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Deterring Rodent Seed Predation Using Seed-Coating Technologies

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Master of Science

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ABSTRACT

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Wildlands across the globe are experiencing increased rates of degradation due to human influence, changing climate patterns, invasive weeds, and high-frequency disturbances. Direct seeding is often implemented to restore wildlands back to their preexisting state. However, these efforts do not always lead to the recovery of native vegetation. Consumption of seeds by rodent granivores has been identified as a major factor limiting seed survival. Emerging technologies seek to reduce rodent damage by coating seeds in products that rodents find aversive. We investigated nine rodent-deterrent coated seed formulations containing: ghost and cayenne pepper powders; essential oils from pine, neem and bergamot, anthraquinone, methyl-nonyl-ketone, beta-cyclodextrin, and activated carbon. We also created a blank coating that contained no active ingredients to serve as our experimental control. We tested the efficacy of these coating products through a series of germination trials with a common restoration seed species, bluebunch wheatgrass (*Pseudoroegneria spicata*); and rodent feeding trials using a common local rodent granivore, Ord's Kangaroo Rat (*Dipodomys ordii*). Germination trials showed that only seeds coated in methyl-nonyl-ketone had substantially lower rates of germination. All other coatings had germination rates similar to the control. Feeding trials used a 2-choice design in which *D. ordii* could choose between consuming uncoated control seeds or seeds coated in one of the above-listed deterrents. The results indicated that *D. ordii* strongly favored control seeds over coated seeds regardless of the active ingredient contained in the coating formulation. Even the blank coating containing no active ingredient reduced seed consumption by 97% indicating that just coating a seed can elicit avoidance behavior in *D. ordii*. However, these results indicate rodent behavior under conditions where rodents have access to a readily available alternative food source, a scenario they would rarely encounter in nature. For this reason, we devised a second feeding trial in which rodents were fasted for 14 hours prior to a 5 hour feeding period and offered only one food choice which they either chose to consume or went hungry for a period of time. Under these more extreme conditions, many *D. ordii* chose to consume coated seeds, but still to a lesser degree than the rodents that were offered control seeds. Seeds coated in ghost pepper powder, neem oil, and activated carbon reduced consumption by 47-50% under calorie-restricted conditions. We recommend these products for implementation in field trials at restoration sites.

Keywords: *Pseudoroegneria spicata*, plant secondary metabolites, capsaicin, *Dipodomys*, granivory, rodent pest management

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CHAPTER 1

Rodent-Avoidance and Germination Rates of Deterrent Coated Seeds

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ABSTRACT

Direct seeding can help restore damaged wildlands, but in many ecosystems, the establishment of planted seeds is limited by seed consumption from granivorous animals. Seed-coating technologies may increase restoration success by covering seeds with products that deter seed predators. We coated seeds in several products that we expected to cause deterrence: powdered ghost and cayenne peppers; essential oils from bergamot, neem and pine; methyl-nonyl-ketone, anthraquinone, activated carbon, and beta-cyclodextrin. We tested the effectiveness of these deterrent-coated seeds using germination trials and two-choice feeding trials with Ord's kangaroo rats (*Dipodomys ordii*). We compared the germination and consumption rates of deterrent-coated seeds, uncoated control seeds, and seeds covered in a blank coating that contained only the clay and polymer binder used in all coating formulations. We found that this blank coating did not negatively affect germination and *D. ordii* consumed 97% less of it than the uncoated control seeds. The addition of rodent-deterrent compounds to the base seed-coating formula did not increase deterrence but, in some cases, reduced germination. Our results suggest that a simple clay and polymer seed-coating can reduce rodent seed predation. Moreover, this base coating is less expensive and safer to prepare than coatings with added deterrent-compounds.

INTRODUCTION

Human activities, climate shifts, and invasive species are disrupting natural vegetation communities around the globe (Tilman & Lehman 2001). Reseeding of native plants is often implemented as a restoration strategy to mitigate ecosystem degradation and can restore native communities (Hobbs & Cramer 2008). However, seeding can also fail to meet intended objectives due to seed and seedling mortality (James et al. 2012; Knutson et al. 2014). Given the high monetary cost of restoration (Taylor et al. 2013), innovative solutions are needed to mitigate seed mortality. Management practices have largely focused on abiotic limitations to germination and establishment (Hardegree et al. 2016), but it is increasingly recognized that biotic limitations are also important (Suding et al. 2004).

One major source of seed loss is seed predation by rodent granivores (Howe & Brown 2001; Orrock et al. 2003; Larios et al. 2017). As it relates to restoration, rodents limit recruitment by eating both native and invasive seeds to varying degrees (Maron et al. 2012) making it difficult to predict how rodents will affect restoration outcomes. Rodent granivory can significantly reduce recruitment during restoration seeding (Nelson et al. 1970; Gurney et al. 2015; Pearson et al. 2018), but rodent removal may negatively affect restoration efforts because rodents can create biotic resistance against invasion as they consume seeds of invasive species (Pearson et al. 2011; St Clair et al. 2016). Rodents also aid in seed dispersal of desirable species (Longland 1996). Hence, management practices need to protect native seeds from rodent consumption while maintaining the benefits of biotic resistance and dispersal that result from normal rodent populations. Seed-coating technologies may offer a management tool that protects sown 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 negative effects on non-target species (Erickson & Urban 2004). Newer approaches include coating seeds in hot pepper derivatives (*Capsicum sp*.) with the goal of non-fatally 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 hot pepper is a skin and respiratory irritant, making it problematic to humans during fabrication, transportation, and application in the field. This drawback may hinder pepper coated seeds from being widely applied 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 made from powdered Bhut Jolokia peppers (*Capsicum chinense*) was included as a positive control. A milder pepper coating was made from ground cayenne peppers (*Capsicum annuum*) to see if a cheaper, less caustic pepper could be effective. We also created seeds coated in the scent reducing compounds: activated carbon and beta-cyclodextrin. To our knowledge planting odor-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 & 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. Lastly, we created a coating comprising only the polymer binder and clay that was used in all of the coating formulations. This coating contained no active ingredient and served as a procedural control (hereon referred to as the blank coating).

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 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 U.S. (Nelson et al. 1970; Lucero & Callaway 2018).

The objective of this study is to identify seed-coating formulations that reduce seed predation by rodents in direct-seeded restoration areas. We explored the following questions: 1) Does applying a rodent deterrent or scent-mask to seed surfaces 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) How do the inactive ingredients of the seed-coating formulations affect rodent consumption? We hypothesize that coating *P. spicata* in the aforementioned rodent repellents will reduce seed consumption by *D. ordii*, without negatively affecting germination.

