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RESEARCH ARTICLE

Deterring rodent seed-predation using seed-coating technologies

Justin B. Taylor[1,2], Kristina L. Cass[1], David N. Armond[1], Matthew D. Madsen[1], Dean E. Pearson[3,4], Samuel B. St. Clair[1]

With many degraded environments undergoing restoration efforts, there is a growing need for the optimization of direct seeding practices. Seeds planted on wildlands are often consumed by rodents, leading to reduced plant establishment. Coating seeds in rodent aversive products may prevent seed-predation. We tested 10 seed-coating formulations containing products expected to deter rodents, namely: ghost and cayenne pepper powders; essential oils from bergamot, neem, and pine; methyl-nonyl-ketone, anthraquinone, activated carbon, beta-cyclodextrin, and a blank coating containing no rodent deterrents to serve as a control treatment. Each treatment was applied to *Pseudoroegneria spicata* (bluebunch wheatgrass) seeds. These seeds germinated similarly to uncoated control seeds unless the coating contained methyl-nonyl-ketone which reduced germination. When seeds were offered to Ord's kangaroo rats (*Dipodomys ordii*), they strongly avoided the treatments in favor of uncoated control seeds. Notably, the blank coating, lacking active ingredients, still elicited 99% avoidance. However, these results indicated behavior when alternative food sources are readily available, a scenario rare in nature. To address this, a second feeding experiment was conducted to observe *D. ordii*'s behavior under calorie-restricted conditions. *D. ordii* were subjected to a fast period, then offered only one treatment. Under these conditions, many subjects chose to consume coated seeds, but to a lesser degree than subjects offered control seeds. Seeds coated in ghost pepper, neem oil, and activated carbon reduced consumption by 47–50%. Given these lab results, we would expect these treatments to increase native plant establishment following the direct seeding of wildlands by protecting seeds from rodent predation.

Key words: ***Dipodomys*, granivory, plant secondary metabolites, *Pseudoroegneria spicata*, rodent pest management, seed enhancement**

Implications for Practice

- Seeds planted during direct seeding may be protected from rodent damage by coating them in ghost pepper powder, neem oil, or activated carbon.
- A simple clay and polymer binder coating is cheaper and safer than coatings containing active ingredients and may cause rodent avoidance under conditions when rodents have other food sources.
- Coated seeds may provide other benefits to seed survival in addition to rodent deterrence, such as bird, insect, and soil parasite deterrence, protection from herbicide, and water and nutrient retention.
- Coated seeds with one exception did not suffer from reduced germination; however, new seed-coating products should still be thoroughly screened for negative impacts on germination.

Introduction

Many native environments have been degraded from their natural state by human activities, resulting in the proliferation of "weedy" species, reduction of native biodiversity, and loss of habitat and ecosystem resources (Tilman & Lehman 2001). Management techniques can be implemented to stabilize and restore ecosystem function (Hardegree et al. 2016). This is often accomplished through direct seeding of native plants which can effectively restore habitat and ecosystem resources (Hobbs & Cramer 2008). However, the native plant community in many ecosystems still struggles to reestablish even after intensive restoration attempts (McIver & Starr 2001; Knutson et al. 2014). This can be attributed to several factors that limit plant survival during early life stages (James et al. 2012). Given the high monetary cost of restoration (Taylor et al. 2013), innovative solutions are needed to increase germination and emergence success. Restoration techniques have often attributed germination and emergence success to abiotic factors (Svejcar et al. 2017), but there is

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[1]Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT, 84602, U.S.A.
[2]Address correspondence to J. Taylor, email justo.mananahi@gmail.com
[3]Rocky Mountain Research Station, USDA Forest Service, 800 East Beckwith Avenue, Missoula, MT, 59801, U.S.A.
[4]Division of Biological Sciences, University of Montana, Missoula, MT, 59812, U.S.A.

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increasing evidence that biotic factors can also become bottlenecks to seedling survival (Suding et al. 2004).

Mounting evidence suggests that consumption of seeds by rodent granivores can strongly regulate the establishment of native and invasive plants (Brown & Heske 1990; Orrock et al. 2003; Maron et al. 2012; Larios et al. 2017). Rodent consumption of planted seeds often limits the success of restoration efforts (Nelson et al. 1970; Gurney et al. 1996; Pearson et al. 2018). To date, few techniques exist for dealing with seed loss from rodent predation (Longland & Ostoja 2013; Pearson et al. 2018). Finding a solution to rodent seed-predation is complex, as the presence of rodents can be beneficial to some aspects of restoration. For example, rodents limit invasions of nonnative plant species by consuming invasive seeds and opening up niche space for natives (Pearson et al. 2011; St Clair et al. 2016). Therefore, solutions to rodent predation during restoration efforts must allow healthy rodent populations to be maintained while protecting native seed.

