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Cheatgrass (*Bromus tectorum*), Native Grasses, and Small Mammals in the Great Basin: a Test of the Apparent Competition Hypothesis Facilitated by a Novel Method of Decanting Seeds from a Flotation Solution

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ABSTRACT

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The effect of shared enemies between invasive and native species has been argued to facilitate biological invasions (i.e., the apparent competition hypothesis or ACH). This study investigated a previously untested possibility: whether granivorous small mammals facilitate cheatgrass (*Bromus tectorum*) invasion by driving food-mediated apparent competition between cheatgrass and native grasses. Specifically, we tested three predictions that must be true if such apparent competition occurs. First, cheatgrass invasion augments total seeds available to granivorous small mammals. Second, density of granivorous small mammals increases in response to increased seed availability (simulated with experimental additions of cheatgrass seeds). Third, granivorous small mammals prefer seeds from native grasses over cheatgrass seeds. We tested these predictions in the Great Basin Desert of Utah, USA. Cheatgrass invasion augmented total yearly seed production. Granivorous small mammals preferred native seeds over cheatgrass seeds. However, neither abundance, richness, nor diversity of granivorous small mammals increased in response to experimental additions of cheatgrass seed. We therefore conclude that granivorous small mammals did not drive food-mediated apparent competition during the study period. The lack of support for the ACH in this study may suggest that the role of small mammal-driven apparent competition is either unimportant in the Great Basin, or that the appropriate indirect interactions between small mammals, cheatgrass, and native grasses have yet to be evaluated. Testing the third prediction required the separation of seeds from the soil matrix. We employed a chemical flotation methodology to recover target seeds from soil, and developed a novel method of decanting target material from the flotation solution. We compared the utility of the novel method to that of a traditional decantation method. Specifically, we compared effectiveness (the proportion of seeds recovered from a known sample), rapidity (the time required to decant that sample), efficiency (the number of seeds decanted per second), and recovery bias (the effect of relative density on seed recovery) between methods. Our proposed method was more effective, more rapid, more efficient, and less biased than the traditional method. Therefore, any future work relying on flotation to analyze seed banks should clearly describe how samples are decanted and should consider the proposed method as a potential means of enhancing the efficiency of chemical flotation.

Keywords: chemical flotation, dietary supplementation, granivory, plant-animal interaction, seed banks, seed enumeration, seed production, recovery bias, relative density, weed invasion

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

CHEATGRASS (*BROMUS TECTORUM*), NATIVE GRASSES AND SMALL MAMMALS IN THE GREAT BASIN: A TEST OF THE APPARENT COMPETITION HYPOTHESIS

ABSTRACT

The mechanisms governing invasion must be described before robust solutions to the many economic and ecologic disruptions associated with biotic invasions become possible. Recently, the effect of shared enemies between invasive and native species has been argued to facilitate biological invasions (i.e., the apparent competition hypothesis or ACH). This study investigated a previously untested possibility: whether granivorous small mammals facilitate cheatgrass (*Bromus tectorum*) invasion by driving food-mediated apparent competition between cheatgrass and native grasses. Specifically, we tested three predictions that must be true if such apparent competition occurs. First, cheatgrass invasion augments total seeds available to granivorous small mammals. Second, density of granivorous small mammals increases in response to increased seed availability (simulated with experimental additions of cheatgrass seeds). Third, granivorous small mammals prefer seeds from native grasses over cheatgrass seeds. We tested these predictions in the Great Basin Desert of Utah, USA. Cheatgrass invasion augmented total yearly seed production. Granivorous small mammals preferred native seeds over cheatgrass seeds. However, neither abundance, richness, nor diversity of granivorous small mammals increased in response to experimental additions of cheatgrass seed. We therefore conclude that, at the time scale of the study, granivorous small mammals did not drive food-mediated apparent competition. The lack of support for the ACH in this study may suggest that the role of small mammal-driven apparent competition is either unimportant in the Great Basin, or that the

appropriate indirect interactions between small mammals, cheatgrass, and native grasses have yet to be evaluated.

INTRODUCTION

Biotic invasions can lead to extensive economic and ecologic damage (Puth and Post 2005). Invasive species account for losses totaling over $137 billion per year in the U.S. alone (Pimentel et al. 2000). These losses stem from reduced crop yields, depleted grazing capacity, costly control measures such as pesticide applications, losses in revenue from ecosystem services including ecotourism, and combating invasive species that have become threats to human health (e.g. parasites and pathogens; Mack et al. 2000). In addition, invasive species can be devastating to biodiversity and ecosystem health, and are implicated in many extinctions worldwide (Mack et al. 2000). Enumerations of these disruptions are plentiful as researchers, conservationists and governments respond to ever-increasing demands to develop robust solutions (Puth and Post 2005). However, developing such solutions is an elusive goal because the general processes conferring exotic invasives such success in novel environs remain poorly understood. Before robust solutions become possible, the mechanisms governing invasion must be described (Barney and Whitlow 2008).

Several hypotheses, which are not mutually exclusive, posit general mechanisms of invasion by exotic species. The Enemy Release Hypothesis argues that when enemies from an invader's native habitat (predators, pathogens, and/or competitors) are absent in the new habitat, invaders can outcompete and overwhelm their new neighbors (Keane and Crawley 2002). The Evolution of Increased Competitive Ability Hypothesis similarly states that invaders released from natural enemies convert energy normally required for defense into biomass, increasing the invaders' competitive ability over time (Blossey and Notzold 1995; Bossdorf et al. 2005; Callaway and

Maron 2006). The Novel Weapons Hypothesis posits that allelopathic plant invaders boast an arsenal of 'novel weapons' in their new habitat against which their naïve neighbors are underequipped (Callaway and Aschehoug 2000). The Biotic Resistance Hypothesis, also known as the Species Richness Hypothesis, states that a habitat's invasibility may be related to its species richness (Elton 1958); some authors report a positive relationship, others a negative (Byers and Noonburg 2003). The Fluctuating Resource Hypothesis describes a positive relationship between the abundance of a habitat's resources and its invasibility (Davis et al. 2000). The Disturbance Hypothesis posits that invasive species are generally better adapted to disturbed sites such as roadsides, with their competitive advantage increasing as the frequency and/or intensity of disturbance increases (Hobbs and Huenneke 1992). More recently, the effect of shared enemies between invasive and native species (apparent competition) has been argued to facilitate biological invasions (Orrock et al. 2008).

