ORIGINAL RESEARCH

Seed mucilage as a defense against granivory is influenced by substrate characters

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Abstract

Many seeds are consumed by granivores despite numerous adaptations to prevent detection or exploitation. The environment can influence the efficacy of these defensive traits. Understanding the mechanisms by which environmental factors modify defensive efficacy is important for understanding spatial patterns of granivory and seed recruitment. Seed mucilage is a sticky coating that binds imbibed seeds to substrates; this attachment has been demonstrated to lessen exploitation by granivores. Seed mucilage as a defense has been recognized for decades, though rarely studied. Here, we investigated whether the environment alters this seed defense by addressing two questions: (1) Does substrate particle size affect attachment strength? (2) Does a change in particle size lead to changes in granivore-related mortality? In the field experiment, ants removed more seeds from finer substrates than their coarser counterparts. Across that same grit range, seeds took less force to dislodge when mucilage-bound to fine sandpaper; however, an investigation across a wider range of grits demonstrated nonlinearities occurred for many species, probably due to structural and chemical mucilage properties. Small differences in substrate grit lead to differential mortality in mucilaginous seeds due to alterations in attachment strength, suggesting that the defensive efficacy of this trait differs across the landscape. This work paves the way for a more integrative look at mucilaginous seeds. Seed mucilage is a widespread trait that is easily studied and has important demographic implications. It represents an ideal system to examine dispersal, germination, and granivory to gain a more holistic view of seed ecology.

Introduction

Few seeds survive from maturation to successful reproduction. Seed predation (granivory) is a major source of pre-germination mortality. Diverse granivores consume a great many seeds both pre- and post-dispersal (Tevis 1958; Palmer et al. 2022). The amount of mortality may be affected by both intrinsic seed traits as well as abiotic factors. Compounds within seeds can make them unpalatable to potential predators (Diaz 1996), physical traits such as size and shape (Long et al. 2014), or the presence of defensive structures

may make seeds more difficult to locate or costly to process (Paulsen et al. 2013). Abiotic factors such as temperature, humidity, proximity to water, soil composition, soil moisture (Vander Wall 1998), solar radiation, substrate type, climate, and seasonal variation may all affect interactions between granivores and the seeds they consume. These factors may operate independently from the seed defense; however, the efficacy of a defensive trait may also be influenced by the abiotic environment, making them more or less effective in different contexts. Here, we studied how substrate characteristics altered the efficacy of a common seed defense.

Environmentally driven context dependency of plant defense is well studied; water availability, temperature (including heat waves), solar radiation, and even wind (Cippolini 1997) alter interactions with herbivores and determine plant performance. These factors are far less-commonly investigated during the seed stage. Nevertheless, a few studies have demonstrated that background substrate color (Nystrand and Granstrom 1997; Porter 2012), temperature (LoPresti et al. 2022), or water availability (Pan et al. 2021;

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LoPresti et al. 2019) alter the efficacy of defenses against granivores. The small size of seeds means that physical aspects of the environment two seeds encounter may be quite different, even if they are in close proximity. These microenvironmental factors have profound impacts on the success of the seed due to substrate, water availability, sunlight, and temperature, in addition to biotic variables (Young and Evans 1973; Rice 1985; Oswald and Neuenschwander 1993). While much of the work done on the effects of microsite variability concerns germination success and seedling survival, the same variables may affect interactions with granivores. Seed predation has profound impacts on spatial structure and demography of plant populations; which, is

why understanding the environmental context-dependency that underlies patterns of granivory is an important goal in functional ecology of seeds.

Across plants, thousands of species have seeds or fruit that swell into a viscid mass when wetted due to the presence of mucilage in outer tissue layers (Grubert 1981; Western 2012; Pan et al. 2021). After wetting, mucilage strands tightly bind a seed to the substrate (Fig. 1; Pan et al. 2022). Mucilage prevents seed predation in several ways. Mucilaginous seeds are unwieldy for insect granivores to handle when imbibed (Yang et al. 2012). Dried mucilage can bind substrate to the seed, making collection and processing difficult or unwieldy (Fuller and Hay 1983; LoPresti et al.

