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Red harvester ant (Order: Hymenoptera) preference for cover crop seeds in South Texas

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11 **Abstract:**

12 Harvester ants are known to selectively forage seeds, potentially impacting nearby plant
13 community composition. In agricultural areas, harvester ants may be viewed as pests by foraging
14 on crop seeds or as beneficials by preferentially removing weed seeds. However, little work has
15 been done on harvester ant preferences for cover crop seeds. Local observations suggest that ants
16 may take cover crop seeds, but no studies have evaluated ant agricultural impacts or seed
17 preferences in the Lower Rio Grande Valley (LRGV). We examined red harvester ant
18 (*Pogonomyrmex barbatus* Smith) preferences for commonly used cover crop seeds in the LRGV
19 (vetch, oat, fescue, sunn hemp, and radish with wheatgrass as a control) and a commonly used
20 bacterial seed inoculation treatment meant to increase root nodulation. We tested seed sets using
21 choice tests housed in seed depots located within the foraging range of ant colonies with no prior
22 exposure to the selected seeds. Of the evaluated cover crop seeds, wheatgrass and oat were the
23 first to be removed entirely from the depot, with vetch remaining after 24 h. When we inoculated
24 the two most preferred seeds to determine if there was a preference for non-inoculated seeds, we
25 found no difference between inoculated and non-inoculated seeds. There were also significant
26 changes in activity over time for both trials. These data indicate that harvester ant foraging
27 preferences and activity can inform grower management recommendations regarding the risks of
28 using certain cover crops and months sowing should be conducted in fields with known harvester
29 ant presence.

30

31 **Key Words:** bacterial inoculum, Lower Rio Grande Valley, seed depot study, seed preference

32 **Introduction**

33 Harvester ants in the genus *Pogonomyrmex* commonly reside in arid to semi-arid regions
34 of the Americas and can be found in a range of habitats including agricultural and peri-urban
35 matrices (Luna et al. 2018; Viera-Neto et al. 2016; Tizón et al. 2010; MacMahon 2000). The
36 state of Texas has 12 species of harvester ants with the red harvester ant (*Pogonomyrmex*
37 *barbatus* Smith) being the most common in the Lower Rio Grande Valley (LRGV), an
38 agriculturally rich location with a semi-arid sub-tropical climate (Martinez et. al., 2020, Davis,
39 2016). Harvester ant foraging occurs mainly along trails that extend from the colony to
40 neighboring food sources within their foraging range (Taber, 1999; Traniello, 1989). While
41 foraging trails average 10 m long, colony-dense areas (which may have over 80 nests/hectare)
42 have trails extending up to 60 m from the nest site (Reed and Landolt, 2019). Harvester ants,
43 primarily granivores, use these trails to collect seeds located on the soil surface, often from or
44 surrounding the parent plant (MacKay and MacKay 2002; Taber, 1999). *P. barbatus*, *P. rugosus*
45 Emery, *P. occidentalis* Cresson, and *P. salinus* Olsen species tend to harvest near the trunk of
46 their foraging trails which are shaped by seed distribution, disturbances, or inter/intra-species
47 interactions (MacMahon, 2000; Traniello, 1989).

48 Harvester ants exhibit seed preferences based on a combination of relative seed
49 abundance, size/shape, and nutritional content of the seeds (Penn and Crist, 2018; MacMahon,
50 2000; Taber 1999). For instance, *P. occidentalis* Cresson prefers to forage with high species
51 fidelity in seed-dense patches, which can reduce local seed bank heterogeneity (Luna et al. 2018;
52 MacMahon, 2000; Crist and MacMahon, 1991). When the seed bank has low seed diversity, ants
53 will collect less preferred seed varieties until more desirable seeds are available (MacMahon,

54 2000). When more preferred seeds return, ants will empty colony seed stores of the less desired
55 seeds to replace them with preferred options (MacMahon, 2000).

56 Harvester ant seed foraging is not limited to natural areas and may occur in agricultural
57 matrices where seed preferences may benefit or harm crop production. Although harvester ants
58 are known to consume weed seeds, their seed preferences may also include consumption of crop
59 seeds (Barbercheck and Wallace, 2021; Baraibar et al. 2011; Taber, 1999). Ant removal of crop
60 seeds and vegetation causes economic loss, especially if the crop is situated within areas of high
61 colony density (Reed and Landolt, 2019; Borth, 1982). Red harvester ants in particular are found
62 in agricultural areas in the LRGV and may have a large impact on the plant community through
63 removal of vegetation surrounding their nest entrance (1-5 m in diameter) or through seed
64 collection (Reed and Landolt, 2019; MacMahon and Crist, 2000)

