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Red Harvester Ant (*Pogonomyrmex barbatus* F. Smith; Hymenoptera: Formicidae) Preference for Cover Crop Seeds in South Texas

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Abstract: Harvester ants often selectively forage seeds, causing these ants to be viewed as pests in agricultural areas where they may forage on crop seeds. While little research has been done on harvester ant preferences for cover crop seeds, grower observations in the Lower Rio Grande Valley (LRGV) suggest that ants may remove these seeds before germination. We examined red harvester ant (*Pogonomyrmex barbatus* F. Smith) preferences for cover crop seeds (fescue, oat, sunn hemp, radish, vetch, and wheatgrass) and the effects of a commonly used bacterial seed inoculant. We evaluated relative preferences using seed depots presented to colonies with no prior exposure to the selected seeds or inoculants. After 24 h, ants had removed oat and radish seeds at the same rate as the preferred wheatgrass control. Fescue, sunn hemp, and vetch seeds were less preferred. The bacterial inoculation of wheatgrass and radish seeds did not alter the removal rates. Further, ant removal of seeds in both trials was dependent on the month and temperature, indicating potential interactions of colony activity levels, availability of seeds in the seed bank, and the intensity of cover crop seed removal. Together, these data indicate that harvester ant foraging preferences and seasonal activity should be considered to help mitigate potential ant predation of cover crops via planting less preferred seeds and at times of lower ant foraging intensity.

Keywords: bacterial inoculum; Lower Rio Grande Valley; *Pogonomyrmex barbatus*; seed depot study; seed preference

1. Introduction

Harvester ants in the genus *Pogonomyrmex* (Hymenoptera: Formicidae: Myrmicinae) commonly reside in arid to semi-arid regions of the Americas and can be found in a wide range of natural areas covered by native vegetation and human use landscapes composed of agricultural and human settlement areas [1–3]. The state of Texas has several species of harvester ants, with the red harvester ant (*Pogonomyrmex barbatus* F. Smith (Hymenoptera: Formicidae: Myrmicinae)) being one of the most common in anthropogenically disturbed habitats of the Lower Rio Grande Valley (LRGV), an agriculturally rich region with a semi-arid subtropical climate [4–8]. Harvester ant foraging occurs primarily along trails that extend from the colony to neighboring food sources within their foraging range [9,10]. While foraging trails average 10 m long, colony-dense areas (which may have up to 80 colonies/ha) can have trails extending up to 60 m from the nest site [11,12].

Harvester ants, primarily granivores, use foraging trails to collect seeds located on the soil surface, often from or surrounding the parent plant [10,13]. Harvester ant species, such as *P. barbatus*, *P. rugosus* Emery, *P. occidentalis* (Cresson), and *P. salinus* Olsen, tend to harvest

near the trunk of their foraging trails which are, in turn, shaped by seed distribution and availability, disturbance frequency, and inter/intra-species interactions [1,9,14,15]. Harvester ants exhibit seed preferences based on a combination of relative seed abundance, size/shape, and nutritional content of the seeds [1,9,16]. For instance, *P. occidentalis* prefers to forage with high plant species fidelity in seed-dense patches, which can reduce local seed bank heterogeneity and plant community composition [1,17,18]. When the seed bank has low seed availability, ants may collect less preferred seeds until more desirable seeds are present [1,19].

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 remove weed seeds, their seed preferences may also include the consumption of crop seeds [20–24]. Ant removal of crop seeds and vegetation may cause economic loss, especially if the crop is situated within areas of high colony density, prompting control measures by some land managers [2,11,25–29]. Red harvester ants (*P. barbatus*) in particular are commonly found in agricultural areas in the LRGV (personal observations within and adjacent to active row crop fields and pastures) and may have a large impact on the local plant community through the removal of vegetation surrounding their nest entrance (1–5 m in diameter) and through seed collection [1,11,30].

In addition to cash crops, harvester ants in agricultural fields may have the opportunity to forage on cover crop seeds. In the LRGV, cover crops are used in row crop fields during fallow periods to prevent soil erosion [5,31–33]. While little research has been done on this, growers in the LRGV have anecdotally reported ant removal of cover crop seeds. Ants may preferentially forage for cover crop seeds before these seeds have had time to germinate, particularly during times of the year when the seed bank has few alternative options. The lack of a root system and above-ground vegetation in the foraged field area can then potentially increase economic loss for the farmer [5,31,32]. Therefore, preventing harvester ant interference with sown seeds by determining ant preferences for commonly used 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 the relative preferences of red harvester ants for commonly used cover crop seeds in the LRGV. Once this is established, we can determine which seeds are most at risk of ant predation and preferentially use different cover crops to prevent the need for farmers to re-seed fields. Seeds belonging to several plant families—Brassicaceae (radish, *Raphanus sativus* L. (Brassicales: Brassicaceae)), Fabaceae (hairy vetch, *Vicia villosa* Roth (Fabales: Fabaceae); sunn hemp, *Crotalaria juncea* L. (Fabales: Fabaceae), and Poaceae (oat, *Avena sativa* L. (Poales: Poaceae)); fescue, *Festuca arundinacea* Schreb. (Poales: Poaceae))—and that are currently being evaluated by farmers in the LRGV, were selected. Wheatgrass (Poaceae: *Triticum aestivum* L. (Poales: Poaceae)) was also included as a known preferred food for harvester ants in more naturalized systems and served as a control for the experiments [34,35]. Based on previously established harvester ant preferences for grasses, we hypothesized that cover crop seeds in the Poaceae family—oat, fescue, and wheatgrass—would be the most preferred [1,10,36,37].