Fig. 1 Seed mucilage, wetted and dried. Wetted seed mucilage of **A** *Plantago erecta* (Plantaginaceae), **B** *Mirabilis nyctaginea* (Nyctaginaceae), **C** *Ocimum americanum* (Lamiaceae), **D** *Camelina sativa* (Brassicaceae). Dried, mucilage-bound seeds, with visible mucilage strands, of **E** *C. sativa*, **F** *O. americanum*, and **G** *M. nyctaginea*. Harvester ants, *Pogonomyrmex barbatus*, clipping dried mucilage strands of (H) *M. nyctaginea* and **I** *Linum usitatissimum* (Linaceae) and struggling with **J** a bound *Salvia azurea* (Lamiaceae) seed. The set-up of the field experiment is depicted in (**K**). Slides with medium (left), fine (middle), and coarse (right) grit, each with 12 mucilage-bound seeds (here: *M. nyctaginea*) were presented to each nest
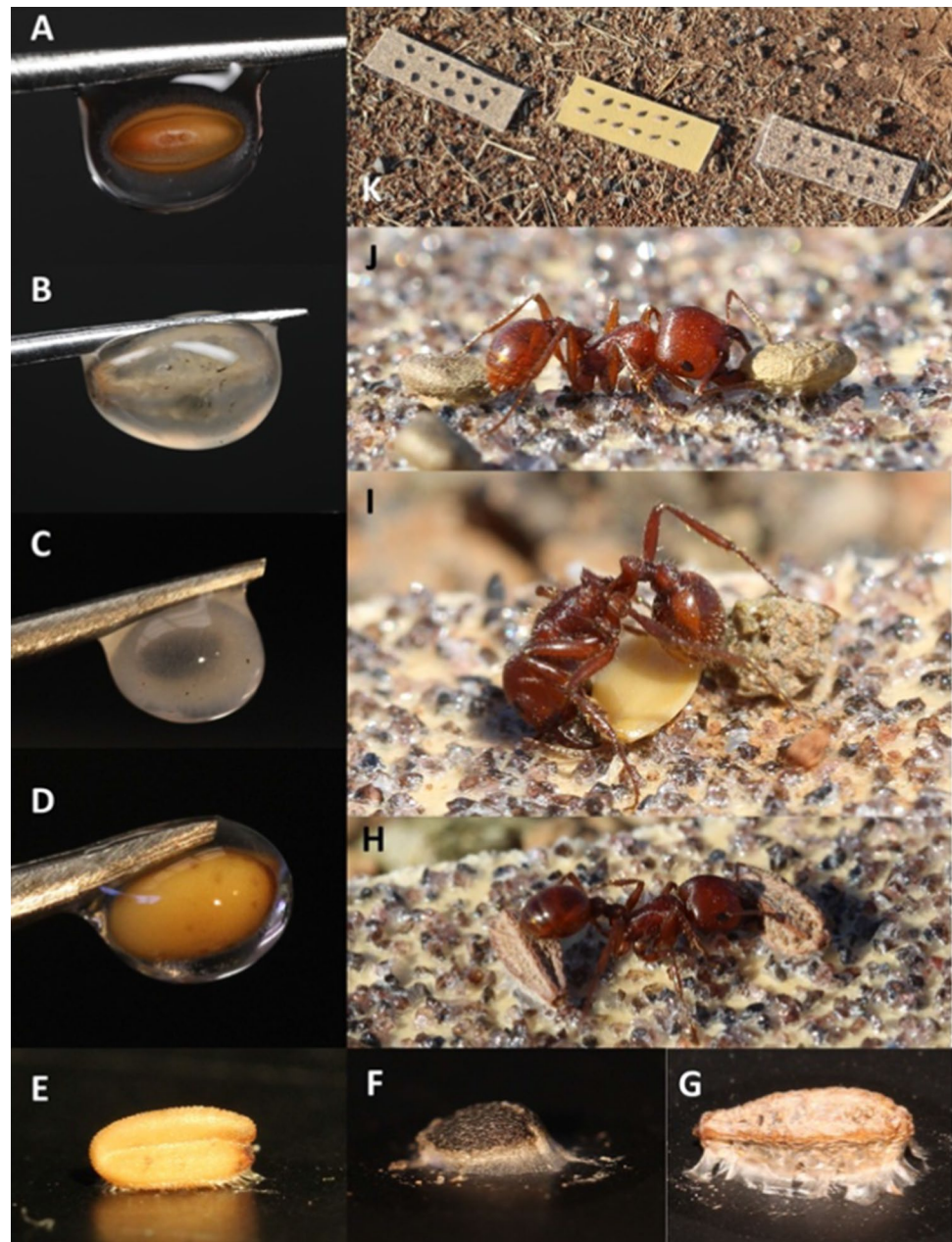
2019, 2023) or it can anchor the seed to the substrate, again making collection and processing difficult (Engelbrecht et al. 2014; Pan et al. 2021). A mucilage-bound seed may require hundreds of grams of force to dislodge, many thousands of times the body mass of small granivores (i.e. ants) (Grubert 1974; Pan et al. 2021, 2022). Therefore, it is unsurprising that seeds more tightly bound to the ground are generally better protected from insect granivores (Pan et al. 2021). However, the strength of attachment is also affected by external factors acting during the drying period. The only investigation in abiotic modification of this interaction thus far found that higher temperatures increased drying speed and decreased the force required to dislodge seeds, leading to higher exploitation by granivorous ants (LoPresti et al. 2022). While a small literature has developed on how seed mucilage affects interactions with granivores, the obvious interplay between this trait and the environment that affects its activity (through wetting and drying) is largely unknown and, therefore, our understanding of mucilage function in realistic interactions is lacking.

The surface characteristics of the interface between the seed and the substrate are critical to the strength of attachment and therefore, survival of the seed when in the presence of granivores (Pan et al. 2021). The variations in substrate that exist across the earth, and even on a very small scale, therefore have the potential to profoundly impact the interactions between seeds and granivores. Here, we asked whether particle size of substrate affects mucilage binding strength and subsequent interactions with granivores. We hypothesized that seeds adhered to finer textured substrate would have a weaker strength of adhesion and would be more likely to be preyed upon by granivores. Using seeds of plant species from five different families—representing independent evolutionary origins of seed mucilage—we tested attachment strength across substrate textures in the laboratory and how that affects exploitation by granivorous ants in the field.

Materials and methods

We used five species, each from a separate family, with varying seed morphologies and mucilage composition to test if substrate differences changed the efficacy of mucilaginous seed defense similarly across a variety of plant families. *Camelina sativa* (Brassicaceae: Adaptive seeds), *Linum usitatissimum* (Linaceae: Premium Gold Flax), *Mirabilis nyctaginea* (Nyctaginaceae: Prairie Moon Nursery), *Plantago erecta* (Plantaginaceae: Larner Seeds), and *Salvia azurea* (Lamiaceae: Prairie Moon Nursery) were used in the field experiment, these species were chosen for phylogenetic diversity and because these species and/or families occurred locally. In addition, we used *Salvia coccinea* (Wildseed

Farms) and *Linum lewisii* (True Leaf Market) for later additional dislodgement assays. For simplicity, we refer to this trait as "seed mucilage", as the diaspores all develop mucilaginous exteriors when wet. However, the external tissue layers of the diaspore which swell up are different—in *C. sativa*, *L. lewisii & usitatissimum*, and *P. erecta* it is the seed coat, in *S. azurea & coccinea*, it is the fruit (a nutlet) coat, and in *M. nyctaginea*, it is remnant perianth (the anthocarp, an accessory fruit). We conducted two experiments, one to directly test the effects of substrate texture on granivory, and one to quantify the amount of force required to dislodge seeds from their place of adhesion.