65 In addition to cash crops, harvester ants in agricultural fields may forage on cover crop
66 seeds (based on personal communications) but have not been well documented. In the LRGV,
67 cover crops are used during fallow periods to prevent soil erosion from wind or water (Soti and
68 Racelis, 2020; Martinez et al., 2020; Nicolas Labrière, 2015; Bodner et al., 2010 Yu et al., 2000).
69 Presumably, if an ant-preferred seed is sown within the foraging range of a colony, it will not
70 have time to germinate before being taken by a forager to the colony granary. The lack of a root
71 system and above-ground vegetation in the foraged field area can then potentially increase
72 economic loss for the farmer (Soti and Racelis, 2020; Martinez et al., 2020; Bodner et al., 2010).
73 So, preventing harvester ant interference with cover crops could potentially reduce soil exposure
74 to erosion as well as save the cost of having to re-seed foraged areas.

75 The primary objective of the study was to determine red harvester ant preferences for
76 commonly used cover crop seeds in the LRGV. We chose members of the families Fabaceae,
77 Poaceae, and Brassicaceae that are currently being evaluated by farmers in the LRGV - hairy
78 vetch (*Vicia villosa*), oat (*Avena sativa*), sunn hemp (*Crotalaria juncea*), radish (*Raphanus*
79 *sativus*), and fescue (*Festuca arundinacea*). Wheatgrass (Poaceae: *Triticum aestivum*) was also
80 included as a known preferred food for harvester ants and served as a control for the
81 experiments (Brito-Bersi et al., 2018; Ryti and Case, 1988). Based on known baseline
82 preferences that harvester ants exhibit towards grasses, we anticipated that oat, fescue, and
83 wheatgrass would be most preferred as they are sugar-rich grasses from the family Poaceae.
84 (MacMahon, 2000; Taber 1999). In addition to the use of cover crops, LRGV farmers may
85 inoculate cover crop seeds with nitrogen-fixing bacteria to facilitate root nodulation to further
86 benefit the soil (Rai et al., 2021; Kasper et al., 2019; Kasper 2019). As such treatments may
87 influence ant foraging decisions, the second objective was to determine if seed inoculation
88 treatments used for increased germination rates would alter the previously established cover
89 crop seed preferences.

90

91 **Methods:**

92 *Site Description*

93 The study site was located within the Lower Rio Grande Valley in South Texas. This area
94 is considered a local steppe climate that is subtropical subhumid marine with an average annual
95 temperature of 24°C (16.3-30.2°C) and 572 mm of precipitation. Soils in these regions of the Rio
96 Grande Plain are considered deep loamy soils with moderately sloped planes and an average
97 altitude of 34 m (USDA, 2008). Specifically, all trials were conducted at the University of Texas

98 at Rio Grande Valley (UTRGV) campus (~ 1.5 km²) in Edinburg, Hidalgo County, TX, USA
99 (26.306667, -98.170944). This site was selected as the ants present would have no prior exposure
100 to the species of seeds presented during the study, but would also still experience disturbance
101 pressures such as irrigation and routine mowing (a proxy for agricultural practices relative to
102 natural settings).

103 On the campus, most vegetation included grasses used for lawns intermixed with weeds
104 (primarily grass burr/sticker burr) and punctuated by standard suburban ornamental plants (such
105 as Tropical Milkweed (*Asclepias curassavica*) and *Lantana sp.*). As of publication of the 2020
106 Tree Campus USA Report, there are 53 different species of trees with Live Oak (*Quercus*
107 *virginiana*), Texas Ebony (*Ebenopsis ebano*), and Honey Mesquite (*Prosopis glandulosa*) being
108 noteworthy examples (UTRGV Office For Sustainability, 2021). The immediate land use
109 surrounding the study site is considered a combination of suburban and peri-urban with
110 intermixed sorghum fields, pasture, and citrus groves. Land use within the LRGV more generally
111 also includes mixed fruit and vegetable crops as well as sugarcane production. Active
112 *Pogonomyrmex* colonies (n = 37) with no prior exposure to cover crop seeds near were mapped
113 throughout the site using an eXplorist 610 GPS unit (Magellan, San Dimas, CA, USA). Colony
114 activity was determined by whether there were foraging trails present with active bidirectional
115 ant traffic.