Apparent competition results from an indirect interaction between focal and alternative prey items sharing a common predator or parasite (Holt 1977; Holt and Lawton 1994). This interaction can influence exotic invasions when the invader increases the local abundance of predators at the invaded site by either increasing food availability (i.e., food-mediated apparent competition; Holt and Lawton 1994) or improving predator habitat (e.g. by providing increased cover; Orrock et al. 2008). The expanding community of predators may then exert disproportionate consumptive pressure on native food items in the presence of invasive species. This foraging behavior can facilitate and help maintain exotic invasion as native individuals are preferentially eliminated (Holt and Lawton 1994; Orrock et al. 2008).

The Great Basin Desert, USA, presents a model system to investigate food-mediated apparent competition as a mechanism of biological invasion (i.e., the Apparent Competition

Hypothesis or ACH) because of the presence of focal prey (native grass species), alternative prey (cheatgrass; *Bromus tectorum*), and an important guild of shared enemies (granivorous small mammals). Although cheatgrass dominates large expanses of the Great Basin (Knapp 1996; Norton et al. 2008), sizeable patches of non-invaded habitat with intact native communities are also common. Cheatgrass-invaded and non-invaded communities often abut along sharp ecotones, facilitating comparison between adjacent community types. Finally, granivores are plentiful and important in this system, consuming a substantial portion of all new seeds produced (Chew and Chew 1970; Soholt 1973; Harper 1977; Brown et al. 1979; Price and Joyner 1997).

Granivorous small mammals may mediate apparent competition between cheatgrass and native grasses in the Great Basin due to increased food provided by cheatgrass invasion. Cheatgrass is a prolific producer of seed (Stewart and Hull 1949). Invaded sites can produce seed banks many times denser than those of adjacent non-invaded sites (Beckstead et al. 2010). An increase in seed production likely translates into an increase in food resources available to granivores (Price and Joyner 1997). This augmentation in seed inputs may allow granivore populations to increase near cheatgrass-invaded sites. Since native granivores often prefer seeds from native plants over seeds from cheatgrass (Kelrick et al. 1986), an inflated granivore community may exert disproportionate pressure on native seeds in the presence of cheatgrass, facilitating cheatgrass invasion and illustrating the ACH.

Our objective was to determine if granivorous small mammals facilitate cheatgrass invasion in the Great Basin by driving food-mediated apparent competition between cheatgrass and native grasses. Specifically, we tested three predictions that must be true if such apparent competition occurs. First, cheatgrass invasion augments total prey availability (seed production). Second, density of native consumers (granivorous small mammals) increases in response to increased

prey availability. Third, native consumers prefer native prey (seeds from native grasses) over exotic prey (cheatgrass seeds).

METHODS

Study site

We conducted our field experiments in Rush Valley, Tooele County, Utah, USA (40° 16' 48.189" N, 112° 15' 24.525" W). Rush Valley is characterized by a mosaic of monocultures of cheatgrass and other invasive plant species (most notably halogeton; *Halogeton glomeratus*) adjacent to intact native communities of big sagebrush (*Artemisia tridentata*), black greasewood (*Sarcobatus vermiculatus*), and black sagebrush (*Artemisia nova*). Intact shrub communities have relatively barren shrub interspaces dotted with forbs and grasses, and little or no visible evidence of invasive species. By contrast, invaded shrubland is typified by intershrub spaces filled by invasive species (most commonly cheatgrass), which replace native forbs and grasses.

Prediction 1: Cheatgrass invasion increases total seed production

We evaluated whether cheatgrass invasion increases an area's seed production by comparing seed rain between adjacent cheatgrass-invaded and non-invaded habitat. We measured seed rain on 3 transects in cheatgrass-invaded (hereafter "invaded") and 3 transects in cheatgrass-noninvaded (hereafter "non-invaded") habitat. Invaded transects were characterized by 50-95% cheatgrass cover in shrub interspace (estimated by an ocular method; Winkworth et al. 1962) while non-invaded transects consisted of 0-5% cheatgrass cover (estimated by the same ocular method). Each transect was 110 m, and consisted of 12 sampling stations spaced 10 m apart. All pairs of transects were separated by ≥1 km. At each station, we placed one seed trap directly

underneath the canopy of the nearest living shrub, and one seed trap in open space ≥ 50 cm from the nearest living shrub.

Seed traps consisted of a 6.7 cm diameter plastic funnel attached to a 118.3 mL specimen jar, with the stem of the funnel measuring 1.2 cm. Two drainage holes were drilled into the bottom of each jar. We buried the seed traps with their rims 3-5 mm above the soil surface (Fig. 1 in Price and Joyner 1997). We installed the traps 18-23 Dec 2009, and collected data on 13-Apri-2010, 6-July- 2010, 25-Aug-2010, and 18-Nov-2010. Samples unearthed for any reason (e.g., wind, water, animal disturbance) were not included in our analyses. After collection, we immediately placed samples in a freezer for storage until sorting and analysis.

