Red harvester ants prefer certain native seeds over others: implications for broadcast seeding restoration

Forest Sheldon

School of Forestry, Northern Arizona University, Flagstaff AZ 86011

May 2026

Major Advisor: Professor Derek A. Uhey

Reader: Professor Richard Hofstetter

Reader: Professor Georgia Roberts

Abstract

Red harvester ants (*Pogonomyrmex barbatus*) are one of the most common ant species in the Southwest United States. Named for their feeding style of foraging and harvesting seeds, these ants are vital ecosystem engineers in arid landscapes. In the Southwest, native seed mixes are often used to help rehabilitate or restore vegetation after disturbances. Harvester ants decrease the number of seeds on the soil surface, posing a threat to these restoration efforts and potentially altering plant species composition in ecosystem. To test seed preferences by harvester ants, seeds from 11 common Arizona native plant species were offered to a harvester ant colony in the lab. The amount of each seed species taken was measured at 1-, 24-, and 48-hour intervals to determine which species were favored by the ants. Ants favored certain seed species; the seeds taken at a higher rate were often smaller, with less extraneous material covering the inner seeds, and higher nutritional content. Favored seeds included bottlebrush squirreltail (*Elymus elymoides*), Sandberg bluegrass (*Poa secunda*), Indian ricegrass (*Achantherum hymenoides*), and scarlet gilia (*Ipomopsis aggregata*), while disfavored seeds include saltbush (*Atriplex canescens*), galleta grass (*Pleuraphis jamesii*), sideoats grama (*Bouteloua curtipendula*), and winterfat (*Krascheninnikovia lanata*). Knowing these preferences, native seed mixes for broadcast restoration can be curated more effectively to avoid interference from red harvester ants.

Keywords: *Pogonomyrmex barbatus*, granivore, broadcast seeding, foraging, native plants

Introduction

Wildfire disturbances in the southwestern United States affect large portions of land and vegetation (Guiterman et al., 2022). While fire is a natural part of many ecosystems in the Southwest, in more recent years climate change has further exacerbated the frequency and intensity of these fires. This has degraded ecosystems by increasing susceptibility to flooding, erosion, and invasive species establishment (Mueller et al., 2020, Peppin et al., 2011). To combat the after-effects of fire, particularly loss of native vegetation, reseeding methods can be used to restore or add plant species to this habitat. Broadcast seeding is a common restoration method used post-fire to rehabilitate ecosystems; restoring grasslands, jumpstarting successional stages, controlling erosion and flooding, and preventing invasion of non-native species (Miller, 2013, Peppin et al., 2011). This method involves scattering an assortment of seeds, most often native to the ecosystem being treated, over the affected land (USDA et al., 2018). Seeds are spread either mechanically or by hand, and treatment is usually followed by tillage to improve seed contact with the soil (USDA et al., 2018). The newly spread seeds combat the long-term effects of the disturbance through regrowth of native vegetation. While this restoration method is used after a variety of disturbances, it is most often after fire.

Another common occurrence in the Southwest is the presence of red harvester ants (*Pogonomyrmex barbatus*), a species of ant both native and extremely abundant within arid regions of the southwestern United States. Harvester ants are named after their foraging, or ‘harvesting’, of seeds from the ground surface and returning those seeds to the colony for storage or consumption. However, because of this foraging behavior red harvester ants can decrease the number of seeds within the seedbank and on the surface (Luna et al., 2018, Pirk & Lopez De Casenave, 2014). For example, compared to areas without red harvester ants, areas with harvester ants have significantly lower abundance of seeds in the soil (Luna et al., 2018). This behavior of collecting seeds can potentially result in reseeding efforts being less successful (Paolini et al., 2020). Failure in these treatments can lead to lower vegetation establishment, slowing regrowth while also making the land more susceptible to erosion and invasive vegetation. This can lead to repeat treatments, provided there is enough funding or labor to do so. Native reseeding is already an expensive treatment (Peppin et al., 2011), so initial treatments being successful is imperative.

Red harvester ants are considered a keystone species in many ecosystems (Uhey & Hofstetter 2022). Harvester ants create hotspots of nutrients around their nests, promoting diverse communities of both flora and fauna around their nests. The flora that grows along many species of harvester ants' nest-rims experience increased growth and resilience to stress (Uhey et al., 2024). Harvester ants' ability to increase biodiversity, plant stress-tolerance, and nutrient/energy flow within the soil, are all factors that increase an ecosystems diversity and stability in turn. Therefore, it is best for restoration to work in conjunction with harvester ants. A proposed solution to negative impacts of red harvester ants on broadcast seeding is to target the plant species ants dislike.

Harvester ants species do not gather seeds indiscriminately. Factors that impact seed preferences by harvester ants include distance the seed is away from the nest, amount of cheatgrass (*Bromus tectorum*) cover, season, and seed morphology (Belchior et al., 2012, Paolini et al., 2020, Pirk & Lopez De Casenave, 2014). Seeds found with more cheatgrass cover and further away from nests are collected less, likely due to the increased amount of effort to forage in these instances. But across distance and coverage, harvester ants have consistent preferences for certain seeds like Indian ricegrass (*Achnatherum hymenoids*) (Paolini et al., 2020). Harvester ants prefer seeds that are easier to handle, smaller, or have simpler morphology, with higher nutritional content (MacMahon et al., 2000). Previous studies have found seed preferences of the western harvester ant (*Pogonomyrmex occidentalis*; Hickey et al. 2016), rough harvester ant (*Pogonomyrmex rugosus*; Martyn et al. 2022), and Owyhee harvester ant (*Pogonomyrmex salinus*; Paolini et al., 2020) for certain plant species; however, the red harvester ant (*Pogonomyrmex barbatus*) remains untested for harvesting preferences. Red harvester ants are widespread in hotter regions of the southwestern United States and understanding their harvesting preferences may be important for broadcast seeding success in these areas.

To examine the preferences of red harvester ants, we constructed a lab experiment that tested ants selections of 11 native plant seeds commonly used in restoration. Based on previous studies on other harvester ant species, we predict that preferred seed species would be smaller, higher in nutritional content, with less extraneous material surrounding the seed. These are features that consume less energy to access and transfer seed material while providing high amounts of nutrition. Our study helps provide better understanding on how to proceed with reseeding efforts in areas with red harvester ants.

Methods

Ant species selection

This study focuses on the dietary preferences for different seed species of the red harvester ant (*Pogonomyrmex barbatus*), a common ant species native to the southwestern United States. To determine preferences of red harvester ants, we offered the seeds of different plant species to a lab colony. This colony originated from a queen collected in Phoenix, Arizona on August 1st, 2022, and the colony was raised on chia seeds in a climate-controlled lab. The colony held roughly ~500 worker ants.

Seed selection and characteristics

"Seed material" is defined as the embryo and food reserve of plants, often surrounded by protective layers formed by maternally derived organs of the plant, resulting in a seed or fruit coat. These seeds are then dispersed through species-specific means for the plant's reproduction. While some of the seeds we tested lack a protective layer, many either have this layer or extraneous plant material surrounding it. Multiple seeds can be found inside these layers or extraneous material but are contained as one unit. Thus, these units, despite multiple seeds or any extraneous material, are still defined as seed material (Table 1).

Table 1. Descriptions of seed material and average values of morphological measurements for each of the 11 native seed species offered to red harvester ants in trials. Boxes that are green indicate species in the "preferred" group while orange indicates species in the "avoided" group. Morphological measurements are given as averages with standard error values in parentheses.

