

Spicing up restoration: can chili peppers improve restoration seeding by reducing seed predation?

Running head: improving restoration seeding with chili peppers

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/rec.12862

Seed predation by rodents presents a significant barrier to native plant recruitment and can impede restoration seeding efforts. In nature, some plants contain secondary defense compounds that deter seed predators. If these natural defense compounds can be applied to unprotected seeds to inhibit rodent granivores, this approach could improve restoration seeding. Capsaicin is the active ingredient in chili-pepper (*Capsicum* spp.) seeds that creates the burning sensation associated with human consumption of hot peppers. This compound has a similar effect on other mammals and is believed to have evolved as a deterrent to rodent seed predators. We used seed-coating techniques to attach powder ground from Bhut Jolokia (*C. chinense*) peppers to native plant seeds and evaluated the efficacy of these seed coatings for deterring rodent seed predation and enhancing native plant recruitment using laboratory and field experiments. Laboratory feeding trials demonstrated that native deer mice (*Peromyscus maniculatus*) consumed far fewer pepper-coated seeds compared with untreated control seeds. Field seed-addition experiments consistently demonstrated that rodent seed predation reduced native plant recruitment over the four year study. Coating techniques used in the first three years were not persistent enough to reduce rodent seed predation effects on plant recruitment. However, a more persistent coating applied in conjunction with late-winter sowing negated rodent seed predation effects on recruitment in year four. Our results demonstrate that coating seeds with natural plant defense compounds may provide an effective, economical way to improve the efficacy of plant restoration by deterring seed predation by ubiquitous rodent granivores.

Key words: biomimicry, capsaicin, chemical defense, chili pepper, reseeded, rodents, seed predation, seed coating, seed sowing

Implications for Practice

- Coating native plant seeds with natural seed defense compounds can reduce rodent seed predation and increase seedling establishment in restoration seeding efforts.
- Applying a capsaicin-coating to spring-germinating seeds sown in late winter can increase native plant recruitment, while reducing the total cost per established seedling.
- The current coating technology is not persistent enough to protect seeds throughout the winter, so future work is needed to extend seed coating efficacy in order to apply this technology for fall applications.

Introduction

The reestablishment of native plant communities previously impacted by natural and anthropogenic disturbances has clear benefits for promoting biodiversity and ecosystem function (e.g., Hobbs & Cramer 2008). Moreover, reestablishing native plants following disturbances and management activities is essential for minimizing exotic plant invasions (D'Antonio et al. 1999; Pearson et al. 2016). Mounting evidence indicates that seed limitation commonly inhibits native plant establishment, a situation that can be exacerbated by disturbances and exotic plant invasions (Seabloom et al. 2003; Clark et al. 2007; Turley et al. 2017). However, reseeding efforts aimed at augmenting natural plant establishment have notoriously low success (James et al. 2012) and can be costly (Taylor et al. 2013). The limited capacity to recover native plants by sowing seeds following disturbances and management activities presents a major gap in restoration technology (Madsen et al. 2016).

Abundant evidence suggests that rodent granivores are an important biotic filter to plant establishment that may inhibit restoration efforts in a variety of plant communities. Rodent seed predation has been shown to suppress recruitment of many functionally important native plants in grassland systems, including perennial grasses, nitrogen-fixing legumes, showy forbs, and threatened plants, with larger seeded species commonly experiencing particularly strong impacts (Brown & Heske 1990; Orrock et al. 2008; Maron et al. 2012; Germain et al. 2013). Despite the deleterious effects of rodent granivores on plant recruitment, there are no feasible methods for excluding rodent granivores at scales relevant to restoration, and poisoning rodents has deleterious side effects (Erickson & Urban 2004).

One approach to reducing rodent granivore impacts in restoration may be to coopt natural plant defense strategies to protect seeds sown for restoration purposes. For example, capsaicin is the active ingredient in chili-peppers (*Capsicum* spp.) that creates the burning sensation associated with consumption of these peppers by humans. This compound has a similar effect on other mammals and is believed to have evolved as a deterrent to rodent seed predators (Tewksbury & Nabhan 2001). Prior feeding trials have demonstrated that capsaicin can deter seed predation by several rodent species (Nolte & Barnett 2000; Willoughby et al. 2011), but to our knowledge no studies have evaluated the efficacy of such treatments for improving plant recruitment. Here, we examine whether newly developed seed-coating technologies (Madsen et al. 2016) can be used to apply capsaicin to native plant seeds in order to reduce rodent seed predation and increase plant recruitment for restoration.