Novel seed-coating technologies may offer a management tool that can protect native seeds without harming rodents. Seed-coating is the process of applying materials to seeds to optimize germination and establishment. Coating techniques have been used to solve numerous constraints to seed survival in both agricultural and natural systems (Sharma et al. 2015; Madsen et al. 2016). Prior to modern environmental laws, poisons were applied to seeds to deter rodent consumption, but these techniques had disputable success (Spencer et al. 1954) and were met with scrutiny for their negative effects on nontarget animal species (Erickson & Urban 2004). Newer approaches include coating seeds in hot pepper derivatives (*Capsicum* sp.) with the goal of nonfatally deterring rodent granivory. The earliest versions of this technique realized only modest success due to the limited durability and potency of the coating products (Barnett 1997; Nolte & Barnett 2000), but recent research has shown that durable seed-coating materials allow pepper products to remain effective even after weathering (Pearson et al. 2018). However, Pearson noted that other deterrents should be explored because ghost pepper powder is an intense skin, respiratory, and ocular irritant, making it problematic to humans during fabrication, transportation, and application in the field. The caustic nature of ghost pepper powder requires more personal protective equipment than what is commonly used when handling other coating products and seeds. This drawback may hinder pepper-coated seeds from being widely accepted as a tool in restoration seeding. To date, no alternative deterrent has been found that is safe and equally effective.

Many plant secondary metabolites have been identified as rodent deterrents and have been used in interior applications, or as area repellents applied to soil and vegetation surfaces (Hansen et al. 2016). However, few of these plant-based products have been applied to seeds to deter rodent granivores. Using these known rodent deterrents, we created multiple seed-coating formulations containing the following products: bergamot oil, neem oil, pine oil, methyl-nonyl-ketone, and anthraquinone. A ghost pepper coating was made from powdered bhut jolokia peppers (*Capsicum chinense*) and milder pepper coating was made from ground cayenne peppers (*C. annuum*) to see if a cheaper, less caustic pepper could be effective.

We also created two seed-coating formulations containing the scent-reducing compounds activated carbon and beta-cyclodextrin. To our knowledge planting scent-reduced seeds as a management practice is novel, but there is evidence that suggests it could be effective (Briggs & Vander Wall 2004; Yi et al. 2016). Briggs and Vander Wall (2004) found that burying seeds in scent-absorbing ash reduced rodents' ability to find seeds. Yi et al. (2016) found that seeds with low odor are less likely to be consumed if found.

The Great Basin region of the United States has become a target of many restoration efforts and provides a relevant study system for testing our coated seeds. Wildfires are threatening to convert the region's native shrublands to an invasive annual grassland (Balch et al. 2013), and the shrub community in some areas may not recover naturally or with current restoration practices (McIver & Starr 2001; Knutson et al. 2014). Rodents have a strong influence in shaping the Great Basin plant community following wildfire (St Clair et al. 2016). Heteromyid rodents such as kangaroo rats seem to have a greater impact on community structure than other clades (Brown & Heske 1990). They also tend to maintain or increase in abundance in areas burned by wildfire (Killgore et al. 2009; Bowman et al. 2017). For these reasons, we chose *Dipodomys ordii* (Ord's kangaroo rat) as our test subjects for experimentally testing coated seeds. Similarly, we chose *Pseudoroegneria spicata* (bluebunch wheatgrass) ([Pursh] Á. Löve.) as the recipient of the seed-coating formulations because it germinates reliably and is one of the most commonly seeded species in the Great Basin and elsewhere. Moreover, the recruitment of this important native species can be substantially reduced by rodent seed-predation across the western United States (Nelson et al. 1970; Lucero & Callaway 2018).

The objective of this study was to identify seed-coating formulations that can reduce rodent seed-predation following restoration efforts. We explored the following questions: (1) Does applying a rodent deterrent or scent-mask to *P. spicata* reduce seed consumption by *D. ordii*? (2) If so, which formulation is most effective? (3) Is the germination success of seeds negatively affected by the application of a seed-coating or its active ingredients? and (4) Do *D. ordii* continue to avoid coated seeds when they have no other food source? We hypothesized that coating *P. spicata* in the aforementioned rodent repellents will reduce seed consumption by *D. ordii* without negatively affecting germination. We also hypothesized that when calories are limited *D. ordii* will consume coated seeds they would otherwise avoid if alternative food sources were available. The results of this study will help us identify the best coating products for deterring rodent seed-predators. The products can then, in turn, be tested by direct seeding of restoration sites to see if the observed aversions continue under field conditions.

Methods

Seed-Coating Procedures

Seed-coating was performed at Brigham Young University Seed Enhancement Laboratory (Provo, UT, U.S.A.). Seeds were treated using a Unicoat 1200 SA centrifugal coating system

(Universal Coating Systems, Independence, OR, U.S.A.). According to standard seed-coating methods, seeds were encrusted with their active ingredients along with powdered bentonite clay as a filler (Swell Clay, Redmond, Inc., Heber City, UT, U.S.A.) and a polymer binder made from polyvinyl alcohol (Selvol 205 s, Sekisui Specialty Chemicals America, Dallas, TX, U.S.A.). The polymer binder was prepared at 15% solid content according to the Sekisui Specialty Chemicals solution preparation guidelines (Sekisui Specialty Chemicals America, 2009).