Plant species	Plant type	Diaspore*	Surface area (mm^2)	Mass (mg)	Surface area to mass ratio (mm^2/mg)
Artriplex canescens	Grass	Abscised fruit	18.83 (0.440)	8.20 (0.26)	2.30
Achnatherum hymenoides	Shrub	Floret – lemma	5.69 (0.001)	4.42 (0.14)	1.29
Bouteloua curtipendula	Grass	Spikelet branch	21.15 (0.026)	0.54 (0.03)	39.16

Bouteloua gracilis	Grass	Spikelet	9.34 (0.006)	0.47 (0.02)	19.87
Elymus elymoides	Grass	Floret -- palea	18.30 (0.010)	6.60 (0.23)	2.77
*Helianthus annuus***	Forb	Individual seed with outer pericarp	16.2 (.885)	62.61 (N/A)	0.26
*Ipomopsis aggregata****	Forb	Individual seed with mucilaginous seed coat	1.45	1.13 (.025)	1.28
Krascheninnikovia lanata	Shrub	Fruiting spikes with long, dense silky hairs	56.70 (0.016)	3.75 (0.5)	15.12
Linum lewisii	Forb	Individual seed with mucilaginous seed coat	10.31 (0.008)	2.29 (0.01)	4.50
Pleuraphis jamesii	Grass	Spikelet cluster	31.36 (0.036)	1.34 (0.05)	23.40
Poa secunda	Grass	Floret -- palea	3.39 (0.002)	0.52 (0.02)	6.52

* diaspore is defined as the plant material that ultimately contains the plant seeds, and when dry and mature, separates from the plant and falls to the ground and is available to harvester ants.
** average mass and surface area measurements were unavailable and instead taken from existing literature. Average surface area was calculated from the documented surface areas of a different sunflower species, due to the unavailability of information regarding *Helianthus annuus* surface area (Perez et al., 2007). Average mass was calculated from the documented masses of different *Helianthus annuus* genotypes, however no standard error is provided (Castillo-Lorenzo et al., 2019).

*** average measurements were unavailable and instead taken from existing literature. Average mass was calculated from the documented masses of *Ipomopsis aggregata* in a different study (Wolf et al., 1986).

Seed material from 11 plant species were selected for the study; 2 shrub, 3 forb, and 6 grasses (Fig. 1), all native to the state of Arizona and general southwest United States. All seed material was purchased from Western Native Seed © (www.westernnativeseed.com). Plant species were chosen based on their status as native plants, as these would be seeds ants encountered naturally, as well as their common use in restoration. Each species selected is commonly used in broad-scale seeding mixes for southwestern drylands , due to their ability to either attract native wildlife, quickly establish post-wildfire, prevent erosion, or a combination of these features (Natural Resource Conservation Service, n.d.). Overall, the seeds selected would be naturally present and commonly seen by ants in their natural conditions.

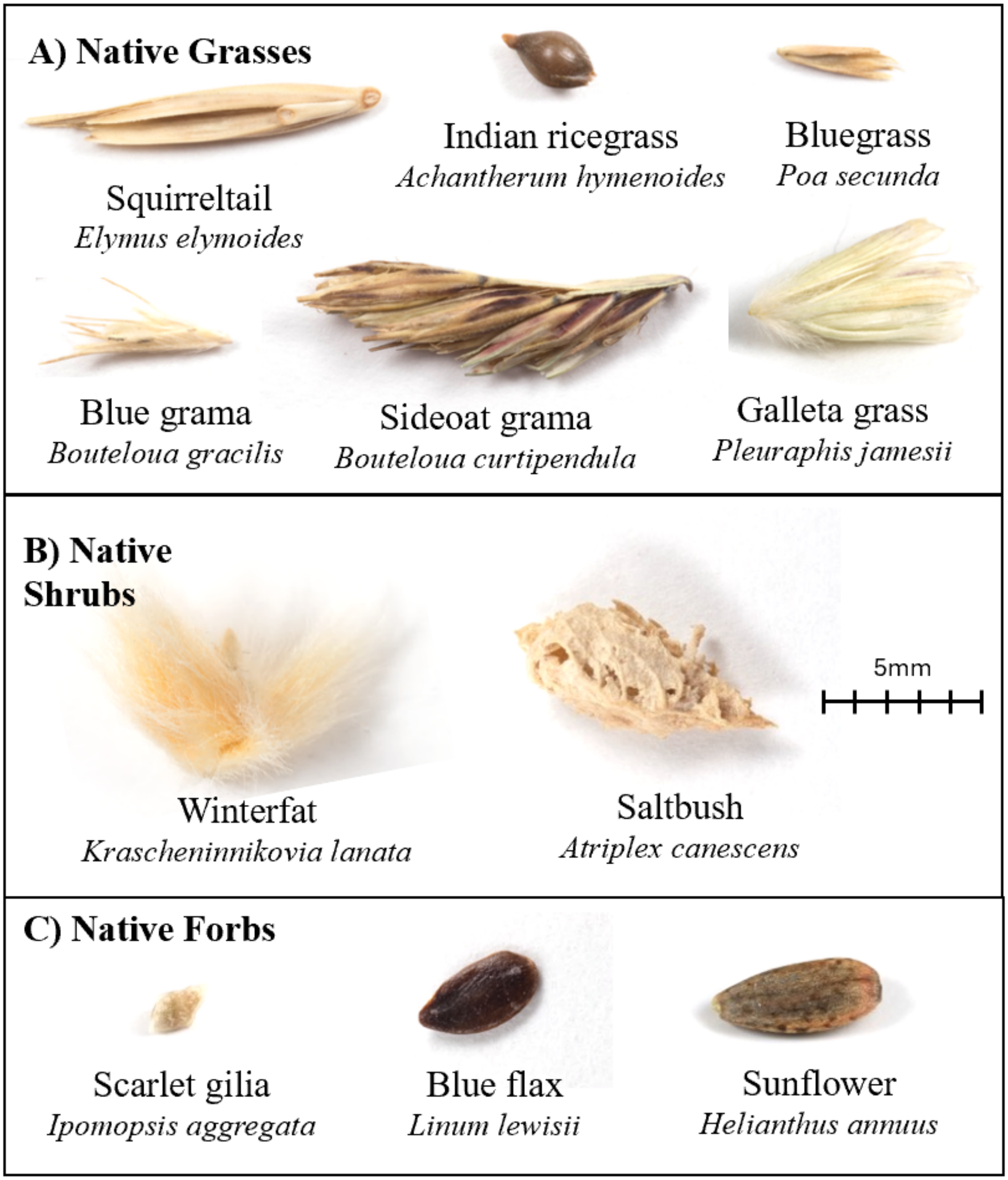

Figure 1. Diaspores of the A) 6 native grass species, B) 2 native shrub species, and C) 3 native forbs species used in red harvester ant seed-selection experiments.

Lab foraging preference trials

A colony of red harvester ants was connected to a separate chamber, filled with the assortment of 11 different seed species that were selected, to test the species overall foraging preferences. The ant colony was connected via a vinyl tube, approximately 10 inches away from the colony to a feeding chamber, 9.5x12x5.5 inches in size, with a constant climate (25-27°C) (Fig. 2). The colony had continuous access to the chamber, which contained seed material from each plant species. The chamber was set up in a “buffet” style, where ants could select whichever seeds they preferred. Samples consisted of 30 units of seed material, separated by species, into a low-lipped 35mm petri dish lid, sized. The petri-dish lids were placed in randomized locations after each trial, to eliminate preferences based on location or proximity to the chamber entrance from the colony. A trial consisted of checking the remaining seed material in each petri-dish at 1-, 24-, and 48-hours. The data collected consists of the number of seeds that were missing from each dish at each time internal. The location of missing seeds is described below. Thirty trials were completed between April 2025 and January 2026.