116 *Seed Preference Trials*

117 To determine whether size differences between seeds could impact preference, 10 seeds
118 of each variety were weighed and averaged and seed texture was noted. For the trial, the cover
119 crop seeds - hairy vetch (Johnny's Selected Seeds, Winslow, ME), oat, sunn hemp (Johnny's
120 Selected Seeds, Winslow, ME), wheatgrass (Todd's Seeds, Livonia, MI), radish (Johnny's

121 Selected Seeds, Winslow, ME), and fescue (GreenCover, Bladen, NE) - were pre-counted in
122 groups of 10 seeds per cover crop and stored in microcentrifuge tubes at room temperature
123 before transport to the field. Seed depots were constructed out of I-plate Petri dishes (100 mm ×
124 15 mm). Petri dishes were sanded to produce a rough surface to increase traction, and 3 U-
125 shaped entrances were created with a soldering iron at 45° and 90° angles on each half of the
126 Petri dish to allow for easy ant entry to the dish.

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

142 Due to a delay in shipping, the colonies observed in the first two days of trials (n=12)
143 were not immediately exposed to fescue seeds. These colonies were re-tested later with a depot

144 mix including fescue seeds. They were compared to colonies that were exposed to fescue from
145 the start and they did not demonstrate any difference in preference. Because of this lack of
146 difference, we decided to use the full data set from the second round of trials from the initial
147 twelve colonies for data analyses.

148

149 *Seed Inoculation Trials*

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

161

162 *Statistical Analysis*

163 R version 3.6.2 (RStudio Team, 2020) was used to conduct all statistical analyses. Within
164 each dataset, each seeds' time to removal was categorized individually with censoring due to
165 external events (e.g., flipped depots due to high wind speeds, removal of the cage prior to the 24
166 hours period, etc.) denoted. The survdiff function from the survival package was used to

167 determine if there was a significant difference in ant cover crop preference (Therneau, 2015;
168 Therneau and Grambsch, 2000). The Kaplan-Meier survival estimator, which estimates the
169 likelihood of an event occurring at a point in time, was used to calculate seed removal event
170 likelihood over time (Johnson, 2018). The log-rank test using the lifelines package (Rickert,
171 2017), a hypothesis test that compares the survival distribution between two samples, was used
172 to compare the survival distribution of cover crop seeds to the wheatgrass and non-inoculated
173 controls. To further investigate these differences while incorporating other variables such as
174 observation month, we used Cox proportional hazard models and preferences compared against
175 the wheatgrass standard using the ggforest function from the survival package (Therneau, 2015;
176 Therneau and Grambsch, 2000).

177

178 **Results**

179 *Seed Preference Trials*

180 Kaplan Meier survival curves were used to compare removal rates of the different cover
181 crop seed varieties (Fig. 1). The Cox proportional hazards model determined the only significant
182 differences in removal were between wheatgrass and vetch ($p < 0.001$), wheatgrass and sunn
183 hemp ($p < 0.001$), and wheatgrass and fescue ($p < 0.050$), (Fig. 2; Table 1). During the trials,
184 ants exhibited a preference for wheatgrass and oat seeds, often removing all the seeds before 24
185 h (Table 1). For differences between seed types outside of wheatgrass, a pairwise log rank test
186 was used.

187 The pairwise log rank test provided differences in survival between the seeds amongst
188 themselves (Table 2). Vetch and Sunnhemp, though not significantly different from one another,
189 were the varieties that were significantly less harvested in comparison to other seed types outside

190 of wheatgrass. Overall, vetch was found to be significantly less harvested when compared to oat
191 ($p < 0.050$), wheatgrass ($p < 0.001$) or radish ($p < 0.050$). Sunn hemp was found to be
192 significantly less harvested when compared to oat ($p < 0.005$), wheatgrass ($p < 0.001$), or radish
193 ($p < 0.003$) (Fig. 2; Table 2). Similarly to the Cox proportional hazards model, Fescue, while not
194 being significantly different from vetch or sunn hemp, was significantly less harvested than
195 wheatgrass ($p < 0.050$), another member of the Poaceae family. Other than seed types, seed
196 collection differed among months (Supplementary Fig. 1). Over time, seed collection
197 significantly decreased from February to June (Supplementary Fig. 1).

198 The physical characteristics of the seeds in the depot did not appear to affect preference
199 as the preferred seeds in the study did not consistently share characteristics. Non-preferred seeds
200 also did not share seed shape or texture, only color and nitrogen-fixing abilities. All the seeds'
201 weights were similar with the exception of fescue and radish, which were significantly lighter
202 than the other varieties (Fig. 3). Vetch and radish shared physical characteristics - both were
203 round and uneven in texture, but they were treated differently by the ants. Sunn hemp was
204 smooth, and bean shaped, while oat and fescue appeared fibrous towards the ends with a thin and
205 elongated shape. Wheatgrass was oblong in shape and relatively smooth.