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 [38,39]. Such treatments may influence ant foraging decisions as ants can discern the presence of microorganisms, such as endophytic fungi in seeds [40]. Alternatively, ants may not exhibit any aversion to bacterial inoculation of seeds, since environmental pathogens do not always alter ant foraging and ants may themselves be able to alter communities of microorganisms on seeds and in nest sites [41–44]. Therefore, the second objective was to determine if bacterial seed inoculation treatments would alter the previously established cover crop seed preferences. Based on prior work establishing that other groups of ants preferentially forage on pathogen-free seeds [40], we hypothesized that the ants would be deterred from foraging on previously preferred cover crop seeds coated in bacterial

inoculation treatments. If such inoculation treatments deter ant foraging, then growers would have another method to prevent ant removal of seeds, regardless of the seed type.

2. Materials and Methods

2.1. Site Description

The study site was located within the Lower Rio Grande Valley in South Texas, USA. This area is considered a local steppe climate that is a subtropical subhumid marine with an average temperature of 24 °C (16.3–30.2 °C) and 572 mm of precipitation annually [45]. 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 [46–48]. Land use within the LRGV includes developed urban and suburban areas, shrubland, grass/pasture/hay, as well as assorted agricultural production, including annual row crops, fruit and vegetable crops, and sugarcane production [6].

Specifically, all trials were conducted at the University of Texas at Rio Grande Valley (UTRGV) campus (~150 ha) in Edinburg, Hidalgo County, TX, USA (26°18′24.0″ N, 98°10′15.4″ W). At the site, most vegetation included grasses used for lawns intermixed with weeds (primarily grasses) and punctuated by ornamental plants (such as *Asclepias curassavica* L. (Gentianales: Apocynaceae) and *Lantana* sp. L. (Lamiales: Verbenaceae)). As at the time of the publication of the *2020 Tree Campus USA Report*, there were 53 different species of trees present with live oak (*Quercus virginiana* Mill. (Fagales: Fagaceae)), Texas ebony (*Ebenopsis ebano* (Berland.), Barneby and J.W.Grimes (Fabales: Fabaceae)), and honey mesquite (*Prosopis glandulosa* Torr. (Fabales: Fabaceae)) being the most common [49]. The immediate land use surrounding the study site is considered to be a combination of suburban and peri-urban with intermixed sorghum fields, pasture, and citrus groves.

This site was selected since it contained many red harvester colonies (Figure 1), all of which had no prior exposure to the species of cover crop seeds presented during the trials but that would still experience frequent disturbance pressures, such as irrigation and routine mowing (a proxy for agricultural disturbance relative to natural settings). In January 2020, all *P. barbatus* colonies at the site (*n*~120) were mapped 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. Nest disc diameter was measured (Figure S1) as an indicator of the colony size. A subset of active, established colonies without overlapping foraging ranges and not likely to be disturbed by passersby (*n* = 37) was used for all trial sets described below.

2.2. Seed Preference Trials

Ant cover crop seed preferences were determined using a choice test with seed depots. Seed depots (see Appendix A for depot design optimization) were constructed out of plastic I-plate Petri dishes (100 × 15 mm) (Figure 2A). All dishes were hand sanded on all surfaces to produce a rough surface to increase traction and facilitate ant movement. Additionally, 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.