To examine the material collected by the seed traps we thawed and dried the samples in an oven at 60° C for 12 hours. We then separated seeds (propagules) from other organic debris with tweezers under a dissecting microscope, prodding each seed to ascertain viability (viable seeds do not crumble when prodded; Price and Joyner 1997). We counted and weighed only viable seeds to calculate total seed rain (measured in terms of number and biomass) for each transect. We identified each seed to species when possible (more often to family). We discarded non-viable seeds and non-target organic material (leaves, twigs, glumes, etc).

We employed general linear models based on a negative binomial distribution (White and Bennetts 1996) using the "MASS" package (Venables and Ripley 2002) in Program R (R Development Core Team 2009) with $\alpha = 0.05$ to compare seed number and biomass between invaded and non-invaded habitat. We elected to use this analysis as our data did not conform to key assumptions made by ANOVA/*t*-test models (i.e., normality, homoscedasticity).

Prediction 2: Granivore density increases with cheatgrass seed supplementation

We tested whether cheatgrass invasion increases density of granivorous small mammals by comparing paired control and experimental (cheatgrass-supplemented) populations of small mammals at three sites. Each site consisted of two paired plots, each measuring 90 x 90 m and separated by 50-100 m. We further subdivided each plot into a 10 x 10 trapping grid with 100 stations spaced every 10 m. All plots were situated > 50 m from any ecotones and anthropogenic structures (i.e. roads and fences).

We used a mark-recapture technique with Sherman live traps placed at each station of each paired site to determine baseline small mammal abundance (new individuals captured), species richness, and diversity (Shannon-Wiener index of diversity; Krebs 1999) prior to supplementation treatment. We conducted pre-treatment trapping sessions during the first 10 days of April and June, 2010. Post-treatment sessions occurred during the first 10 days of August and October of 2010, and April and June of 2011. Trapping sessions lasted 3 nights at each site during which the site's 200 traps (100 at each plot) were baited < 1 hour before sunset with commercially available gerbil feed, and checked the following morning at sunrise. Traps were closed during the day. We placed 5 g of polyfil batting in the back of each trap during sessions when overnight temperatures were expected to dip below 5° C to reduce mortality from exposure. We marked captured individuals with uniquely numbered ear tags and recorded tail and hind-foot length to assist in species identification. We divided captured species into Heteromyid (family Heteromyidae) and non-Heteromyid functional groups. The Heteromyids are primarily obligate granivores (Brown et al. 1979) and therefore expected to exhibit a stronger response to seed supplementation. Non-Heteromyids were considered facultatively granivorous.

We randomly selected one plot at each site to receive cheatgrass supplementation. We outfitted each experimental plot with 81 feeding trays (placed in the center of each cell of the trapping grid), spaced 10 m apart, alternately placed either directly under the canopy of the nearest living shrub or in shrub interspace. Feeding trays consisted of 2.84 L aluminum casserole tins buried with the rim flush to the ground. We placed a 3 cm x 20 cm wooden ramp running from the bottom to the rim and punctured 3 drainage holes in the bottom of each tray. We filled each feeding tray with approximately 100 g of cheatgrass seed (filled by volume) the first week of every month, including winter, from July 2010 – June 2011. Seed escape from feeding trays due to wind was considered minimal (Saba and Toyos 2003). We obtained all cheatgrass seed used for supplementation during June and July 2010, on land managed by the Bureau of Land Management in Rush Valley and Skull Valley, UT. We took care to avoid harvesting seed from diseased patches.

We used a repeated-measures analysis of variance in Program R (R Development Core Team 2009) with $\alpha = 0.05$ to elucidate the effect of supplementation on abundance, species richness, and diversity of small mammals over time relative to pre-treatment baseline data. We expected abundance, species richness, and diversity of granivores on control plots to decrease or remain constant over time following treatment. Conversely, we expected abundance, species richness, and diversity to increase over time on experimental plots following treatment (Fig. 2a).

Prediction 3: Granivores prefer native seeds over cheatgrass seeds

To test whether granivorous small mammals prefer seeds from native plants over seeds from cheatgrass (Kelrick et al. 1986), we used modified giving-up density (GUD) experiments (Valone and Brown 1989). We conducted experiments during October 2010, on 8 - 550 m transects (5 transects in non-invaded big sage communities and 3 in non-invaded black

greasewood communities). Each transect consisted of 12 stations separated by 50 m. At each station, we placed 2 - 45 x 45 x 2 cm aluminum trays filled with 3 L of on-site soil, sieved through a 1 cm mesh. We placed trays directly on the soil surface, side by side. We designated one tray "native," the other "cheatgrass." The native tray contained 3 g (dried at 60° C for 12 hours) of either Indian ricegrass (hereafter "ricegrass;" *Achnatherum hymenoides*) or bottlebrush squirreltail (hereafter "squirreltail;" *Elymus elymoides*) seeds. The cheatgrass tray contained 3 g (dried at 60° C for 12 hours) of cheatgrass seed. At each transect, we randomly selected the order in which the species presented in the native tray would alternate. We raked the seed into the soil of each tray by gently passing the fingers of one hand through the soil surface 10 times. The trays were left undisturbed in the field for 1 week, after which we transferred the contents of each tray into paper sacks and oven-dried them for 1 week at 60° C. After drying, we stored the samples at room temperature until analysis.

To separate the seed from the soil for GUD calculation, we first passed each sample through 1680 μm (to remove rocks and large debris) and 500 μm (to retain seeds and small debris) sieves stacked on top of a solid base for 12 minutes. After sieving, we floated the seeds from the soil using Malone's procedure (1967). We then dried all matter (including leaf and root litter and other organic debris) recovered from flotation at 60° C for 12 hours, and picked the seeds out with tweezers. We redried the recovered seeds at 60° C for 12 hours and weighed them to calculate GUD for each sample. We compared mean GUD for each seed type using analysis of variance in Program R (R Development Core Team 2009) with $\alpha = 0.05$ after square root-transforming data for normality.

