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RESEARCH ARTICLE

Promoting acorn survival using capsaicin seed coatings is strengthened by the removal of invasive shrubs

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Promoting regeneration of native trees, like *Quercus* spp., is a priority for land managers given the ecological and economic importance of oak woodlands. Although direct seeding may promote recruitment of *Quercus* spp., the effectiveness of direct seeding may be greatly reduced in environments where the activity of granivorous rodents is high. Importantly, the activity of granivorous rodents may be highest in environments where oak restoration is most desired, such as habitats invaded by non-native woody shrubs. Implementing chemical deterrents to granivory should promote direct seeding success; yet it is essential to understand if those deterrents are effective in challenging restoration situations (e.g. areas with dense invasive shrub cover). Moreover, it is important to determine whether chemicals that deter granivory have undesired effects on beneficial ecological interactions, such as animal-mediated seed dispersal. We used multi-field site experiments in shrub-invaded and shrub-cleared forest plots to compare the removal and dispersal of *Quercus rubra* acorns with seed coats treated with a pepper-based capsaicin extract versus acorns treated with control solutions (i.e. water and ethanol). Seed removal was quantified for 37 days, and seed survival and dispersal were quantified by relocating nail-tagged acorns after 8 weeks. We found that capsaicin-treated seeds had a significantly higher probability of survival compared to seeds treated with control solutions; the presence of the invasive shrub *Rhamnus cathartica* increased post-dispersal seed consumption regardless of seed-coat treatment; capsaicin did not affect acorn dispersal distance; and the concentration of capsaicin coatings on acorns declined over time in the field.

Key words: capsaicin, invasive shrubs, *Quercus rubra*, *Rhamnus cathartica*, seed dispersal, seed predation

Implications for Practice

- Pepper-based capsaicin-extract coated acorns have higher survival probabilities compared to acorns treated with controls (i.e. water and ethanol), thereby lengthening the window of time that treated acorns can escape predation.
- The presence of invasive shrubs increases rates of acorn loss regardless of seed coatings, suggesting that the removal of invasive shrubs should be a top priority for managers seeking to restore oak woodlands.
- Application of capsaicin coatings on acorns should occur immediately prior to sowing to ensure the highest concentration of deterrent remains on seed coats for as long as possible.
- Managers applying capsaicin to acorns can do so without concerns regarding altering essential seed dispersal by scatter-hoarding rodents, potentially increasing future establishment success.

Introduction

Despite their ecological significance and economic value (e.g. supporting high levels of biodiversity, providing ecosystem services) (McShea & Healy 2002; Dey & Kabrick 2015; Dey et al. 2017), oak-dominated forests have experienced significant declines (McShea et al. 2007; Fox et al. 2010; Dey 2014). A primary impediment to the restoration of oak forests is a lack of successful recruitment of oaks from seed (Dey et al. 2008; Leverkus et al. 2013; Löf et al. 2019). Direct seeding, whereby seeds of oaks are sown directly into a site targeted for restoration, may be a promising means of increasing oak recruitment because it may be more efficient and cost-effective for oak woodland restoration compared to growing and planting oak seedlings (Atondo-Bueno et al. 2018; Villalobos et al. 2020; García-Hernández & López-Barrera 2024). Although direct seeding may be a promising means for increasing oak recruitment, the outcome of direct seeding can be highly variable depending on the magnitude of other factors that affect the survival and recruitment of oak seed.