For the preference trial, the selected cover crop seeds—fescue (GreenCover, Bladen, NE, USA), oat (Johnny's Selected Seeds, Winslow, ME), radish (Johnny's Selected Seeds, Winslow, ME), sunn hemp (Johnny's Selected Seeds, Winslow, ME, USA), vetch (Johnny's Selected Seeds, Winslow, ME, USA), and wheatgrass (Todd's Seeds, Livonia, MI, USA)— were pre-counted in groups of 10 seeds per cover crop and stored in microcentrifuge tubes at room temperature before transport to the field. Further, to determine whether size differences among seeds could impact ant preference, 50 seeds of each type were randomly selected and weighed with seed texture, noted for later comparison.
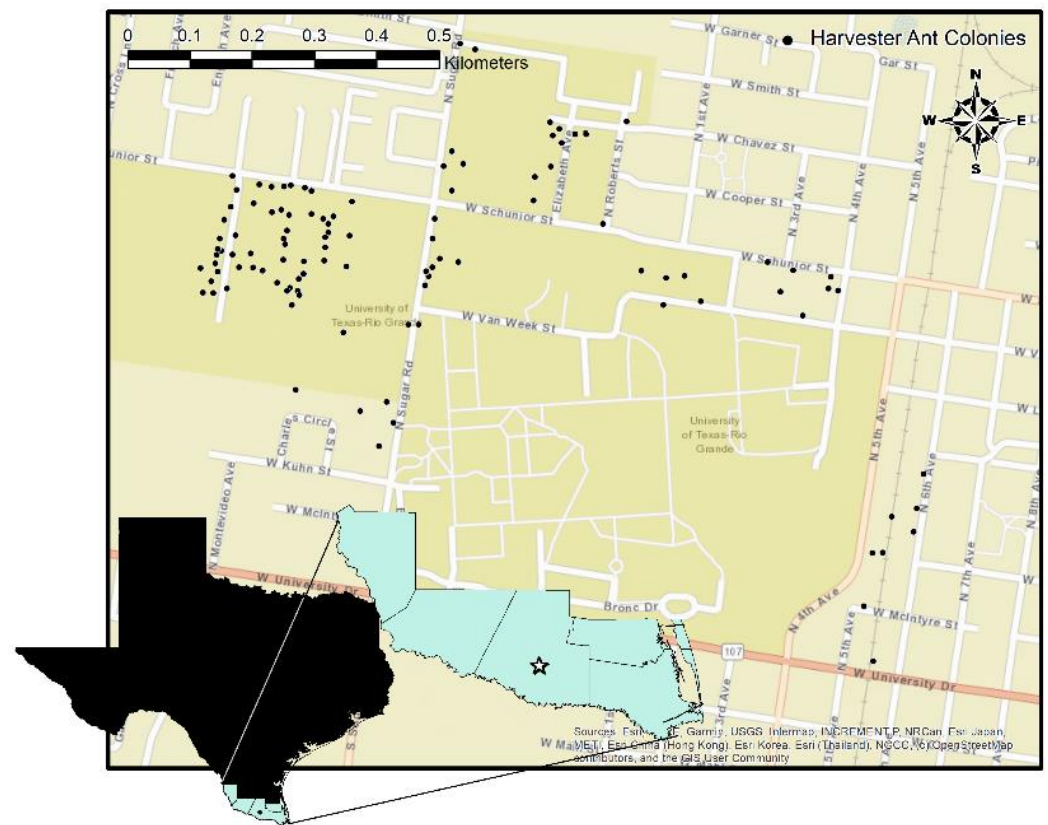
Figure 1. Map of *Pogonomyrmex barbatus* F. Smith (Hymenoptera: Formicidae: Myrmicinae) nest sites (represented by black dots) within the study area located in Edinburg, TX, USA (yellow star).
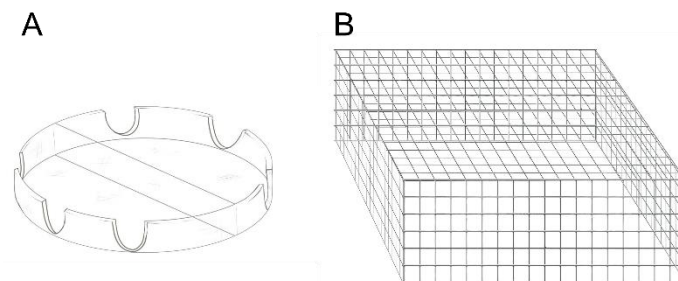
Figure 2. Diagrams of (**A**) the I-plate Petri dish used as the seed depot modified with entrances; and (**B**) the depot cage constructed from wire to protect seeds from larger granivores.

The seed depot was placed 2 m from the nest entrance along the primary foraging trail, with seed depot entrances facing the foraging trail (see Appendix B for the optimization of the depot placement). Upon initiation of each trial, a mix of all seed types (with 10 seeds per cover crop) was placed into a depot, with the same number of seeds per type per side of the Petri dish. After the addition of the seeds, cages (1 × 1 cm hardware cloth (Everbilt, The Home Depot, Atlanta, GA, USA) shaped into a 23 × 23 cm square, Figure 2B) were placed over the depots and secured into the ground with 3 cm fence staples to prevent vertebrate removal of the seeds [50–52].

Seed removal was documented at intervals of 1, 2, 4, and 24 h. During each inspection, temperature, wind speed, and estimated cloud cover were measured and the seeds within and outside of the depots were counted. Seed preference trials were conducted continuously from the beginning of February 2020 through to the end of June 2020 in groups of 8–10 colonies per observation period. The tested colonies were a minimum of 10 m apart to prevent an 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 activity (temperature) but to minimize the risk of the wind overturning the seed depots. Depots were cleaned to remove ant trail pheromones in between use. In total, 34 unique colonies were tested (initially we observed 37 colonies but 3 were excluded due to external interference), with 28 of these colonies tested twice for a total of 62 colony × sampling date combinations. Due to a delay in shipping, the colonies observed in the first two sets of trials (*n* = 12) were not immediately exposed to fescue seeds. These colonies were tested again when fescue seeds were available. The subsequent observations of these colonies with fescue were compared to colonies that had only been observed once with fescue. No differences in seed preferences were found, so these data were combined with the larger dataset.

2.3. Seed Inoculation Trials

To determine if bacterial seed inoculation alters ant cover crop seed preference, we examined uninoculated and inoculated seeds of radish and wheatgrass. These seeds were chosen as they were both preferred seed types from the seed preference trials to ensure that any inoculation effects would not be confused with lack of preference and because they represent different plant families (Brassicaceae and Poaceae). Seeds for the inoculation treatment were inoculated in the laboratory with the Guard-N Omri Seed Inoculant (Johnny's Selected Seeds, Winslow, ME, USA) via a slurry method. For every 90 g of seeds, 0.7 g of inoculant was added to the container and shaken. Seeds were then stored separately to prevent the contamination of clean seeds at room temperature in a marked microcentrifuge tube until used in the field.