206

207 *Seed Inoculum Trial*

208 Unlike the seed preference trials, inoculum trials did not indicate significant differences
209 in preference. The Kaplan Meier curve created from the collected data further demonstrated the
210 visual lack of preference between inoculated versus non-inoculated seed between the same seed
211 type (Fig. 4). Additionally, the Cox proportional hazards data demonstrated that the difference in
212 preference between the inoculated and non-inoculated seeds was not significant (Fig. 5; Table 3).

213 This lack of overall preference also meant that there was no preference between one another
214 (Table 4). Surprisingly, the only significance found within the trial was a change in seed removal
215 (Supplementary Fig. 2). Depot harvesting was significantly higher in July in comparison to June
216 or August.

217

218 **Discussion**

219 The goal of the study was to determine if red harvester ants exhibit preferences among
220 different cover crop seed varieties and whether inoculating preferred seed types with nitrogen-
221 fixing bacteria would inhibit the desirability of the seed. We introduced naive harvester ants to
222 agricultural seeds via seed depots deployed over 24 h. We found that harvester ants had a
223 significant preference for grass seeds and radish seeds compared to nitrogen-fixing sunn hemp
224 and vetch seeds. However, we did not observe any difference in preference between inoculated
225 and non-inoculated seeds of either wheatgrass or radish.

226 We had assumed harvester ants would prefer to forage on certain seeds based on physical
227 characteristics and family (Poaceae) (Penn and Crist, 2018; MacMahon, 2000; Taber 1999). As
228 anticipated due to prior work on seed preferences in natural areas, all grass seeds were similarly
229 preferred. However, the attributes of radish overlapped with the less preferred seeds in terms of
230 shape, color, or weight, indicating these physical traits were not the only driver of preference
231 within this context (MacMahon, 2000; Taber 1999).

232 Alternatively, seed preferences could have been based on seed availability in the
233 surrounding habitat, which likely changed from February 2020 to August 2020. During the
234 study, we observed native seed burrs (Genus *Cenchrus* L.) being taken into the colony often as
235 well as smaller grass seeds. Prior documentation of burrs in and around Hidalgo county indicates

236 that burrs are annual grasses with an affinity for frequently disturbed sites such as roadsides,
237 similar to the study sites (Goel et al., 2011; Shaw, 2011). *Cenchrus echinatus* L. begin to
238 germinate in the late spring, continuing through the fall (Smith et al., 2012; Cope & Gray, 2009).
239 The decrease in seed removal from trials that occurred from spring to summer could be a change
240 in priority from depot seeds to collecting recently germinated seeds from the surrounding
241 *Cenchrus sp.* Given these observations, the interactions of cover crop seeds with weed banks
242 within agricultural settings needs to be evaluated further, particularly in regards to sowing
243 timing. Outside of seed preference changes due to the surrounding seed pool, *P. barbatus*
244 activity is closely related to rainfall, peaking in the summer months and correlated with overall
245 seed availability. With additional rainfall, more grasses outside of drought resistant varieties such
246 as *Cenchrus sp.* potentially germinated, allowing for more diversity in the seed pool
247 (MacMahon, 2000; Smith et al., 2012; Cope & Gray, 2009). The additional surrounding native
248 seeds could have been another cause for the reduction in depot harvesting over time from
249 February to June. Alternatively, during the sudden increase in depot harvesting from June to July
250 could be in preparation for August, which is usually known for its higher temperatures. In
251 August, activity significantly decreased in comparison to both June and July, implying that high
252 amounts of collection in June could have been done to avoid excess water loss for the colonies in
253 August (Supplementary Fig. 2)

254 Another interesting, isolated event was recorded on July 23rd, 2020, two days prior to the
255 touch down of Hurricane Hanna in the LRGV. Within one hour, 8 of 9 colonies had completely
256 emptied the depots. The impacts of such weather events are known to affect insect behavior in
257 response to changes in barometric pressure; many insects exhibit sudden insatiable appetites
258 likely preparing for weather events that follow. (Fernando R. Sujimoto, 2019; Flitters, 1963).

259 Leaf-cutter ants have been observed to significantly increase foraging during periods of low
260 barometric pressure, and harvester ants may do the same (Fernando R. Sujimoto, 2019). Future
261 studies regarding the correlation between harvester ant foraging intensity and barometric
262 pressure could help determine risk during certain planting dates in regions along the gulf coast
263 that have the potential to experience tropical cyclones annually.