RESULTS

Prediction 1: Cheatgrass invasion increases total seed production

Cheatgrass-invaded habitat produced more seeds than non-invaded habitat in terms of both number ($P < 0.01$; Table 1) and biomass ($P < 0.01$; Table 2). As expected, cheatgrass accounted for the greatest proportion of seeds produced on invaded habitat (69.10% of seed number and 77.90% of seed biomass; Tables 1 and 2 respectively). In addition, invaded habitat produced 1184.78% more squirreltail ($P < 0.01$) than non-invaded habitat. Non-invaded habitat produced 291.00% more Asteraceous seeds ($P = 0.02$; Table 1).

Prediction 2: Granivore density increases with cheatgrass seed supplementation

We captured 20.87 ± 2.59 deer mice (*Peromyscus maniculatus*), 6.10 ± 1.11 Great Basin pocket mice (*Perognathus parvus*), 5.10 ± 1.65 chisel-toothed kangaroo rats (*Dipodomys microps*), 2.93 ± 0.62 Ord's kangaroo rats (*D. ordii*), 1.90 ± 0.72 least chipmunks (*Tamias minimus*), 1.73 ± 0.77 house mice (*Mus musculus*), 0.23 ± 0.16 grasshopper mice (*Onychomys leucogaster*),0.15 ± 0.07 sagebrush voles (*Lemmiscus curates*), and 0.07 ± 0.04 desert woodrats (*Neotoma lepida*) $plot^{-1}$ $sampling\ period^{-2}$ ± SE. We classified these species into Heteromyid and non-Heteromyid groups as previously described. We captured sufficient numbers of the deer mouse, pocket mouse, chisel-tooth kangaroo rat, and Ord's kangaroo rat to perform species-specific analyses.

Neither obligate granivores as a whole ($P = 0.78$) nor any individual species of obligate granivore numerically increased in response to cheatgrass supplementation over time ($P = 0.59$, 0.92, and 0.32 for pocket mice, Ord's kangaroo rats, and chisel-toothed kangaroo rats respectively). Facultative granivores were similarly unaffected ($P = 0.99$), even after the

exclusion of deer mice (P = 0.76). Cheatgrass supplementation also failed to influence either species richness (P = 0.97) or diversity (P = 0.94; Fig. 2).

Prediction 3: Granivores prefer native seeds over cheatgrass seeds

Giving-up density between ricegrass (0.82 g ± 0.03 SE) and squirreltail (0.82 g ± 0.04 SE) did not differ significantly (P = 0.91). However, small mammals drove both native species to significantly lower GUDs (P < 0.01) than cheatgrass (1.04 g ± 0.05 SE; Fig. 3).

DISCUSSION

Our data partially support the hypothesis that granivorous small mammals facilitate cheatgrass invasion by driving food-mediated apparent competition. Cheatgrass invasion augmented total yearly seed production, supporting our first prediction. Granivorous small mammals preferred native seeds over cheatgrass seeds, supporting our third prediction. However, cheatgrass supplementation did not elicit a significant increase in abundance of obligate granivores at the time scale of this study, not supporting our second prediction. Since consumers (granivorous small mammals) did not increase in response to alternative prey items (cheatgrass seeds), we conclude that granivorous small mammals did not drive food-mediated apparent competition (Holt 1977) during the study period.

Cheatgrass invasion augmented seed production (Tables 1 and 2), theoretically increasing food resources available to granivores. Of the seeds added to invaded habitat, cheatgrass itself was/is probably the most numerically important to potential seed consumers (Table 1). Several species of granivorous small mammals are known to at least facultatively consume cheatgrass seeds (e.g., Flake 1973; Kritzman 1974), and are therefore presumably morphologically and physiologically capable of extracting calories from this food source (Schreiber 1979). Thus, the

additional seed resources provided by cheatgrass invasion should be at least provisionally valuable to seed consumers living on or near cheatgrass-invaded habitat.

The additional calories (seeds) supplied by cheatgrass invasion do not appear to benefit granivorous small mammals. It is possible that small mammals do not respond to this increased food source, but other explanations exist. The study period may have been too short to detect a response, as suggested by the trends observed in Fig. 2c and 2d. The response of obligate granivores (the most biologically relevant group of small mammals; Fig. 2c) and all small mammals combined excluding deer mice (Fig. 2d) visually if not statistically conform to the pattern predicted by the ACH (Fig. 2a). For these groups, the change in abundance over time on cheatgrass-supplemented plots appears to diverge from that of control plots. If the study period were extended or if additional experimental units were added, this apparent divergence may have become statistically significant over time. Moreover, granivorous small mammals at our study sites may not have been food limited. Granivores may have little incentive to consume seeds from less-preferred species like cheatgrass (Fig. 3; Kelrick et al. 1986) until seeds from preferred species become scarce. Precipitation is a crucial determinant of yearly seed production in arid environments like the Great Basin (Brown et al., 1979). Since precipitation during the study period (2010) was approximately 35% greater than the area's 30 year average (27.3 cm $year^{-1}$; Gardner and Kirby 2011), the availability of preferred seeds may never have dwindled sufficiently to induce appreciable consumption of cheatgrass seeds. It may be reasonable to only expect treatment effects involving less-preferred, nutritionally meager supplements like cheatgrass (Fig 3; Kelrick and MacMahon 1985; Kelrick et al. 1986) on particularly poor habitat or during periods of pronounced resource scarcity such as drought (Boutin 1990; McMillan et al. 2005).