The experimental design for the seed inoculation trials was conducted in a similar manner to the seed preference trials discussed above (Section 2.2). The same colonies (*n* = 33 colonies and 49 colony × date combinations) and the number of colonies per observation period (*n* = 8–10) were used. The only difference in this trial was that one side of the depot was used to house only inoculated seeds (of both wheatgrass and radish) and one side housed only uninoculated seeds (of both wheatgrass and radish), with equal numbers of each cover crop's seeds on both sides. To differentiate which depot side held inoculated seeds and which held uninoculated, the undersides of depots were marked with a small section of tape. Seeds trials were completed between June and August 2020, according to the previously used seed preference methods.
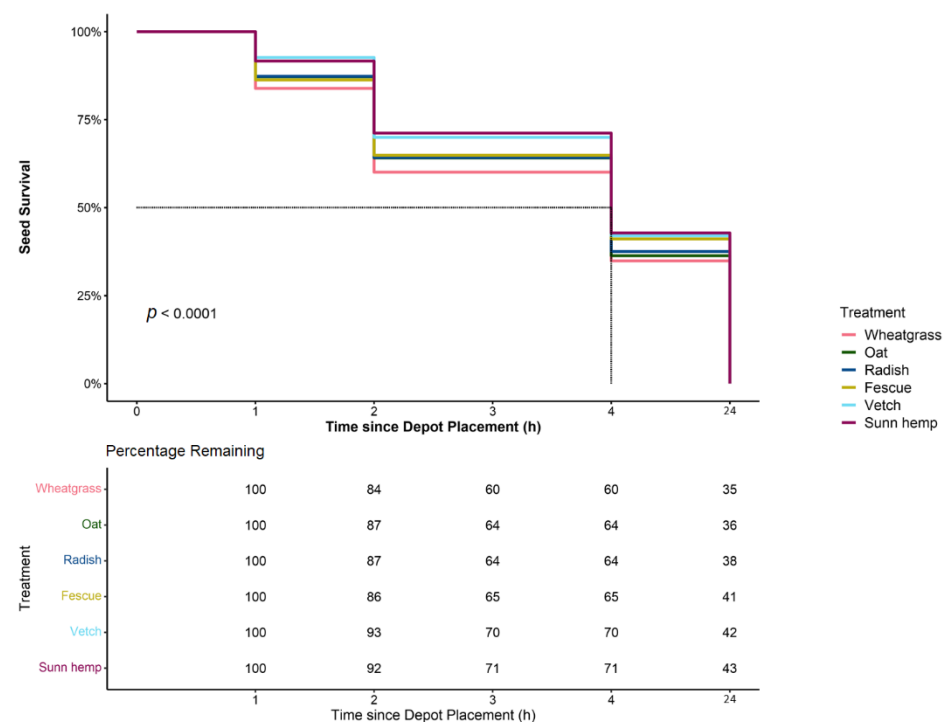
2.4. Statistical Analysis

R version 3.6.2 was used to conduct all statistical analyses [53]. Within each dataset (seed preference and seed inoculation), each individual seed's time to removal was recorded where individual seeds were the unit of observation but clustered based on the colony and observation date. Observations were recorded as censored due to external events (e.g., flipped depots due to high wind speeds, removal of the cage prior to the 24 hours period, etc.) and lack of removal by the end of the observation period was denoted. Survival analysis to determine the time to event (seed removal), using the survdiff function from the survival package, was used to determine if there was a significant difference in ant cover crop seed preference over time [54,55]. The Kaplan–Meier survival estimator, which estimates the likelihood of an event occurring over time, was used to calculate seed removal event likelihood over time grouped by cover crop seed type and seed inoculation treatment [56]. Kaplan–Meier survival curves also allow for a greater ease of treatment comparisons when examining seed removal over time. The log-rank test, a hypothesis test that compares the survival distribution between two samples using the lifelines package, was used to compare the survival distribution of cover crop seeds to the preferred wheatgrass control in the seed preference trial and the uninoculated wheatgrass control in the bacterial inoculation trial [57]. To further quantify ant preference differences while incorporating other potentially influential variables, such as the observation month and temperature, we used Cox proportional hazard models. Ant preferences for cover crop seeds were compared against the wheatgrass standard using the ggforest function from

the survival package [54,58]. Using this model, uninoculated wheatgrass (seed type), the month of June, and an ambient temperature of 28 °C were specified as model intercept values for ease of comparison between trial types, as both of these values were present in the seed preference and inoculation trials. Finally, differences in seed weights among cover crops were evaluated separately from the other models due to high levels of correlation with cover crop type using ANOVA and Tukey HSD comparison.

3. Results

3.1. Seed Preference Trials

Kaplan–Meier survival curves were used to compare the removal rates of the different cover crop seed types (Figure 3) and indicated significant differences among cover crops. The wheatgrass control was the most preferred seed throughout the entire observation period, indicating that it worked well as a known preferred control. The survival curves also indicated that fescue, oat, radish, and wheatgrass had similar removal through hour 4, with fescue then grouping with the less preferred sunn hemp and vetch after 24 h.


Percentage Remaining	1	2	3	4	24
Wheatgrass	100	84	60	60	35
Oat	100	87	64	64	36
Radish	100	87	64	64	38
Fescue	100	86	65	65	41
Vetch	100	93	70	70	42
Sunn hemp	100	92	71	71	43

Figure 3. Kaplan–Meier curve of seed type (colors) likelihood of survival over the course of the seed preference trial (*n* = 34 colonies). The dashed line indicates the overall median removal time. The mean percentage of remaining seeds per type at each time point is given in the table below, with colors matching each seed type's Kaplan–Meier curves color.

The pairwise log-rank test indicated differences in survival between the cover crop seeds (Table 1). Vetch and sunn hemp, though not significantly different from one another, were removed significantly less compared to other seed types (aside from the preferred wheatgrass control). Vetch was found to be significantly less preferred when compared to oat, radish, or wheatgrass (Table 1). Sunn hemp was also found to be significantly less preferred when compared to oat, radish, or wheatgrass (Table 1). Fescue, while not removed at different rates than vetch or sunn hemp, was removed less often than wheatgrass (Table 1), another member of the Poaceae family.