MATERIALS AND METHODS

Germination Trials

P. spicata seeds were coated in each of the rodent-deterrent compounds mentioned previously according to standard seed-coating procedures (See Appendix 1). Seeds of each coating type were germinated under controlled lab conditions to test whether coatings have negative effects on germination. Eight replicates of twenty-five seeds from each treatment were placed in separate 7 x 7 x 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 to the coated seeds relative to uncoated seeds. The feeding trials were conducted at a temporary field camp within the burn scar of the Stage Wildfire that burned June 2017 northwest of Vernon, UT, USA. Prior to the fire, the site contained a mix of *Tridentata sp*. (Wyoming big sagebrush) and mixed *Atriplex spp*. (saltbush) intermingled with patches of cheatgrass monoculture (*Bromus tectorum* L.). 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 x 46 x 40 cm clear plexiglass bins with wire mesh tops and kept under a shade canopy for the duration of the trial. A 10 x 18 x 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 2.5cm 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. All healthy adult *D. ordii* were transferred to individual plexiglass bins. The test subjects were offered oats and water ad libitum until 12:00. Rodents were then subjected to a 7-hour fasting period until 19:00 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 coated 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 is 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 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 6 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, USA) 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.

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 seeds that had 100% germination, a small constant of 1^{-6} was negated to adjust for 1 inflation. A Dunnett's 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*, we performed a series of paired t-tests on the data collected from the 2-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 9 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 increased avoidance by *D. ordii* beyond the avoidance induced by the seed-coating process (blank coating), we performed an ANOVA with seed-coating type as our explanatory variable and the difference in consumption as our response variable (control seeds consumed – coated seeds consumed). This metric served as a measure of avoidance where a high value represents aversion to coated seeds. A Dunnett's post-hoc test was then performed to compare each difference in consumption to the difference in consumption of the blank control. All statistical analyses were performed using JMP® (Version *14.2*.0 SAS Institute Inc., Cary, NC, USA).

RESULTS

The germination trial analysis revealed that seed viability was not affected by the seed-coating process (Fig. 1.1); the control seeds and the blank-coated seeds exhibited similar germination at 97% and 96% respectively (p = 0.597, t = -0.53; 95% CI: [95%, 98%], and [94%, 98%] respectively). 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 the two choice feeding trials. 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 feeding trials revealed that all coated seed treatments were consumed less than their paired control seeds ($p \leq 0.047$; $t \geq 3.218$) (Fig.1.2). *D. ordii* that were offered blank and control seeds together showed a 97 ± 2% preference for the control seeds ($p < 0.001$; $t = 11.9531$) (Fig. 1.2a). The blank coating's level of deterrence was similar to the deterrence of all other coated seeds ($p \geq 0.895$), excluding pine oil (Fig. 1.3). The pine oil treatment reduced seed consumption by 59%, which was marginally significantly lower than the blank control ($p = 0.055$).

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). In evaluating the efficacy of various seed-coating formulations for reducing *D. ordii* seed predation, we found that all formulations strongly reduced seed predation (Fig. 1.3), most without inhibiting germination of *P. spicata* (Fig. 1.1). Importantly, we also found that the blank coating, containing only clay and polymer binder, reduced seed predation as much as the coatings containing deterrents or scent masks. These results suggest that a simple clay and polymer binder seed-coating may be a strong seed-predator deterrent that could improve restoration efforts.

Our finding that blank-coated seeds reduced *D. ordii* seed consumption by 97% (Fig. 1.2a), and that additional deterrent compounds did not improve on this effect, was unexpected (Fig. 1.3). 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). Finally, *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 (Fig. 1.3); *D. ordii* would be familiar with the similar oils of pinion pine (*Pinus monophylla* Torr. & Frém.) and Utah juniper (*Juniperus osteosperma* (Torr.) Little), which are common in their environment. Alternatively, 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 us to develop coated seeds that target specific avoidance behaviors and potentially lead to more effective restoration efforts.

Our finding that blank coatings may strongly deter rodent seed predation could have significant ramifications for improving restoration. However, it is important to note the limitations of the current study and highlight future research directions. Our study involved a laboratory experiment using a single seed type, a single rodent species, and a two-choice feeding trial that allowed *D. ordii* a valuable alternative food source. Rodents may act differently under natural conditions and when food resources are scarce. The active ingredients we tested may become more important under scarce conditions. For example, when conducting laboratory feeding trials in conjunction with seed sowing trials in the field, Pearson et al. (2018) found that several hot pepper coating applications strongly deterred deer mouse (*Peromyscus maniculatus*) seed predation in the lab, but that only one of these treatments was successful in maintaining deterrence in the field long enough to increase seedling recruitment. Moreover, they found that the same blank coating that we used in the present study appeared to increase seed predation by deer mice in the field. The importance of chemical and physical defenses can vary depending on seed predators (Kuprewicz 2013), such that rodent responses to coatings may be species-specific (Nolte & Barnett 2000). Hence it is necessary to test seed-coatings against multiple rodent species. It is also necessary to test the seeds of multiple plant species, since seed-coatings may differentially affect germination across species and seeds differ in their appeal to rodent seed predators (Henderson 1990) making highly desirable seeds more difficult to protect. The efficacy of seed-coatings for restoration must be tested using seed sowing experiments in the field to determine their ultimate effects on seedling recruitment. Nonetheless, our results suggest that seed-coatings could provide strong deterrents to rodent seed predators that may greatly improve restoration seeding success. If future experimentation verifies the in-field effectiveness of blank-coated seeds it has the potential to lower the cost of sowing coated seeds since the clay and polymer binder are relatively cheap components of the seed-coating formulations. Additionally, blank-coated seeds would be benign to human applicators and minimize impacts to non-target species.