Methods

We conducted a series of experimental trials in an effort to develop pepper seed coating techniques that could increase seedling recruitment of dominant native plant species commonly used for restoration in Intermountain grasslands of western Montana (Table 1). In all lab and field experiments, capsaicin was applied to seeds as finely ground powder derived from Bhut Jolokia (*Capsicum chinense*) fruits and seeds rather than pure extracted capsaicin because pure capsaicin is expensive, whereas pepper powder is relatively inexpensive and readily available. Bhut Jolokia peppers were chosen because they have extremely high capsaicin concentrations (>1,000,000 Scoville Heat Units). We tested different coating techniques in each year in an iterative effort to develop an effective method (Appendix S1).

Laboratory experiments

We conducted laboratory feeding trials to assess the effect of pepper coatings on seed predation by the deer mouse (*Peromyscus maniculatus*), a widespread seed predator across North America (Wilson and Ruff 1999). In each feeding trial, one adult wild-captured deer mouse was placed overnight (from 1900 to 0700) in a 10-gallon glass terrarium and offered locally collected, native *Balsamorhiza sagittata* (Asteraceae) seeds that were either uncoated or pepper-coated. Seeds were set out in a 60 ml plastic cup (2-3 g of seed per cup, standardized in each trial) on opposite sides of the cage and separated by a center partition (after Pearson et al. 2011). The mouse was provided a shelter at the cage center with equal access to both offerings. Seeds were air dried for 24 hours at ambient lab conditions before weighing prior to and following trials. Residual seed mass was used to estimate seed consumption. Mice were freshly captured each day using Sherman live traps within natural grasslands in the Missoula valley, and they were returned to their capture sites on the morning after trials ended (Pearson et al. 2011). All human safety and animal handling protocols were vetted by the University of Montana Institutional Animal Care and Use Committee.

We conducted feeding trials in year one (n=3 trials) and two (n=16 trials) of the study. Because year one field trials suggested that precipitation and hot temperatures might erode seed coatings, we expanded feeding trials in year two to examine the effect of weathering on pepper-coated seeds. The weathering treatment involved spreading fresh pepper-treated seeds on a metal tray and placing them outdoors for a week when average temperatures ranged from 19-35 °C (night vs day, respectively). Each day, seeds were collected into a colander and rinsed with cold water from a garden hose for five minutes to simulate periodic heavy rain. They were then returned to the tray. Weathered pepper-treated seeds and untreated seeds from the same seed batch were offered to mice in feeding trials as described above. These results were then evaluated against trials using unweathered pepper-treated seeds and untreated seeds from the same seed batch.

Field experiments

We conducted seed addition experiments to evaluate whether pepper coating seeds increased seedling recruitment under field conditions in each of four years (seedling count years were 2013-2016). In the first three years, we compared pepper-treated versus untreated seeds, as this most closely reflects standard restoration scenarios where seeds are sown without coatings. In the final year, we added a coating control treatment to evaluate how seed coatings without pepper affected seed predation and recruitment. In years 1-3, seeds were set out in a randomized split plot design, with two 1-m² subplots with either pepper-coated or uncoated control seeds paired inside and outside of rodent exclosure plots (\pm pepper nested in \pm rodent exposure). Rodent exclosures were 5 x 3 m within which subplots were placed 1 m apart and 1 m from exclosure walls. The walls were constructed of aluminum flashing anchored by wooden stakes on the inside, with 15 cm buried in the ground and 45 cm extending above the ground. Unexclosed subplots with rodent access were located two meters from the exclosures and similarly spaced 1 m apart. This design was fully randomized

and replicated at three locations at ≥ 100 m intervals at each of four sites located >1 km apart around the Missoula valley (Fig. 1). All seeds were purchased from local vendors. Seeds were added in the fall (October) in the first and second year. In these years, 100 seeds of each species was added to each plot, with 10 and 8 species used in each year, respectively. Two species were dropped in the second year due to poor germination the prior year. In the third year, seeds were added in late winter (February) in an effort to evaluate how reducing the time that seeds were exposed to weathering affected outcomes. Two of the prior species were used in this year, with 50 seeds per species added to each plot (Table 1). In all years, the same plots were used and all vegetation was removed from the plots in fall prior to seeding. Seeds were sown on the ground surface as is done in broadcast seeding.