Substrate effects on granivory

We attached seeds to sandpaper of different grit and conducted field trials with red harvester ants *Pogonomyrmex barbatus (Smith),* in order to determine whether substrate texture affects granivory. Harvester ants were an ideal subject for this portion of the experiment because they are hugely important granivores in many arid systems worldwide (Tevis 1958; Johnson 2000, 2001). We prepared the substrate by gluing strips of silicon carbide sandpaper (3M: 40, 80, 180 grit; i.e. 0.43, 0.19, 0.08 mm particle size, respectively) to standard glass microscope slides. We followed the mucilage attachment procedures of Pan et al. (2021, 2022); briefly, seeds were submerged in water and allowed to imbibe for a minimum of one hour. The imbibed seeds were then individually placed on the sandpaper-covered microscope slides with 12 seeds of the same species per slide. We then allowed them to dry overnight, which tightly bound the seed to the sandpaper (Fig. 1).

Using methods adapted from Pan et al. (2021), we conducted field trials of granivory from harvester ants, *Pogonomyrmex barbatus* (Smith), at the Oklahoma State University Botanical Garden in Stillwater, OK. Harvester ants were chosen as the subject for this experiment because their high abundance in the region and their granivorous diet make them locally-important granivores. These nests are also likely to have direct experience with two of the study species (*M. nyctaginea, S. azurea*) which are locally common, as well as close relatives of others (congeners of tested species: *L. lewisii & sulcata, P. aristata, lanceolata, major, patagonica,* and *virginica*), and several genera of weedy mustards closely related to *C. sativa*. In the morning of July 15th, 2022, between ~ 8:30 and 11:00, slides were placed an equal distance (~ 30 cm) from the entrance to each of 14 harvester ant (*Pogonomyrmex barbatus*) nests, each nest was given the same combination of the 5 species and 3 grit treatments, totaling to 15 slides per nest. We monitored the nests regularly by walking between them, rechecking each at least once every 5 min. A final census of surviving seeds of each

species were taken once a treatment group of that species was completely consumed or the 2-h trial had passed.

Substrate effects on attachment strength—OSU tests of the field grits

In order to determine whether attachment strength differed between the substrate textures, and whether this might explain any granivory differences, we measured the amount of force needed to dislodge the seeds from the sandpaper-covered slides in two sets of tests. We followed the protocol of Pan et al. (2022) closely; briefly, a force meter (Pesola brand, 300, 600, or 1000g, depending on plant species) was used to apply pressure and dislodge the seeds from the slide. Force was applied parallel to the flat surface of the slide and perpendicular to the longest length of each seed; the force was recorded after the seed was dislodged. This resulted in a quantifiable value for the effectiveness of dried mucilage as an adhesive for each substrate-plant species combination, in order to determine the mechanistic relationship between adhesion strength and survival rates against granivory. We did this in two separate sets of tests. In the laboratory at Oklahoma State University, we tested the same three grits and five species as in the granivory test, in order to have a direct comparison to the field granivory data. For each species/grit combination, we tested 60 seeds (though two species had 2 and 4 seeds total missing), for a total of 176–180 seeds/species and 894 seeds total.

Substrate effects on attachment strength—USC tests of a wider range of grits

While the particle sizes used in the field and previous dislodgement force assays were ecologically appropriate for realistic substrates, realistic substrates also encompass a far larger range than we tested. Therefore, we conducted a second test in the laboratory at the University of South Carolina, where we used sandpaper with ten different grit values (Hercules Ceramic: 60, 80, 100, 120, 150, 180, 220, 320, 400, 600 grit: 0.25–0.01mm), and six plant species (*Linum usitatissimum, L. lewisii, Plantago erecta, Mirabilis nyctaginea, Camelina sativa* & *Salvia coccinea*), with contrasting mucilage compositions (Phan and Burton 2018). For *P. erecta*, we used 60 seeds per grit level, for a total of 600 seeds. For all the other species, we used 36 seeds/grit level for a total of 360 seeds/species.

Analysis

For the field granivory experiment, we analyzed the effect of substrate texture on survival using a binomial mixed model, with survival (0,1) as the response variable, species and substrate texture (coarse, medium, fine) as interactive

categorical predictors and nest as random intercept to account for varying activity levels across the nests used. We then performed a post hoc comparison of means across the substrate textures to determine whether survival differed between grits.

We analyzed the Oklahoma State dislodgement force data using a linear regression, with dislodgement force as the response variable, substrate texture and species as interactive categorical predictors. We then performed a post hoc comparison of means across the substrate textures to determine whether the dislodgement force varied (both within and among species). For the University of South Carolina tests, we analyzed each species separately, with the ten values of particle size as a continuous variable, with slide ID as a random effect, in a series of linear models with particle size as a 1st, 2nd, and 3rd order polynomial predictor, to explore a nonlinear response. We also used a null model without particle size as a predictor to ascertain whether particle size had a measurable effect. We did a stepwise deletion approach to find the minimum adequate model for each species. We did these comparisons both for the overall (10 grits) data, as well as a reduced sample size (4 grits) spanning the range sampled in the OSU trials, in order to make a more direct comparison of the two.

All stats were performed and all non-photographic figures made in R vers. 4.2.1, using the packages *lme4, lmertest,* and *emmeans*. The analysis scripts, and data, are available on Figshare at doi: https://figshare.com/projects/Sandpaper_Mucilage/156597.