264 Harvester ants have been previously observed to have contradictory behavior regarding
265 the same seed species based on other aspects such as seed germination or fungal infection
266 (MacMahon et al., 2000; Taber, 1999; Crist and Friese, 1993). However, in our trials, inoculated
267 and non-inoculated seeds were not treated differently, indicating that the presence of nitrogen-
268 fixing bacteria did not inhibit or encourage harvester ant predation. Regardless, there is
269 conflicting data regarding the amount of microbial diversity/biomass within the soil around ant
270 colonies (Ginzburg et al., 2008; Boulton et al., 2003; Wagner et al., 1997). *Pogonomyrmex*
271 *barbatus* in the study showed no preference towards or against inoculated seeds, hinting that
272 their granaries could be potentially rich in microbial activity. Alternatively, harvester ants do
273 partake in seed cleaning behavior that could occur at any point prior to introduction to the
274 granary.

275 In subtropical areas such as the LRGV, prior studies recommend the use of warm season
276 cover crops due to subtropical climate and promotion of native mycorrhizal fungi (Soti et al.
277 2016; Rugg, 2016). Based on the data collected in this study, harvester ants were exhibited lower
278 levels of preference towards certain seed varieties such as sunn hemp. The benefits that these
279 nitrogen fixing varieties, such as sunn hemp, hold towards the soil can be extremely beneficial.
280 Sunn hemp, for example, conserves phosphorus in the soil, increases nitrates, and has the
281 potential to improve soil health in subtropical agroecosystems such as the LRGV (Soti et al.

282 2016; Rugg, 2016; Mansoar et al. 1997). Not only does sunn hemp have the potential to be an
283 excellent South Texas cover crop, but it is also increasing in popularity in other southern areas of
284 the U.S. like Florida and Louisiana. Similarly, hairy vetch also has potential to be a great cover
285 crop due to the low ant preference and its weed suppression and nitrogen-fixing abilities (Moran
286 and Greenberg, 2008). Given these cover crops are not preferred over grasses in the seed depot
287 study, which are common in the non-crop habitats surrounding LRGV crop fields, harvester ants
288 would likely predate on surrounding weeds and grasses instead of the chosen cover crop.

289 Harvester ants can be a substantial disturbance agent in arid to semi-arid regions of the
290 United States and Mexico. *Pogonomyrmex sp.* have a pest status for seed collection and plant
291 removal in agricultural areas and can remove up to 100% of a preferred seed within their
292 foraging range (Crist and MacMahon, 1992; Tabber, 1999). Our data suggests we can
293 recommend nitrogen-fixing cover crops like sunn hemp and vetch to farmers as a potential cover
294 crop during fallow periods and could be paired with the fact that seed inoculation is neither
295 preferred or rejected by harvester ants. Inoculating these nitrogen-fixing seeds could help with
296 nodulation, nitrogen-fixing processes, and benefit the soil health below ground while protecting
297 topsoil from erosion. Not only that, using the pair for a cover crop trial, could in turn encourage
298 harvester ant predation on weed species or surrounding native plants that could limit crop yields
299 (Baraibar et al., 2011). Additional research should be conducted regarding harvester ant
300 preferences. For example, conducting preference studies with rural harvester ants that have more
301 exposure to different agricultural seed varieties and in turn, potential differences in preferences.
302 A better understanding of harvester ant seed preferences can be used to encourage predation on
303 native or weed seeds while reducing the need to eradicate native harvester ant colonies.

304

305 **Acknowledgements**

306 Funding support for LE was provided by the Dean's Graduate Research Assistantship
307 from UTRGV. We would like to thank the Racelis' Agroecology Lab at UTRGV for providing
308 the seeds used in the experiment, as well as the inoculum.

309

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432 **Figure Labels**

433 **Figure 1.** Kaplan Meier curve of seed types' likelihood of survival over the course of the seed
434 preference trial based on selected data. (n=37 colonies). The dashed line indicates the overall
435 median removal time.

436 **Figure 2.** Hazard Proportional Ratio test demonstrating differences in preferences between seed
437 types. Reference is wheatgrass. Means on the right side of the chart indicate a larger number of
438 seeds that were removed during the trial. Differences in n (observed seed number) were due to
439 seeds that were censored for external events.

440 **Figure 3.** Differences in seed weight between the six cover crop seeds (n=50/seed type) used in
441 the study. Boxplots are in the style of Tukey where the box limits represent the lower 25% and
442 upper 75% quantile with the line representing the median. Tukey HSD was used to determine
443 significance differences (denoted by letters) among seed weights.