Coating treatments included ground ghost pepper powder (Butterfly Herbs, Missoula, MT, U.S.A.), cayenne pepper powder (The Great American Spice Co., Rockford, MI, U.S.A.), anthraquinone (Sigma-Aldrich, St. Louis, MO, U.S.A.), methyl-nonyl-ketone (Sigma-Aldrich, St. Louis, MO, U.S.A.), pine needle essential oil (*Pinus sylvestris*), bergamot essential oil (*Citrus bergamia*) (Bulk Apothecary, Aurora, OH, U.S.A.), neem oil (*Azadirachta indica*) (GreenHealth Brand, WFmed Quality Control, Lorton, VA, U.S.A.), activated carbon powder (Nuchar, Ingevity Corporation, SC, U.S.A.), and beta-cyclodextrin (Chem Center, RND Center, Inc., La Jolla, CA, U.S.A.). A blank coating was also created that lacked active ingredients but still contained the polymer binder and bulking agent used in the other coating formulations; this coating served as a procedural control to observe the effects of the coating alone without the effects of deterrents or scent masks.

Efforts were made to create uniformity between the seed-coating formulations (see Table 1); however, some variation in product quantities was necessary to maintain a similar coating thickness between treatments containing different active ingredients. Each seed-coating formula was applied to 100 g batches of *P. spicata* (variety: Anatone, pure live seed 93%, Granite Seed Company, Lehi, UT, U.S.A.). Each batch of seeds received 195 g of clay, except the activated carbon formulation which adhered well to the seeds without clay. All coating formulations contained 90 mL of polymer binder except those containing activated carbon or ghost and cayenne pepper which are highly absorbent and required more binder to adhere to the seed; 180 mL and 270 mL of the polymer binder was applied to these batches respectively. The liquid active ingredients (bergamot oil, neem oil, pine oil, and methyl-nonyl-ketone) were applied to their respective batches at 25 mL. Due to a large variation in physical characteristics and potency, dry products were applied at the following variable amounts: ghost and cayenne pepper powders (170 g), anthraquinone (8 g), activated carbon (200 g), and beta-cyclodextrin (50 g). The blank procedural control coating received only the polymer binder and clay bulking agent but lacked any active ingredient. All batches of seeds were placed on a forced air dryer at 20°C for 8 minutes following the seed-coating procedure. For the bergamot oil, pine oil, neem oil, and methyl-nonyl-ketone coatings the active ingredients were applied after drying to minimize the evaporative loss of the volatile active ingredient. This was done by first coating the seeds in only polymer binder and clay, drying them as per usual method, returning the seeds to the coating machine and applying an atomized mist of their respective liquid products. The similarities between seed-coating recipes resulted in 10 batches of seeds coated in a unique active ingredient while maintaining similar coating thickness and robustness.

Germination Trials

Seeds of each coating type were germinated under controlled lab conditions to test whether coatings would have negative effects on germination. Eight replicates of 25 seeds from each treatment were placed in separate 7 × 7 × 2.5 cm containers filled with 100 g of fine sand wetted with 20 mL of water. The containers were then covered with a lid and enclosed in a plastic bag to minimize moisture loss. These containers were then placed inside a germination chamber at 20 °C with 12-hour day-night cycles. The arrangement of the trays within the chamber followed a complete randomized block design. The trays were inspected every three days and the number of germinated seeds was recorded until germination ceased and final germination counts were recorded.

Two-Choice Feeding Trials

We conducted a series of two-choice feeding trials after Pearson et al. (2018) to observe the level of aversion that *D. ordii* have towards the coated seeds relative to uncoated seeds. The feeding trials were conducted at a temporary field camp within the burn

Table 1. A description of the formulae of the 10 seed-coating formulations containing either: ghost pepper powder, cayenne pepper powder, pine oil, bergamot oil, neem oil, methyl-nonyl-ketone (MNK), anthraquinone (AQ), beta-cyclodextrin (BCD), or activated carbon. A blank coating was created that contained no active ingredient.

Treatment	*Product Quantity*	Pseudoroegneria spicata *Seeds (g)*	*Swell Clay (g)*	*Selvol 205s 15% (mL)*
Blank	–	100	195	90
AQ	8 g	100	195	90
BCD	50 g	100	195	90
Bergamot	25 mL	100	195	90
Carbon	200 g	100	–	270
Cayenne	195 g	100	195	170
Ghost	195 g	100	195	170
MNK	25 mL	100	195	90
Neem	25 mL	100	195	90
Pine	25 mL	100	195	90

scar of the Stage Wildfire that burned June 2017 northwest of Vernon, UT, U.S.A. Prior to the fire, the site contained *Artemisia tridentata* spp. *wyomingensis* [Beetle & A. Young] S.L. Welsh (Wyoming big sagebrush) and several *Atriplex* L. spp. (saltbush) intermingled with patches of *Bromus tectorum* L. (cheatgrass). Trapping showed that *D. ordii* was the dominant seed predator within the boundary of the Stage Wildfire.

D. ordii test subjects were caught and housed in 24 × 46 × 40 cm clear plexiglass bins with wire mesh tops and kept under a shade canopy for the duration of the trial. A 10 × 18 × 8 cm PVC nest box was placed at a central location within the bin. The nest box had two exits facing feeding trays on opposite sides of the bin. Water was provided ad libitum in a 500-mL watering bottle over the nest box between the two feeding trays. A 2.5-cm tall divider was installed at the center of the cage to minimize the mixing of seeds from opposite sides of the cage.