FIGURES

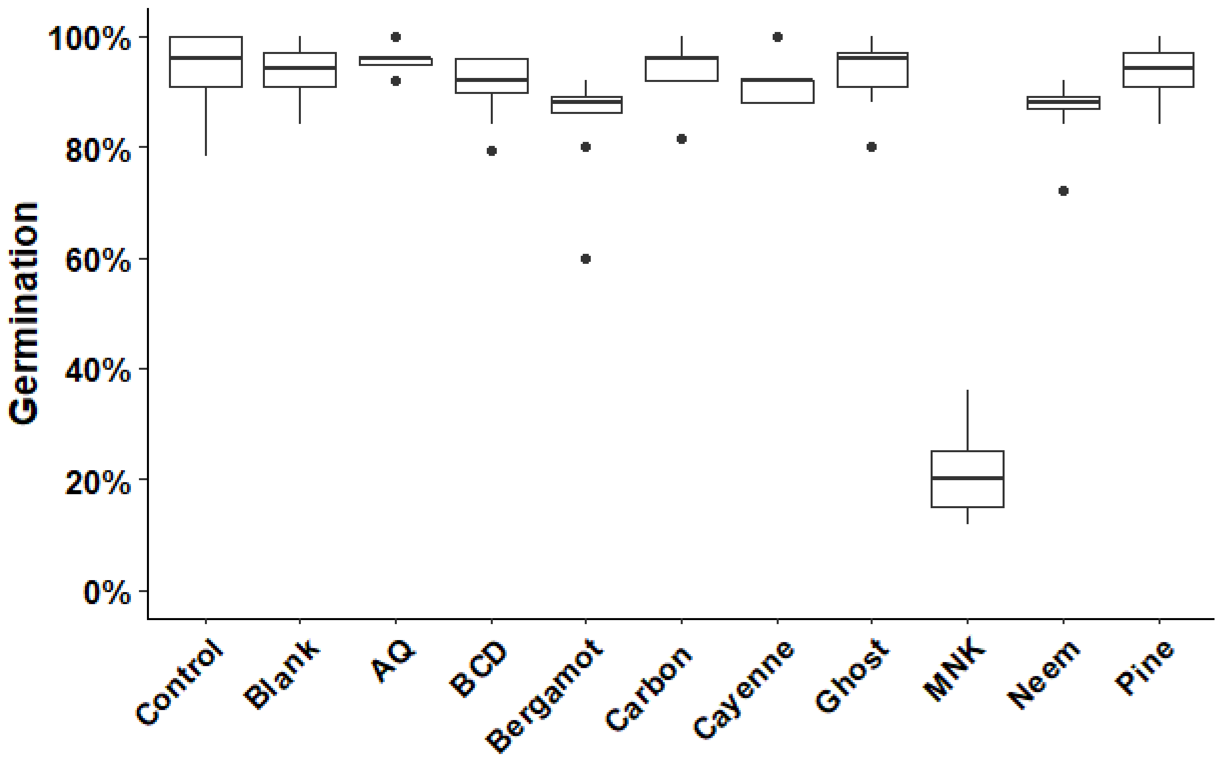

Figure 1.1. The distribution of percent germination under laboratory conditions for 10 seed-coatings: ghost pepper powder, cayenne pepper powder, pine oil, bergamot oil, neem oil, methyl-nonyl-ketone (MNK), anthraquinone (AQ) beta-cyclodextrin (BCD), activated carbon, a blank coating, and an uncoated control.

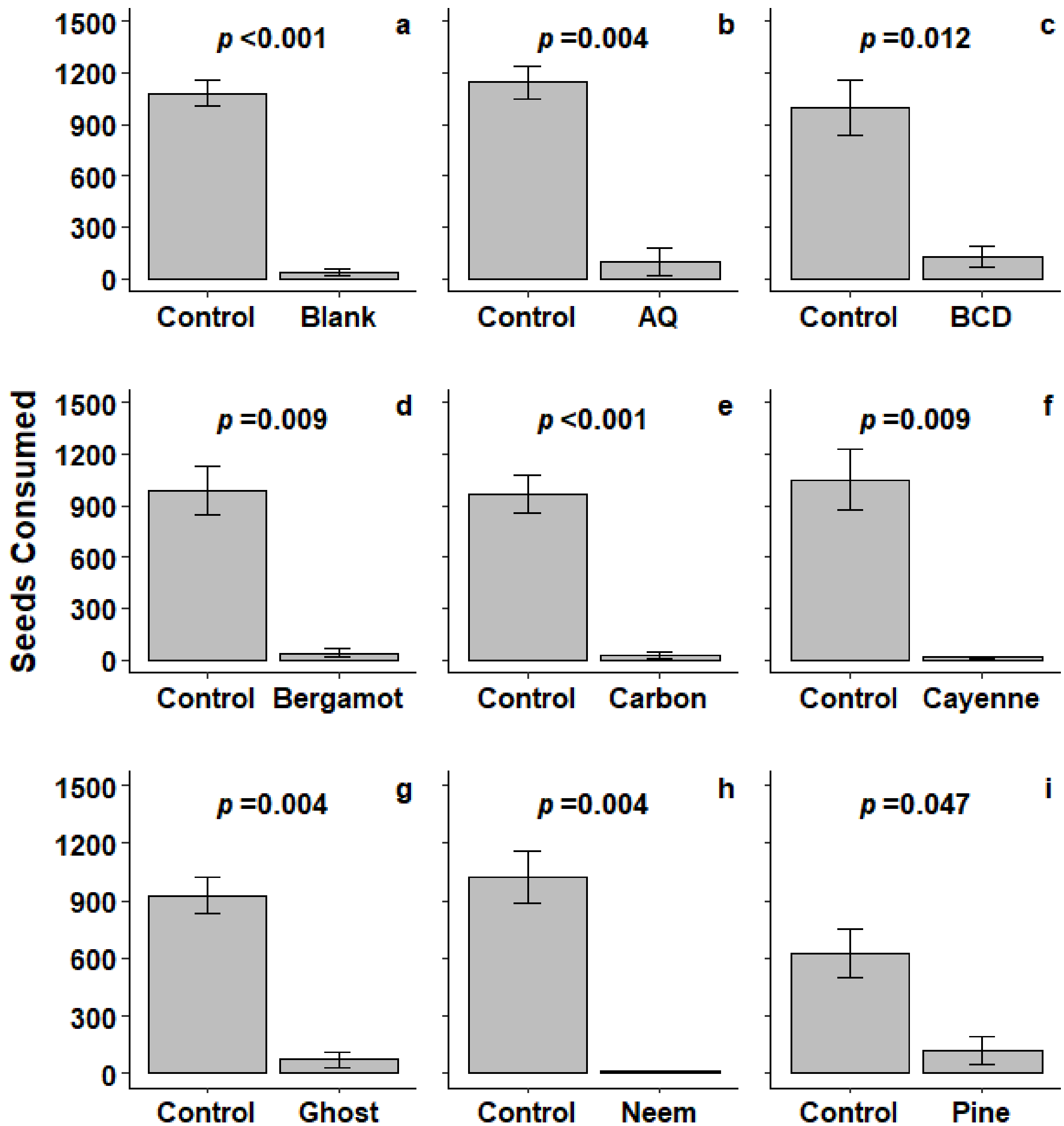

Figure 1.2. The results of the two-choice feeding trials for the coatings containing: ghost pepper powder, cayenne pepper powder, pine oil, bergamot oil, neem oil, methyl-nonyl-ketone (MNK), anthraquinone (AQ) beta-cyclodextrin (BCD), activated carbon and blank-coated seeds, showing the average number of coated seeds consumed by Ord's kangaroo rats 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.