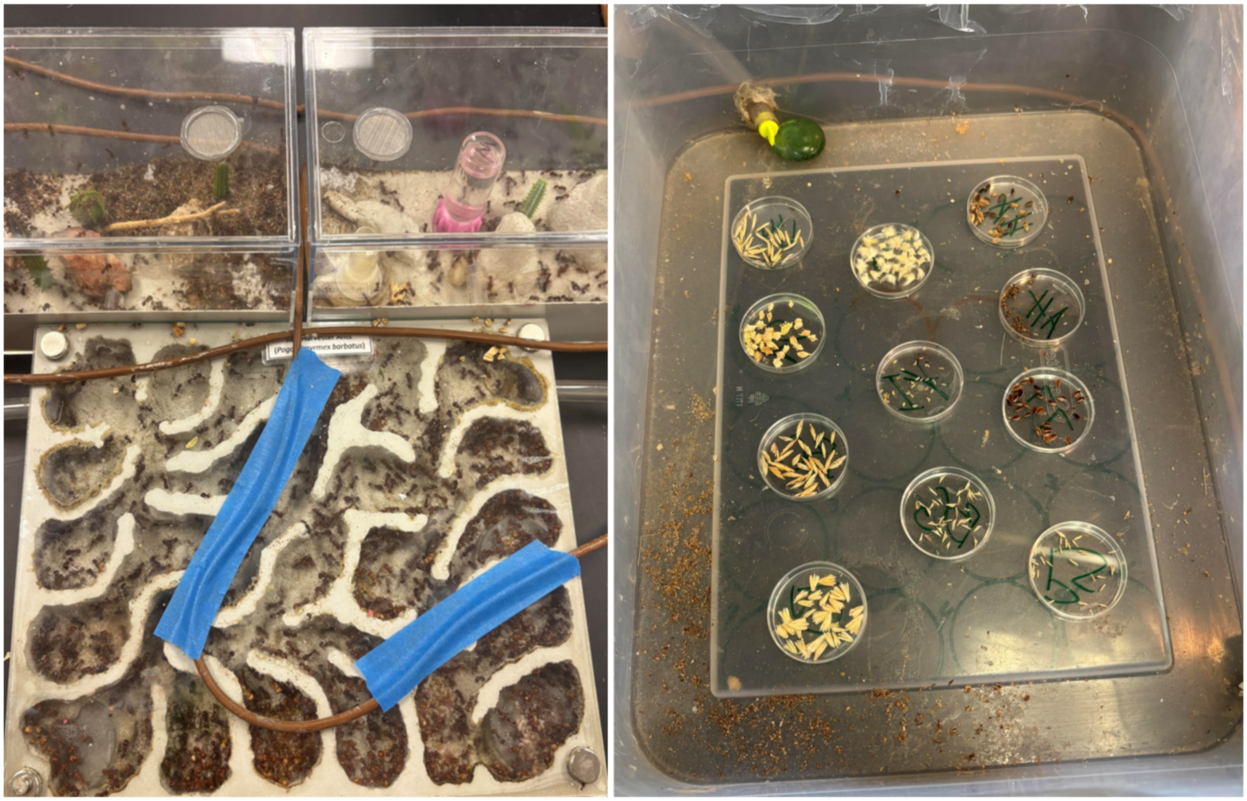

Figure 2. Lab setup of red harvester ant colony (left) and connected seed chamber containing petri-dishes of 11 different seed species (right).

While conducting trials we noted certain behaviors. Ants would remove seed material from their petri-dish but wouldn't take it back to the colony; instead, they would discard it somewhere else in the chamber, often a corner. Ants would also discard stray material, bodies of other deceased ants, or other "trash" outside of the main colony and into the feeding chamber. Both of these events would occur often enough that after the first few trials, a secondary, smaller, chamber was connected to the feeding chamber. No petri-dishes containing seed material were placed in this offshoot chamber, but instead it was used as a "dumping ground" for the seeds ants discarded, or other types of trash from inside the colony. We observed ants occasionally taking seed material into the main colony but would later return it to the feeding chamber. In these instances, seeds discarded from the original petri-dish, or brought back and then discarded, were not counted as "taken", as they did not actually consume or store the seed in the nest.

Statistical Analysis

To test preferences in harvesting by ants, average numbers of different seed species taken into the nest were compared using ANOVA followed by a post-hoc Tukey test for pairwise comparisons. All analyses are completed in R v.4.5.1.

Results

Lab experiment results

Ants took the majority of seeds within the first hour of the trials; 31% of total seeds averaged across all species. At 24^{th} hours, 20.23% of the total seeds were removed, and at 48^{th} hours, 3.14% of the total seeds were removed (Fig. 3).

Averages for each species taken were calculated for the 1-, 24-, and 48- hour intervals. By the 48-hour check over 90% of seeds were taken for four species (squirreltail, Indian ricegrass, scarlet gilia, and bluegrass), between 50-75% for three species (blue flax, blue grama, and sunflowers), and under 10% for four species (winterfat, sideoat grama, saltbush, and galleta grass) on average (Fig. 4). Species where less than 10% of total seeds were taken were often left in their original containers, untouched, or were put in a 'refuse' piles and discarded.

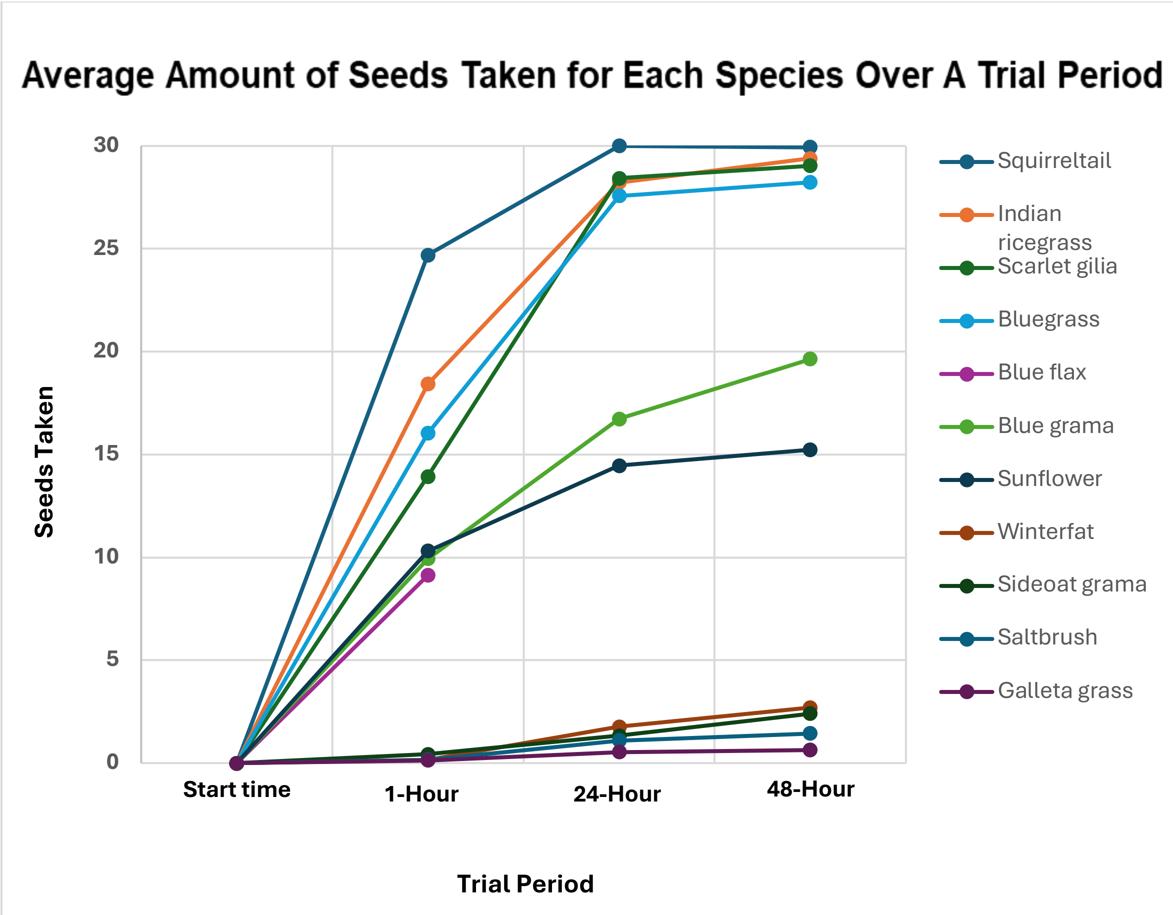

Figure 3. Average amount of seeds taken for each species over time.