In the final year, we employed a different experimental design that used a shortened seed exposure time while ensuring consistent seed predator presence during the exposure period. In February, seeds of only four species not requiring extensive cold stratification were set out in paired wire mesh cages (40 L x 40 W x 20 H cm) that either allowed or precluded rodent access, with 50 seeds added per species and cage (Table 1). Cages were dug into the ground 10 cm and filled with soil to ground level (Pearson et al. 2014). Rodent-access cages had two holes (5 cm H x 10 cm W) cut in each side at ground level, and all cages had removable tops for access. Three pairs of cages were set out at each location, with each pair assigned to either pepper-coated seeds, uncoated seeds, or coated seeds with no pepper added. Paired cages were 50 cm apart and 50 cm from the next pair. This design was replicated three times at approximately 12-m spacing inside of six 15 x 15 m rodent enclosures, each of which had two wild deer mice introduced just prior to seed addition. This mouse density reflects moderately high natural mouse densities commonly attained in this habitat (Pearson & Fletcher 2008). Rodent enclosure design followed that of Maron et al. (2012), with mice enclosed within the enclosures instead of excluded from them. The study

site was located at the University of Montana Avian Studies Center Field Site in the Missoula valley. In all field experiments, seedlings were counted in plots 2-3 times over the spring (late March-early June), with the timing of censuses based on annual variation in phenology and weather effects on emergence. Finally, we evaluated the cost per established seedling for pepper-coated versus uncoated seeds based on the field results from the coating technique which successfully reduced rodent seed predation in the final year of the study (Appendix S2).

Statistical Analyses

All analyses were conducted using generalized linear mixed models (GLMMs) in SAS (version 9.4, PROC GLIMMIX, SAS Institute 2013). Years were analyzed separately due to differences in seed coating methods. For each year of lab experiments, the proportion of seeds consumed by mice was treated as the response variable (with the addition of a small constant to eliminate zeroes) in a model fit with a beta distribution and containing pepper (\pm) as a fixed factor and trial as a random factor. In the model for the second year of lab experiments, weathering (\pm) was also included as a fixed factor.

In our main analysis of each year of field experiments, we treated the number of seedlings counted in each visit across species as the response variable in a model fit with a negative binomial distribution. For comparison, we also ran separate models for each year that evaluated the response for 1) the species with the highest recruitment level in that year and 2) remaining species pooled. Data from four rodent enclosure plots (one per site) in year two were excluded from analysis given evidence of rodent entry (seed predation), and one location in year three was lost due to disturbance. Models for years 1-3 contained rodent exposure (\pm), pepper (\pm), visit (n=3 in years 1 and 4, n=2 in years 2 and 3), and all interactions among these variables as fixed factors, while random factors included site,

location nested within site, rodent exposure plot nested within location (to account for split-plot design), and subplot nested within rodent exposure plot (to account for repeated sampling). The model for year 4 contained the same fixed factors, but the pepper treatment included a seed coating control, and random factors were enclosure and cage nested within enclosure (to account for repeated sampling). To evaluate effects of pepper treatment on recruitment in the absence of rodent predation in each year of field experiments, we contrasted least squares means between pepper treatments in plots with no rodent access. Across the 4 years of field experiments, rodent and pepper effects did not vary significantly among visits in most cases ($p < 0.05$), and in the few exceptions, effects varied in magnitude but not direction. For this reason, we focus on overall rodent and pepper effects as evident across visits, but present test statistics for all model terms including those examining variation among visits in Table S1.