The day before each feeding trial, Sherman live traps were set out overnight and baited with birdseed and peanut butter. Traps were checked the following morning at 07:00 hours. All healthy adult *D. ordii* were transferred to individual plexiglass bins. The test subjects were offered oats and water ad libitum until 12:00 hours. Rodents were then subjected to a 7-hour fasting period until 19:00 hours during which they were given only water. The tray on one side of the bin was then filled with 1,500 uncoated seeds, while the tray on the opposite side was filled with an equal number of seeds coated with one of the seed treatments. Seeds were counted using an Elmor C1 seed counting machine (Elmor Ltd., Schwyz, CHE). A pretrial run of the experiment determined that 1,500 seeds are more than what a single *D. ordii* could consume during a 12-hour feeding period. The seeds were left for the *D. ordii* to consume for the next 12 hours until 07:00 hours the following morning (the day after capture). Rodent's cheek pouches were inspected for seeds between each step of the experiment to ensure that the seeds were actually consumed. Rodents were then marked and released at their capture sites to ensure that each individual was used in only one trial. Human safety and animal handling protocols were approved by the Brigham Young University Institutional Animal Care and Use Committee, Protocol Number: 18-0403. We repeated each trial six times for each of the seed-coating formulations for a total of 54 individual two-choice trials.

Seeds remaining at the end of each trial were separated from consumed seed chaff using a Seedburro General Seed Blower (Seedburro Equipment Co., Des Plaines, IL, U.S.A.) and then sorted further by hand. Seeds were then counted using the same Elmor C1 seed counting machine to determine how many seeds were consumed. To minimize variability in the accuracy of the counting machine we used a set aperture, speed, and sensor sensitivity both before and after the trial.

One-Choice Trials

In order to observe *D. ordii*'s level of aversion to deterrent-coated seeds under calorie-restricted conditions, we conducted a second series of feeding trials. The design followed closely to the two-choice trials but was modified to one-choice similar to Nolte and Barnett (2000). To focus our efforts on the coating formulations that we felt were most promising, the number of treatments was reduced to ghost and cayenne pepper; neem oil, activated carbon, beta-cyclodextrin, and the blank coating. The feeding trials were conducted at the Brigham Young University Veterinary Clinic (Provo, UT, U.S.A.). Sixty-six *D. ordii* were captured either from within the burn scar of the Stage Wildfire (Vernon, UT, U.S.A.) (n = 11) or from a small section of sand dunes east of Little Sahara Recreation Area (Lynndyl, UT, U.S.A.) (n = 55). Test subjects were housed in the same cages described in previous trials, but with the ceiling height raised to minimize injuries from attempted cage escape. A sandy soil from the Stage Wildfire capture site was sifted to 1 mm and placed in the bottom of the cage to mimic *D. ordii's* natural environment and reduce stress behaviors that excluded some test subjects from the previous study. A single feeding tray was placed on one side of the cage with a hydration tray on the opposite side. Because *D. ordii* do not typically consume liquid water, hydration was instead provided by offering rodents fresh segments of celery (Suckow et al. 2012).

Feeding trials were conducted across several weeks from May to July 2019, At the beginning of each week, rodents were captured in the same manner as described previously and transported from their capture site to the housing facility. Test subjects had ad libitum access to birdseed during transport. The first 2 days of captivity were acclimation days followed by a one-night experimental period. Acclimation days allowed test subjects to adjust to their new environment and allowed us time to observe and remove individuals that exhibited poor health or abnormal behavior. On the first acclimation day, *D. ordii* were introduced to their cages at approximately 10:00 hours (the morning of their capture). Rodents were fasted during daytime hours and fed oats and celery from 21:00 hours to 07:00 hours each night matching the test subjects' natural foraging time. Lights were turned on and off at these hours to maintain a normal circadian rhythm with bright daytime lights, and dim lights at night to simulate moonlight and provide rodents sufficient light to forage. The air temperature was maintained between 20 and 21°C. Temperature, humidity, and light cycles were monitored using an Element-A environmental monitor and data-logged using the Elemental Insights Software (Elemental Machines, Cambridge, MA, U.S.A.) (see Fig. S1).

On day 3 the sand in the bottom of the cage was changed and the subjects' cheek pouches were checked in order to certify that they had no access to alternative food sources. *D. ordii* were then subjected to a daytime fast as per the usual schedule. At 21:00 hours subjects were instead given 1,500 *P. spicata* seeds in place of oats. These seeds were either uncoated or coated with one of the six seed treatments. Each test subject received only one offering which was assigned at random. The seeds were left for the individual to consume for the next 5 hours until 02:00 hours. Test subjects were then given ad libitum access to birdseed and celery until 07:00 hours to recoup any lost calories that may have resulted if rodents chose not to eat their previous food offering. Rodents were then marked and released as in previous trials. Seeds and cage sand were separated using a

1 mm sieve and then counted using the same techniques described in the previous trial. We repeated each trial 10 times for each of the seed-coating formulations for a total of 70 individual one-choice trials. (Some replicates were removed from the final analysis due to poor health or abnormal behavior of the test subjects. This resulted in a few treatments with only nine replicates.) Human safety and animal handling protocols were approved by the Brigham Young University Institutional Animal Care and Use Committee, Protocol Number: 19-0306.

Data Analysis

To test if the coating formulations had negative effects on germination, we performed a generalized regression fit to a beta distribution on the data collected during the germination trial. We used treatment type as the explanatory variable and percent germination as the response variable. Because beta distributions require data to fall on the unit interval [0,1] and we had several batches of seed that had 100% germination, a small constant of 1^{-6} was negated to adjust for 1 inflation. A Dunnett post hoc test was then performed to evaluate differences in germination between each of the coated seed treatments, and the uncoated control seeds.