Ants preferred (i.e. harvested) particular plant species ($F = 2142.8$, $p<0.001$). There was no significant statistical difference in number of seeds taken between 17 (30.91%) species comparisons, but there was a significant statistical difference between the remaining 38 (69.09%) species comparisons. All comparisons between species of the same average grouping (e.g. over 90%, between 50-75%, and under 10% seeds taken) had no significant difference between them in number of seeds taken (Fig, 4). Most species that were in different groupings had significant differences. There were two exceptions to this in which a species lacked significant differences between different groupings, both with blue flax. While its seeds taken average was between 50-

75%, it had no significant difference in seeds taken with blue grass and scarlet gilia, both species where over 90% of seeds were taken. All other comparisons of species in different groupings held significant differences with number of seeds taken.

Three different preference groups were created based off of these averages of seeds taken for each species and significant statistical differences in number of seeds taken when comparing species. Species where over 90% of seeds were taken and they lacked significant differences with each other were categorized as preferred (squirreltail, Indian ricegrass, scarlet gilia, and bluegrass). Similarly, species taken between 50-75% and lacking significant differences were categorized as moderated (blue flax, blue grama, and sunflowers), and species taken under 10% and lacking significant differences were categorized as avoided (winterfat, sideoat grama, saltbush, and galleta grass). Although blue flax lacked differences with two species in a different group, scarlet gilia and bluegrass, it still had significant differences with the other two species where over 90% of seeds were taken, squirreltail and Indian ricegrass. Additionally, blue flax was closer to the other “moderate” species in both average taken and the fact it lacked significant differences with all species in the 50-75% group, instead of just half of them. Because of these factors, blue flax was put into the moderate preference group. These preference groups were consistent among time intervals, with highly preferred, moderate, and avoided species staying in the same groups as trials progressed.

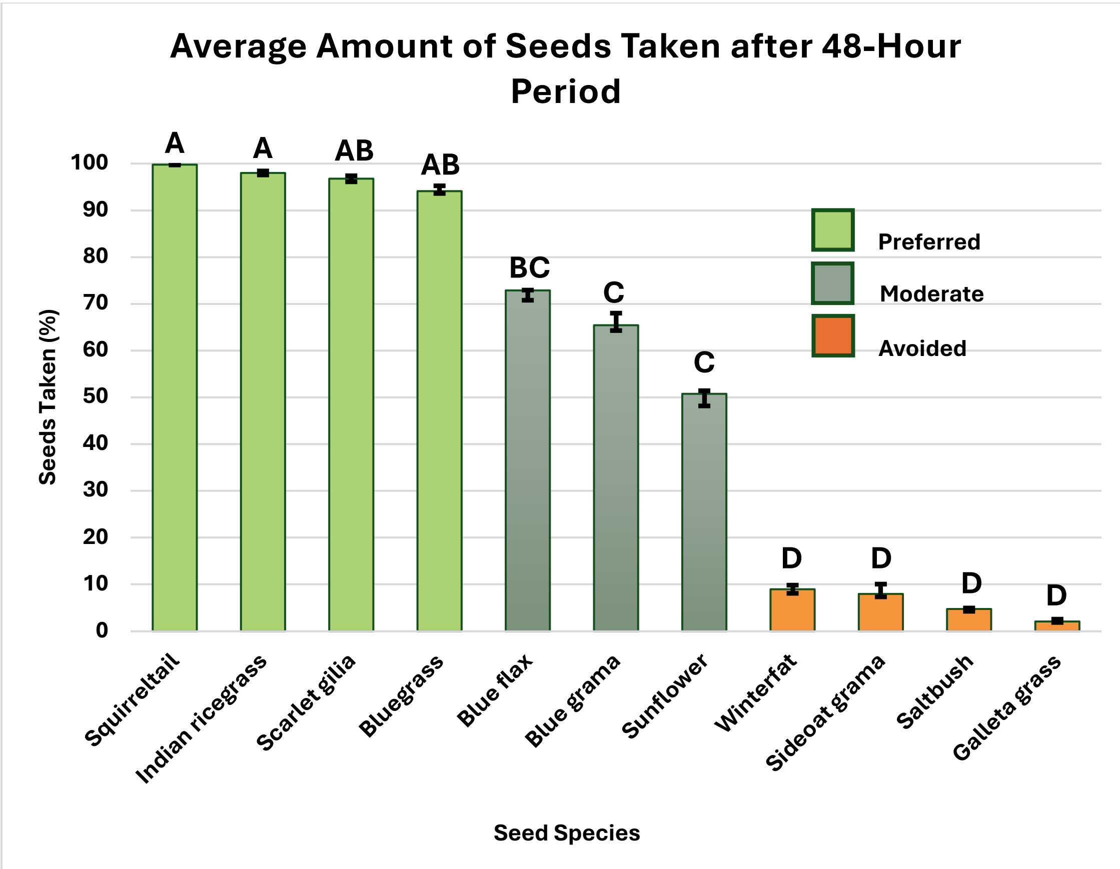

Figure 4. Average amount of seeds taken at the 48-hour mark during trials, displayed as a percentage. Standard errors are shown as a line above each bar. Species that were considered preferred are colored in a bright green, species taken in moderate amounts are colored in gray, and species that were considered avoided by ants are colored in orange. Species which share a letter lack a significant statistical difference between each other in number of seeds taken. Supplemental table for statistical differences (Table 2) located in Appendix.

Amounts of seeds taken for each respective species remained consistent across the trial period—April 2025 through January 2026—with the exception of a few species. One of these was sunflower. Ants heavily preferred sunflowers, taking all 30 seeds, and on one occasion 29, for the first 13 trials from April 25th to September 24th, 2025. Transitioning from September to October, rates of sunflower seeds taken were inconsistent; on the 14th and 15th trial the number of seeds taken dropped from 30 to 14 to 0. They dropped suddenly before rising back up and dropping again. These rates would continue to go up and down, although now at much smaller levels, before tapering off to none being taken by the end of the experiment in January for the remaining 15 trials (Fig. 5). To a lesser extent, this also occurred with blue flax seeds. Flax seeds were heavily foraged in the early trials, before dropping and becoming more inconsistently preferred around the same time.