The success of oak restoration via direct seeding may depend strongly on the activities of small mammals. Small mammals can negatively affect oak recruitment by consuming oak seeds (Peters et al. 2003; Siepielski & Benkman 2008). On the other

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hand, small mammals may increase oak recruitment via the dispersal of seeds to microsites favorable for germination and establishment (Lichti et al. 2014; Pesendorfer et al. 2016; Bogdziewicz et al. 2020). Seed consumption and dispersal by small mammals are closely linked with habitat structure (Mattos et al. 2013; Bartowitz & Orrock 2016; Guiden & Orrock 2017) and changes in habitat structure that alter understory woody shrub density, such as the encroachment of invasive shrubs, may amplify the effects small mammals have on oak acorn survival and dispersal. For example, small mammals can consume twice as many native seeds in shrub-invaded versus shrub-cleared plots (Mattos et al. 2013; Bartowitz & Orrock 2016; Keller & Orrock 2023), and invasive shrubs can modify both the magnitude and spatial distribution of rodent seed predation and dispersal via multiple means (e.g. refuge, supplemental food and extended seasonal activity) (Orrock et al. 2015; Keller & Orrock 2023; Connolly et al. 2024). Therefore, the removal of invasive woody shrubs (e.g. Common buckthorn [*Rhamnus cathartica*], Amur honeysuckle [*Lonicera maackii*]) should increase the efficacy of direct seeding as a restoration strategy by reducing acorn seed consumption and safeguarding historical secondary dispersal dynamics.

One approach that may improve seed survival following direct seeding is the practice of applying taste-averting chemical coatings to seeds (Pearson et al. 2019; Javidi & Hargreaves 2021; Lanni et al. 2023). Capsaicinoids (i.e. dihydrocapsaicin and capsaicin) extracted from *Capsicum chinense* have been shown to deter granivory when applied to seed coats because these active ingredients generate a burning sensation in the mouths of mammals (Tewksbury & Nabhan 2001; Pearson et al. 2019; Lanni et al. 2023). Capsaicin-based seed coatings can effectively deter granivory for some smaller-seeded plant species (Nolte & Barnett 2000; Pearson et al. 2019; Taylor et al. 2020). Despite this, there is a current gap in our knowledge regarding the efficacy of coating large, highly desirable seeds, such as oak acorns, with capsaicin extract to reduce granivory from rodents. Because of the large size and high nutrient content of acorns (Chambers & MacMahon 1994; Dylewski et al. 2020; Moore et al. 2021), rodent acorn consumption is often high and has been shown to be at or near 100% consumption of experimentally deployed seeds (Bartowitz & Orrock 2016; Guiden & Orrock 2017; Connolly et al. 2024). Additionally, because past studies examining the efficacy of capsaicin seed coatings focus solely on seed survival, it is unknown whether or how capsaicin modifies secondary seed dispersal, which is an important component of oak life history and regeneration success (Steele et al. 2004; Dey et al. 2019).

We examined the efficacy of using capsaicin extracted from *C. chinense* as a seed coating to reduce rodent granivory on *Quercus rubra* acorns. Using a field-based manipulation of invasive shrubs, we tested the effects that invasive shrubs may have on the efficacy of capsaicin-coated seeds to successfully escape predation. To address how forest habitat structure may interact with this seed-coating treatment to influence direct seeding outcomes, we used a field-based seed removal assay followed by an acorn tracking experiment in forest plots where invasive shrubs had been removed versus plots where invasive shrubs were present. Due to its ecological value in supporting wildlife and commercial value in producing high-quality timber, *Q. rubra* is an important species throughout woodlands in the northern hemisphere, and *Q. rubra* survival is often indicative of the survival of other oak species (e.g. *Q. alba* and *Q. macrocarpa*) (Burns & Honkala 1990; Aldrich et al. 2005; Flores-Cano et al. 2012). We hypothesized that *Q. rubra* acorns coated in capsaicin extract would be (1) removed significantly less than uncoated acorns, (2) more likely to be found intact, and (3) that these effects would be amplified in the absence of invasive shrubs.