Results

Substrate effects on granivory

Seeds attached to a fine grit surface across all species had the highest mortality rate (Fig. 2, overall). Seeds on coarser grit surfaces (medium and coarse) survived at ~50% higher rates than their fine grit counterparts (44% survival in the fine-grit group, 64% & 63% in coarse and medium, respectively). This interactive (species*grit) model fit significantly better than an additive model (Likelihood ratio test, $X^2 = 32.6$, $p < 0.01$), or null models without grit and species as predictors. Post hoc comparisons of overall survival between fine and medium grit groups, and between fine and coarse grit groups were statistically significant ($p < 0.01$); however, comparisons of survival rates between medium and coarse grit groups did not differ significantly ($p = 0.68$). When split by species, the same pattern was found in all species except *Camelina sativa*, where no treatments differed in their post-hoc comparisons (Fig. 2, individual species comparisons).
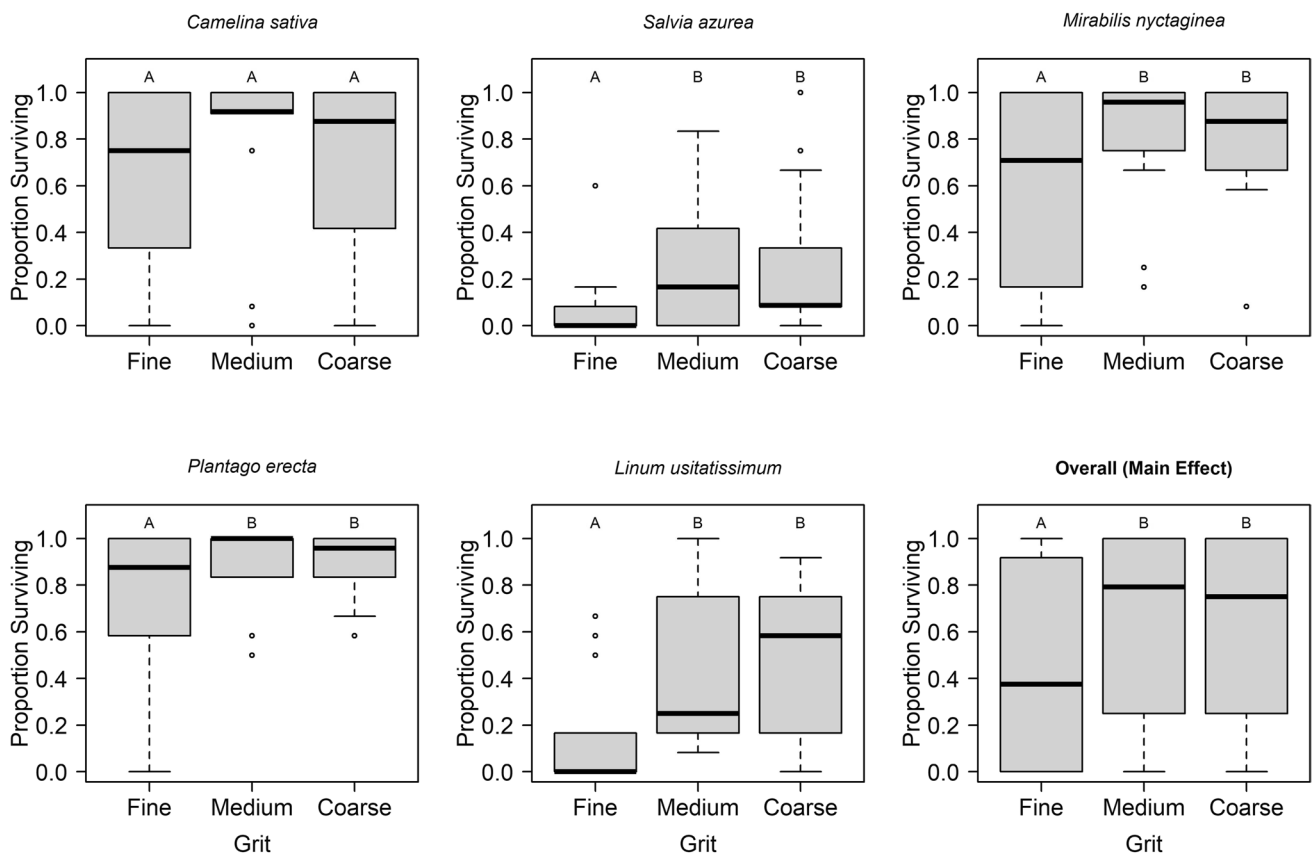
Fig. 2 Proportion seeds which survived the granivory trials, split by species and overall (bottom right). Each species had 168 replicates per grit type. Letters indicate significant post hoc differences (all $p < 0.01$) from the best fitting binomial mixed-model. The center line represents the median value while the box of the graph encompasses the quartiles, the whiskers denote the minimum and maximum values, and the dots represent outliers of the data

Substrate effects on attachment strength

Predictably, for those species that we tested against ants (i.e. the Oklahoma State University tests), attachment strength of seeds on the fine grit surface was significantly weaker than the attachment strength of seeds to medium and coarse grit sandpaper (Fig. 3, overall). These results were qualitatively consistent across all test species except *Salvia azurea*, though they were only significant in three species (Fig. 3, individual species comparisons). However, the overall post hoc comparisons of fine grit to the two coarser grits were highly significant. This interactive (grit*species) model fit significantly better than an additive model (Likelihood ratio test, $X^2 = 1.98$, $df = 2$, $p < 0.05$). The reduced attachment strength directly correlates with the reduced rate of survival for seeds in the field trial on those same low grit surfaces (Fig. 4).

