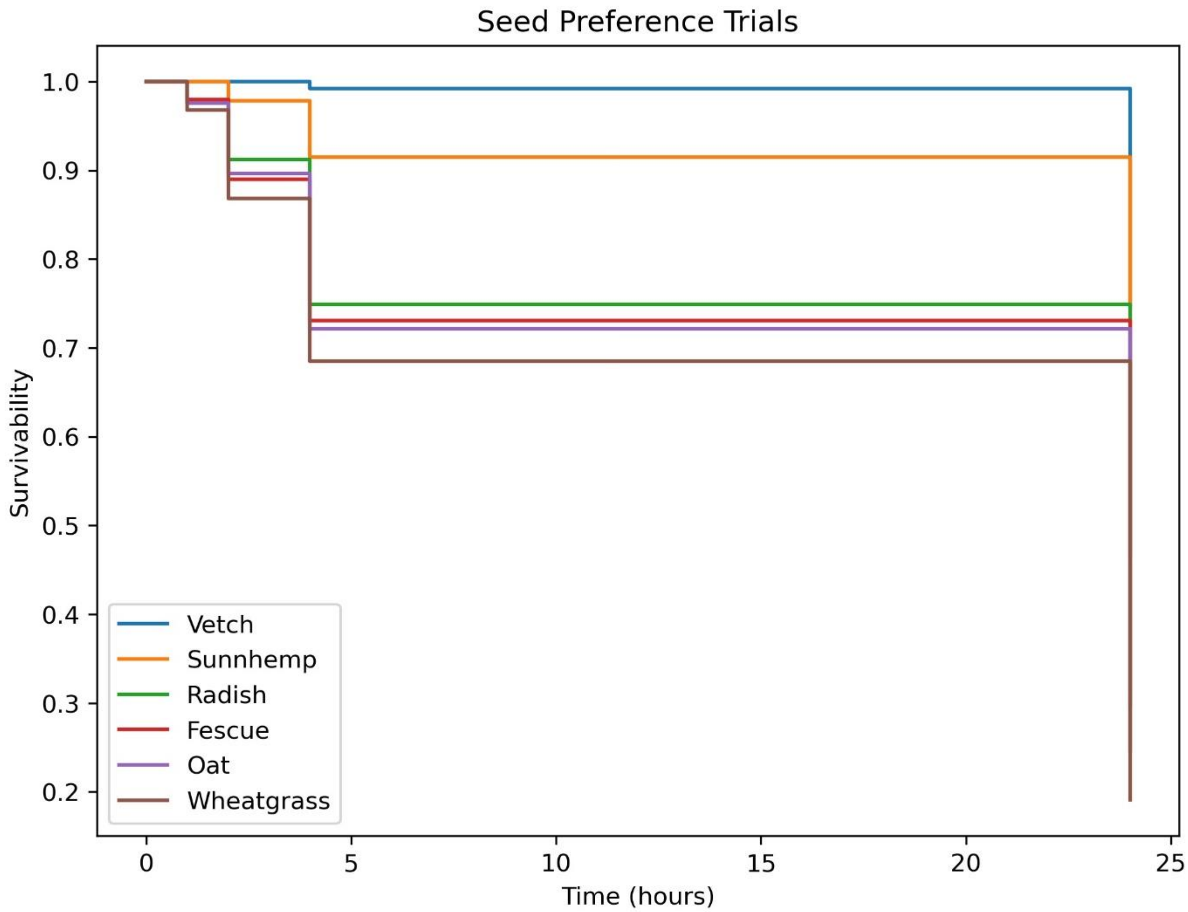

Figure 1. Kaplan Meier Graph of seed types' likelihood of survival over the course of the trial based off selected data. n = 37.

Visually in figure 1, the largest difference seemed to occur between Sunnhemp/ Vetch vs. the other four seed varieties. In order to see whether this difference was significant, we ran a Log Rank Test to compare the seeds' survivabilty against one another. Below is the significance between Wheatgrass and Vetch, the two visually farthest samples in the trial (Table 1).

Table 1. Log Rank Test to determine the statistical significance between survival likelihood of Vetch and Wheatgrass seeds. p < 0.005

test_statistic	p	log2(p)
81.19	<0.005	62.08

The log rank test is an hypothesis test that compares the survival distribution between two samples. The point of the test is to reject the null hypothesis of: "These two survival distributions are not significantly different from one another". Similar to an ANOVA test, this is specific to survival distributions in the lifelines package. So a p-value < 0.05 (5%) is significant. In table 1, we see that there was a significant difference between the survival distributions, meaning there was a significant difference in preference between those seed types. We can determine ant's can have a significant preference difference between seed types, and its visible between Vetch/Sunnhemp and the other four seed types (Oat, Wheatgrass, Radish, and Fescue). Now we move to the question of whether making physical changes to the seed affects the desirability to harvester ants.