To test the level of deterrence each seed-coating formulation had on *D. ordii* during the two-choice trials, we performed a series of paired *t* tests on the data collected from the two-choice trials. These *t* tests were run using the difference between the number of treatment seeds consumed and the number of paired control seeds consumed. This method was repeated for each deterrent coating for a total of nine tests. A Holm correction was applied to the *p* values to avoid type 1 error for multiple tests.

In order to determine if the addition of deterrents or scent masks to the seed-coating formulation reduced consumption during two-choice trials relative to the blank control, we performed an analysis of variance (ANOVA) with seed-coating type as our explanatory variable and the difference in consumption as our response variable (control seeds consumed minus coated seeds consumed). This metric served as a measure of avoidance where a high value represents aversion to coated seeds. A Dunnett post hoc test was then performed to compare each difference in consumption to the difference in consumption of the blank control.

To evaluate which coating formulations reduced consumption during the calorie-limited one-choice trial, we created a linear model with seeds consumed as the response variable. The initial model included seed treatment, sex, weight, Δ weight, trap location, oats consumed during acclimation nights, and trial week as explanatory variables. Interaction terms between seed treatment and all other variables were also included. Using a stepwise elimination procedure nonsignificant terms were removed from the model. Our final model was chosen using the lowest Akaike information criterion correction (AICc) value and contained seed treatment as the only explanatory variable (the AICc of the top model was 913 with all other models >917). We performed an ANOVA on this simplified model, followed by a Tukey post hoc test to compare all seed treatments. All statistical analyses were performed using JMP (version 14.2.0 SAS Institute, Inc., Cary, NC, U.S.A.).

Results

Germination Trial

The germination trial analysis revealed that seed viability was not affected by the application of a seed coating (Fig. 1); the control seeds and the blank-coated seeds exhibited similar germination at 97 and 96%, respectively ($p = 0.597$, $t = -0.53$; 95% confidence interval [CI]: 95–98 and 94–98, respectively). The formulation containing methyl-nonyl-ketone had a strong negative effect, with germination around 24% (95% CI: 15–34), a reduction of roughly 73% ($p < 0.001$, $t = -11.66$). For this reason, we excluded methyl-nonyl-ketone coated seeds from further investigation. The bergamot oil, neem oil, and beta-cyclodextrin coated seeds germinated at 82, 83, and 87%, respectively (95% CI: 73–89, 74–90, and 80–93) reducing germination by roughly 14, 13, and 8%, respectively ($p \leq 0.008$, $|t| \geq 3.62$). There was little evidence that anthraquinone, activated carbon, cayenne, ghost pepper, and pine oil coated seeds substantially reduced germination relative to uncoated seeds ($p \geq 0.062$, $|t| \leq 1.8$).

Two-Choice Trial

Two-choice feeding trials revealed that all coated seed treatments were consumed less than their paired control seed ($p \leq 0.047$; $t \geq 3.218$) (Fig. 2). *D. ordii* that were offered blank and control seeds together showed their preference for uncoated seeds by choosing, on average, a diet consisting of 99% control seeds and 1% blank coated seeds ($p < 0.001$; $t = 11.9531$) (Fig. 2A). This avoidance of coated seeds in favor of uncoated control seeds was similar regardless of which seed treatment the rodent was offered ($p \geq 0.895$), excluding pine oil ($p = 0.055$) (Fig. 3). Rodents offered pine oil coated seeds

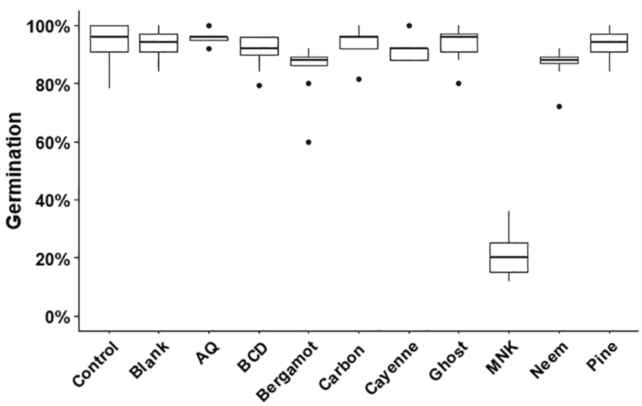

Figure 1. The distribution of percent germination under laboratory conditions for 10 seed-coatings: ghost pepper powder, cayenne pepper powder, pine oil, bergamot oil, neem oil, methyl-nonyl-ketone (MNK), anthraquinone (AQ), beta-cyclodextrin (BCD), activated carbon, a blank coating, and an uncoated control.