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445 **Figure 4.** Kaplan Meier curve of seed types' likelihood of survival over the course of the seed
446 inoculation trial based on selected data. (n=34 colonies). The dashed line indicates the overall
447 median removal time.

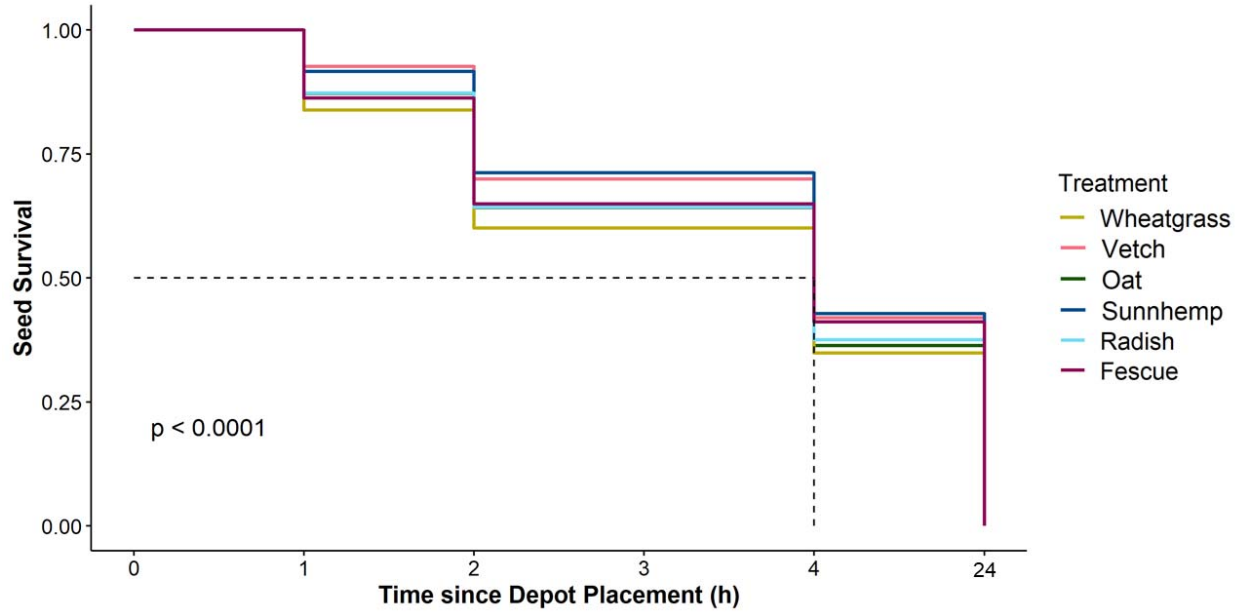
448 **Figure 5.** Hazard Proportional Ratio test demonstrating differences in preferences between
449 inoculated and uninoculated seed types. Reference is wheatgrass. Means on the right side of the
450 chart indicate a larger number of seeds that were removed during the trial. Differences in n
451 (observed seed number) were censored for external events.

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454 **Figures**

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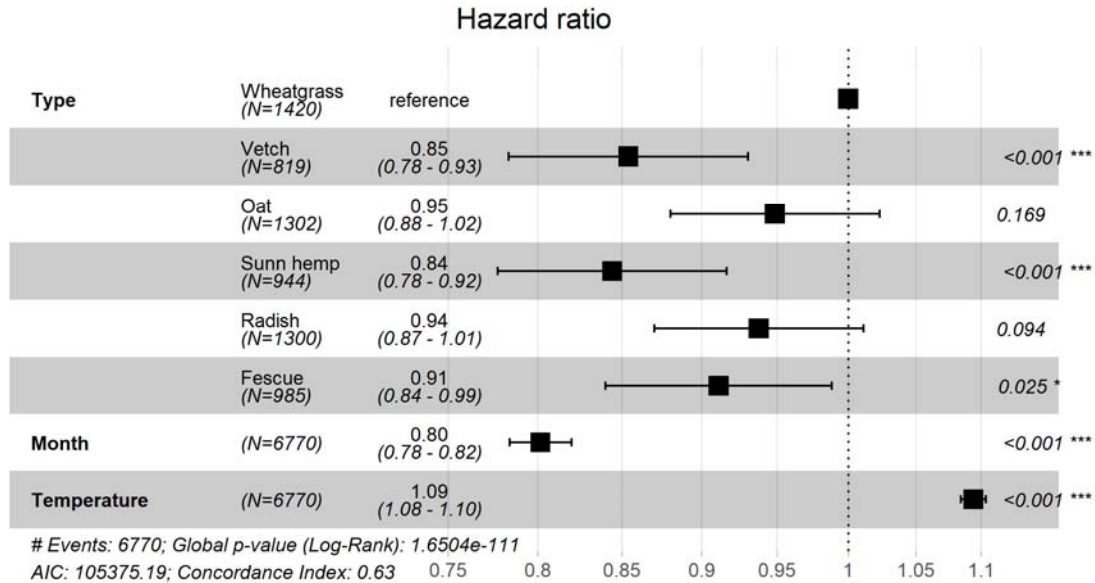