Results

In laboratory feeding trials, *P. maniculatus* consumption of pepper-coated *B. sagittata* seeds was dramatically reduced compared with uncoated seeds in both year one ($\bar{x} = 0.06\% \pm 0.03$ vs. $\bar{x} = 48\% \pm 0.09$, respectively; $F(1, 2) = 40.83$, $p = 0.025$) and year two ($F(1, 14) = 103.91$, $p < 0.01$; Fig. 2). There was no evidence that experimental weathering reduced the effect of pepper treatment on seed predation levels (weathering x pepper treatment interaction, $F(1, 14) = 0.45$, $p = 0.51$). In field experiments, the most consistent pattern across all years was that of a significant reduction in seedling recruitment across species when rodents had access to seeds ($p < 0.01$; Table S1; Fig. 3). In the absence of rodent predation, pepper treatment had a marginally significant negative effect on recruitment across species in year one ($t(22) = -1.78$, $p = 0.09$), but elevated recruitment in year three ($t(22) = 3.91$, $p < 0.01$), with no evidence that patterns diverged with rodent exposure in these years (rodent exposure x pepper

interactions, $p > 0.3$, Table S1). However, in year four, pepper treatment enhanced recruitment only in plots with rodent access, mitigating the suppressive effect of rodents (pepper x rodent interactions, $p < 0.01$, Table S1). Results for the primary species recruiting in each year generally conformed to patterns seen across species with one exception: in year one, the inhibitory effect of pepper was not evident for the main species, *Lupinus sericeus* ($t(22) = 0.08$, $p = 0.94$), but was evident for the remaining nine species pooled ($t(22) = 2.21$, $p = 0.04$; Fig. 3). Except in years one and three when recruitment was relatively high for remaining species pooled, experimental patterns were generally weaker for this group. The cost per established plant when using *C. chinense* coated versus uncoated seeds was 66% lower for all species and 28% lower for the primary species (Appendix S2, Table S2, S3).

Discussion

Reseeding is a cost effective approach to recovering native plant communities when it works, however, its efficacy is unpredictable due to strong abiotic and biotic limitations on seedling establishment (James et al. 2012). Here, we provide evidence that natural plant chemical defenses against seed predators can be applied to native seeds to reduce seed predation and increase success of native plant reseeded efforts, while also reducing restoration costs per established plant.

The degree to which protecting seeds from predation will benefit restoration depends on the strength of seed predation as a biotic constraint to plant establishment. Across grasslands and semi-arid systems of North America, many studies have demonstrated that rodent seed predation can suppress a range of dominant native plant species, particularly larger seeded species (Brown & Heske 1990; Orrock et al. 2008; Maron et al. 2012; Germain et al. 2013). In our system, despite the fact that rodent population fluctuations can drive variation in rodent seed predation (Pearson & Callaway 2008), we found that rodents

significantly suppressed establishment of restoration species by two to four-fold across study years. Hence, reducing the effects of rodent seed predators has the potential to substantially increase plant recruitment in this system. The fact that studies across North America demonstrate high levels of rodent impacts on plant establishment suggests that rodent seed predation is a substantial biotic filter that must be addressed in restoration and, more generally, in plant ecology (Larios et al. 2017).

Developing seed coatings capable of inhibiting seed predators and increasing plant establishment presents several challenges. Chief among these: 1) the deterrent must be potent and maintain potency when bound to seeds by coating compounds, 2) the deterrent and coating must not inhibit seed germination, and 3) the treatment must endure weathering effects under field conditions long enough to inhibit seed predation until germination. Our laboratory feeding trials indicated that the deterrent effects of fresh chili powder treatments were substantial, with pepper treatments reducing *P. maniculatus* seed predation by 86%. Moreover, repeated heating, cooling, and washing of seeds did not diminish this effect in the short-term. Willoughby et al. (2011) found that capsaicin was the most effective of five repellents that were applied to seeds that were offered to both wood mice (*Apodemus sylvaticus*) and gray squirrels (*Sciurus carolinensis*), whereas Nolte and Barnett (2000) found capsaicin to be effective in reducing seed predation by house mice (*Mus musculus*) but not deer mice. The cause of the weak response by deer mice to capsaicin in this study was unclear, but could be linked to the coating that was used or capsaicin concentrations. Neither of these studies experimentally isolated the effects of repellents versus rodent seed predation to evaluate the independent effects of each on seedling establishment in the field.