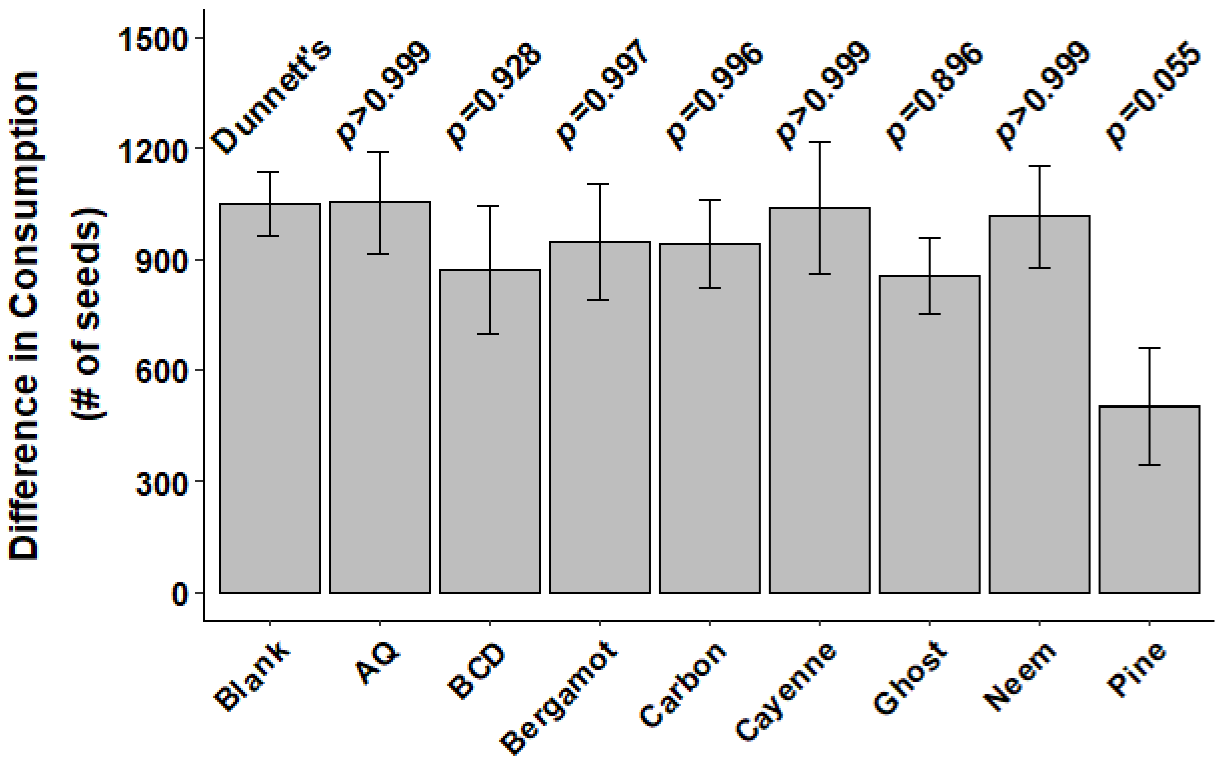

Figure 1.3. The results of the two-choice feeding trials showing the difference in seed consumption between control and coated seeds (control seeds consumed minus treatment seeds consumed). The difference serves as a measure of deterrence with a high value representing strong avoidance of the treated seed by Ord's kangaroo rats. The *p*-values were obtained from a Dunnett's 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.

TABLES

Table 1.1. 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

	Blank	**AQ**	**BCD**	**Bergamot**	**Carbon**	**Cayenne**	**Ghost**	**MNK**	**Neem**	**Pine**
Product Quantity	0	8g	50g	25ml	200g	195g	195g	25ml	25ml	25ml
P. spicata Seed (g)	100	100	100	100	100	100	100	100	100	100
Swell Clay (g)	195	195	195	195	0	195	195	195	195	195
Selvol 205s 15% (ml)	90	90	90	90	270	170	170	90	90	90

SUPPLEMENTAL MATERIAL

Appendix 1: Seed-Coating Procedures

Seed-coating was performed at Brigham Young University Seed Enhancement Laboratory (Provo, Utah, USA). Seeds were treated using a Unicoat 1200 SA centrifugal coating system (Universal Coating Systems, Independence, OR, USA). According to standard seed-coating methods we used powdered bentonite clay as a filler (Swell Clay®, Redmond Inc. Heber City, UT, USA) and a polymer binder made from polyvinyl alcohol (Selvol 205s, Sekisui Specialty Chemicals America, Dallas, TX, USA). 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 Bhut jolokia (*Capsicum chinense*) powder (Butterfly Herbs, Missoula, MT, USA), cayenne (*Capsicum. annuum*) pepper powder (The Great American Spice Co., Rockford, MI, USA), anthraquinone (Sigma-Aldrich, St. Louis, MO, USA), methyl-nonyl-ketone (Sigma-Aldrich, St. Louis, MO, USA), pine needle essential oil (*Pinus sylvestris*) (Bulk Apothecary, Aurora, OH, USA) bergamot essential oil (*Citrus bergamia*)(Bulk Apothecary, Aurora, OH, USA), neem oil (*Azadirachta indica*) (GreenHealth brand, WFmed Quality Control, Lorton, VA, USA), activated carbon powder (Nuchar, Ingevity Corporation, SC, USA) and beta-cyclodextrin (Chem Center, RND Center Inc. La Jolla, CA, USA).

Efforts were made to create uniformity between the seed-coating formulations (see Table 1.1). Each seed-coating formula was applied to 100 g batches of *P. spicata* (variety: Anatone, pure live seed 93%, Granite Seed Company, Lehi, UT, USA). 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 for 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 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 a polymer binder and clay without an 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.

CHAPTER 2

Seed-Coating Technologies Deter Kangaroo Rats Under Calorie Restricted Conditions

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ABSTRACT

With many natural landscapes undergoing restoration efforts around the globe, there is a growing need for the optimization of direct seeding practices. The consumption of sown seeds by rodents limits the effectiveness of such restoration attempts. Seeds coated in rodent aversive products are a viable solution for preventing the consumption of sown seeds. We tested six seed-coating formulations containing products expected to cause rodents to avoid seeds, namely: ghost pepper, cayenne pepper, neem oil, activated carbon, beta-cyclodextrin, and a blank coating that was coated but lacked any rodent deterrent product. Each of these treatments was applied to *Pseudoroegneria spicata* (bluebunch wheatgrass) seeds and then offered to a local granivorous rodent species, Ord's kangaroo rat (*Dipodomys ordii*) in a single choice design under calorie-restricted laboratory conditions. Coatings containing cayenne pepper, beta-cyclodextrin, and blank coatings failed to substantially reduce the consumption of seeds. However, seeds coated in ghost pepper, activated carbon, or neem oil elicited a 47-50% reduction in consumption even when *D. ordii* was deprived of alternate food for 19 hours. These coating products show promise to reduce the limitations of rodent consumption during direct seeding of restoration sites.