Mirroring this result, in the continuous grit measurements (the University of South Carolina test), spanning the same particle size range as the OSU tests, we found a significant positive relationship between particle size and attachment force for four of the six tested species to the right of the vertical dotted lines in Fig. 4. However, in the larger sample—i.e. the ten grit levels—the results were more nuanced. For *S. coccinea*, we found the second-order polynomial fit better than a linear relationship (LRT: $X^2 = 14.3$, $df = 1$, $p < 0.001$), showing a nonlinear increase in dislodgement force on larger particles sizes. The same result was found for *C. sativa* ($X^2 = 22.8$, $df = 1$, $p < 0.001$) and *M. nyctaginea* ($X^2 = 31.0$, $df = 1$, $p < 0.001$). For *P. erecta*, a second-order polynomial did not improve on the fit of a linear model (LRT: $X^2 = 1.57$, $df = 1$, $p = 0.21$), but the linear model fit significantly better than the null model ($X^2 = 14.9$, $df = 1$, $p < 0.001$). A linear model also fit best in *L. lewisii* ($X^2 = 6.6$, $df = 1$, $p < 0.01$), however, the relationship was opposite, with weaker attachment at higher particle sizes. For *L. usitatissimum*, a model with particle size fit no better than a null ($X^2 = 0.06$, $df = 1$, $p = 0.81$).
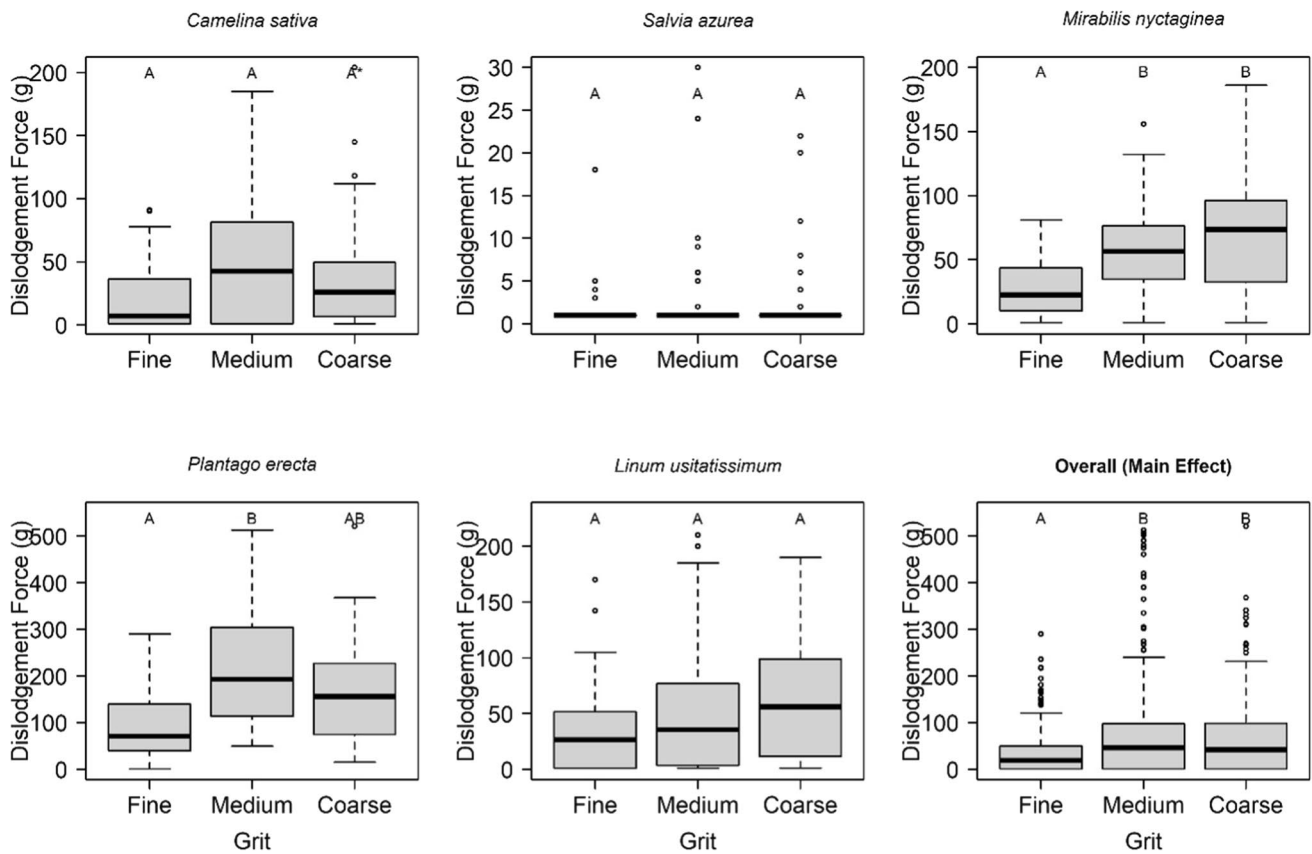
Fig. 3 Dislodgement force, by species and grit, measured on 60 seeds per species per grit level. Letters indicate significant post-hoc differences (all $p < 0.01$) from the best fitting Gaussian mixed-model. The center line represents the median value while the box of the graph encompasses the quartiles, the whiskers denote the minimum and maximum values, and the dots represent outliers of the data

Discussion

Mucilaginous seeds adhered to the fine grit surfaces of the field tests generally required significantly less force to dislodge for most species tested, mechanistically explaining the field results that seeds bound to finer substrates were significantly less likely to survive harvester ant granivory. This finding aligns well with previous findings that seeds less tightly bound to the substrate (due to species or temperature differences) were easier for granivorous ants to exploit. However, when we examined dislodgement force across a wider range of particle sizes, we found rather strikingly different relationships across species, indicating that there is no single common force–grit relationship—and indeed, no relationship at all for one species. Therefore, unquantified mucilage chemical and physical characters probably play large roles in determining the more specific species effects. The finding of some idiosyncrasies is probably unsurprising; 'mucilage' is an extremely varied phenomenon across its many independent evolutionary origins, with strong differences in physical structure (Grubert 1974; Kreitschitz et al. 2021) chemistry (Viudes et al. 2020; Cowley et al. 2021;

Phan and Burton 2018, etc.), and even tissue layers (i.e. *M. nyctaginea* produces mucilage in its accessory fruit, *S. azurea* in true fruit coat, and *C. sativa*, *L. lewisii & usitatissimum*, and *P. erecta* all in seed coats). Other multi-species studies of the ecological functions of mucilage have also found certain species (or whole families) were outliers from the general trends (LoPresti et al. 2019; Pan et al. 2021, 2022). The mechanisms underlying the force-grit relationship need to be understood in order to properly apply these findings to future research.