Methods

Study Location and Plot Design

We conducted a seed-removal study and an acorn-tracking experiment within the University of Wisconsin-Madison Arboretum in Madison, Wisconsin. We used ten 400 m^2 plots, with half containing intact *Rhamnus cathartica* shrubs and half with all shrubs mechanically removed. Experimental plots were established in 2014 (Bartowitz & Orrock 2016) and have been used to measure both small mammal seed removal and dispersal in the past (Bartowitz & Orrock 2016; Guiden & Orrock 2017; Keller & Orrock 2023). Invasive shrubs were removed using the cut-stem method followed by herbicide stem application (Bartowitz & Orrock 2016). Routine monitoring of plot-level *R. cathartica* encroachment has taken place since initial establishment to maintain treatment differences and prevent invasive shrub encroachment.

Capsaicin Extraction and Concentration

Capsaicins (dihydrocapsaicin and capsaicin) were extracted from ground *Capsicum chinense* powder following the protocol in Lanni et al. (2023). Application of pepper extract to seed coatings was done by pouring the pepper extract into the bottom of a brass sieve (U.S. Standard Sieve Series) and dropping batches of approximately 200 acorns into each sieve before attaching a stainless-steel mesh cover with 4.75 mm drainage holes. Seeds were fully submerged in the pepper extract for approximately 20 seconds while simultaneously swirled in the extract. Following this soaking period, all capsaicin extract was poured from the sieve, and the remaining acorns were individually placed, using forceps, onto insulated baking pans to air-dry for 2 days prior to field deployment. To measure the concentration of capsaicin present on seeds, we used a series of colorimetric assays to examine differences in capsaicin concentrations between recently treated acorns and those collected following field deployment (González-Zamora et al. 2015; Lanni et al. 2023). Using spectrophotometric analysis with stock concentrations of capsaicin extract (displaying peaks at 280 nm) and creating various dilutions using ethanol, we were able to develop a standard curve of average absorbance that allowed us to accurately estimate the capsaicin concentration present on seeds (Lanni et al. 2023). The range of concentrations that were created spanned a maximum of 75% capsaicin extract to a minimum of 0.01% pepper extract.

Acorns were stripped of capsaicin by soaking acorns in ethanol for 48 hours, followed by a 1-minute vortex prior to spectrometric analysis.

Seed Removal

To test the efficacy of capsaicin-coated seeds to reduce rodent granivory, a seed-removal study was conducted starting in August 2023. A total of 300 *Quercus rubra* acorns were coated with either capsaicin extract, an ethanol control, or a water control. The use of an ethanol control is due to ethanol being used in the capsaicin extraction protocol (Lanni et al. 2023). Once dried, 10 acorns were placed into individual seed removal depots based on seed-coat treatment in the center of each of the 10 experimental plots, totaling 30 depots. Seed removal depots, which are 3.78 L buckets with 5 cm^2 openings on each side to allow for small mammal access, have been used in previous studies to measure seed removal within both invaded and uninvaded systems (Mattos et al. 2013; Chandler et al. 2020; Keller & Orrock 2023). Once established on August 23, depots were monitored for 17 consecutive days, followed by monitoring every 3 days until all seeds were removed; this monitoring regime facilitates comparison with other seed-removal studies (Bartowitz & Orrock 2016; Fuka & Orrock 2024).