INTRODUCTION

Many native environments have been degraded from their natural state as a result of human activities (Tilman & Lehman 2001). State changes related to human alterations of ecosystems often lead to the loss of diversity and invasions, creating the need for management approaches that can stabilize and restore ecosystem function (Hardegree et al. 2016). Direct seeding of native species is one of the most common restoration techniques, and often effectively reestablishes native plant cover (Hobbs & Cramer 2008). However, some environments struggle 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). Restoration techniques have often focused on seed mortality due to abiotic factors (Svejcar et al. 2017), but there is increasing evidence that biotic factors can also become bottlenecks to seed survival (Suding et al. 2004).

Mounting evidence suggests that consumption of seeds by rodent granivores can cause drastic changes to the composition of native environments as rodents consume both native and invasive seeds (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, due to the fact that the presence of rodents can be beneficial to some aspects of restoration. For example, rodents limit invasions of non-native plant species by consuming invasive seeds (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 techniques may be a viable solution. Seeds coated in aversive compounds such as ghost pepper powder (*Capsicum chinense*) have been shown to limit rodent consumption and increase establishment when sown in native habitats (Pearson et al. 2018). Due to the difficulties of safely handling ghost pepper powder, other rodent-deterring coating products have been investigated as alternative solutions (see Chapter 1). Chapter 1 demonstrated that a common rodent granivore in the Great Basin, Ord's kangaroo rat (*Dipodomys ordii*), strongly avoided all of the 9 coated seed products tested, including a blank coating that did not contain any ingredients. In Chapter 1 we postulated that avoidance of blank coated seeds was the result of the novelty of the new food source, the physical barrier created by the seed coating, or the suppression of visible, olfactory, and tactile cues that made it difficult for *D. ordii* to identify the seeds as food. While this study provided evidence for the effectiveness of seed-coatings, it did so under 2-choice conditions where *D. ordii* had ample access to an alternative food source (uncoated control seeds). It is assumed that in nature alternative food sources are scarce and rodents would likely consume food items they otherwise wouldn't when their caloric needs aren't being met. Under calorie-restricted conditions novelty, physical barriers, and reduction of identifying traits may not be enough to cause avoidance. The chemical characteristics of seed-coatings may begin to play a more important role in causing avoidance when rodents must either eat the coated seeds or go hungry. Nolte & Barnett (2000) proposed that assessing rodents' food choices under calorie-restricted conditions may be obtained through single-choice tests which are believed to more stringently assess the strength of avoidance than 2-choice tests (Nolte & Mason 1998).

Our study seeks to answer the following question: 1) Do *D. ordii* continue to avoid deterrent coated or blank coated seeds under calorie-restricted conditions? We hypothesize that under calorie-restricted laboratory conditions the active ingredients of the seed-coating formulations will allow seeds to avoid predation by *D. ordii* even when calories are limited. We also hypothesize that the physical qualities of blank coatings that have been observed to deter rodents during 2-choice trials will not result in a significant reduction in seed consumption under calorie-restricted conditions. The results of this study will help us identify the best coating products for deterring rodent seed-predators. These seed-coating products can then, in turn, be tested by direct seeding of restoration sites to see if the observed aversions continue under field conditions. We expect that seed-coating techniques will allow seeds to overcome the seed survival limitations caused by rodent granivores and lead to more successful restoration efforts.

MATERIALS AND METHODS

Single-Choice Trials

In order to observe rodents' level of aversion to deterrent coated seeds under calorie-restricted conditions, we conducted a series of feeding trials using *Dipodomys ordii* (Ord's Kangaroo Rat) as test subjects and *Pseudoroegneria spicata* (bluebunch wheatgrass) as recipients of the coating formulations. The design followed closely to Chapter 1 but was modified from 2-choice to 1-choice similar to Nolte & Barnett (2000). The feeding trials were conducted at the Brigham Young University Veterinary Clinic (Provo, Utah, USA).

Seed-Coating Procedure

Seed-coating was performed at Brigham Young University Seed Enhancement Laboratory (Provo, Utah, USA). Seeds were treated using a Unicoat 1200 SA centrifugal coating system (Universal Coating Systems, Independence, OR, USA). According to standard seed-coating methods we used powdered bentonite clay (Swell Clay®, Redmond Inc. Heber City, UT, USA) as a filler and a polymer binder made from polyvinyl alcohol (Selvol 205s, Sekisui Specialty Chemicals America, Dallas, TX, USA). The polymer binder was prepared at 15% solid content according to the Sekisui Specialty Chemicals Solution preparation guidelines (Sekisui Specialty Chemicals America, 2009).

We tested the following six coatings: Bhut jolokia powder (*Capsicum chinense*), and cayenne pepper powder (*Capsicum annuum*) (The Great American Spice Co., Rockford, MI, USA), neem oil (*Azadirachta indica*) (GreenHealth brand, WFmed Quality Control, Lorton, VA, USA), activated carbon powder (Nuchar, Ingevity Corporation, SC, USA) and beta-cyclodextrin (Chem Center, RND Center Inc. La Jolla, CA, USA). A blank coating was also created containing only the same polymer binder and clay bulking agent that were used in all coatings, only this blank coating contained no active ingredient.

Efforts were made to create uniformity between the seed-coating formulae (see Table 2.1), but some variation was necessary to maintain even coating thickness. Each seed-coating formula was applied to 100 g batches of *P. spicata* (variety: Anatone, pure live seed 93%, Granite Seed Company, Lehi, UT, USA). Each batch of seeds received 195 g of clay, except for the activated carbon formulation which adhered well to the seeds without clay. All coating formulations contained 90 ml of polymer binder except for those containing activated carbon, ghost, or cayenne pepper which were highly absorbent and required more binder to adhere to the seed; These respective batches instead received 180 ml, 270 ml, and 270 ml of the polymer binder. Due to a large variation in the physical characteristics and potency of the products, they were applied at the following variable amounts: Ghost and cayenne pepper powders (170 g), activated carbon (200 g), beta-cyclodextrin (50 g) and neem oil (25 ml). The blank procedural control coating received only a polymer binder and clay without an active ingredient. Following the seed-coating procedure, all batches of seeds were placed on a forced air dryer at 20 °C for 8 minutes. However, neem oil was applied to its coating after drying to minimize 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 the usual method, returning the seeds to the coating machine and applying an atomized mist of neem oil. The similarities between seed-coating recipes resulted in 6 batches of seeds coated in a unique active ingredient while maintaining similar coating thickness and robustness.