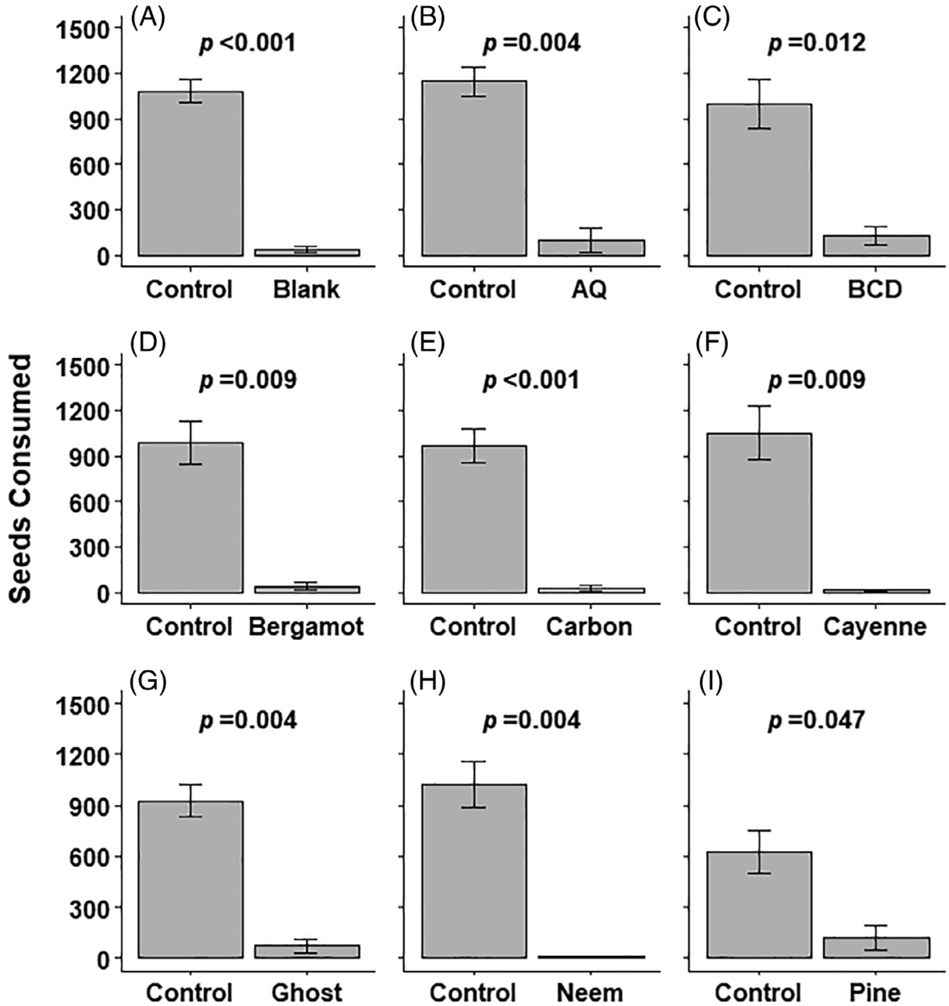

Figure 2. The results of the two-choice feeding trials for the coatings containing: ghost pepper powder, cayenne pepper powder, pine oil, bergamot oil, neem oil, methyl-nonyl-ketone (MNK), anthraquinone (AQ), beta-cyclodextrin (BCD), activated carbon, and blank-coated seeds, showing the average number of coated seeds consumed by *Dipodomys ordii* compared to the average number of paired uncoated control seeds consumed. The *p* values were obtained from paired *t* tests and have been adjusted for multiple tests with a Holm correction. Error bars represent ±SE.

alongside control seeds chose a diet consisting of 86% control seeds, still showing a preference for the control ($p = 0.047$, $t = 3.218$), but with a comparatively lower level of deterrence than the trials where blank coated seeds were offered (Fig. 3).

One-Choice Trial

During the one-choice trials, *D. ordii* consumption of *P. spicata* seeds was reduced by the application of several of the seed-coating formulations (ANOVA, $F = 4.3416$, $p = 0.0011$) (Fig. 4). The Tukey post hoc test showed that rodents offered seeds coated in neem oil, ghost pepper powder, and activated carbon consumed around half the number of seeds on average compared to the rodents that were offered uncoated seeds (50, 43, and 43%, respectively) ($p = 0.0009$, 0.0086, 0.0087). Seeds coated in beta-cyclodextrin, cayenne pepper powder, or the blank coating were not consumed differently from the control according to a Tukey test ($p = 0.3435$, 0.4541, 0.1154). The Tukey test was not able to detect differences in consumption between the different types of coated seeds ($p \geq 0.1821$).

Discussion

Extensive research demonstrates that rodent seed-predation can greatly reduce native plant recruitment (Brown & Heske 1990; Howe & Brown 2001; Larios et al. 2017) thereby hindering restoration efforts (Nelson et al. 1970; Gurney et al. 2015; Pearson et al. 2018). We evaluated the efficacy of various seed-coating formulations for reducing *D. ordii* seed-predation on Great Basin wildlands. We found that all formulations strongly reduced seed consumption when *D. ordii* were not calorie limited and that the formulations containing ghost pepper, neem oil, and activated carbon reduced seed consumption by 47–50% compared to uncoated seeds even when rodents were calorie limited.