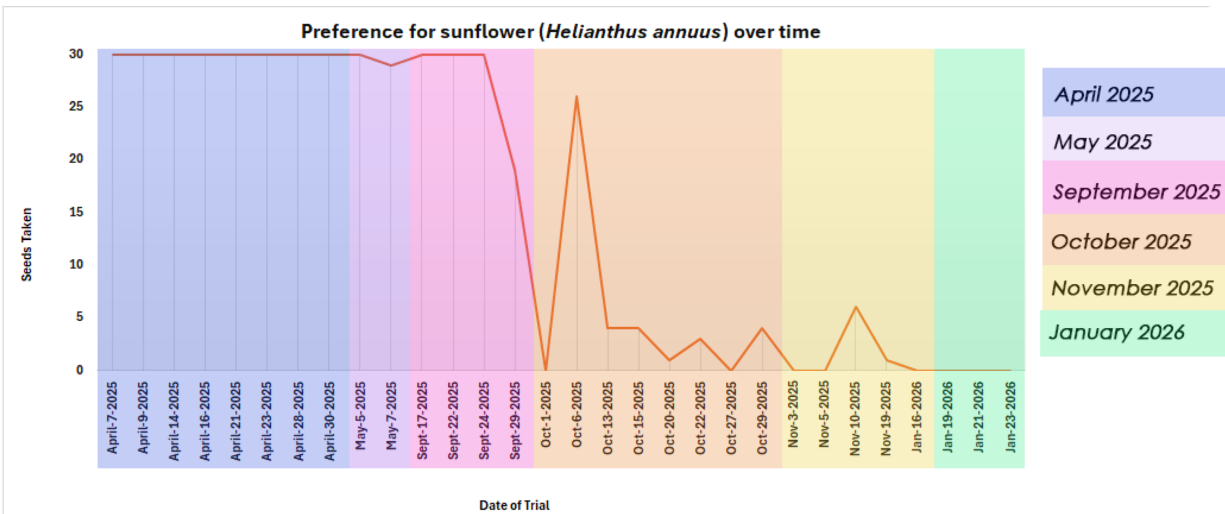

Figure 5. Number of sunflower seeds (out of 30) red harvester ants for each trial over the course of the experiment; separate colors indicate month trial took place.

Standard errors for most species' averages were relatively low; the only species with slightly higher standard errors were sunflower, blue flax, blue grama, and sideoat grama. Ants' preferences for these species experienced some fluctuations throughout the course of the experiment (Fig. 6). Sunflower and blue flax were both taken heavily in early trials before fluctuating later in the experiment, after September. Blue grama was taken inconsistently throughout the entire experiment. Sideoat grama experienced fluctuations but to a lesser extent; those that did occur were more moderate and only occurred a few times throughout the trials.

Neither species' fluctuations seemed to be particularly affected by season. Due to fluctuations in sideoat grama being minor, and seasonality not affecting avoided seeds, it is still considered an "avoided" seed. Fluctuations occurred regardless of time and to not enough of an extent to dramatically alter Sideoat grama's levels outside of the same range as other avoided species.

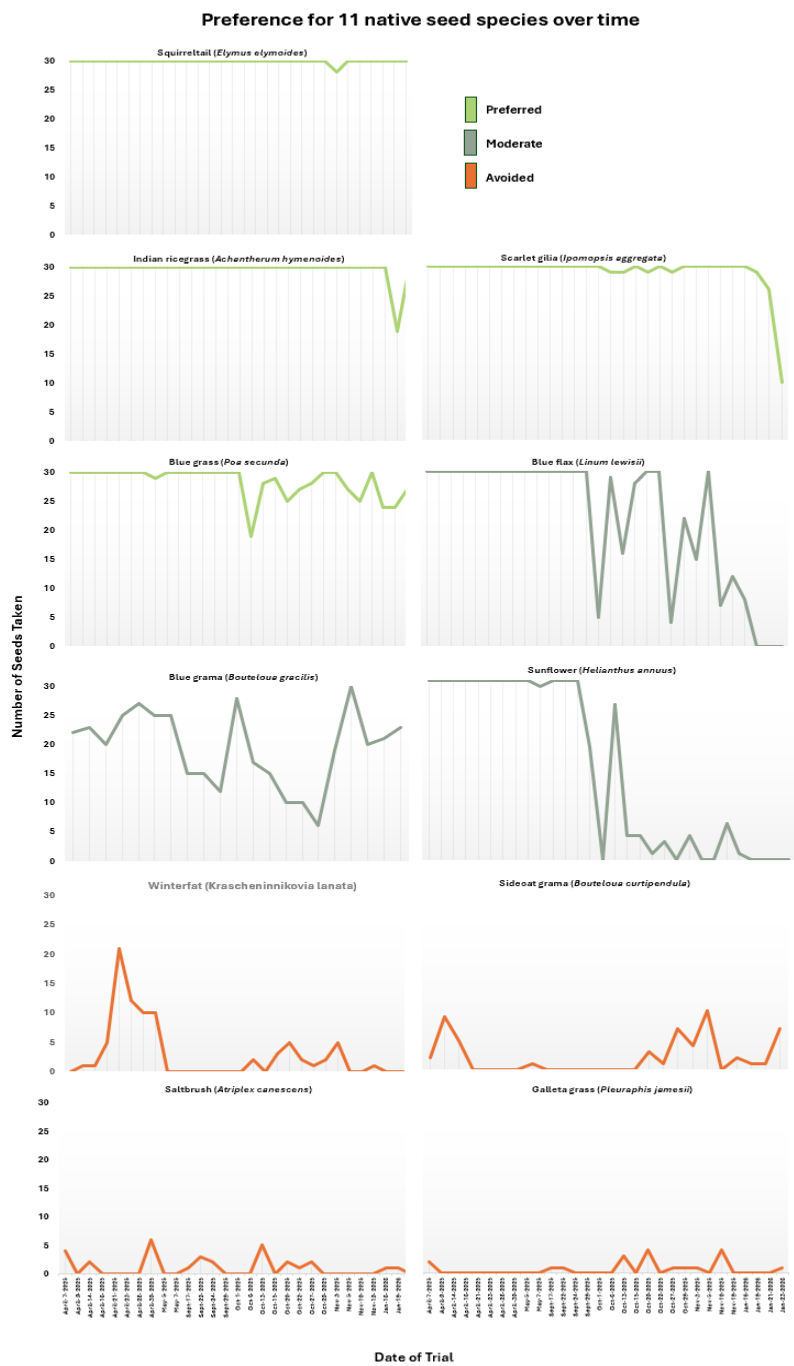

Figure 6. Number of seeds for each of the 11 native species that red harvester ants took for each trial over the course of the experiment. A total of 30 seeds per species per trial. Species that were considered preferred are colored in a bright green, species taken in moderate amounts are colored in gray, and species that were considered avoided by ants are colored in orange.

Seed Morphology

Three of the four (75%) preferred species were grasses, with the one (25%) remaining preferred species being a forb. Two of the four (50%) of the avoided species were grasses, the remaining two (50%) avoided species being both shrubs. Shrubs were less preferred, while preference for grasses or forbs generally varied.

Morphological traits are examined to classify certain traits as being preferable or avoided to red harvester ants. Morphological traits are visually observed and described (Table 1). Two of the preferred species, squirreltail and bluegrass, are both grasses with palea sheaths protecting small, oval shaped inner seeds. These inner seeds contain the nutritive tissue past seed coats and is what harvester ants seek out as food. The other two species, scarlet gilia and Indian ricegrass, have no external seed material at all. Indian ricegrass seeds have a lemma and palea covering, but this falls off before maturity, leaving only a hard seed coat over their nutritive tissue. Scarlet gilia has a mucilaginous seed coat but otherwise no outer protection. Avoided seeds varied more morphologically. Saltbush is an abscised fruit with a hard outer lemma coating, that is both grainy in texture and spiked. Getting through the lemma, palea, and seed coat is required to reach nutritive tissue. It is the only one of the avoided seeds to have a hard lemma, rather than a fluffy spikelet. Sideoat grama seed material consists of a spikelet, multiple lemmas connected to a stem, with both a palea and seed coat inside. Galleta grass seeds consists of a cluster of multiple spikelet, but the external material is generally fluffy. Winterfat is similar in morphology, with multiple spikelet that consist of long silky hairs. All avoided seeds also average on the larger side than preferred seeds.

Surface area to mass ratio (mm^2/mg) is also determined for each species and described numerically (Table 1). Lower ratios indicate smaller yet more densely packed seeds, while higher ratios indicate large seeds that lack density, and instead may be porous or have large amounts of light, external material. Ratios of all species ranged from 0.26 to 39.16 (Fig. 7); the highest and lowest value belonging to sunflower and sideoat grama, respectively. Preferred species held a smaller range of ratios, from 1.29 to 6.52. Avoided species ranged from 2.3 to 39.16, while moderately taken species ranged from 0.26 to 19.87. The difference between ratios was 5.23 for preferred species, 36.86 for avoided species, and 19.11 for moderate species. Preferred seeds range tended to be smaller, while moderately taken and avoided seeds tended to have much larger ranges.