Seed Fate Following Removal

To examine whether capsaicin affects the fate of removed seeds, we used an acorn-tracking experiment. Following the conclusion of the *Q. rubra* seed removal in late September 2023, we nail-tagged 2400 *Q. rubra* acorns using 12-mm brad nails, a method used by previous studies to successfully measure seed dispersal and track seed fate (Sork 1984; Guiden & Orrock 2017; Bartel & Orrock 2020). Half of all acorns were coated in capsaicin extract while the other half were treated in a water control; we did not use an ethanol control in this study because our seed-removal experiment revealed that there was no difference between water controls and ethanol controls (see Section 3). Fluorescent-colored paint was applied to each nail to distinguish between seed-coat treatments, and each color was randomized between the coating treatment and plot. At each of the 10 experiment plots, 120 *Q. rubra* acorns of each seed-coat treatment were placed into a 0.5 m^2 hardware cloth exclosure with 7 cm^2 openings on each side to allow small mammal access, totaling 240 total acorns in two exclosures per plot. After 8 weeks, we used a metal detector (Bounty Hunter Platinum, First Texas Products, El Paso, TX, U.S.A.) to search for remaining acorns within a 25 m radius of each exclosure (Guiden & Orrock 2017). The condition of the seed (intact or destroyed), the seed-coat treatment (capsaicin or control), the distance from the depot, and the cardinal direction each found acorn was recorded. Ancillary trials were also conducted to ensure that recovery rates did not differ in plots with *R. cathartica* intact vs. plots with *R. cathartica* removed and that we were able to detect acorns buried to at least 15 cm, a depth that exceeds known maximum caching depths for scatter-hoarding rodents (Lichti et al. 2014; Bartel & Orrock 2020). We assume that any unrecovered *Q. rubra* acorns were consumed and destroyed outside of our search radius or brought to arboreal caches (hereafter referred to as seed loss), a finding that is consistent with other seed-tracking studies (Steele et al. 2001, 2011; Bartlow et al. 2018).

Statistical Analyses

For our seed-removal experiment, we performed a survival analysis using a Cox proportional hazards regression model (package: "survival," Therneau et al. 2024) to examine potential differences in the time to removal for acorns based on both seed-coat treatment (capsaicin vs. ethanol vs. water) and invasion (*R. cathartica* intact vs. *R. cathartica* removed). For our seed-dispersal experiment, we compared acorn dispersal distance and acorn condition (intact vs. destroyed) between invasion treatments (*R. cathartica* invaded vs. *R. cathartica* removed) using *t* tests. We used a linear mixed effects model package ("lme4," Bates et al. 2015) to test for the effects that invasion (*R. cathartica* invaded vs. *R. cathartica* removed) and seed-coat treatment (water vs. capsaicin) had on *Q. rubra* dispersal distance. We used a Bayesian generalized linear mixed model with a binomial response distribution (package "brms," Bürkner 2017) to test for the effects that invasion (*R. cathartica* invaded vs. *R. cathartica* removed) and seed-coat treatment (water or capsaicin) had on *Q. rubra* survival following dispersal.

Results

Seed Removal

Once deployed, 100% ($n = 200$) of control *Quercus rubra* acorns (ethanol and water) were removed after 8 days, and 100% ($n = 100$) of capsaicin-treated acorns were removed after 37 days. In *Rhamnus cathartica* invaded plots, all control acorns were removed after 5 days following deployment, and all capsaicin-treated acorns were removed after 23 days (Fig. 1). In *R. cathartica* removed plots, all control acorns were removed after 8 days following deployment, and all capsaicin-treated acorns were removed after 37 days (Fig. 1). Both water-control and ethanol-control treated seeds had significantly higher hazard values (i.e. were more likely to be removed) when compared to capsaicin-treated seeds ($W = 95.87$, $p < 0.001$; $W = 100.9$, $p < 0.001$). Irrespective of seed-coat treatment, seeds were removed sooner in *R. cathartica* invaded plots compared to *R. cathartica* removal plots ($z = -5.94$, $p < 0.001$), while there was no significant interaction between seed-coat treatment and *R. cathartica* invasion on the rate of seed loss ($z = 0.226$, $p = 0.821$) (Fig. 1).