The role of small mammals in facilitating cheatgrass invasion in the Great Basin remains unclear. Cheatgrass invasion may increase fitness of small mammals in other ways besides augmenting food availability. For example, herbaceous cover in shrub interspace may provide refuge for small mammals and decrease risk of predation (Orrock et al. 2008). This possibility could be evaluated by sampling small mammal populations across a gradient of variously-invaded habitat. Small mammal abundance would be expected increase with proximity to cheatgrass invasion (Orrock et al. 2008). Positive results would oppose the widely-held view that cheatgrass invasion adversely affects abundance of small mammals (e.g. Gano and Rickard 1982; Gitzen et al. 2001; Ostoja and Schupp 2009; Hall, in press).

Other generalist predators besides small mammals have the potential to facilitate cheatgrass invasion through apparent competition. For example, the seed pathogen black fingers of death (BFOD; *Pyrenophora semeniperda*) is a fungal pathogen common in the soils of the Great Basin that can cause substantial mortality to the seeds of both cheatgrass and native grasses (Beckstead et al. 2010). Beckstead et al. (2010) showed that seed banks in cheatgrass-dominated habitat support higher levels of BFOD than do seed banks in non-invaded habitat, and posit that cheatgrass can negatively affect native grasses at the seed stage by acting as pathogen reservoirs for BFOD. In addition, common herbivorous grasshoppers (e.g., *Xanthippus corallipes* and *Melanoplus confuses*) may also facilitate cheatgrass invasion by driving apparent competition. In a study conducted in the Great Basin, Beckstead et al. (2008) noted that squirreltail established in highly-invaded habitat (cheatgrass cover > 85%) experienced 43% greater herbivory on vegetative structures and produced 11 times fewer reproductive structures than squirreltail established in less-invaded habitat (cheatgrass cover < 15%). Correspondingly, grasshopper density was greater on highly-invaded habitat relative to less-invaded habitat

(Beckstead et al. 2008). Finally, ants (genera *Pogonomyrex, Pheidole*, and *Veromessor*) are an important group of granivores in arid regions of North America (Brown et al. 1979), like the Great Basin, and may also drive apparent competition between cheatgrass and native plants. Many ant species are central-place foragers that prefer resources from high-density seed patches (Brown et al. 1979). Not surprisingly, habitat dominated by cheatgrass is often dotted with anthills (Lucero, personal observation). If ant abundance increases with proximity to cheatgrass invasion, and if ants prefer seeds produced by native plants persisting in the invaded habitat (e.g., squirreltail; Hironaka and Tisdale1963; Humphrey and Schupp 2004), they may facilitate cheatgrass invasion by mediating apparent competition between cheatgrass and native plants at the seed stage. The list of indirect interactions that may support the ACH in the Great Basin is too long for exhaustive consideration here. Any generalist consumer that attacks both cheatgrass and native species has the potential to mediate apparent competition. The lack of support for the ACH in this study may suggest that the role of small mammal-driven apparent competition is either unimportant in the Great Basin, or that the appropriate indirect interactions between small mammals, cheatgrass, and native grasses have yet to be evaluated.

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Table 1 – Relative contributions to the mean number of viable seeds captured transect^{-1} (n = 3; 0.0846 m^2 sampling area transect^{-1}) in cheatgrass-invaded ("Invaded") and non-invaded ("Non-Invaded") habitat ± SE (some SE are relatively large because data were pooled at the transect level). Differences in mean number of seeds (% Diff) between habitat types are also reported, with statistical significance ($P \leq 0.05$; determined using general linear models based on a negative binomial distribution) denoted by an asterisk (*). Cheatgrass (*Bromus tectorum;* Poaceae) and squirreltail (*Elymus elymoides*; Poaceae) were sufficiently common to merit specific consideration.

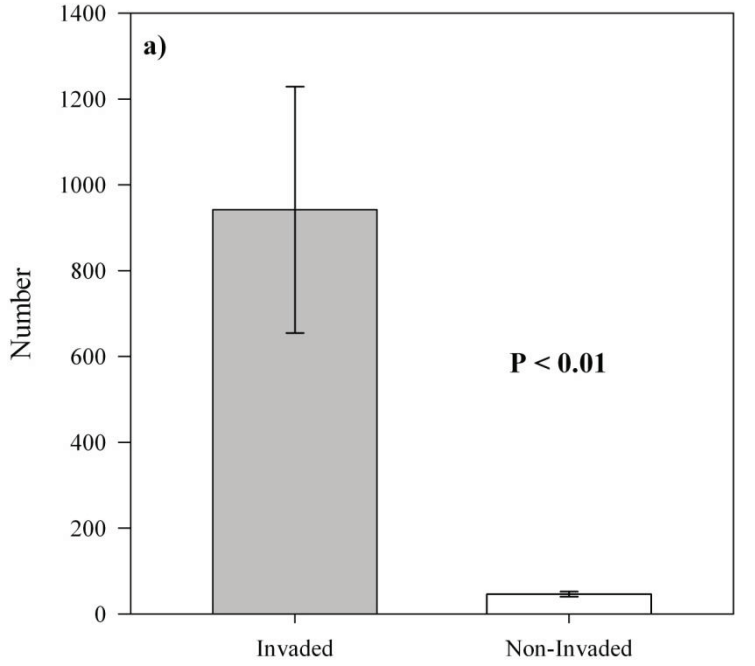
Seed Source	No. Seeds Non-Invaded	SE	No. Seeds Invaded	SE	% Diff	P-Value
B. tectorum	2.33	1.86	650.67	271.23	27925.62*	< 0.01
E. elymoides	15.33	7.87	181.67	102.59	1184.78*	< 0.01
Asteraceae	21.33	4.70	7.33	3.39	-291.00*	0.02
Brassicaceae	5.00	5.00	85.00	84.00	1700.00	0.99
Chenopodiaceae	0.00	0.00	10.00	10.00	-	1.00
Malvaceae	0.33	0.33	0.00	0.00	-	0.99
Unknown	0.33	0.33	1.33	1.33	403.03	0.99
Total	45.67	5.78	941.67	287.03	2061.90*	< 0.01