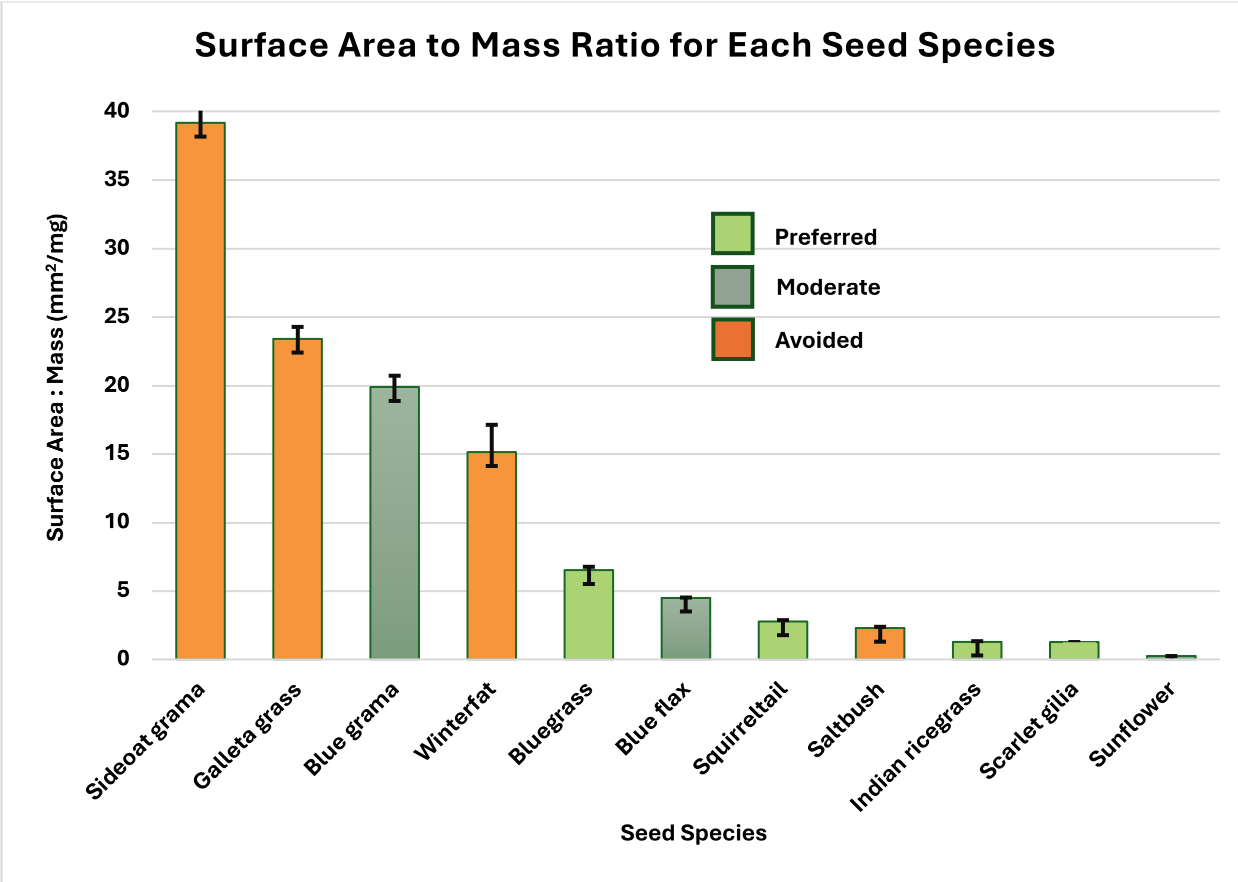

Figure 7. Surface area to mass ratios for each seed species, ordered by highest ratio value to lowest. Standard errors are shown as a line above each bar. Scarlet gilia and sunflower do not have standard error bars due to data being sourced from alternative studies. Species that were considered preferred are colored in a bright green, species taken in moderate amounts are colored in gray, and species that were considered avoided by ants are colored in orange.

Discussion

Red harvester ants have a strong preference and aversion for certain traits in seeds. The red harvester ants appeared to not prefer seeds with certain traits and strongly avoided or discarded these seeds in 'refuse' piles. Preferences, while complex, seemed to be rooted in different features of seed species, notably morphology, but surface area, mass, and time of the year may also affect how ants interact with seed species (Uhey et al., unpublished). Utilizing seeds with more “cumbersome” morphology, multiple layers, chaff, or hardened shells, in broadcast seeding may lead to less interference from red harvester ants foraging seeds (Martyn et al., 2022). Organizing broadcast seedings to take place during fall, winter, or early spring may also reduce interference, as red harvester ants may prefer seeds during late spring and summer than other types of prey items (like insects or sugar sources) (Pirk et al., 2009).

Seed trait preferences

Morphological traits, including physical features and composition, played a large role in preferences. Red harvester ants avoided seeds with large amounts of chaff, fluffy spikelet and lemma exteriors. Seeds with these traits include sideoat grama, winterfat, and galleta grass. Alternatively, saltbush had thick, spikey lemmas, which requires additional effort from ants to reach the nutritive seed inside. Ants must clear away multiple layers around the seed, which can be cumbersome, before reaching the nutritive tissue. Balancing energy of transport and energy of the seeds contributes to ant preferences of seeds (Holldobler, 1976). The additional time and energy required for opening chaff-heavy seeds with external layers make some species less appealing for red harvester ants.

Plant species preferred by red harvester ants sometimes lacked seed protective layers. Scarlet gilia lacked any protective layers and provided direct access to the nutritive tissue inside. Indian ricegrass only has a hard seed coat. Squirreltail and bluegrass have a palea layer covering their seed, but this layer is relatively thin and easy to open for ants. Harvester ant species generally prefer both small and unprotected seeds (Martyn et al., 2022). These thin coverings, or lack of covering at all, make it easier and more energy efficient for red harvester ants to open these seeds to access the same nutritive tissue as avoided seeds. Seed morphology and ease of access appear to drive red harvester ant seed preference or avoidance.

The relationship between preference and seed surface area and mass is complex. There is no obvious pattern suggesting red harvester ants heavily prefer higher or low surface areas to mass ratio values. Preferred seeds had a more conservative range in value between seeds, while the range for avoided and moderately taken species were both much larger. This could imply red harvester ants prefer seeds within a specific value range for surface area/mass, and not strictly high or low values. However, it should be noted that the large range in ratios for avoided seeds is largely created by one species, saltbush. While the other three avoided seeds hold high values, saltbush skews the range by having a relatively low value. This low value for saltbush is caused by its relatively large weight compared to its surface area, but it is possible a significant portion of this weight comes from its outer shell, rather than the seed inside. All avoided seeds have external layers, but saltbush's external layer is a thick shell, rather than light chaff like the other three avoided species have. This may be why saltbush has a considerably larger weight than the other avoided species. If we exclude saltbush, ants appear to avoid high area to mass ratio seeds and prefer lower ratios seeds. Other harvester ant species have been shown to avoid seeds with high surface area and low mass (Uhey et al., unpublished), but there doesn't seem to be as clear of connection with red harvester ants. Additional preference trials and surface area/mass values should be examined on additional species to better determine if either of these two patterns is truly accurate for red harvester ants.