Table 1. *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

The Cox proportional hazards model was used to incorporate the sampling month and daily temperature fluctuations into the foraging preferences output. Using this model, the only significant differences in removal were between wheatgrass and vetch ($p < 0.001$), wheatgrass and sunn hemp ($p < 0.001$), and wheatgrass and fescue ($p = 0.023$), (Figure 4; Table 2 and Table S1). During the seed preference trials, ants exhibited a consistent preference for wheatgrass and oat seeds, sometimes removing all of these seeds before 24 h. These model results generally supported seed preferences indicated by the other analyses. Aside from the seed types, seed collection intensity differed among the observation months, decreasing from February to June (Figure S1; Table 2 and Table S1). Additionally, the temperature was a significant factor in the model, potentially due to the interactions of ant activity with their thermal tolerance (Figure S1; Table 2 and Table S1).
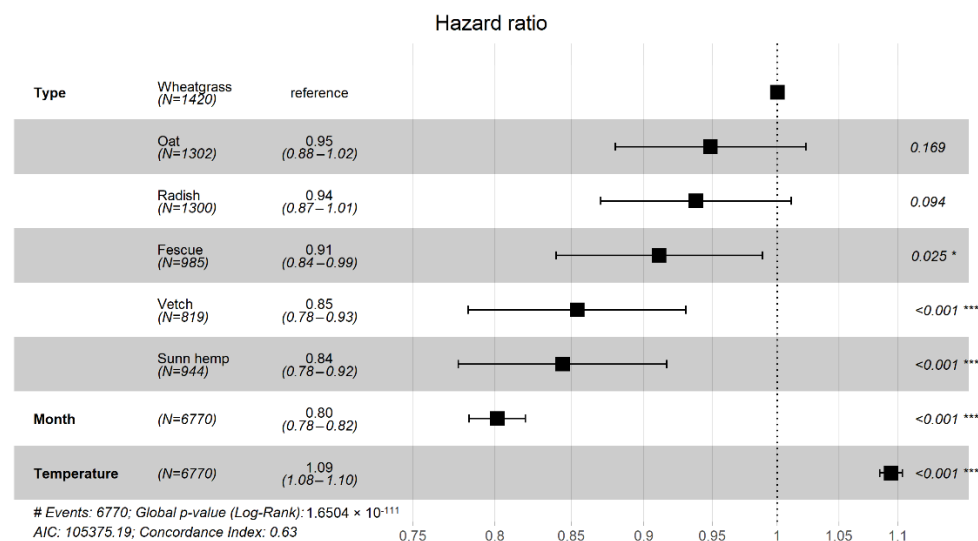

Figure 4. Hazard ratio test demonstrating differences in preferences between seed types. Reference is wheatgrass. Means and standard errors on the right side of the chart indicate a larger number of seeds that were removed during the trial compared to wheatgrass (control), June (month), and 28 °C (temperature). Differences in month *n* (observed seed number) were due to differences in numbers of trials per month and temperatures at the time of seed count as well as censorship due to external events. Expanded hazard ratios for month and temperature can be seen in the Supplemental Materials (Figure S2). Asterisks indicate the relative *p*-values indicating statistical significance where * indicates $p < 0.05$ and *** indicates $p < 0.001$.

Preferred seeds did not always share the same plant family. For instance, fescue was less preferred than oat and wheatgrass, other members of Poaceae. Preferences were also not fully aligned with the physical characteristics of the cover crop seeds (Figure 5). All the seeds' weights were similar except for fescue and radish, which were significantly lighter than the other types (Figure 5). While both radish and fescue seeds weighed less than the other seeds, radish was preferred more than fescue. Similarly, vetch and sunn hemp weighed the same as oat and wheatgrass, yet neither were preferred as much as

the latter two. These two seeds also did not share seed shape or texture, only color and nitrogen-fixing abilities. 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.

Table 2. Summary of the fitted Cox proportional hazards model for cover crop seed preferences. Coef indicates variable coefficients, SE indicates the standard error, *Z* is the test statistic used, and *P* indicates the significant values. Model output for month and temperature can be found in the Supplemental Materials (Table S1).