Table 2 – Relative contributions to the mean biomass of viable seeds captured transect^{-1} (n = 3; 0.0846 m^2 sampling area transect^{-1}) in cheatgrass-invaded ("Invaded") and non-invaded ("Non-Invaded") habitat ± SE (some SE values are relatively large because data were pooled at the transect level). Differences in mean biomass of seeds (% Diff) between habitat types are also reported, with statistical significance ($P \leq 0.05$; determined using general linear models based on a negative binomial distribution) denoted by an asterisk (*). Cheatgrass (*Bromus tectorum;* Poaceae) and squirreltail (*Elymus elymoides*; Poaceae) were sufficiently common to merit specific consideration.

Seed Source	Biomass Non-Invaded (g)	SE	Biomass Invaded (g)	SE	% Diff	P-Value
B. tectorum	0.005	0.004	1.469	0.671	29380.00*	< 0.01
E. elymoides	0.030	0.015	0.332	0.188	1106.67*	< 0.01
Asteraceae	0.013	0.003	0.004	0.002	-325.00*	0.03
Brassicaceae	0.004	0.004	0.065	0.064	1625.00	0.99
Chenopodiaceae	0.000	0.000	0.011	0.011	-	1.00
Malvaceae	0.000	0.000	0.00	0.000	0.00	-
Unknown	0.000	0.000	0.00	0.000	0.00	-
Total	0.052	0.013	1.884	0.674	3623.08*	< 0.01

LIST OF FIGURES

Fig. 1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1000
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600
400
200
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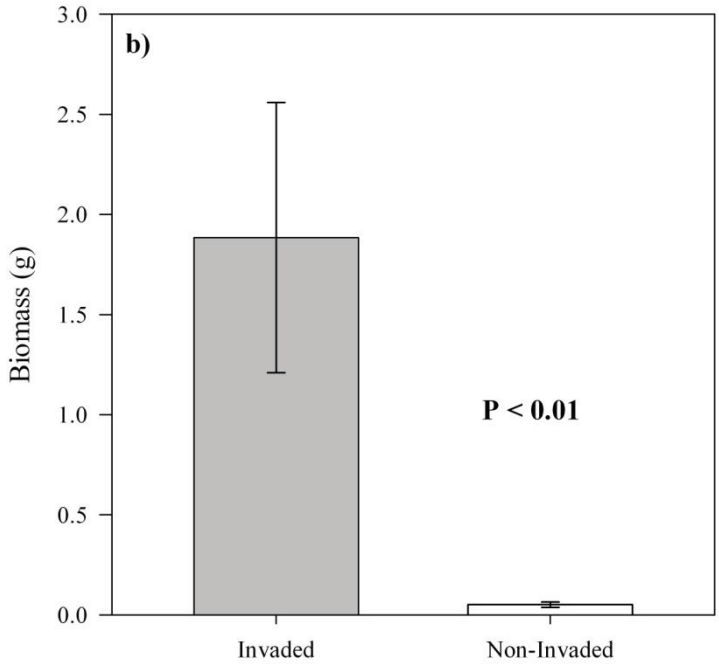
Invaded
Non-Invaded


b)
Biomass (g)
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2.0
1.5
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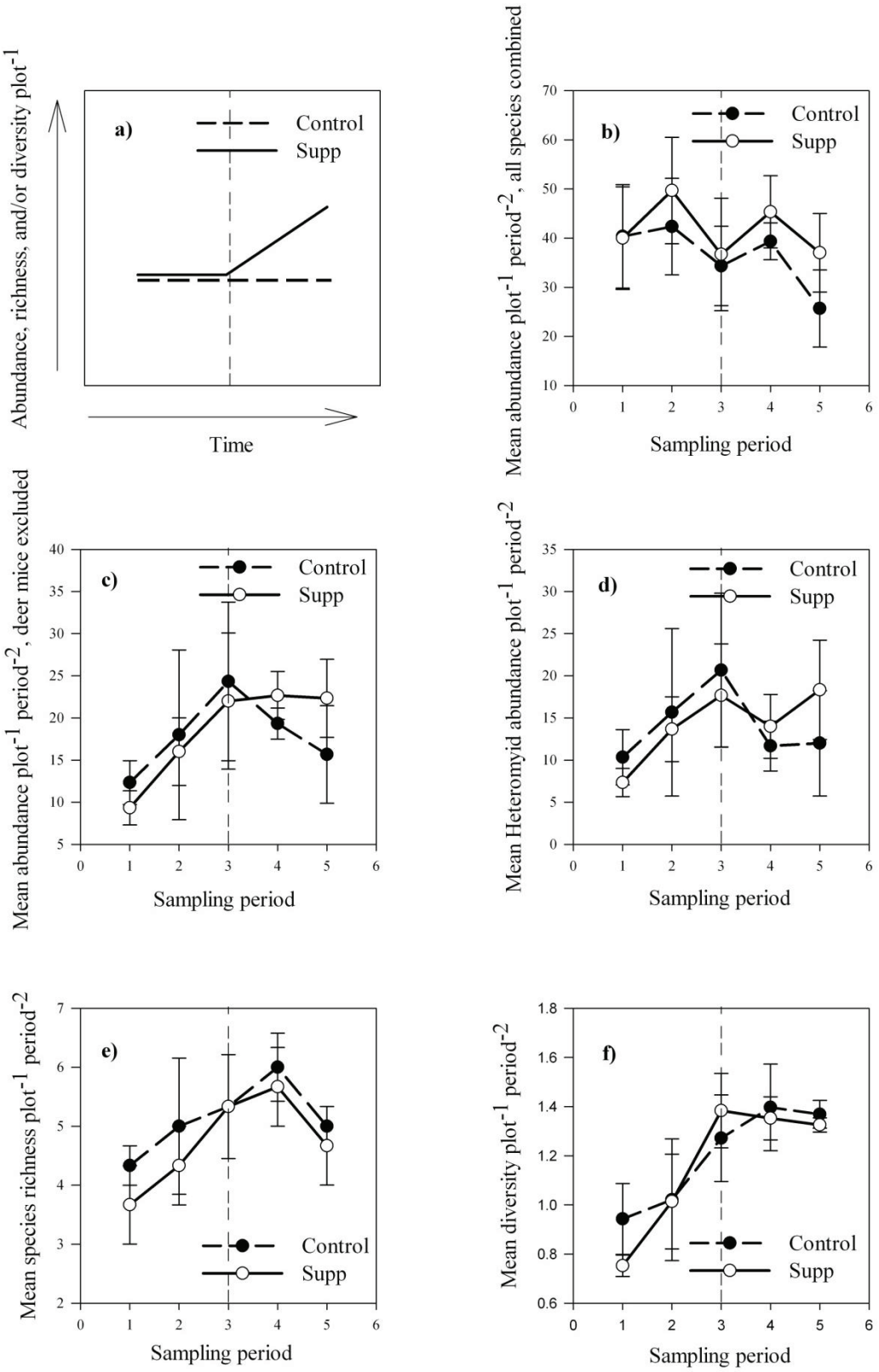
Invaded
Non-Invaded