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457 **Figure 1.** Kaplan Meier curve of seed types' likelihood of survival over the course of the seed
458 preference trial based on selected data (n=37 colonies). The dashed line indicates the overall
459 median removal time.

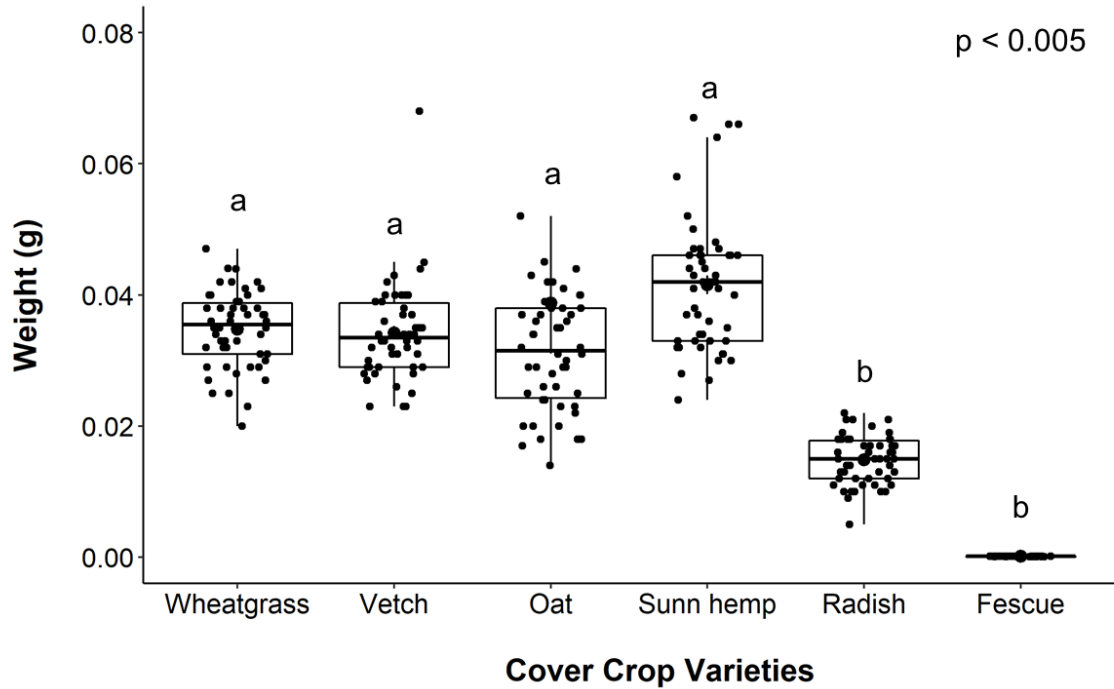
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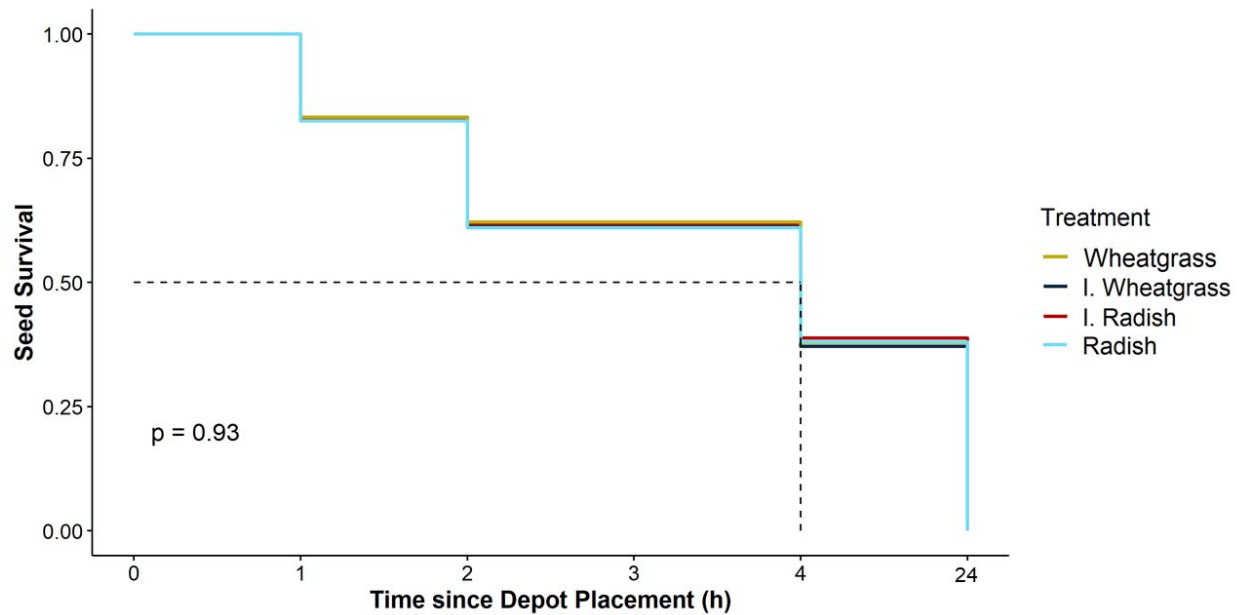
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463 **Figure 2.** Hazard Proportional Ratio test demonstrating differences in preferences between seed
 464 types. Reference is wheatgrass. Temperature is in Celsius. Means on the right side of the chart
 465 indicate a larger number of seeds that were removed during the trial. Differences in n (observed
 466 seed number) were due to censoring for external events.