Coating effects on seedling germination/establishment were variable, ranging from negative to positive, as indicated by results from field cages that protected seeds from

rodents. The pepper treatment effects on seedling establishment in the absence of rodent seed predation were presumably due to coatings inhibiting seed germination in some cases and in other cases interacting with abiotic factors like moisture uptake to favor germination (Madsen et al. 2016). Interestingly, in year four, seeds with coatings but no pepper added established more poorly than did pepper coated seeds or uncoated seeds when *P. maniculatus* had access to them (Fig. 3), suggesting that the coating itself increased seed predation, even though this effect was countered by the addition of pepper. This preference for clay-coated seeds could arise if mice were seeking the clay as a source of limited minerals or as an “antacid” for alleviating discomfort associated with the capsaicin consumed in adjacent plots (Wilson 2003). Collectively, these results indicate the necessity of fully understanding how seed coating treatments interact with both abiotic and biotic factors in order to engineer coatings that maximize restoration efficacy (Madsen et al. 2016).

Maintaining the efficacy of pepper treatments from initial sowing to seed germination in the field proved to be the most difficult challenge to overcome. Field experiments in years one and two indicated that these coating methods were insufficient to maintain rodent deterrence from fall until spring germination. Visits to the experimental plots in the fall to monitor the sown seeds indicated that coated seeds were initially ignored by rodents while uncoated seeds were consumed. However, by late fall, after multiple daily cycles of heating and cooling and multiple rains, it was apparent that coatings were eroding and rodents were beginning to consume treated seeds in rodent-access plots. In year three, we hired a company specializing in agricultural seed coatings to treat the seeds using commercial machine techniques. We also set out seeds in late winter to shorten exposure to weathering. This seed-coating treatment elevated seedling recruitment in the absence of seed predation, but the benefit of the coating was overcome when seed predators had access to seeds. In the final year, we applied coating techniques specifically designed for restoration applications

(Madsen et al. 2016). We again sowed seeds in late winter to minimize exposure to weathering, while also placing seed offerings within rodent enclosures where we could ensure seed predators were present during the short experiment. In this experiment, mouse access reduced overall recruitment of uncoated seeds by 55% relative to levels in mouse enclosures, but recruitment of pepper-treated seeds was not significantly affected by mouse access, indicating that the pepper treatment functionally negated seed predation effects on seedling recruitment. This effect on plant recruitment was driven largely by the community dominant, *P. spicata*, which was the strongest recruiting plant species in that year.

Our results demonstrate that natural plant defense compounds can be adapted for improving restoration seeding efforts. However, we note that additional studies are needed to refine this restoration tool. We found that the greatest hurdle to overcome is developing seed coatings that can maintain treatment efficacy under field conditions over longer periods. While the current technology can be applied by sowing treated seeds in late winter for species not requiring stratification and/or seeds that can be stratified in advance, ideally treatments would be done in the fall in temperate systems like ours in order to incorporate the full complement of species with varying stratification requirements. Additionally, other deterrents should be explored. While capsaicin can be an effective nontoxic deterrent, it is difficult to work with under laboratory conditions because it is a skin and respiratory irritant to humans. Finally, the degree to which rodent seed predation impacts plant recruitment is a function of the interaction between seed predation and abiotic conditions that can stress or kill the seeds and seedlings that survive predation (Pearson et al. 2014). As a result, ideal seed coatings will combine seed predation deterrents with compounds that also allow seeds to overcome abiotic filters (Madsen et al. 2016).

Acknowledgements

We thank W. Hansen and D. Landres for lab work, and S. Anderson, R. Bell, L. Ehlen, D. Gundlach, H. Schultz, K. Sousa, and Native Yards Inc. for help with field experiments and seed coating. Markus Hamm provided pepper power in 2012. The authors of this work have no conflicts of interest to declare.