Rodent Housing Procedure

Sixty-six *D. ordii* test subjects were captured either from within the burn scar of the Stage Wildfire (Vernon, Utah, USA) (n=11) or from a small section of sand dunes east of Little Sahara Recreation Area (Lynndyl, Utah, USA). (n=55). Test subjects were housed in 24 x 46 x 40 cm clear plexiglass bins with wire mesh. A sandy soil from the Stage Wildfire capture site was sifted to 1 mm and placed in the bottom of the cage to mimic their natural environment. A 10 x 18 x 8 cm PVC nest box was placed at a central location within the bin and had two exits facing either side of the cage. A feeding tray was placed on one side of the cage and a hydration tray on the opposite side. Because *D. ordii* does not typically consume liquid water, hydration was 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, Sherman live traps were set out overnight and baited with birdseed and peanut butter. Traps were checked the following morning at 07:00. All healthy adult *D. ordii* were transported from their capture site to the housing facility. Test subjects had ad libitum access to birdseed during transport. The first two days of captivity were acclimation days followed by a 1-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 (the morning of their capture). Rodents were fasted during daytime hours and fed oats and celery from 21:00 to 07:00 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 20-21°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, Massachusetts, USA) (See Fig. 2.S1).

On day three the sand was changed in the bottom of the cage 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 usual schedule. At 21:00 subjects were instead given 1,500 *P. spicata* seeds in place of oats. Seeds were counted using an Elmor C1 seed counting machine (Elmor Ltd., Schwyz, CHE). These seeds were either uncoated or coated with one of the 6 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. Test subjects were then given ad libitum access to birdseed and celery until 07:00 to recoup any lost calories that may have resulted if rodents chose not to eat their previous food offering. Rodents were then marked by shaving a patch of hair on their rump and transported back to their capture sites and released. Markings allowed us to avoid retesting previously used individuals. Human safety and animal handling protocols were approved by the Brigham Young University Institutional Animal Care and Use Committee, Protocol Number: 19-0306. 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 the health or abnormal behavior of the test subjects. This resulted in a few treatments with only 9 replicates).

Seeds and cage sand were separated using a 1mm sieve. Chaff from consumed seeds was removed using a Seedburro General Seed Blower (Seedburro Equipment Co., Des Plaines, IL, USA) 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 the same aperture, speed, and sensor sensitivity both before and after the trial.

Data Analysis

To evaluate which coating formulations reduced consumption of *P. spicata* by *D. ordii*, 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 non-significant terms were removed from the model. Our final model was chosen using the lowest 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. Statistical analyses were performed using JMP® (Version *14.2*.0 SAS Institute Inc., Cary, NC, USA).

RESULTS

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. 2.1). 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

This study adds to the growing body of evidence that seed-coating is an effective technique for limiting the consumption of seeds by rodents (Barnett 1997; Nolte & Barnett 2000; Pearson et al. 2018). We hypothesized that the active ingredients of the seed-coating formulations will allow seeds to avoid predation by *D. ordii* even when calories are limited. Consistent with this hypothesis, we found that seeds coated in neem oil, activated carbon, or ghost pepper powder were consumed by nearly half the amount of the control (Fig. 2.1). The aversive effects of ghost pepper are well documented (Nolte & Barnett 2000; Hansen et al. 2016; Pearson et al. 2018)(Chapter 1), 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 prevent granivory from birds (Mason & Matthew 1996), and insects (Ogbuewu et al. 2011), and prevents plant-parasitic nematodes (Akhtar & Mahmood 1996). These compounding advantages 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). Conversely, activated carbon, to our knowledge has not been investigated as a rodent repellent prior to our Chapter 1 experiments where we postulated its potential effectiveness based on the work of Briggs & Vander Wall (2004) and Yi et al. (2016). They discovered that seeds covered in carbon (ash) substrates were difficult for rodents to locate through olfaction (Briggs & Vander Wall 2004), and seeds with low odor were less likely to be consumed (Yi et al. 2016). Given our results and the surrounding literature, we recommend ghost pepper, neem oil, and activated carbon for field testing at restoration sites to verify their effectiveness under practical applications.

Cayenne pepper and beta-cyclodextrin coated seeds did not reduce consumption compared to the control (Fig. 2.1). 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.

We also hypothesized that blank coatings that have deterred rodents during 2-choice trials in Chapter 1 will fail to reduce seed consumption under our calorie-restricted conditions. This hypothesis is somewhat supported by our data as our Tukey comparison between the blank coated seeds and the control was not significant ($p = 0.1154$). However, since this p-value is approaching significance it is possible that the physical properties of the seed coating may to some degree reduce the consumption of seeds by *D. ordii.* The relative mean difference we observed between blank coated seeds and the control was 32% which is markedly lower than the 97% avoidance reported in a similar study (Chapter 1). Since the primary difference between these two studies 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.

One 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 (see error bars in Fig. 2.1). 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 1060, 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 likely contain individuals immune to the deterrent effects of the products we tested.

It is important to note that our analysis could not 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 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.

Field testing of these coatings is necessary to verify their effectiveness under practical applications. Chapter 1 showed that the same formulations of 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., we expect our coatings to be effective under field conditions. However, their study 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 the continued effectiveness under field conditions, we expect this technology to become a valuable tool for wildland restoration managers.