| Cover Crop	Coef	SE	Z	P
Vetch	0.000	0.040	−0.040	0.970
Oat	0.000	0.040	−0.080	0.930
Sunn hemp	0.000	0.040	−0.110	0.910
Radish	0.010	0.040	0.150	0.880
Fescue	−0.170	0.040	−4.130	0.000
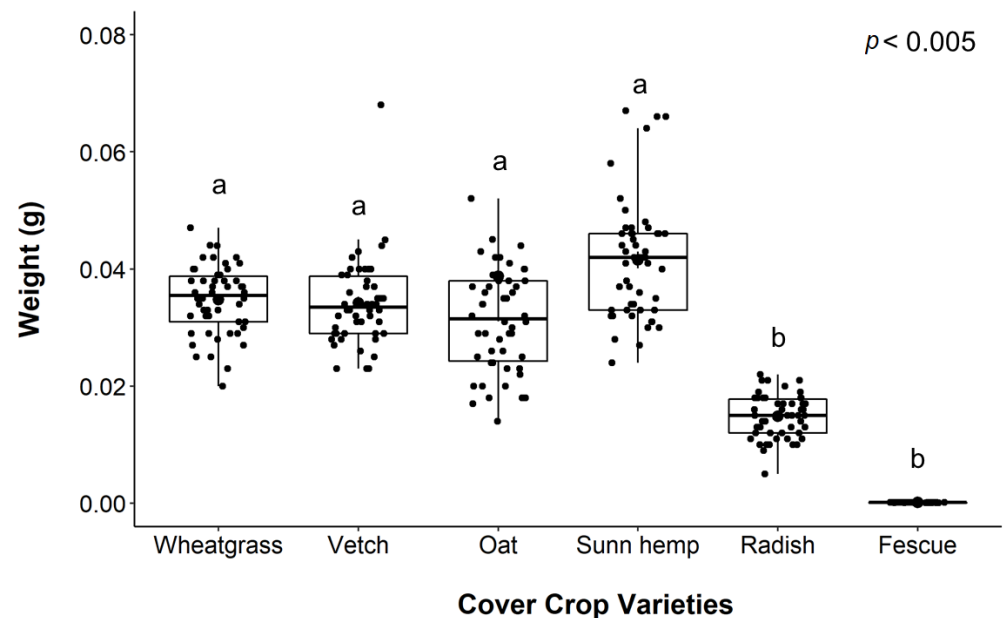
Figure 5. Differences in seed weight among the six cover crop seeds (*n* = 50/seed type) used in the study. Boxplots are in the style of Tukey where the box limits represent the lower 25% and upper 75% quantile, with the line representing the median. Tukey HSD was used to determine significant differences (denoted by letters) among seed weights.

3.2. Seed Inoculum Trial

Unlike the seed preference trials, bacterial inoculum trials did not indicate significant differences in ant preference. The Kaplan–Meier survival curve, created from the collected data, further demonstrated the lack of preference between inoculated versus uninoculated seeds between the same seed type over the 24 h period (Figure 6).
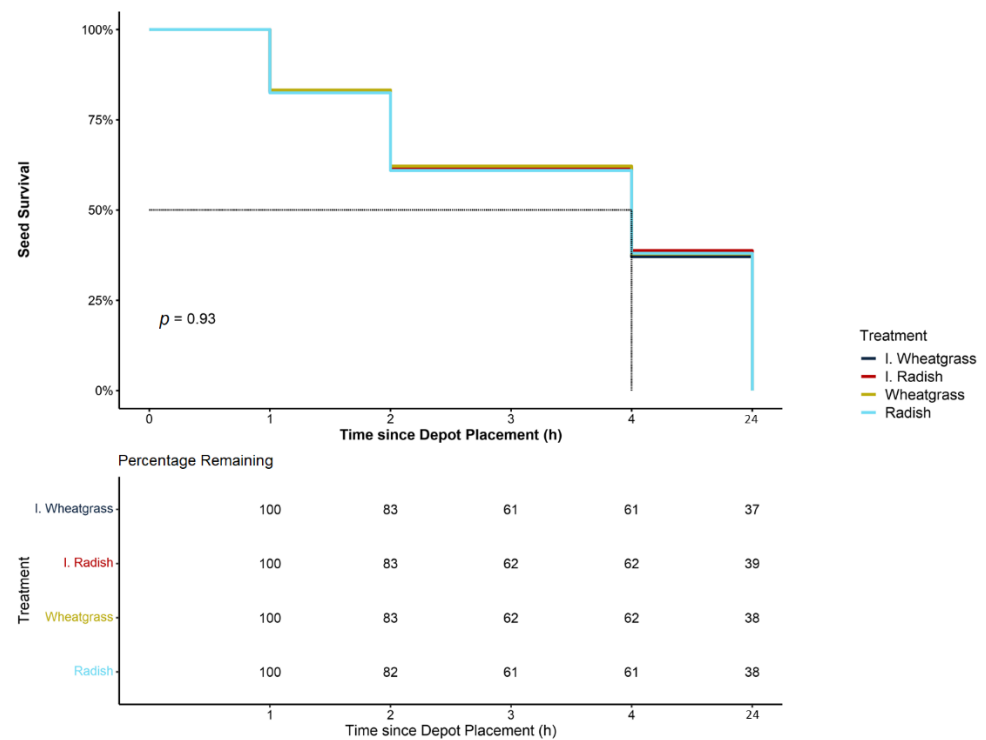
Figure 6. Kaplan–Meier curve of seed types' likelihood of survival over the course of the seed inoculation trial based on selected data. (*n* = 33 colonies). The dashed line indicates the overall median removal time. "I" indicates the inoculated seed treatments for each tested seed type. The mean percentage of remaining seeds per type at each time point is given in the table below, with colors matching each seed type's Kaplan–Meier curves color.

Both the log-rank test and the Cox proportional hazard model indicated that there was no difference in preference between the inoculated and uninoculated seeds. This was true of both the preferred wheatgrass control and the radish seed (Figure 7; Tables 3 and S2). Similar to the seed preference trial, the observation month and temperature were significant predictors of seed removal within the seed inoculation Cox proportional hazard model (Figure S2; Tables 3 and S2).

Table 3. Summary of the fitted Cox proportional hazard model for inoculated seed preferences. Coef indicates variable coefficients, SE indicates the standard error, *Z* is the test statistic used, and *P* indicates the significant values. Model output for month and temperature can be found in the Supplemental Materials (Table S2).