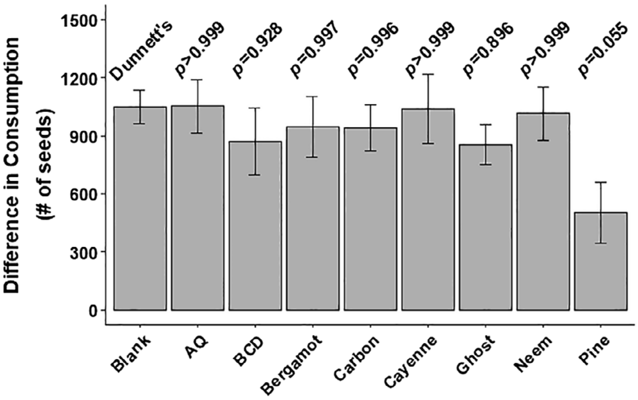

Figure 3. The results of the two-choice feeding trials showing the difference in seed consumption between control and coated seeds (control seeds consumed − treatment seeds consumed). The difference serves as a measure of deterrence with a high value representing strong avoidance of the treated seeds by *Dipodomys ordii*. The *p* values were obtained from a Dunnett post hoc test comparing the treatments ghost pepper powder, cayenne pepper powder, pine oil, bergamot oil, neem oil, methyl-nonyl-ketone (MNK), anthraquinone (AQ), beta-cyclodextrin (BCD), and activated carbon to the blank coating. Error bars represent ±SE.

The aversive effects of ghost pepper are well documented (Nolte & Barnett 2000; Hansen et al. 2016; Pearson et al. 2018), as are the effects of neem (Oguge et al. 1997; Hansen et al. 2015). The success of the neem oil coating is particularly exciting because neem has also been shown to repel insects (Ogbuewu et al. 2011), prevent plant-parasitic soil pathogens (Akhtar & Mahmood 1996), and deter feeding by birds (Mason & Matthew 1996). However, the compounds in hot peppers do not deter bird granivores (Schulze & Spiteller 2009). These compounding advantages of neem make the coating particularly attractive for use in restoration efforts. Similarly, activated carbon coatings have multiple uses in addition to the rodent deterrence we observed; it protects planted seeds from herbicide (Madsen et al. 2014) and is considered a beneficial soil amendment for water and nutrient retention (Sohi et al. 2010). To our knowledge, our study is the first to demonstrate activated carbon as a rodent deterrent which we hypothesize deters rodent predation by masking the scent of the seeds (Briggs & Vander Wall 2004). Both activated carbon and neem coated seeds are relatively benign to humans compared to seeds coated in ghost pepper powder. Given our results and the surrounding literature, we recommend coatings containing neem oil and activated carbon for field testing at restoration sites to verify their effectiveness under practical applications.

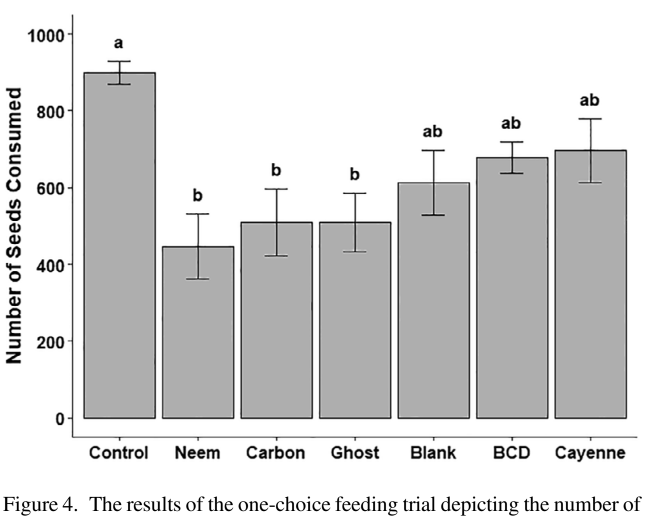

Figure 4. The results of the one-choice feeding trial depicting the number of seeds consumed by *Dipodomys ordii* that were assigned to receive one of seven seed-coating types: uncoated seeds (control), neem oil, activated carbon, ghost pepper powder, beta-cyclodextrin (BCD), cayenne pepper powder, or the blank coating containing no active ingredient. Connecting letters indicate significance according to a Tukey test (α = 0.05). Error bars represent ±SE.

Our finding that blank-coated seeds were hardly consumed when an uncoated alternative was present was unexpected. Why might a coating containing only clay and polymer binder have such a strong deterrent effect? First, the shell-like physical barrier of the coating may reduce utilization by increasing handling time (Jacobs 1992). This explanation seems likely given that we observed *D. ordii* using their forelimbs and incisors to break apart the clay coating before consuming seeds. Second, these ingredients could have an aversive smell or taste. However, this seems unlikely since clay is used in animal feed to increase appetite (Bringe & Schultz 1969), and the polymer binder we used is readily consumed by lab rats when mixed into experimental feed (DeMerlis & Schoneker 2003). Alternatively, *D. ordii* may have avoided the blank-coated seeds due to novelty. Novel food avoidance, or neophobia, has been noted in many rodent species (Barnett 1988), including kangaroo rats (Daly et al. 1982). Such neophobia could also explain why *D. ordii* avoided pine oil to a lesser extent than other coatings; *D. ordii* would be familiar with the similar oils of *Pinus monophylla* Torr. & Frém. (pinion pine) and *Juniperus osteosperma* [Torr.] Little (Utah juniper), which are common in their environment. Lastly, the odor-absorbing properties of clay may serve as a scent-mask (Zhong 2002; Opaliński & Dobrzański 2007) or alter the visual and tactile presentation of the seeds (Lawhon & Hafner 1981), such that coated seeds may smell, look, or feel like small aggregates of soil rather than actual seeds. Rodent foraging habits are complex and likely influenced by many factors. Therefore, further experimentation is necessary to determine the exact mechanisms that cause avoidance by *D. ordii* and other rodents. Understanding these mechanisms would enable the development of coated seeds that target specific avoidance behaviors and potentially lead to more effective restoration efforts.