Seed Fate Following Removal

Following the 8-week deployment, we recovered 2.3% ($n = 113$) of deployed tags. Among all recovered tags, 83% ($n = 93$) were found without an acorn, an indication of seed predation. The total number of recovered tags from destroyed acorns did not differ between invasion treatments ($t_4 = 0.39$, $p = 0.71$). All relocated intact acorns (100%, $n = 20$) were located within *R. cathartica* removed plots; no intact acorns were found in plots with *R. cathartica* intact. On average, twice

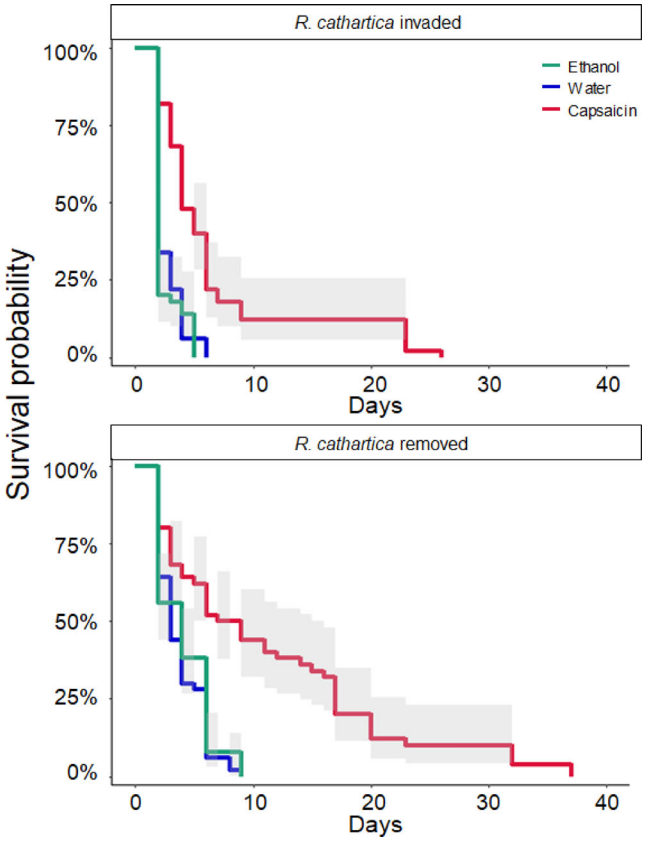


Figure 1. Effects of invasive shrub removal (*Rhamnus cathartica* invaded vs. *R. cathartica* intact) and seed-coat treatment (capsaicin vs. ethanol vs. water) on days to *Quercus rubra* acorn removal. Survival curves are calculated from proportional hazards analysis split by invasion treatment. Steeper decreases in survival curves indicate more rapid acorn predation over time.

as many acorns were found consumed in *R. cathartica* intact plots (6.2 ± 3.8 SE) compared to *R. cathartica* removed plots (3.1 ± 0.85 SE). As such, post-dispersal seed survival was significantly affected by invasion ($\beta = 38.19$, 95% CI: 2.50, 174.90), but not seed-coat treatment ($\beta = 24.87$, 95% CI: −21.88, 149.81) nor the interaction between invasion and seed-coat treatment ($\beta = -24.43$, 95% CI: −149.50, 22.18) (Fig. 2). Tag distance (nails found without an acorn) did not differ by invasion treatment ($F = 0.89$, $p = 0.372$) or seed-coat treatment ($F = 0.55$, $p = 0.457$), and there was no effect of the interaction between invasion and seed-coat treatment on tag distance ($F = 1.16$, $p = 0.283$) (Fig. 2). The mean dispersal distance for all acorns was 2.06 ± 0.29 m (SE), with the furthest distance dispersed recorded as 11.6 m. Following initial capsaicin application, peak capsaicin concentration detected by spectrophotometric absorbance was 0.50% present on seed coats. Once collected from the field following the 8-week dispersal experiment, a significant reduction in capsaicin concentration was detected, measuring below our minimum threshold value of 0.01% ($t = 11.15$, degrees of freedom [df] = 80, $p < 0.001$) (Fig. 3).