FIGURES

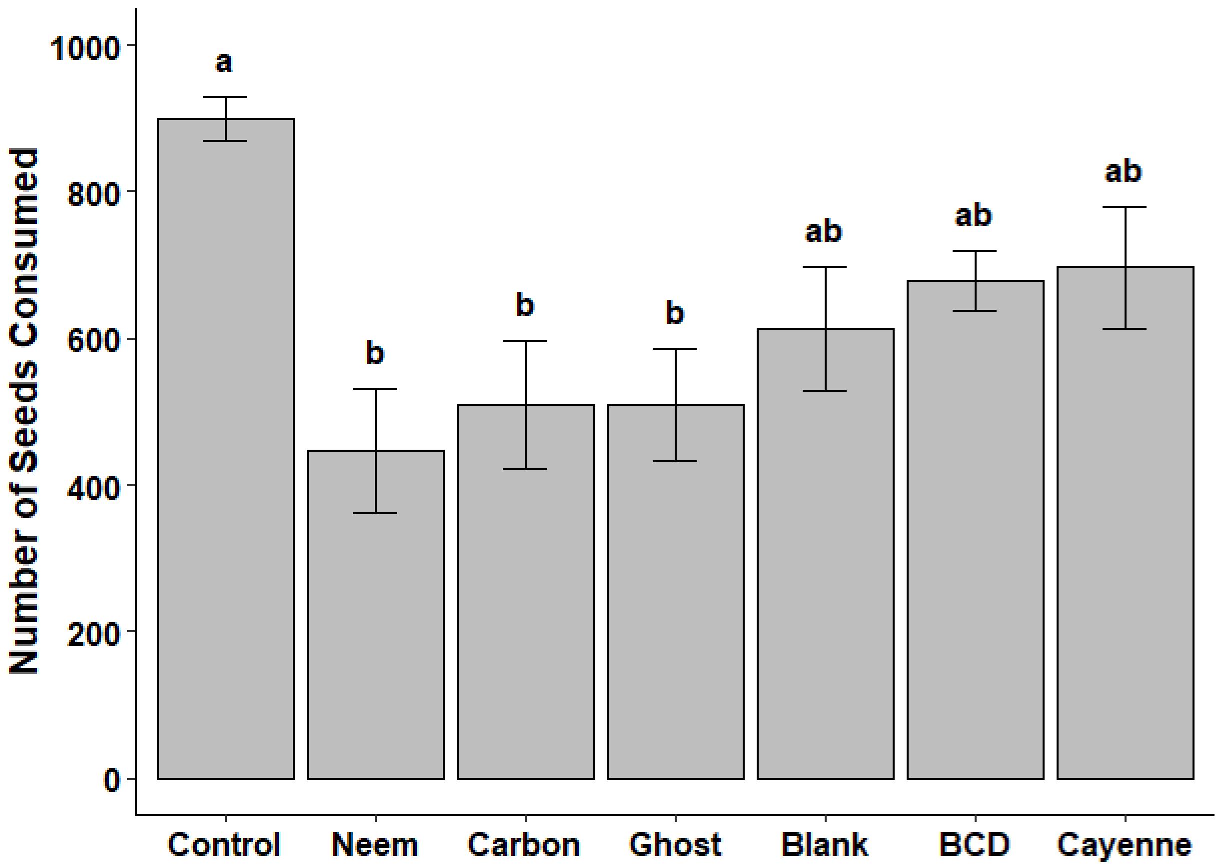

Figure 2.1. The results of the 1-choice feeding trial depicting the number of *Pseudoroegneria spicata* seeds consumed by *Dipodomys ordii* that were assigned to receive one of seven seed-coating types: uncoated seeds (control), neem oil, activated carbon, ghost pepper powder, beta-cyclodextrin (BCD), cayenne pepper powder, or the blank coating containing no active ingredient. Connecting letters indicate significance according to a Tukey test ($\alpha = 0.05$).

TABLES

Table 2.1. The formulae of the 7 seed-coating formulations containing either: ghost pepper powder, cayenne pepper powder, neem oil, beta-cyclodextrin (BCD), or activated carbon. A blank coating was created that contained no active ingredient

	Blank	**Neem**	**Carbon**	**Ghost**	**BCD**	**Cayenne**
Product Quantity (g)	0	20	200	195	50	195
P. spicata Seeds (g)	100	100	100	100	100	100
Swell Clay (g)	195	195	0	195	195	195
Selvol 205s 15% (ml)	90	90	270	170	90	170

SUPPLEMENTAL MATERIAL

Supplemental Figure 2.S1. Graphs of temperature (C°), percent humidity, and light (lux) within the housing room over the duration of the study as recorded by an Element-A environmental monitor and data-logged using the Elemental Insights Software (Elemental Machines, Cambridge, Massachusetts, USA).

LITERATURE CITED

Akhtar M, Mahmood I (1996) Control of plant-parasitic nematodes with organic and inorganic amendments in agricultural soil. Applied Soil Ecology 4:243-247

Balch JK, Bradley BA, D'antonio CM, Gomez-Dans J (2013) Introduced annual grass increases regional fire activity across the arid western USA (1980-2009). Global Change Biology 19:173-183

Barnett JP (1997) Oleoresin capsicum has potential as a rodent repellent in direct seeding longleaf pine. Proceedings of The Ninth Biennial Southern Silvicultural Research Conference:326-328

Barnett S (1988) Exploring, sampling, neophobia, and feeding. Rodent Pest Management:296-317

Bowman TR, Mcmillan BR, St Clair SB (2017) A comparison of the effects of fire on rodent abundance and diversity in the Great Basin and Mojave Deserts. Plos One 12:12

Briggs JS, Vander Wall SB (2004) Substrate type affects caching and pilferage of pine seeds by chipmunks. Behavioral Ecology 15:666-672

Bringe A, Schultz L (1969) Effects of roughage type or added bentonite in maintaining fat test. Journal of Dairy Science 52:465-471

Brown JH, Heske EJ (1990) Control of a desert-grassland transition by a keystone rodent guild. Science 250:1705-1707

Daly M, Rauschenberger J, Behrends P (1982) Food aversion learning in kangaroo rats - a specialist generalist comparison. Animal Learning & Behavior 10:314-320

Demerlis C, Schoneker D (2003) Review of the oral toxicity of polyvinyl alcohol (PVA). Food and Chemical Toxicology 41:319-326

Erickson WA, Urban DJ. (2004) Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. US Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances Washington, DC,

Gurney CM, Prugh LR, Brashares JS (2015) Restoration of native plants is reduced by rodent-caused soil disturbance and seed removal. Rangeland Ecology & Management 68:359-366, 358

Gurney JE, Watkins RW, Gill EL, Cowan DP (1996) Non-lethal mouse repellents: Evaluation of cinnamamide as a repellent against commensal and field rodents. Applied Animal Behaviour Science 49:353-363

Hansen SC, Stolter C, Imholt C, Jacob J (2016) Plant secondary metabolites as rodent repellents: a systematic review. Journal of Chemical Ecology 42:970-983

Hansen SC, Stolter C, Jacob J (2015) The smell to repel: The effect of odors on the feeding behavior of female rodents. Crop Protection 78:270-276

Hardegree SP, Jones TA, Roundy BA, Shaw NL, Monaco TA (2016) Assessment of range planting as a conservation practice. Rangeland Ecology & Management 69:237-247

Henderson CB (1990) The influence of seed apparency, nutrient content and chemical defenses on dietary preference in Dipodomys ordii. Oecologia 82:333-341

Hobbs RJ, Cramer VA (2008) Restoration ecology: interventionist approaches for restoring and maintaining ecosystem function in the face of rapid environmental change. Annual Review of Environment and Resources 33:39-61

Howe H, Brown J (2001) The ghost of granivory past. Ecology Letters 4:371-378

Jacobs LF (1992) The effect of handling time on the decision to cache by grey squirrels. Animal Behaviour 43:522-524

James JJ, Rinella MJ, Svejcar T (2012) Grass seedling demography and sagebrush steppe restoration. Rangeland Ecology & Management 65:409-417