Cover Crop	Coef	SE	Z	P
Inoculated Wheatgrass	0.010	0.040	0.260	0.750
Inoculated Radish	−0.010	0.040	0.680	0.820
Radish	0.000	0.040	0.410	0.930
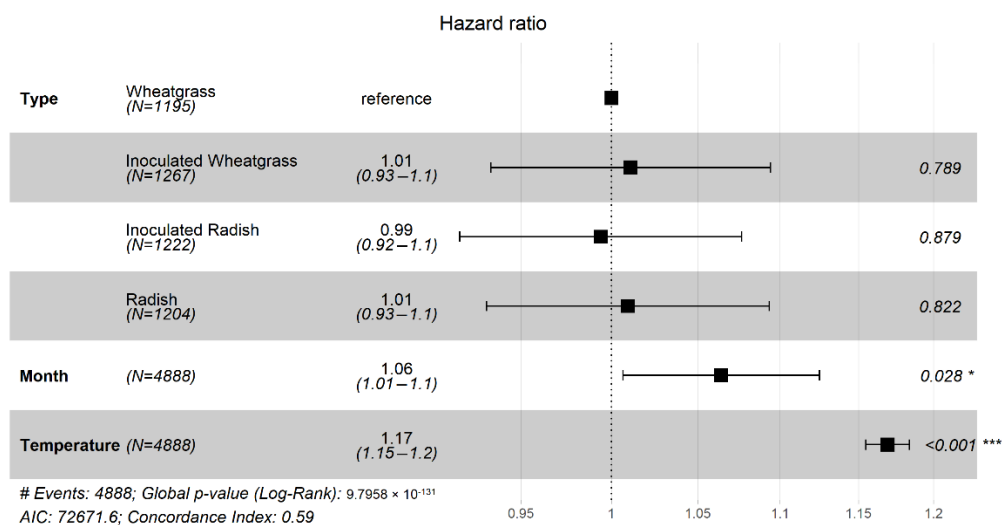
Figure 7. Hazard ratio test demonstrating differences in preferences between inoculated and uninoculated seed types. Reference is wheatgrass. Means and standard errors on the right side of the chart indicate a larger number of seeds that were removed during the trial compared to wheatgrass (control), June (month), and 28 °C (temperature). Differences in month *n* (observed seed number) were due to differences in numbers of trials per month and temperatures at the time of seed count as well as censorship due to external events. Expanded hazard ratios for month and temperature can be seen in the Supplemental Materials (Figure S3). Asterisks indicate the relative *p*-values indicating statistical significance where * indicates $p < 0.05$ and *** indicates $p < 0.001$.

4. Discussion

The two goals of this study were to determine if red harvester ants *Pogonomyrmex barbatus* exhibit relative preferences among different cover crop seeds commonly used in the Lower Rio Grande Valley, and whether inoculating preferred seeds with a nitrogen-fixing bacteria treatment would alter the desirability of the seed. To evaluate this, we introduced selected cover crop seeds via seed depots deployed over 24 h to naïve harvester ant colonies. We found that harvester ants had a significant preference for grass seeds, such as wheatgrass as well as radish seeds, when compared to nitrogen-fixing sunn hemp and vetch seeds. When ants were presented with inoculated and uninoculated wheatgrass or radish seeds, we did not observe any changes in seed preference between inoculated and uninoculated seeds of either wheatgrass or radish.

We had hypothesized that harvester ants would prefer to forage on certain seeds based on physical characteristics and plant family, with a preference for Poaceae [1,10,16]. As anticipated, based on prior work evaluating harvester ant seed preferences in natural areas, grass seeds were similarly preferred to each other at 4 h. However, fescue was removed less than oat and wheatgrass after 24 h. Further, physical attributes did not always appear to explain the differences in preferences. For instance, radish and fescue were both of lower weight compared to all other seeds, but radish was preferred more than fescue. Additionally, the attributes of radish seeds overlapped with the less preferred seeds of sunn hemp and vetch in terms of shape and color, indicating these physical traits were not the only drivers of preference within this study [1,10].

Observed ant seed preferences could have been influenced, in part, by seed availability in the surrounding habitat, which likely changed throughout the observation period of February to August [36,59]. Throughout the study, we observed native seed burrs (*Cenchrus* sp. L. (Poales: Poaceae)) as well as other smaller grass seeds being taken into the study colonies. Prior documentation of burrs in and around Hidalgo County, TX, indicates that, like harvest ants, burrs are often found in highly disturbed locations such as roadsides [60,61]. Further, burrs are known to germinate in the late spring, continuing through the fall [62,63], corresponding to the timing of the seed depot trials. Given these

observations, the interactions of cover crop seeds with other seed availability, particularly within agricultural settings, need to be evaluated.

Harvester ant activity is also closely related to weather patterns such as rainfall and humidity, which peak in the summer months in the LRGV [64–66]. Rain events may positively correlate with overall seed availability in the local seed bank since non-drought resistant grasses such as *Cenchrus* sp. L. (Poales: Poaceae) germinate after rainfall, generating greater diversity and abundance in the seed bank [1,63,65]. The changes in the surrounding seed bank might further explain the changes in ant foraging intensity at seed depots over the observation period. Alternatively, the sudden increase in depot harvesting from June to July could have been in preparation for August. During August, ant activity significantly decreased in comparison to both June and July, potentially to avoid excess water loss during periods of high temperatures (Figure S2) [64]. Examples of changes in ant foraging intensity varying with temperature even after a month is accounted for, as seen in the Cox proportional hazard models, which have been documented in other harvester ant species as well [67].