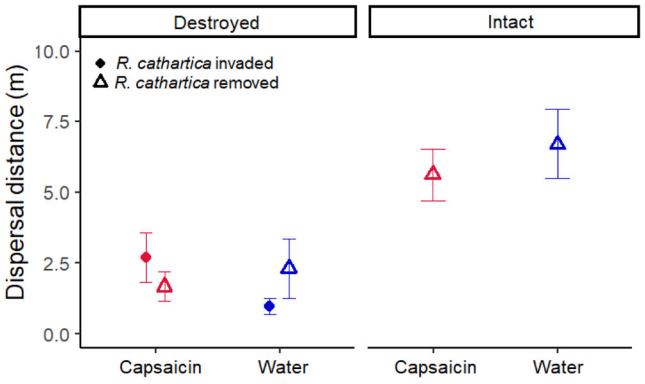


Figure 2. Effects of invasive shrub removal (*Rhamnus cathartica* invaded vs. *R. cathartica* removed), seed-coat treatment (capsaicin vs. water), and acorn condition (found destroyed vs. found intact) on mean *Quercus rubra* acorn dispersal distance. Because no intact acorns were found in *R. cathartica* invaded plots, these points are missing from the right (intact) panel.

Discussion

Because seed survival is essential to oak recruitment, the success of direct seeding to increase oak recruitment may depend upon whether seed coatings can reduce rodent granivory (Birkedal et al. 2010; Löf et al. 2019; Leverkus et al. 2021), whether coatings are effective in high-granivory environments (i.e. habitats with invasive shrubs present; Utz et al. 2020; Bartowitz & Orrock 2016; Mattos et al. 2013) and whether seed coatings have undesired effects on other important elements of regeneration from seed (e.g. secondary dispersal; Bartel & Orrock 2020; Vander Wall et al. 2005). Our findings underscore the primacy of habitat structure in estimating seed fate, but also demonstrate how coating seeds with pepper-extracts containing capsaicin may provide an important temporal benefit to seed survival. The presence of invasive shrubs significantly increased the rate of seed removal, but the rate of seed removal for capsaicin-treated acorns was lower in comparison to untreated acorns in both invaded and uninvaded forest plots. While pepper-extract coatings did not modify seed dispersal distance, capsaicin concentration decreased over time, and the effect of capsaicin ultimately had no effect on an acorn being found intact: seed survival was largely attributable to whether invasive shrubs were removed prior to seed deployment. Our results have several implications. First, although applying capsaicin as a seed-coat defense against rodent granivores may provide a window for seeds to escape predation, the removal of invasive shrubs prior to implementing seed addition may ultimately prove more effective for increasing the magnitude and duration of seed survival while also reducing belowground competition with established seedlings. Second, because the use of capsaicin seed coatings does not hinder the important process of secondary seed dispersal, facultative dispersal is not likely to be hindered by the use of capsaicin-coated seeds. Finally, additional studies to explore ways to maintain high concentrations of capsaicin by developing novel application techniques are likely to further improve the efficacy of capsaicin seed coatings in promoting restoration.