What mechanisms are behind the grit-dislodgement force relationship?

The force required to dislodge a seed that has been bound to the ground is driven by many variables—the mucilage mass and volume (Pan et al. 2021, 2022), the mucilage composition (Kreitschitz et al. 2015, 2021), the temperature at which it dries (LoPresti et al. 2022), the imbibition status of the mucilage (Kreitschitz et al. 2015; Pan et al. 2022), and, as demonstrated here, the substrate grit. The only mechanistic hypothesis put forth thus far is that the area of attachment
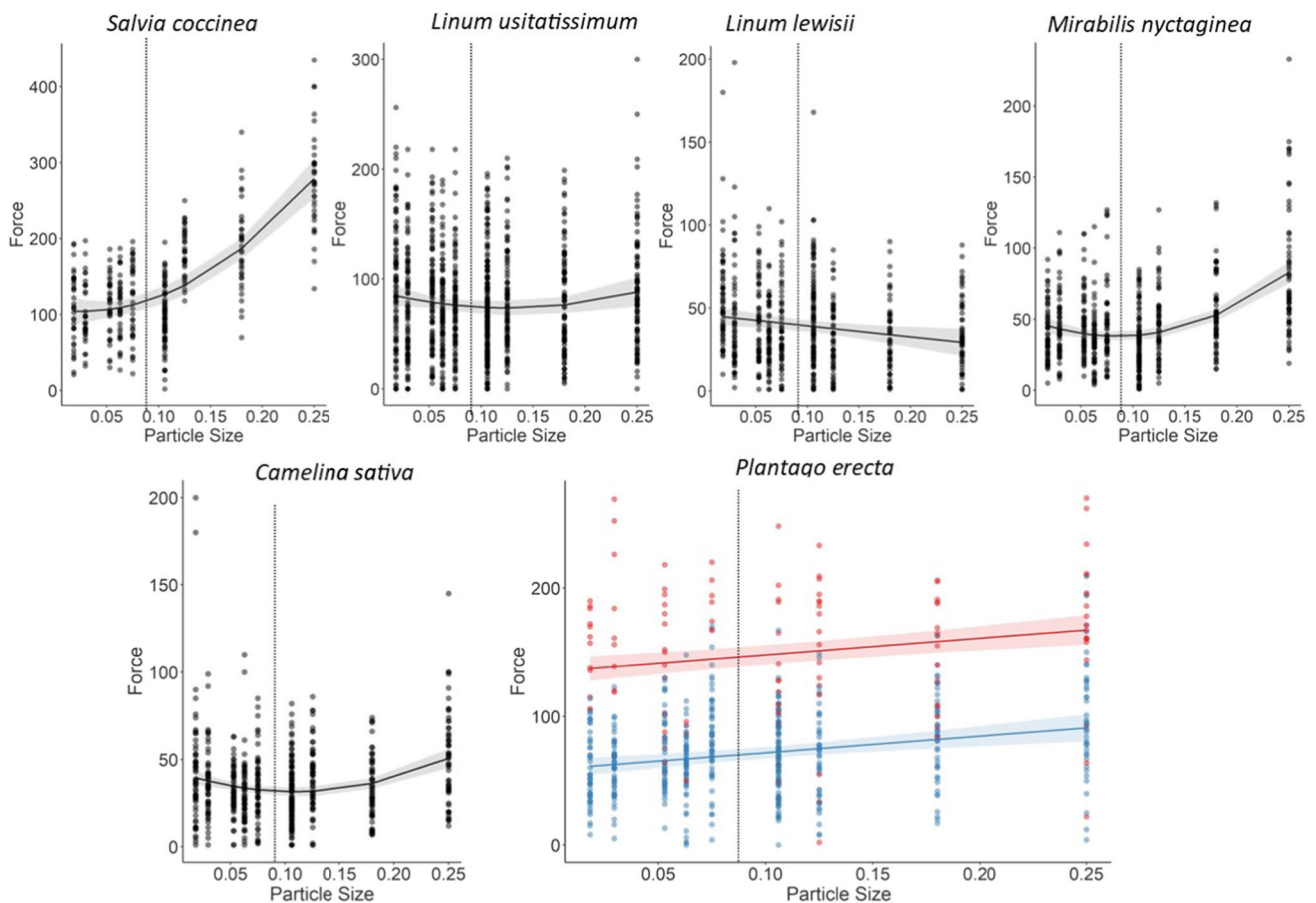
Fig. 4 Predictions of dislodgement force required from linear models for the species tested across the 10 grits at USC. All species had 36 replicates per grit level, except for *P. erecta*, which had 60 replicates per grit level. The best fitting models are plotted for all except the two *Linum* species. For those two species, a null fit better than the linear model plotted (i.e. the slope of the plotted linear model did not differ from zero). Data for *P. erecta* is split between concave up (blue) and concave down (red) orientation of the seeds, which differ in attachment strength but have the same slope (the orientation was additive in the best fitting model). Investigations into the ecological importance of this difference in orientation on germination and survival are ongoing and will be reported in a later paper

correlates positively with the force necessary to dislodge the seed (LoPresti et al. 2022). Mucilage strands only extend so far beyond a seed (measured for many mints in Ryding 2001). Therefore, the basal area of mucilage attachment for a given species is likely similar between substrates. However, within that area, the coarser grit substrate offers a much higher surface area, and therefore, more attachment points for the mucilage strands to bind. This particular rationalization explains well the increasing force at coarser grits found in the narrower range of the OSU tests (0.08–0.43 mm) and the results within that same range from *P. erecta, S. coccinea, C. sativa*, and *M. nyctaginea* in the USC tests.