Fig. 2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Time
b)
Control
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Sampling period
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Control
Supp
Mean abundance plot-1 period-2, deer mice excluded
Sampling period
d)
Control
Supp
Mean Heteromyid abundance plot-1 period-2
Sampling period
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f)
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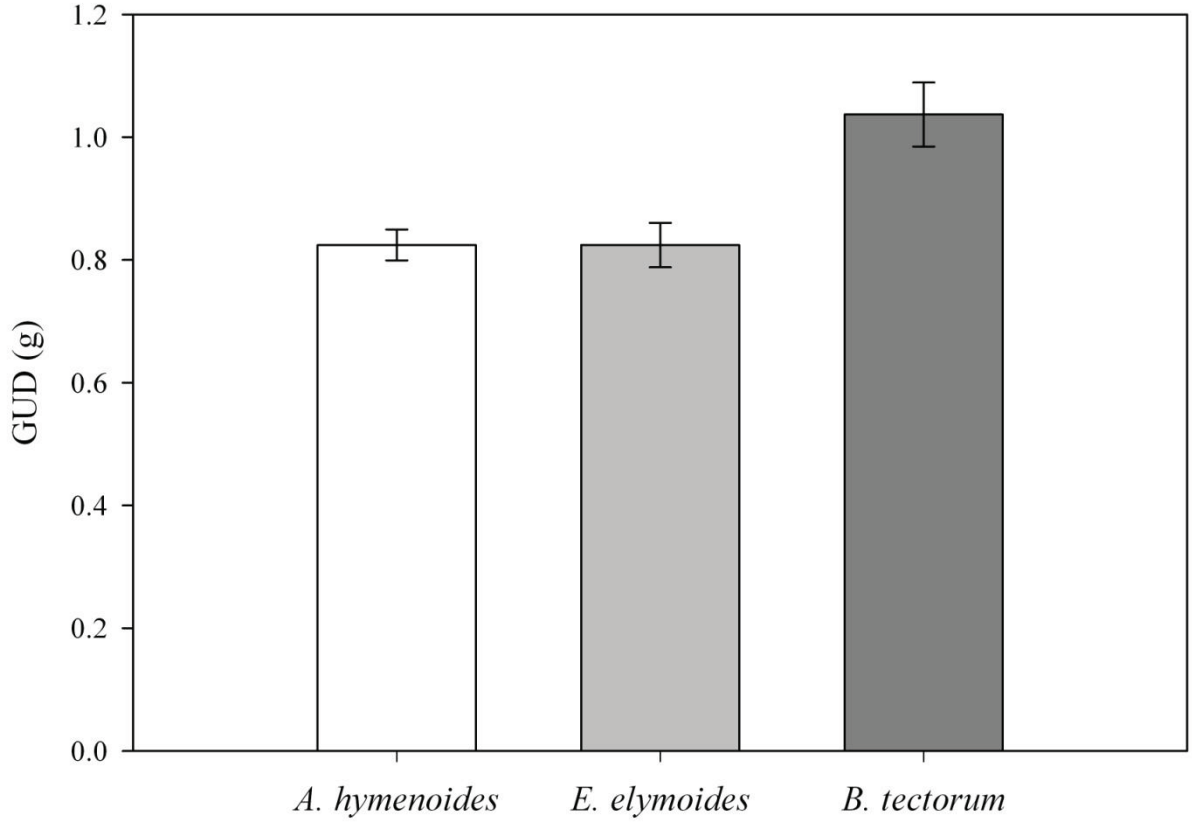
Supp
Mean diversity plot-1 period-2
Sampling period