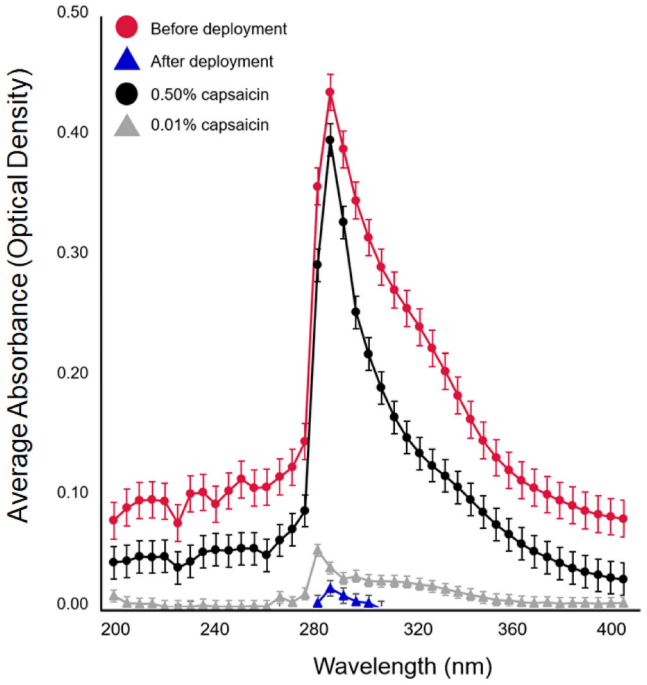


Figure 3. Summary of the absorbance spectra (range: 200–400 nm at 5 nm increments) for: (1) the pepper-extract solutions applied to acorns before field deployment ("Before deployment"), (2) the pepper-extract solutions collected from pepper-extract coated seeds after 8 weeks of deployed in the field ("After deployment"), and (3) two reference concentrations (0.5 and 0.01%) of pepper extract.

Capsaicin Seed Coatings May Prolong the Survival of Acorns, Even in the Presence of Invasive Shrubs

Seed survival is a critical life stage in the plant life cycle (Vander Wall et al. 2005; Clark et al. 2007; Crawley 2013), and studies have linked the survival of seeds to the future establishment of plant communities (Howe & Brown 2001; Larios et al. 2017; Dylewski et al. 2020). As such, a crucial management practice involves the ability to protect seeds from granivorous rodents to assist in the future recruitment of native trees (Villalobos et al. 2020, 2023; García-Hernández & López-Barrera 2024). Our findings support the use of capsaicin-based seed coatings as an economical and efficient means to promote native tree seed survival and increase the efficacy of direct seeding, even in the presence of invasive shrubs. For many large-seeded tree species, like *Quercus rubra*, seed burial is an essential stage for successful germination (Grime 2001; Flores-Cano et al. 2012). Once germination initiation has begun, acorns become much less palatable to granivorous rodents as energy-reserve degradation within the acorn decreases the nutritional value of the seed during radicle formation (Steele et al. 2004; Perea et al. 2011; García-Hernández & López-Barrera 2024). As such, our results suggest that coating acorns with capsaicin immediately prior to spring sowing could allow acorns to escape predation from rodent granivores by giving acorns enough time to germinate.

Although our findings suggest that capsaicin was effective at increasing the probability of acorn survival in both invaded and uninvaded plots, competition is a major driving force structuring tree seedling survival upon germination (Aronson & Handel 2011; Dillenburg et al. 1993); following oak germination, invasive shrubs may negatively affect the development of oaks through both above- and belowground competition (Gorchov & Trisel 2003). Studies have shown that the shallow root systems of invasive shrubs (e.g. *Lonicera maackii* and *Rhamnus frangula*) can directly lead to nearby native tree seedling mortality (Collier et al. 2002; Gorchov & Trisel 2003; Fagan & Peart 2004). As such, there is strong evidence that the removal of invasive shrubs can significantly reduce the negative effects associated with competitive interactions (Gorchov & Trisel 2003; Fagan & Peart 2004). Therefore, regardless of seed-coating techniques, our findings suggest that the removal of invasive shrubs is likely to have the dual benefit of increasing the number of favorable microsite conditions suitable for seedling growth and reducing granivore pressure by modifying foraging patterns. Our results emphasize that invasive shrub removal should remain a primary strategy prior to tree-seed sowing during restoration projects.

Acorn Dispersal Was Not Affected by Capsaicin

Seed dispersal, specifically dispersal done by scatter-hoarding rodents, is an essential process for the recruitment of trees, including oaks, throughout many ecosystems (Chambers & MacMahon 1994; Steele & Smallwood 2002; Vander Wall et al. 2005). Moreover, in certain species of oak (i.e. *Q. montana*, *Q. alba*, *Q. variabilis*, and *Q. mongolica*), rapid germination following caching has been shown to be an evolutionary strategy designed to help acorns escape predation (Fox 1982; Jansen et al. 2006; Yi et al. 2012). Our findings reveal that applying capsaicin extract to acorns did not affect acorn dispersal distance, suggesting that capsaicin may provide benefits in terms of seed survival, without having a negative effect on the beneficial process of seed dispersal. Although our results have promising implications for the use of capsaicin to improve direct-seeding efforts, we also note that additional studies are needed to understand the fate of seeds. For example, although our seed-recovery rate is similar to that in other studies on oaks (López-Barrera et al. 2005; Moore et al. 2007; Lichti et al. 2014, but see Guiden & Orrock 2017), recovery rates are often low; studies that use more sophisticated tracking methods (e.g. radio transmitters; Bartlow et al. 2018) will be important for exploring the full extent of the effect of capsaicin on seed dispersal.