This hypothesized mechanism is nonlinear: starting from extremely fine grit, as grit gets larger, the attachment area under the mucilage envelope would increase, yet at some point, the surface area available would decrease again—at least by the point that the particle size of the grit approaches the seed size. Substrate particle sizes of a reasonably large

size—even small pebbles—get larger than many seeds, and thus, the surface roughness of those become the particle size experienced by the seed. For example, many mucilaginous *Arabidopsis thaliana* seeds could stick to a single 4-mm diameter pebble. Particle size, for the purposes here, is not exactly that which one could measure with a soil sieve set, or that which could be given as a single number, instead it is a relative value to seed size. Therefore, this hypothesis would predict highest attachment at a species-specific intermediate grit level, though we were not able to sample at the point where grit approaches seed size for any of our species (it would not have been possible with our force assay). However, while that mechanism was probably sufficient to explain much of why more rapid drying decreased attachment force (its original formulation in LoPresti et al. 2022), it is certainly insufficient to explain the totality of results here, especially given the opposite relationship in *L. lewisii*! We believe that a more realistic model must involve

not just surface area for attachment, but also the direction of the mucilage strands resisting the sheer force dislodging the seed. This hypothesis may help explain high dislodgement forces for many species on smooth glass slides (as reported in Pan et al. 2021, 2022, and LoPresti et al. 2022). It is likely that the mucilage strands line up more uniformly on those flatter substrates, resisting sheer force dislodgement more effectively. Faster drying reduces the strength of attachment and alters mucilage structure (LoPresti et al. 2022; Pan and LoPresti, pers. obs.); this result may be due to a slow alignment of strands.

Additionally, and probably most importantly, while it is easy to consider "seed mucilage" to be a discrete trait, which thousands of plant species have (Grubert 1981), it is far more complicated than that simple view. Phan and Burton (2018) compare the known chemical compositions of different seed mucilages; that of *Salvia hispanica* is formed of entirely different polysaccharide classes than that of *Arabidopsis thaliana*! The chemical and structural differences, even across species of the same genus may be substantial (Cowley et al. 2021). Flax, which bucked all the trends found across the other species in this study, has a very slimy mucilage composed primarily of arabinoxylan (Cui et al. 1994), without the really strong envelope or well-defined strands that most other species have (see also Western 2012), and it seems to flow into finer cracks more easily. All these mechanistic explanations, however, are simply hypotheses and demand more biophysical explanation than this ecological investigation can offer. Integration of the chemical and physical aspects has the potential to explain the many non-uniform responses across species reported here and in other studies (LoPresti et al. 2019; Pan et al. 2021).

Ecological realism and applicability

While our results clearly demonstrated that seeds of the same species in close proximity but on different substrates were differentially preyed upon, the ecological realism of this assay is worthwhile to consider. Field observations of diverse mucilaginous seeds cemented to various substrates, including rocks, dried leaves, other plants (and the mother plant itself), dead wood, and the soil surface (pers. obs. and Beal 1898, Pan et al. 2021; images in Pan et al. 2022, LoPresti et al. 2022) suggest that the actual "substrate" that seeds adhere to is a highly heterogeneous category on a very small scale. Therefore, a gross characterization of soil texture may miss ecologically important realism at the scale of a seed. Many seeds are very tiny—with some on the same order as the substrate particle size—and therefore, two seeds on the same substrate may have very different adhesive environments. Furthermore, especially on sloped or windblown substrates, soil texture changes on an extremely fine scale. Our results here demonstrate that substrate alters interactions

with granivores, however, attachment is also highly important in preventing erosion into unsuitable habitats (Grubert 1974; Ellner and Schmida 1981; Engelbrecht et al. 2014; Pan et al. 2022) and therefore, these small-scale mucilage attachment differences may alter germination patterns and distributions through mechanisms besides granivory.

Sandpaper represents a particularly amenable and standardized surface for these trials with ecological realism for a great many seeds. Our previous studies (Pan et al. 2021, 2022; LoPresti et al. 2022) used standardized dislodgement force on a glass slide, which is highly repeatable and easy to measure, and correlates well with dislodgement force on sandpaper and tile (Pan et al. 2021; V.S. Pan and LoPresti, pers. obs.). However, glass is less-ecologically appropriate—with surfaces that smooth being rare in nature (ice and obsidian being two possible exceptions, though largely unimportant substrates for plant growth)—especially for field trials. Ants struggle for grip on glass slides, hence why we used bare ground in Pan et al. (2021) and sandpaper in LoPresti et al. (2022). Nevertheless, because of its replicability, we still advocate for using the glass slide attachment force in inter- and intra-specific lab assays. Dislodgement assays from glass slides do predict multiple functional aspects of the mucilage (Grubert 1974; Pan et al. 2021, 2022; LoPresti et al. 2022) and they represent an easy way to test mechanisms of attachment, which is important for linking laboratory and field results into a useful framework. Yet, sandpaper—of appropriate grit—represents a standardized and ecologically appropriate substrate for field trials.

Might substrate affect the interaction independent of attachment strength?