Killgore A, Jackson E, Whitford W (2009) Fire in Chihuahuan Desert grassland: short-term effects on vegetation, small mammal populations, and faunal pedoturbation. Journal of Arid Environments 73:1029-1034

Knutson KC, Pyke DA, Wirth TA, Arkle RS, Pilliod DS, Brooks ML, Chambers JC, Grace JB (2014) Long-term effects of seeding after wildfire on vegetation in Great Basin shrubland ecosystems. Journal of Applied Ecology 51:1414-1424

Kuprewicz EK (2013) Mammal abundances and seed traits control the seed dispersal and predation roles of terrestrial mammals in a Costa Rican forest. Biotropica 45:333-342

Larios L, Pearson DE, Maron JL (2017) Incorporating the effects of generalist seed predators into plant community theory. Functional Ecology 31:1856-1867

Lawhon DK, Hafner MS (1981) Tactile discriminatory ability and foraging strategies in kangaroo rats and pocket mice (Rodentia: Heteromyidae). Oecologia 50:303-309

Longland WS (1996) Desert rodents in disturbed shrub communities and their effects on plant recruitment. Pages 209-215 Proceedings: Wild Land Shrub and Arid Land Restoration Symposium, INT-GTR

Longland WS, Ostoja SM (2013) Ecosystem services from keystone species: Diversionary seeding and seed-caching desert rodents can enhance indian ricegrass seedling establishment. Restoration Ecology 21:285-291

Lucero JE, Callaway RM (2018) Native granivores reduce the establishment of native grasses but not invasive Bromus tectorum. Biological Invasions 20:3491-3497

Madsen MD, Davies KW, Boyd CS, Kerby JD, Svejcar TJ (2016) Emerging seed enhancement technologies for overcoming barriers to restoration. Restoration Ecology 24:S77-S84

Madsen MD, Davies KW, Mummey DL, Svejcar TJ (2014) Improving restoration of exotic annual grass-invaded rangelands through activated carbon seed enhancement technologies. Rangeland Ecology & Management 67:61-67

Maron JL, Pearson DE, Potter T, Ortega YK (2012) Seed size and provenance mediate the joint effects of disturbance and seed predation on community assembly. Journal of Ecology 100:1492-1500

Mason JR, Matthew D (1996) Evaluation of neem as a bird repellent chemical. International Journal of Pest Management 42:47-49

Mciver J, Starr L (2001) Restoration of degraded lands in the interior Columbia River basin: passive vs. active approaches. Forest Ecology and Management 153:15-28

Nelson JR, Wilson A, Goebel CJ (1970) Factors influencing broadcast seeding in bunchgrass range. Rangeland Ecology & Management/Journal of Range Management Archives 23:163-170

Nolte DL, Barnett JP (2000) A repellent to reduce mouse damage to longleaf pine seed. International Biodeterioration & Biodegradation 45:169-174

Nolte DL, Mason JR (1998) Bioassays for mammals and birds. Pages 326-395 In: Methods in Chemical Ecology Volume 2. Springer

Ogbuewu I, Odoemenam V, Obikaonu H, Opara M, Emenalom O, Uchegbu M, Okoli I, Esonu B, Iloeje M (2011) The growing importance of neem (Azadirachta indica A. Juss) in agriculture, industry, medicine and environment: A review. Res J Med Plant 5:230-245

Oguge N, Ndung'u D, Okemo P, Schockaert E (1997) Effects on neem plant (Azadirachta indica juss, Meliaceae) products on maize grain consumption by three common rodent pests in Kenya. Belgian Journal of Zoology 127:129-136

Opaliński S, Dobrzański Z (2007) Odours identification in the laying hen's experimental room and the use of aluminosilicates to deodorization. Pages 942-946 Proceedings of XIII International Congress in Animal Hygiene ISAH (17-21 June 2007, Tartu, Estonia), Estonian University of Life Sciences

Orrock JL, Danielson BJ, Burns MJ, Levey DJ (2003) Spatial ecology of predator–prey interactions: corridors and patch shape influence seed predation. Ecology 84:2589-2599

Pearson DE, Callaway RM, Maron JL (2011) Biotic resistance via granivory: establishment by invasive, naturalized, and native asters reflects generalist preference. Ecology 92:1748-1757

Pearson DE, Valliant M, Carlson C, Thelen GC, Ortega YK, Orrock JL, Madsen MD (2018) Spicing up restoration: can chili peppers improve restoration seeding by reducing seed predation? Restoration Ecology 27(2):254-260

Sharma K, Singh U, Sharma P, Kumar A, Sharma L (2015) Seed treatments for sustainable agriculture-A review. Journal of Applied and Natural Science 7:521-539

Sharma N, Baldi A (2016) Exploring versatile applications of cyclodextrins: an overview. Drug delivery 23:729-747

Shaughnessy R, Sextro R (2006) What is an effective portable air cleaning device? A review. Journal of Occupational and Environmental Hygiene 3:169-181

Sohi SP, Krull E, Lopez-Capel E, Bol R (2010) A review of biochar and its use and function in soil. Pages 47-82 In: Advances in agronomy. Elsevier

Spencer DA, Kverno NB, Shaw EW, Casebeer RL, Harmon WH (1954) Symposium on rodent control research in the direct seeding of forest and range lands. Journal of Forestry 52:824-831

St Clair SB, O'connor R, Gill R, Mcmillan B (2016) Biotic resistance and disturbance: rodent consumers regulate post-fire plant invasions and increase plant community diversity. Ecology 97:1700-1711

Suckow MA, Stevens KA, Wilson RP. (2012) The laboratory rabbit, guinea pig, hamster, and other rodents. Academic Press,

Suding KN, Gross KL, Houseman GR (2004) Alternative states and positive feedbacks in restoration ecology. Trends in Ecology & Evolution 19:46-53

Svejcar T, Boyd C, Davies K, Hamerlynck E, Svejcar L (2017) Challenges and limitations to native species restoration in the Great Basin, USA. Plant Ecology 218:81-94

Taylor MH, Rollins K, Kobayashi M, Tausch RJ (2013) The economics of fuel management: Wildfire, invasive plants, and the dynamics of sagebrush rangelands in the western United States. Journal of Environmental Management 126:157-173

Tilman D, Lehman C (2001) Human-caused environmental change: impacts on plant diversity and evolution. Proceedings of the National Academy of Sciences 98:5433-5440

Yi X, Wang Z, Zhang H, Zhang Z (2016) Weak olfaction increases seed scatter-hoarding by Siberian chipmunks: implication in shaping plant–animal interactions. Oikos 125:1712-1718

Zhong JJ (2002) Preparation of bentonite deodorant for refrigerator. Jiangxi Chemical Industry 2