Post-Dispersal Seed Survival May Be Reduced by Invasive Shrubs and the Loss of Capsaicin Coating

Invasive shrubs have been shown to amplify the activity and subsequent granivory of small mammals, directly leading to increases in seed loss within invaded forests (Bartowitz & Orrock 2016; Guiden & Orrock 2019; Connolly et al. 2024). Following dispersal, our results reveal that *R. cathartica* invaded habitat was associated with higher amounts of acorn consumption compared to *R. cathartica* removed habitat, further reinforcing previous findings associated with the link between increased seed loss based on available shrub cover (Mattos et al. 2013; Keller & Orrock 2023;

Connolly et al. 2024). In particular, the presence of invasive shrubs, not coating seeds with capsaicin, was a primary predictor of ultimate seed survival. This suggests that a top priority for managers seeking to restore invaded woodlands should be to first remove invasive shrubs before implementing seed additions of native trees. Differences in results from our seed-removal experiment (where capsaicin decreased seed removal rate) and our seed-fate study (where capsaicin did not increase seed survival) may be due to the loss of capsaicin from seeds as seeds are exposed to natural weathering and rainfall. The longevity of capsaicin seed coatings may therefore be a factor related to both exposure to environmental conditions as well as the application method (Nolte & Barnett 2000; Pearson et al. 2019; Lanni et al. 2023). Moreover, previous studies have demonstrated a link between the duration of seed deployments and seed loss (Lanni et al. 2023; Fuka et al. 2024), indicating that seed loss may increase as a result of decreasing capsaicin concentration over time. Our results, viewed considering other studies, suggest the development of effective, long-lasting capsaicin seed-coating methods may be a promising avenue of future research.

Future Directions

While direct seeding oak acorns to assist in facilitating oak woodland regeneration has shown promising results (Grossnickle & Ivetić 2017; Leverkus et al. 2021; García-Hernández & López-Barrera 2024), the need to protect sown acorns continues to be a top priority for restoration projects, as granivorous rodents pose a severe threat to seed survival (Madsen & Löf 2005; Löf et al. 2019; Villalobos et al. 2023). Our study further reinforces the strong effect that invasive shrub removal can have on increasing seed survival, demonstrating a powerful restoration tool that should be a priority for managers. Additionally, our study introduces a cost-effective and efficient seed defense technique to be used prior to direct seeding of acorns to reduce granivory by rodents by deterring seed consumption without affecting seed dispersal. Our results highlight future areas of research that will be important to fully understand the potential for the use of capsaicin as a granivore deterrent when applied to seeds. For example, because *Q. rubra* acorns are typically sown in the spring based on manager preference (Fox 1982; Hopper et al. 1985; Bartlow et al. 2018) future studies might examine the efficacy of capsaicin when applied to acorns during the spring, as well as when sown into soil rather than placed within seed depots. While studies have examined the germination success of capsaicin-treated acorns using various species of oaks (e.g. *Q. germana* and *Q. ilex*) spanning multiple systems (e.g. montane forests and Mediterranean forests) (Fox 1982; Hopper et al. 1985; Bartlow et al. 2018), future studies should consider the physical characteristics of the acorns used, as well as the microhabitat and climate in which capsaicin is applied that could affect both its ability to deter granivorous rodents and impact germination.

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