We decided inoculating the seed with nitrogen fixing bacteria might alter the opinion of harvester ants. Some ants are extremely particular about which seeds they bring into their underground granaries, going as far to clean each individual seed prior to introducing it to the rest of the stock. Some ants periodically scrape through their stock to remove 'sick' or germinating seeds, our thought process including whether harvester ants were as particular about cleanliness of their seeds as they are about seed types.

The data includes inoculated and uninoculated versions of the two preferred seed types from the seed preference trials: Radish and Wheatgrass. Surprisingly, there didn't seem to be a difference in preference between inoculated and uninoculated seed, and instead were slightly differenciated by seed type rather than amendment.

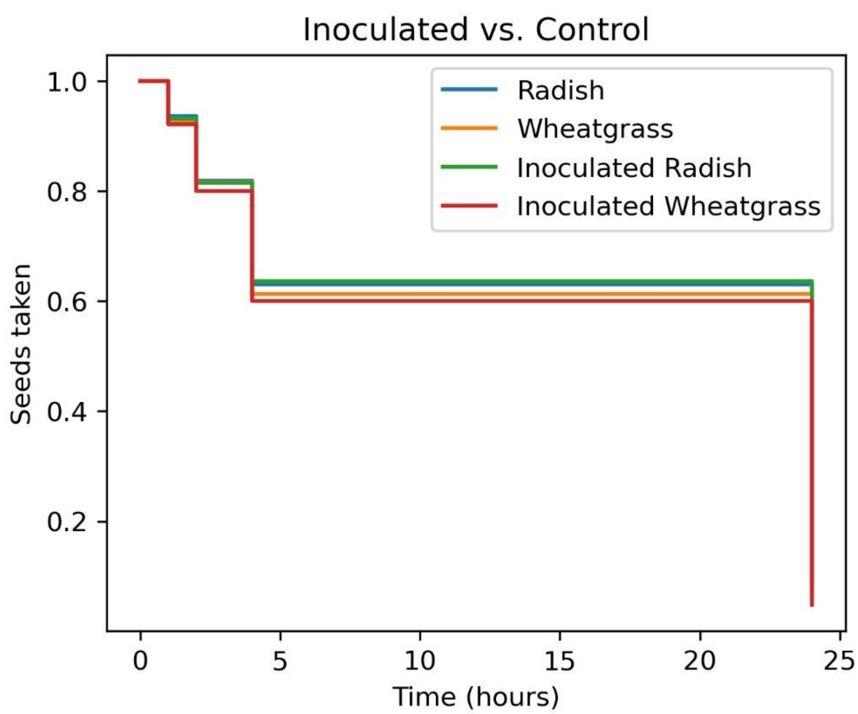

Figure 2 Kaplan Meier Graph of seed groups' (inoculated vs. control) likelihood of survival over the course of the trial based off selected data.

Below is the table determining whether there was a significant preference for the inoculated seeds vs. the control. Unsurprisingly (given the lack of visual difference), there was no significant difference between radish vs. inoculated radish or wheatgrass vs. inoculated wheatgrass.

Table 2Using a Log Rank test, we were able to determine there was no significant difference of survival rates between inoculated radish and uninoculated radish seeds.

test_statistic	p	log2(p)
0.33	0.6	0.82

So, coating the seeds in bacteria didn't change their preference for them. How would harvester ants react to a legitimate diseased seed? Dr. Choudhury collected diseased seed heads and healthy seeds from a local sourgum field. We used these for the diseased seed trials. Given harvester ants didn't statistically mind bacterial coated seeds, how would they feel about disease? If they prefer them to healthy seeds, could they become allies to farmers in removing disease from fields? Or further pests by removing healthy seeds and fascilitating disease distribution?

Below is the results for the Kaplan Meier graph for the diseased seed trials (figure 2).