Temporal preferences

Visualizing these seed-taking behaviors over time revealed a possible pattern of seasonal preferences for some plant species. Three separate studies found that a variety of species of harvester ants have shown seasonal preferences (Belchior et al., 2012, Robertson & Bossart, 2024, Pirk et al., 2009). Ants had larger home ranges and sought seeds more actively and from further away during the late spring and summer. Once shifting to autumn, foraging activity decreased (Belchior et al., 2012, Robertson & Bossart, 2024, Pirk et al., 2009). Despite studying different harvester ant species and in different locations, seasonal patterns of high and low foraging rates were similar. The seasonal change in red harvester ants (i.e. sudden decline in interest in sunflowers and blue flax) may mimic patterns found in natural observations of harvester ants. However, there are many hypotheses as to why preferences changed in October, additional species-specific studies in the southwest are needed to support the hypothesis that preferences changed because of season patterns (rather than a change in seed quality, seed contamination, or a change in lab conditions). Harvester ants alter foraging behavior once their prime foraging season ends; sometimes changing the preferences for seeds and other food resources (Pirk et al., 2009). In any case, harvester ants likely change their preferences for seed species throughout the seasons.

Red harvester ants may have strict guidelines for avoiding certain seeds. Another study on a different species of harvester ant determined seasonal preferences only affected preferred seeds, while avoided species remained the same regardless of time or season (Crist & MacMahon, 1992, Pirk et al., 2009). This appears true for our study as well. Avoided seeds were avoided throughout the entire course of the experiment, unaffected by seasonal preferences. Their findings further support the idea that examination of seasonal preferences of red harvester ants is needed to understand their preferences and avoidance of seeds.

Moderate seed preference

Despite blue flax lacking significant differences with two preferred species, scarlet gilia and blue grass, blue flax was categorized as a moderate species. However, because of blue flax's lower average and the fact it lacks statistical difference with all the other moderate species, instead of just half with the preferred, it is categorized as a moderately preferred species.

Although categorized as a moderate species, blue flax is the most irregular of its grouping. It has the most seeds taken of the moderate species, and since scarlet gilia and blue grass have the lowest number of seeds taken from the preferred category, they are close together. This may explain why they lacked a significant difference. Blue flax also notably shares certain morphological traits and surface area/mass values similar to preferred species. Like scarlet gilia, it lacks a seed coat at all, making it easy to access the nutritive tissue inside. While surface area/mass value isn't an explicit indicator of preference for red harvester ants, blue flax's value does fall in the same range as the other preferred species (Fig. 7). Of the other species examined, it is the most similar to the preferred species.

In the early experiment trials, all 30 blue flax seeds were taken each trail, until October, when ants avoided them. Sunflower foraging also followed this pattern (Fig. 6). As previously mentioned about seasonal preferences for harvester ants, blue flax is likely a species that was preferred in spring and summer, but as prime foraging seasons shifted it becomes less preferred. There are no obvious reasons for this with regard to morphology or surface area/mass values for blue flax or sunflower. To determine if a species is actually preferred, it is necessary to test preferences only in prime foraging months, or moderate, longer-term studies that examine preference through both spring, summer, and fall. Alternatively, examining different traits between moderate and preferred species may reveal clearer distinctions between their categorizations. There is also the possibility this occurrence only occurred in the lab setting, due to certain species seeds being infected with fungal agents. This may cause ants to suddenly avoid infected seeds. Re-testing in a field, and not lab setting, may clarify if outside pathogens are a contributing factor to seed avoidance.

Implications for broadcast seeding

Red harvester ants, which are extremely common in arid landscapes and known to remove seeds, pose a risk of interfering with broadcast treatments (Luna et al., 2018, Uhey & Hofstetter 2022). Taking of seeds or moving them to unfavorable locations for germination, likely lessens the success of reseeding and thus would require repeat treatments. Using species like saltbush, galleta grass, sideoats grama, winterfat, or species with similar morphological traits can reduce red harvester ant interference with broadcast seeding. Our findings can aid land managers and restoration projects to reduce interference via granivory by red harvester ants, while still benefitting from their presence as a keystone species and creation of 'refuse' piles. We also identify patterns of seed removal in both temporal and surface area/mass relationships that provide direction for future research on harvester ants. Knowing how these factors relate may help understand red harvester ants' seed preferences, and help managers minimize their reseeding cost.

Conclusion

Red harvester ants are keystone species in their ecosystems and provide a myriad of benefits to vegetation and wildlife within these ecosystems (Uhey & Hofstetter, 2022). While ants do collect seed (granivory) in response to broadcast seeding, there are strategies to mitigate their interference. Knowledge of when to curate broadcast seedings and strategies for avoiding harvester ant foraging preferences, we can reduce their interference in restoration efforts.

Although we know that arid ecosystems benefit from the presence of both red harvester ants and native vegetation, there are still many unexplored facets and complexities to red harvester ant preferences and avoidance of seeds.

References

Belchior, C., Del-Claro, K., & Oliveira, P. S. (2012). Seasonal patterns in the foraging ecology of the harvester ant *Pogonomyrmex naegelii* (Formicidae, Myrmicinae) in a Neotropical savanna: Daily rhythms, shifts in granivory and carnivory, and home range. *Arthropod-Plant Interactions*, *6*(4), 571–582. https://doi.org/10.1007/s11829-012-9208-1

Bulot, A., Provost, E., & Thierry, D. (2016). Refuse pile turnover by harvester ants (Hymenoptera: Formicidae) increases seed density and seedling species richness in dry grasslands. *Myrmecological News*, *23*, 91–100.

Castillo-Lorenzo, E., Pritchard, H. W., Finch-Savage, W. E., & Seal, C. E. (2019). Comparison of seed and seedling functional traits in native *Helianthus* species and the crop H. annuus (sunflower). *Plant Biology*, *21*(3), 533–543. https://doi.org/10.1111/plb.12928

Crist, T. O., & MacMahon, J. A. (1992). Harvester ant foraging and shrub-steppe seeds: Interactions of seed resources and seed use. *Ecology*, *73*(5), 1768–1779. https://doi.org/10.2307/1940028

Guiterman, C. H., Gregg, R. M., Marshall, L. A. E., Beckmann, J. J., van Mantgem, P. J., Falk, D. A., Keeley, J. E., Caprio, A. C., Coop, J. D., Fornwalt, P. J., Haffey, C., Hagmann, R. K., Jackson, S. T., Lynch, A. M., Margolis, E. Q., Marks, C., Meyer, M. D., Safford, H., Syphard, A. D., … Stevens, J. T. (2022). Vegetation type conversion in the US Southwest: Frontline observations and management responses. *Fire Ecology*, *18*(1), 6. https://doi.org/10.1186/s42408-022-00131-w

Hickey, A., Hollander, J., & Peacock, M. (2016). Assessing microhabitat cover and distance effects on harvester ant intraspecific seed preference. *Journal of Insect Behavior*, *29*(5), 535–547. https://doi.org/10.1007/s10905-016-9580-2

Hölldobler, B. (1976). Recruitment behavior, home range orientation and territoriality in harvester ants, Pogonomyrmex. *Behavioral Ecology and Sociobiology*, *1*(1), 3–44. https://doi.org/10.1007/BF00299951

Luna, P., García-Chávez, J. H., & Dáttilo, W. (2018). Complex foraging ecology of the red harvester ant and its effect on the soil seed bank. *Acta Oecologica*, *86*, 57–65. https://doi.org/10.1016/j.actao.2017.12.003

MacMahon, J. A., Mull, J. F., & Crist, T. O. (2000). Harvester ants (*Pogonomyrmex* spp.): their community and ecosystem influences. *Annual Review of Ecology, Evolution, and Systematics*, *31*(Volume 31, 2000), 265–291. https://doi.org/10.1146/annurev.ecolsys.31.1.265

Martyn, T. E., Christina L. Kwapich, Albert Kline, & Elise S. Gornish. (2022). Granivorous ants prefer small and unprotected seeds—Implication for restoration in arid ecosystems. *Restoration Ecology*. https://doi.org/DOI: 10.1111/rec.13759