Another notable, weather-related change in ant foraging intensity was recorded on 23 July 2020, two days prior to the touchdown of Hurricane Hanna in the LRGV. Within one hour, 8 of 9 colonies actively being observed completely removed all seeds from the depots, with an unusually high intensity of foraging. The impacts of such weather events are known to affect insect behavior via changes in barometric pressure; many insects exhibit increased foraging, likely in preparation for a time of no foraging during the weather events that follow [68,69]. For instance, leaf-cutter ants have been observed to significantly increase foraging during periods of low barometric pressure, and harvester ants may do the same [69]. Future studies regarding the correlation between harvester ant foraging intensity and barometric pressure linked to large storm events could help to determine seed risk for planting dates during hurricane season in regions along the Gulf Coast that have the potential to experience tropical cyclones annually.

Harvester ants have been previously observed to exhibit contradictory behavior regarding the same seed species based on other factors, such as seed germination or fungal infection [1,10,70]. However, in our trials, inoculated and uninoculated seeds were not treated differently, indicating that the presence of nitrogen-fixing bacteria on the seed surface did not deter or encourage harvester ant predation. Regardless, there is conflicting data regarding the amount of microbial diversity/biomass within the soil around ant colonies and little data on how seed microbial communities impact harvester ant preferences [71–73]. The red harvester ants in this study showed no preference towards or against inoculated seeds, indicating that their granaries could potentially be rich in microbial activity. Alternatively, red harvester ants may conduct seed cleaning behavior that could occur at any point prior to storage in the granary [43,74,75].

Harvester ants can cause substantial disturbance in arid to semi-arid regions of the United States and Mexico. Harvest ants in the genus *Pogonomyrmex* may be considered agricultural pests due to their seed collection and plant removal in agricultural areas, where they can remove up to 100% of a preferred seed within their foraging range [10,70]. In subtropical agricultural areas such as the LRGV, prior studies have recommended the use of warm-season cover crops due to a subtropical climate and the promotion of native mycorrhizal fungi [76,77]. Although cover crops may be extremely beneficial for soil health, cover crop seeds may be at risk of predation by harvester ants residing in agricultural areas. Our study evaluated which commonly used cover crop seeds in this area are preferred by red harvester ants, with the harvester ant most found in LRGV agricultural fields. Further, we determined if the addition of bacterial inoculum used to promote root nodule formation in some crops may deter ant removal of preferred seeds. The ant seed preference data suggest we can potentially recommend sunn hemp and vetch to farmers as potential cover crops during fallow periods. These cover crops can also be used in conjunction with bacterial seed inoculation since inoculation does not change harvester ant preferences.

Using non-preferred cover crop seeds may also encourage harvester ant predation on weed species or surrounding native plants, which could limit crop yields [23]. Given that sunn hemp and vetch seeds were not preferred over grasses, which are common in the non-crop habitats surrounding LRGV crop fields, harvester ants may likely predate on surrounding weeds and grasses instead of these particular cover crops. However, additional research should be conducted regarding harvester ant preferences for alternative cover crop seeds, within the different agricultural contexts (e.g., crops, tillage regimes, and weed management practices), and at different times of the year to account for changes in ant activity and seed abundance and diversity. 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.

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Appendix A

Depot Cage Construction and Depot Optimization

To ensure that bird and rodent predation of seeds did not occur in between observation periods, depot cages were created out of metal wire. Sections of 1×1 cm hardware cloth (Everbilt, The Home Depot, Atlanta, GA, USA) were cut into 4 sections of 7×23 cm pieces and one 23×23 cm cube. The ends of the wire were tripped neat to prevent injury to members of the lab. The 4, 7×23 cm sections were tied together at the short ends to one another using zip-ties to make the outer form of the box. Excess ends of the zip-tie were trimmed off to facilitate transport. When the four sections were secure their long ends were secured to the perimeter of the 23×23 cm base using zip-ties. The use of these cages was monitored in the field during preliminary trials to ensure that the only organisms foraging on the selected cover crop seeds were red harvester ants.

Depots with cages were deployed during multiple stages of depot development to ensure that ants were able to easily enter the depots, find seeds, and remove seeds. All preliminary trials utilized ant colonies not used for the main trials and data analysis, preventing prior exposure from skewing the data. During preliminary trials, it was observed that unsanded depots did not allow ants to easily move within the dish without slipping.

Additionally, ants foraging in depots with standard Petri dish sides (even with sanding) exhibited difficulty in removing seeds. These ants would start to remove seeds from the dish but end up leaving the seeds after several minutes of failed attempts to climb the sides of the dish while holding the seed. Given these observations, sanded depots with entrance holes were used for the final trials.

Appendix B

Optimization of Depot Placement

As red harvester ants forage along trunk trails, depots need to be placed along existing colony trails. However, to optimize where along this trail depots were placed, a separate set of preliminary trials were conducted using colonies not included in either of the main trial sets. Seed depots were placed $\frac{1}{2}$, 1, 2, and 3 m away from each colony only on the main trail to monitor ant interactions with the depots. Visual observations were made to see where along the trail seeds were most often noticed by foraging harvester ants. When the depots were located too close to the colony ($\frac{1}{2}$ to 1 m away), other members of the colony, such as members of the waste disposal unit, would interfere with foragers or be disrupted in their disposal tasks by the depot being in their path [78]. In the latter case, disposal ants were seen to place small stones and other debris in and around the depots. Alternatively, depots located too far (3 m) had fewer interactions with foragers as the foraging trail had begun to branch off into smaller sections, and foragers were more widely dispersed [15]. Placing the depot 2 m away from the colony allowed for the highest concentrations of foraging ants to interact with the depot on their way to and from the colony without interference from ants performing other colony duties.

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