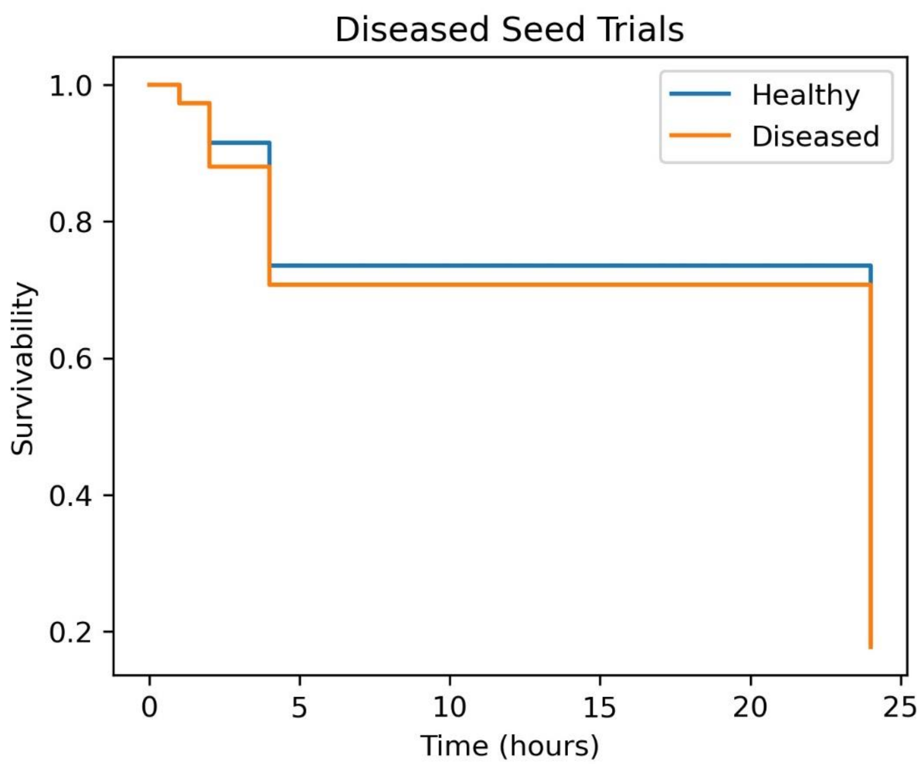

Figure 3. Kaplan Meier Graph of seed groups' (diseased vs healthy) likelihood of survival over the course of the trial based off selected data.

Figure 3 shows a slight preference for diseased seeds over healthy seeds, but table 3 says there is no signficicant preference between the two samples. Meaning the presence of disease in the sourgum seed does not change it's

Table 3. Using a Log Rank test, we were able to determine there was no significant difference of survival rates between healthy and unhealthy seeds.

test_statistic	p	-log2(p)
0.19	0.66	0.6

Conclusions

In conclusion, though there is significant preference in some seed types over others, inoculating these seeds of presence of disease in a seed doesn't change the preference in the seed type to harvester ants.

We can make recommendations to local farmers regarding planting Vetch seeds for cover crops but given this was a cafeteria study and not a no-choice option, there is no certainty that harvester ants will not choose the vetch seeds over native seeds. Alternatively, if their feelings for Vetch seeds haven't changed in comparison to their usual diet of native seeds (though it was not preferred to other grasses in the cover crop pool so this may be likely), we cannot rule out that they won't react negatively and look elsewhere for more food variety. All we can conclude is that harvester ants have seed preferences and given more trials and analysis we can draw further conclusions on how to prevent their predation on cover crop seeds via practice recommendations to local farmers.

Timeline

April 4th - 9th, 2021: Complete and submit proposal. Search for aid in using Jupyter Notebook to run analysis. Write down methodology to run a survival analysis using Python via the lifelines package.

April 12th -16th, 2021: Begin analysis with current preference data on hand, organize to ease process of survival analysis.

April 19th- 23rd, 2021: Complete draft figures and evidence to support or disprove hypothesis. Begin first draft of analysis explanation and its effects.

April 26th- 29th, 2021: Complete final figures, ensure written portion is done properly, insert graphs and reference them properly throughout the text.

Citations

Davis, J. M., & Parks, T. (n.d.). *Management of the Red Harvester Ant*. 9.

Henke, S. E., & Fair, W. S. (n.d.). *MANAGEMENT OF TEXAS HORNED LIZARDS*. 8.

Hook, A. W., & Porter, S. D. (1990). Destruction of Harvester Ant Colonies by Invading Fire Ants in South-Central Texas (Hymenoptera: Formicidae). *The Southwestern Naturalist*, *35*(4), 477–478. https://doi.org/10.2307/3672056

Johnson, R. A. (2001). Biogeography and Community Structure of North American Seed-Harvester Ants. *Annual Review of Entomology*, *46*(1), 1–29. https://doi.org/10.1146/annurev.ento.46.1.1

Reed, H. C., & Landolt, P. J. (2019). Chapter 22—Ants, Wasps, and Bees (Hymenoptera). In G. R. Mullen & L. A. Durden (Eds.), *Medical and Veterinary Entomology (Third Edition)* (pp. 459–488). Academic Press. https://doi.org/10.1016/B978-0-12-814043-7.00022-4

Team T. (2021, March 16). Survival analysis with python tutorial - how, what, when, and why. Retrieved May 05, 2021, from https://pub.towardsai.net/survival-analysis-with-python-tutorial-how-what-when-and-why-19a5cfb3c312