Miller, C. F. (2013). The evolving understanding of grassland restoration seeding protocols. *Ecological Restoration*, *31*(2), 127–129. https://doi.org/10.3368/er.31.2.127

Mueller, S. E., Thode, A. E., Margolis, E. Q., Yocom, L. L., Young, J. D., & Iniguez, J. M. (2020). Climate relationships with increasing wildfire in the southwestern US from 1984 to

2015. *Forest Ecology and Management*, *460*, 117861.
https://doi.org/10.1016/j.foreco.2019.117861

Natural Resource Conservation Service. (n.d.). *PLANTS database*. United States Department of Agriculture. Retrieved https://plants.usda.gov/

Paolini, K. E., Matthew Modlin, Alexis A. Suazo, David S. Pilliod, Robert S. Arkle, Kerri T. Vierling, & Joseph D. Holbrook. (2020). Harvester ant seed removal in an invaded sagebrush ecosystem: Implications for restoration. *Ecology and Evolution*, *10*(24), 13731–13741.
https://doi.org/10.1002/ece3.6963

Peppin, D. L., Fulé, P. Z., Sieg, C. H., Beyers, J. L., Hunter, M. E., & Robichaud, P. R. (2011). Recent trends in post-wildfire seeding in western US forests: Costs and seed mixes. *International Journal of Wildland Fire*, *20*(5), 702–708. https://doi.org/10.1071/WF10044

Perez, E. E., Crapiste, G. H., & Carelli, A. A. (2007). Some physical and morphological properties of wild sunflower seeds. *Biosystems Engineering*, *96*(1), 41–45.
https://doi.org/10.1016/j.biosystemseng.2006.09.004

Pirk, G. I., De Casenave, J. L., Pol, R. G., Marone, L., & Milesi, F. A. (2009). Influence of temporal fluctuations in seed abundance on the diet of harvester ants (*Pogonomyrmex* spp.) in the central Monte desert, Argentina. *Austral Ecology*, *34*(8), 908–919.
https://doi.org/10.1111/j.1442-9993.2009.01999.x

Pirk, G. I., & Lopez De Casenave, J. (2014). Effect of harvester ants of the genus Pogonomyrmex on the soil seed bank around their nests in the central Monte desert, Argentina. *Ecological Entomology*, *39*(5), 610–619. https://doi.org/10.1111/een.12140

Robertson, A., & Bossart, J. (2024). Seasonal diet change of the Florida harvester ant (*Pogonomyrmex badius*) and competition with the Red imported fire ant (*Solenopsis invicta*). *Southeastern Naturalist*, *23*(3), 351–365. https://doi.org/10.1656/058.023.0306

Uhey, D. A., Aguilar, M. B., Balazs, K. R., Vissa, S., Gehring, C. A., Stevens, B. M., & Hofstetter, R. W. (n.d.). *Harvester ants (Pogonomyrmex spp.) vary in their preference for native seeds but reject nonnatives: Implications for seed restoration*. Unpublished.

Uhey, D. A., & Hofstetter, R. W. (2022). From pests to keystone species: ecosystem influences and human perceptions of Harvester ants (*Pogonomyrmex, Veromessor,* and *Messor* spp.). *Annals of the Entomological Society of America*, *115*(2), 127–140. https://doi.org/10.1093/aesa/saab046

Uhey, D. A., Vissa, S., Haubensak, K. A., Ballard, A. D., Aguilar, M. B., & Hofstetter, R. W. (2024). Increased cover of native and exotic plants on the rims of harvester ant (Hymenoptera: Formicidae) nests under grazing and drought. *Environmental Entomology*, *53*(1), 180–187. https://doi.org/10.1093/ee/nvad113

USDA, Natural Resource Conservation Service, & Plant Materials Center. (2018). *Seed establishment techniques and seeding specifications*.

Wolf, L. L., Hainsworth, F. R., Mercier, T., & Benjamin, R. (1986). Seed size variation and pollinator uncertainty in *Ipomopsis aggregata* (Polemoniaceae). *Journal of Ecology*, *74*(2), 361–371. https://doi.org/10.2307/2260260

Appendix

Table 2. Pairwise comparisons between each species, showcasing difference between 48-hour averages for each seed species and the significance level of that difference. Asterisks denote significant differences. Comparisons between two avoided species are marked in orange, two preferred in green, and two moderate in gray. Comparisons where there was no significant difference but the species had different groupings are marked in blue.

Species 1	Species 2	Estimated difference	Adjusted p-value
Saltbush	Indian ricegrass	27.97	<0.001*
Saltbush	Sideoat grama	0.9667	.979
Saltbush	Blue grama	18.20	<0.001*
Saltbush	Squirreltail	28.50	<0.001*
Saltbush	Sunflower	13.80	<0.001*
Saltbush	Scarlet gilia	27.60	<0.001*
Saltbush	Winterfat	1.267	.971
Saltbush	Blue flax	20.43	<0.001*
Saltbush	Galleta grass	-0.8000	.916
Saltbush	Blue grass	26.80	<0.001*
Indian ricegrass	Sideoat grama	-27.00	<0.001*
Indian ricegrass	Blue grama	-9.767	<0.001*
Indian ricegrass	Squirreltail	0.5333	.973
Indian ricegrass	Sunflower	-14.17	<0.001*
Indian ricegrass	Scarlet gilia	-0.3667	1.00
Indian ricegrass	Winterfat	-26.70	<0.001*
Indian ricegrass	Blue flax	-7.533	.0450*
Indian ricegrass	Galleta grass	-28.77	<0.001*
Indian ricegrass	Blue grass	-1.167	.782
Sideoat grama	Blue grama	17.23	<0.001*
Sideoat grama	Squirreltail	27.53	<0.001*
Sideoat grama	Sunflower	12.83	<0.001*
Sideoat grama	Scarlet gilia	26.63	<0.001*
Sideoat grama	Winterfat	0.3000	0.999
Sideoat grama	Blue flax	19.47	<0.001*
Sideoat grama	Galleta grass	-1.767	0.252
Sideoat grama	Blue grass	25.83	<0.001*
Blue grama	Squirreltail	10.30	<0.001*
Blue grama	Sunflower	-4.400	0.890
Blue grama	Scarlet gilia	9.400	<0.001*
Blue grama	Winterfat	-16.93	<0.001*
Blue grama	Blue flax	2.233	0.997
Blue grama	Galleta grass	-19.00	<0.001*
Blue grama	Blue grass	8.600	<0.001*
Squirreltail	Sunflower	-14.70	<0.001*
Squirreltail	Scarlet gilia	-0.9000	0.955
Squirreltail	Winterfat	-27.23	<0.001*
Squirreltail	Blue flax	-8.067	0.022*
Squirreltail	Galleta grass	-29.30	<0.001*
Squirreltail	Blue grass	-1.700	0.057
Sunflower	Scarlet gilia	13.80	<0.001*

Species 1	Species 2	Estimated difference	Adjusted p-value
Sunflower	Winterfat	-12.53	0.002*
Sunflower	Blue flax	6.633	0.655
Sunflower	Galleta grass	-14.60	<0.001*
Sunflower	Blue grass	13.00	<0.001*
Scarlet gilia	Winterfat	-26.33	<0.001*
Scarlet gilia	Blue flax	-7.167	0.078
Scarlet gilia	Galleta grass	-28.40	<0.001*
Scarlet gilia	Blue grass	-0.8000	0.996
Winterfat	Blue flax	19.17	<0.001*
Winterfat	Galleta grass	-2.067	0.460
Winterfat	Blue grass	25.53	<0.001*
Blue flax	Galleta grass	-21.23	<0.001*
Blue flax	Blue grass	6.367	0.152
Galleta grass	Blue grass	27.60	<0.001*