During one-choice trials the deterrent effect of the blank coating was less drastic, causing a reduction in consumption of only 32%, markedly lower than the 99% reduction noted during two-choice trials. Since the primary difference between these two trials is the presence of an alternative food source, we feel it reasonable to deduce that the blank coating in its current formulation is only substantially effective at preventing seed-predation by *D. ordii* that are not calorie limited. This same coating formulation also performed quite poorly under field conditions (Pearson et al. 2018) making it an undesirable candidate for application in restoration settings. However, a blank coating could be designed with a more robust binding agent and increased coating thickness; such a coating may provide a physical barrier substantial enough to deter granivory even under calorie-restricted conditions. Such a coating would be desirable since a lack of active ingredients would lower cost and minimize product safety concerns.

During the one-choice trials, cayenne pepper and beta-cyclodextrin coated seeds did not significantly reduce consumption compared to the control as they did during two-choice trials. The lack of success with cayenne pepper is surprising when compared to the effectiveness of the ghost pepper coating. Both contain the same primary active ingredient capsaicin which is usually measured in Scoville heat units (SHU). The cayenne pepper product, however, had a much lower concentration of capsaicin (90,000 SHU vs. 1,000,000 SHU). From this, we can deduce that potency plays an important role when attempting to elicit an aversion response in *D. ordii*. This may also explain why early attempts by Pearson et al. (2018) to use capsaicin-derived coatings were not as successful as the results they obtained the final year of their study when they used seeds with a substantial amount of ghost pepper covering the seed surface. The lack of success with the beta-cyclodextrin coating can similarly be contrasted to the success of the carbon coating; both of these coatings were selected for testing based on their ability to capture odor molecules (Shaughnessy & Sextro 2006; Sharma & Baldi 2016). The lack of success with beta-cyclodextrin covered seeds could imply that whatever odor molecules *D. ordii* use to identify seeds as food are not substantially absorbed by beta-cyclodextrin but might be absorbed by activated carbon. However, this explanation is difficult to substantiate since there is no easy way of determining the rodents' reasons for avoiding or consuming these two coatings and the avoidance could have been caused by taste aversion or some other factor. Regardless of the mode of action, given our results, we do not recommend coatings containing cayenne pepper or beta-cyclodextrin for continued investigation as rodent deterrents in coating formulations.

Another surprising observation from our study is the low variability in seed consumption by rodents from our control group relative to the high variability observed in the groups that received coated seeds. For example, the groups of rodents from our three most effective coatings (neem oil, activated carbon, and ghost pepper) each contained at least one individual that consumed 0 seeds, but within those same groups were individuals that consumed 1,060, 886, and 751 seeds respectively. These values are not far off from the mean of the control group 898. This is an indication that avoidance behavior is somewhat individual-specific, and wild populations may contain individuals that are less sensitive to the deterrent effects of the products we tested.

It is important to note that neither of our analyses could detect differences between any two of the seed-coating formulations we tested. Decisive studies could be conducted to determine which deterrent elicits the strongest aversion response; for example, a two-choice study where rodents must choose between two types of coated seeds. Such a study would increase our ability to determine whether neem oil, activated carbon, or ghost pepper is the most effective. As it stands our results promote all three as viable solutions to the seed-predation problem. Future studies could evaluate coatings containing a combination of both neem oil and activated carbon, since both have desirable added benefits beyond rodent deterrence, and there could be some degree of synergism when used in combination.

It is important to note the limitations of this study and inspire future research opportunities. The effectiveness of the products we tested may vary against other granivorous species (Nolte & Barnett 2000). Hence it is necessary to test the products against multiple rodent species to verify that the effects apply to all species present at a restoration site. It is also necessary to test the coating formulations on the seeds of multiple plant species, since seed-coatings may differentially affect germination across species. Also, because seeds differ in their appeal to rodent seed predators (Henderson 1990), it is likely that highly desirable seeds are more difficult to protect.

Additional field testing of these coatings is necessary to verify their effectiveness under natural conditions. Our germination trials showed that seeds coated in neem, ghost, and carbon had negligible effects on germination under laboratory conditions. However, only the ghost pepper coating has been tested and demonstrated to increase seedling emergence under field conditions (Pearson et al. 2018). Given that we used similar coating formulations to those of Pearson et al. (2018), we expect our coatings to be similarly effective under field conditions. However, Pearson et al. only demonstrated increased emergence when seeds were sown in late winter, which they did to minimize weathering of the coatings before spring emergence. In the Great Basin and in many areas in the West, it is common practice to seed in the fall to allow for cold stratification of dormant seeds, and because fall soil conditions are often more favorable for operating planting equipment. Hence, the coating formulations may need to be adjusted to prevent degradation over longer periods of exposure in order to last under more traditional seeding practices. As future investigations optimize the coating formulations and verify effectiveness under field conditions, this technology will likely become a valuable tool for restoration managers looking to mitigate rodent predation of native seeds following direct seeding.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Graphs of temperature (°C), percent humidity, and light (lux).

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