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468 **Figure 3.** Differences in seed weight between the six cover crop seeds (n=50/seed type) used in
469 the study. Boxplots are in the style of Tukey where the box limits represent the lower 25%
470 quantile and upper 75% quantile with the line representing the median. Tukey HSD was used to
471 determine significance differences (denoted by letters) among seed weights.



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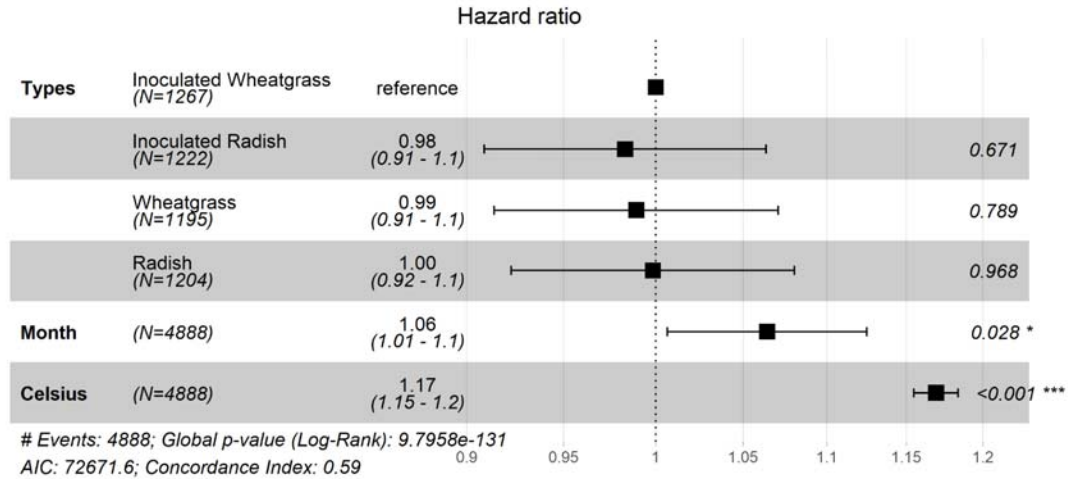
473 **Figure 4.** Kaplan Meier curve of seed types' likelihood of survival over the course of the seed
474 inoculation trial based on selected data (n=34 colonies). The dashed line indicates the overall
475 median removal time.

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481 **Figure 5.** Hazard Proportional Ratio test demonstrating differences in preferences between
 482 inoculated and uninoculated seed types as well as seed consumption differing between months..
 483 Reference is wheatgrass. Means on the right side of the chart indicate a larger number of seeds
 484 that were removed during the trial. Differences in type n (observed seed number) were due to
 485 censoring for external events. Differences in Month n (observed seed number) was differences in
 486 numbers of trials per month.

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495 **Tables**

496 **Table 1.** Summary of the fitted cox model for cover crop seed preferences.

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

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500 **Table 2.** Pairwise comparisons using Log-Rank Test between seed types for the seed preference
501 study (n = 6770 total seeds). Levels of significance indicated by asterisks.

	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

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514 **Table 3.** Summary of the fitted cox model for inoculated seed preferences.

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

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