When interacting with adhered mucilaginous seeds, ants markedly changed their behavior in order to collect the seed. Despite the ants' considerable strength—plus *P. barbatus* is larger than many other species of harvester ants (Taber 1998)—most seeds are adhered too tightly to dislodge with brute force alone (see Grubert 1974; Pan et al. 2021; LoPresti et al. 2022). Oftentimes, the ants wedged their mandibles between the seed and the substrate and cut the strands of mucilage that were holding it in place. Once enough strands had been severed, the ant was able to dislodge the seed and carry it into the nest for further processing and consumption (Fig. 1; also shown in supplementary videos in Pan et al. 2021). We expect that this behavior itself may be altered by grit size. It is probably far harder to wedge mandibles between a seed and a completely flat surface than one with some relief; as grit size increases, this relief may allow mucilage strands to be more easily clipped (Imagine, at an extreme, a seed bound horizontally between two pebbles; clipping the strands would be quite easy). However, we do not have any reason to believe that the limited difference

between the finest and coarsest grit sizes in the field study affected the ants' behavior greatly, as these represent a rather narrow range.

Utility of this seed mucilage system to tease apart foraging decisions.

In our experiments, ants showed strong preferences for *L. usitatissimum* and *S. azurea* seeds (Fig. 2). These seeds were exploited more heavily, even though *L. usitatissimum* was by far the hardest seed to remove, indicating a strong preference by the worker ants. We can speculate that the preference for *L. usitatissimum* may be due to the seeds' high oil content, and that the ants' preference for *S. azurea* may be due to its very low attachment strength. Removal of seeds regularly required long time periods spent clipping mucilage strands to dislodge the seed (see supplementary videos in Pan et al. 2021). However, the increased exploitation of more weakly bound seeds of even the less preferred species (*C. sativa, M. nyctaginea* & *P. erecta*) demonstrates the importance of this defense. It would be informative to manipulate attachment strength in greater detail (by manipulating temperature and substrate grit, and/or by demucilaging seeds); an estimate of the 'benefit' of the seed could be made by determining at what attachment strength the ants no longer exploit the seed (somewhat analogous to using "giving up density" in patch foraging). Behavioral studies could also incorporate meaningful observation, in LoPresti et al. (2019), we timed some ants which took > 10 min to remove a sand coating from highly desirable *L. usitatissimum* seeds, and quantifying exploitation time could allow a more precise examination of the foraging decisions, as well as the value of increasing attachment strength. Further, it is easy to find, or create, this situation in a far less artificial manner using different species and removing, or adding mucilage (*Plantago ovata* mucilage, i.e. psyllium powder, is widely available and inexpensive).

A plea to study seed mucilage

Seed mucilage is a common trait across plants. Having independently evolved in over 100 lineages, mucilage is found in thousands of species (Grubert 1974, 1981; LoPresti, unpublished data). In most urban, agricultural, desert, prairie, scrubby, and disturbed temperate areas, there are many herbaceous plants with mucilaginous seeds (LoPresti, pers. obs.). The model plants *Arabidopsis* and *Plantago* have been thoroughly investigated from mucilage chemistry, development, and production angles (see Western 2012; Cowley et al. 2021 for reviews), the effect on germination in the lab has been well investigated (summarized in Western 2012, Table 1), and there is excellent work on the physical properties of mucilage (Kreitschitz et al. 2015, 2016,

2021). Yet how mucilage affects seed ecology in the field is seriously understudied (but see: Gutterman and Shem-Tov 1997; Engelbrecht et al. 2014; Yang et al. 2012; LoPresti et al. 2019; Pan et al. 2021) and every multi-species study conducted—including this one—has unexpected results for certain species, indicating much more work is necessary to understand this trait.

Mucilaginous seeds are not difficult to work with nor are they difficult to procure. Wherever you are, worldwide, you are likely within a short walk of a *Plantago, Cardamine, Capsella*, *Chaemasyce*, *Ruellia, Salvia, Veronica, Viola*, or *Lepidium* species, and if in the right season, you can crouch or better yet lie on your belly, and find seeds firmly attached to hard substrates. Running your hands through loose sand of vegetated coastal or inland dunes, or even loose roadsides, you can often find the mucilage-bound sand balls made by a wetting and drying cycle (Fuller and Hay 1983; LoPresti et al. 2019, 2023). One can easily obtain seeds of chia or flax from a grocery store, a great many more species from seed suppliers (rosemary, basil, cress, rock rose, *Ruellia*, *Salvia*, *Ipomopsis, Gilia*, *Croton*, etc.), and possibly some *Arabidopsis*, *Capsella*, or *Plantago* seeds from nearby researchers. There are numerous ecological hypotheses put forth that have been untested, or tested in one or a few species, which could be examined (see Grubert 1974, 1981; Ryding 2001; Western 2012; and Pan et al. 2021). Our understandings of the defensive value of mucilage would benefit greatly from studies of more diverse granivores in more locations, as well as explicit incorporation of chemical composition. The methods of Fuller and Hay (1983), Gutterman and Shem-Tov (1997), Yang et al. (2012), Engelbrecht et al. (2014), LoPresti et al. (2019), Pan et al. (2021, 2022), and this study require no specialized scientific equipment and can be easily adapted to any location or granivore.

Conclusions

Substrate effects are well known to affect germination success and subsequent seed survival. Here, we demonstrate that small differences in substrate grit lead to differential mortality in mucilaginous seeds due to alterations in attachment strength. The small range of grit sizes which we used suggest that realistic field conditions, including soil and all other substrates that seeds attach to, could lead to even larger survival differences than we documented. The documented, though unexplained, nonlinearities in dislodgement force across grit size present a fruitful avenue for mechanistic structure to function investigations. Seed mucilage is a widespread and easily studied trait with important demographic implications where dispersal, physiology (i.e. germination), and granivory can be integrated into a more holistic view of seed ecology.

Availability of data and materials All data and code is available on Figshare in the link in the document.

Code availability All data and code is available on Figshare in the link in the document.

Declarations

Conflicts of interest Not applicable.

Ethics approval Not applicable.

Informed consent Not applicable.

Consent for publication Not applicable.

Highlighted student paper statement Many plant species have seed mucilage, yet the environmental affects its ecological role is understudied. I detail how substrate alters seed/granivore interactions; refining our views on seed ecology.

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