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Figure 1. Map of the Missoula valley showing the field experimental sites (yellow circles), the enclosure experiment site (yellow square), and associated study designs. At each experimental field site, there were three locations ≥ 100 m apart containing pairs of 3 x 5 m rectangular plots, with one plot that precluded (solid black rectangle) and one that allowed rodent access (broken black rectangle). Each of these plots contains a 1-m² subplot with pepper treated (red square) or untreated (blue square) seeds. The enclosure site is comprised of six adjoining 15 x 15 m enclosures with three plots embedded within each (grey circles). At each plot there are three pairs of 40 x 40 x 20 cm wire mesh cages, buried 10 cm into the ground and spaced 50 cm apart, that preclude (solid squares) or allow (broken squares) rodent access, containing either pepper treated seeds (red squares), untreated seeds (blue squares), or seeds coated as pepper-treated seeds without pepper added (grey squares).

Figure 2. Results from laboratory feeding trials showing effects of pepper treatments and simulated weathering on deer mouse seed consumption for uncoated seeds and pepper-coated seeds. Summary statistics represent least squares means back-transformed from the scale used in analysis (± 1 SE).

Figure 3. Seedling recruitment results from seed-addition experiments conducted in the Missoula valley of western Montana over four years where native seeds were either uncoated, coated with pepper, or coated without pepper (2016 only), and sown within enclosures that allowed or precluded rodent seed predators. Results are reported for all species combined, the primary recruiting species for that year (*L. sericeus* from 2013-2015 and *P. spicata* in 2016), and all species other than the primary recruiting species combined. Coating techniques and experimental approaches differed among years as described in the methods. Summary statistics represent least squares means back-transformed from the scale used in analysis (± 1 SE).

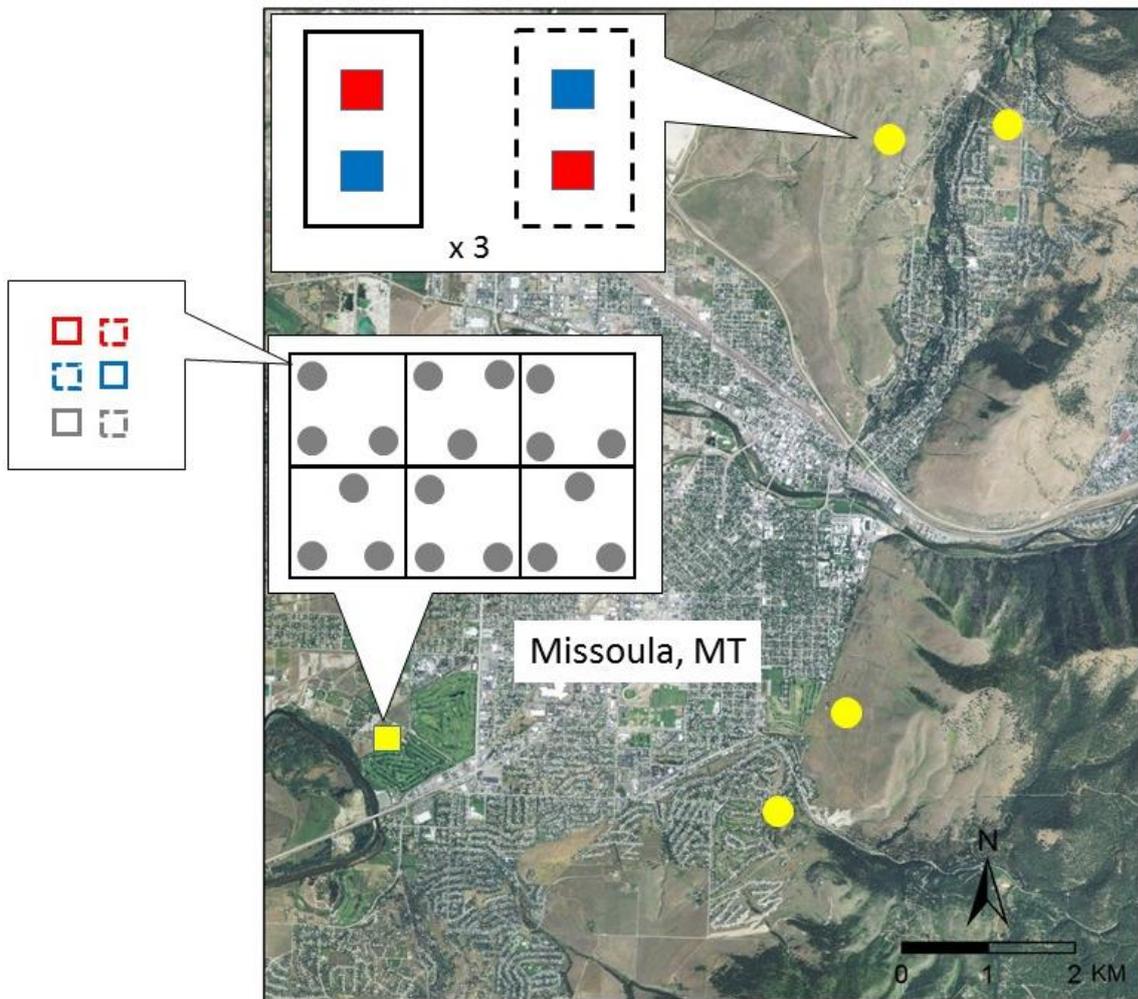


Fig. 1

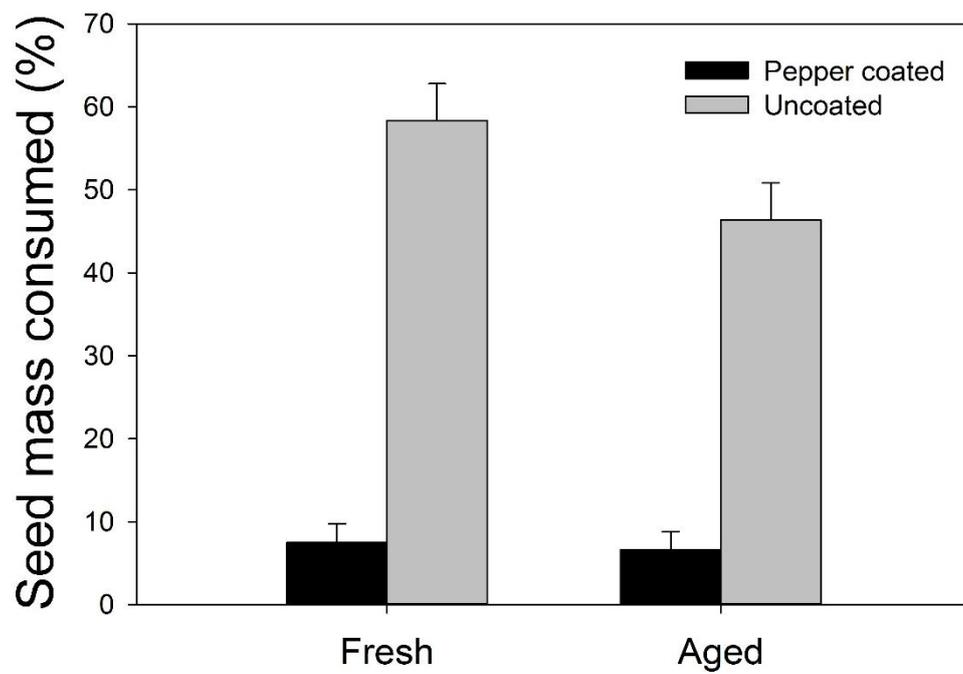


Fig. 2

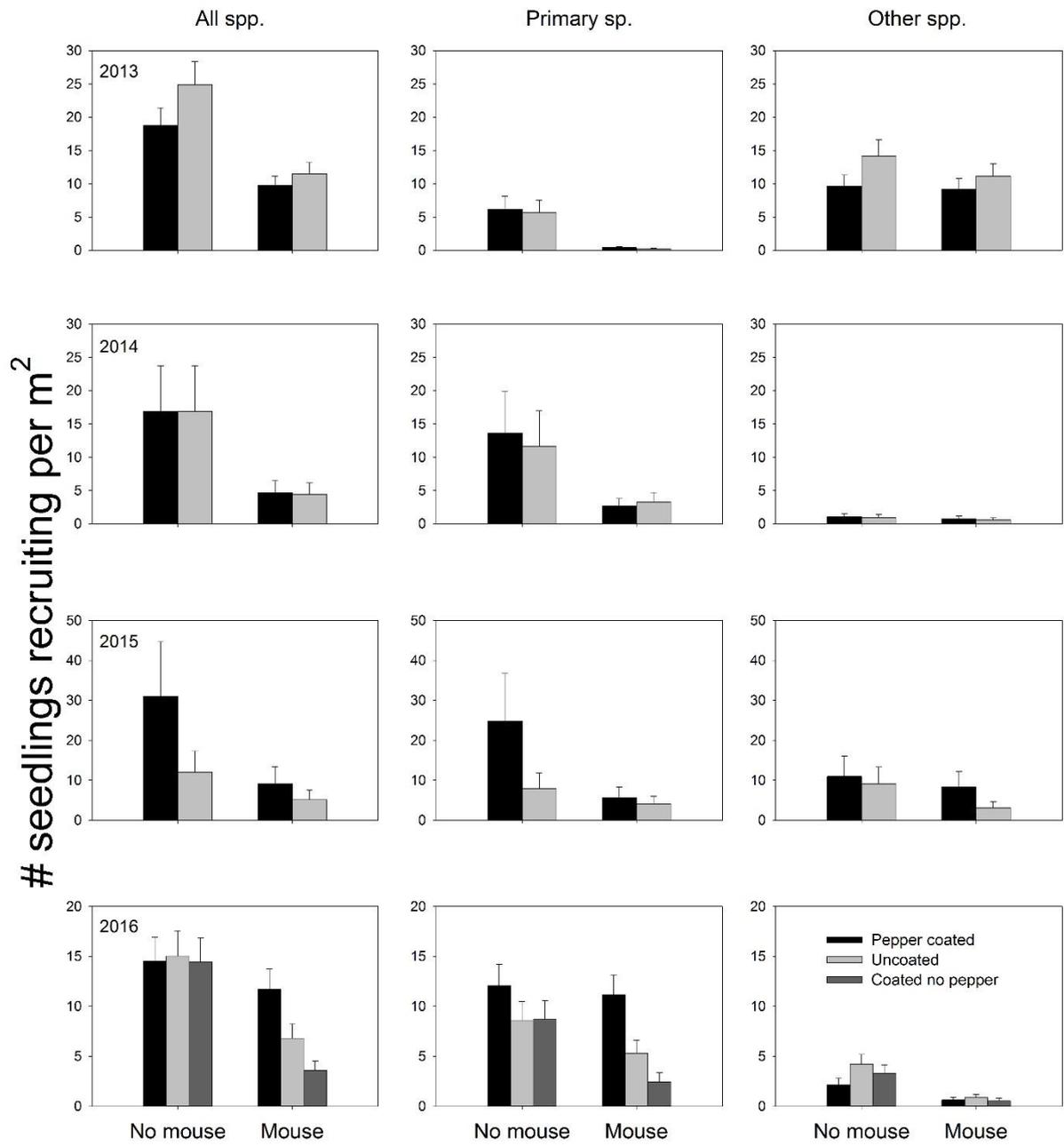


Fig. 3

Table 1. Species of native seeds used in seed addition experiments by year. Seed masses are provided for all but two species based on five measures of 100 seeds for each species taken in 2013. Functional group codes are P = perennial, A = annual, G = grass, F = forb, S = Shrub, N = nitrogen fixer. Asterisks indicate the primary species recruiting in each year.

Species	Functional		
	group	Seed mass (g)	Recruitment year
<i>Pseudoroegneria spicata</i>	PG	0.0043	2013, 2014, 2015, 2016*
<i>Balsamorhiza sagittata</i>	PF	0.0098	2013, 2014
<i>Festuca idahoensis</i>	PG	0.0015	2013, 2014
<i>Gaillardia aristata</i>	PF	0.0033	2013, 2014
<i>Heterotheca villosa</i>	PF	0.0004	2013, 2014
<i>Lupinus sericeus</i>	PFN	0.0031	2013*, 2014*, 2015*, 2016
<i>Penstemon wilcoxii</i>	PF	0.0002	2013, 2014
<i>Poa secunda</i>	PG	0.0006	2013, 2014
<i>Clarkia pulchella</i>	AF	0.0002	2013
<i>Monarda fistulosa</i>	PF	0.0003	2013
<i>Helianthus annuus</i>	AF		2016
<i>Purshia tridentata</i>	PS		2016