Fig. 3



CHAPTER 2

THE VALVE METHOD: AN EFFICIENT AND INEXPENSIVE MEANS OF DECANTING SEEDS FROM A FLOTATION SOLUTION

ABSTRACT

Enumerating seeds present in the soil is an element of many studies in disparate fields. Chemical flotation, in which target material is floated to the top of a liquid solution, is a common methodology for recovering seeds from the soil. Traditionally, target material is then decanted from the flotation solution by using a net to repeatedly skim the surface. However, this traditional method of decantation can be biased toward species with low relative densities. This study reports on the development of a novel method of decantation (i.e., the valve method), and compares performance of the valve method to the traditional net-skimming methodology. Specifically, we compare effectiveness (the proportion of seeds recovered from a known sample), rapidity (the time required to decant that sample), efficiency (the number of seeds decanted per second), and recovery bias (the effect of relative density on seed recovery). Our new method is more effective (98% ± 0.37 SE seed recovery vs. 92% ± 1.03 SE recovery), more rapid (68 ± 1.90 SE seconds per sample vs. 289 ± 12.03 SE seconds per sample), more efficient (2.19 ± 0.05 seeds per second vs. 0.49 ± 0.02 seeds per second), and less biased than the traditional method. The differential results obtained using disparate decantation methods highlight the importance of decantation in the flotation procedure and underscore the necessity of specifying the method of decantation used in any research employing chemical flotation. Any future work relying on flotation to analyze seed banks should clearly describe how samples were decanted and should consider the valve method as a potential means of enhancing the effectiveness and efficiency of chemical flotation.

INTRODUCTION

Enumerating seeds present in the soil (i.e., seed bank) is an element of many studies in disparate fields. Community ecologists study seed banks to quantify resources available to granivores (e.g. Price and Joyner 1997) or to understand and predict regeneration of plant communities (Kalamees and Zobel 2002; Olano et al. 2005; Hassan and West 1986). Other researchers inspect seed banks to explain the success of invasive species (Blaney and Kotanen 2002). Agronomists may study seed banks to examine the effectiveness of herbicidal treatments (Kanampiu et al. 2002) or to quantify the extent to which weed seeds contaminate commercially important soils (Mogensen et al. 2005). Regardless of the study, enumerating the seed bank requires the successful separation of seeds from the mineral fraction of the soil.

Chemical floatation is a common methodology used to recover seeds from the soil. Flotation involves preparing a liquid with a density greater than that of target seeds but less than the mineral fraction of the soil (e.g. Malone 1967; Hayashi and Numata 1971; Hayashi 1975; Roberts and Ricketts 1979; Hassan and West 1989; Price and Joyner 1997). Therefore, the mineral portion of the soil sinks while seeds float to the top. Floated seeds are then decanted from the solution for analysis.

Net-skimming is a traditional method of decanting target material from a flotation solution that has yielded useful data (e.g., Hassan and West 1986; we are unaware of more recent publications expressly stating that net-skimming was used) despite certain complications. The methodology uses a net with an appropriate mesh-size to repeatedly skim the surface of the flotation solution to collect target material. However, net-skimming can be ineffective at collecting relatively dense seeds, which may not rise all the way to the surface of the solution via

chemical flotation (Lucero, personal observation). This may be especially problematic if target material consists of seeds with differential relative densities (Gross 1990), as is often the case. Such complications can introduce unintended biases and compromise the results of subsequent analyses, especially among seeds with varying relative densities.

The objectives of this study were to 1) develop a novel method of decantation and 2) compare its performance to the traditional net-skimming methodology. Specifically, we compared effectiveness (the proportion of seeds recovered from a known sample), rapidity (the time required to decant that sample), efficiency (the number of seeds decanted per second), and recovery bias (the effect of relative density on seed recovery) between the proposed and traditional methodologies.

METHODS

We developed a novel method of decanting target seeds from a flotation solution. We named the new method the "valve method" because of the incorporation of a ball valve in the design of the simple and inexpensive device. Specifically, we joined a plastic, 2 L bottle with threaded lips to a plastic, 600 mL bottle with threaded lips using a threaded, copper, 3/4-inch ball valve (Fig. 1). We applied polytetrafluoroethylene tape to the threads of both bottles to ensure watertight joints. We removed the base of the 2 L bottle, which becomes the top of the device once assembled (Fig. 1), so that chemical flotation could later be performed directly in the top of the device. The 600 mL bottle was left intact and filled with 480 mL of room-temperature tap water (80% of the volume of the of flotation solution later used to process soil/seed samples; Fig. 1). Assembly required less than 5 minutes.

Once assembled, we used the device to decant target material from a flotation solution (Fig. 2). With the valve completely closed, we added the flotation solution to the top of the device,

after which we immediately but slowly added the soil/seed to be processed (Fig. 2a). Agitation was avoided as much as possible. After 30 seconds, we opened the valve to 2/3 of maximum flow, permitting the mineral portion of the soil (and some solution) to flow out of the 2 L bottle and into the 600 mL bottle. We intended this action to maximize the amount of target material separated from the soil while avoiding the aforementioned complications potentially associated with net-skimming. We allowed flow to continue until the 600 mL bottle was completely filled (i.e., with liquid and/or mineral debris; Fig. 2b). If flow did not begin immediately upon opening the valve, we used a thin stirring rod to coax flow. Once the 600 mL bottle had been full for 30 seconds, we closed the valve. We then unscrewed the 600 mL bottle and discarded its contents (Fig. 2c), setting aside the 2 L bottle and valve assembly. We transferred the contents of this assembly (decanted target material, remaining solution, and fine-grained mineral debris) to an empty sieve by flushing material out the bottom of the assembly through the valve (as opposed to pouring). We completely rinsed all contents of the assembly into the sieve using a gentle stream of water. We washed fine soil particles through the sieve with the same stream of water and transferred remaining target material to Petri dishes with a spoon. We dried collected target material at 60° C for 24 hours in preparation for analysis. Negligible quantities of the mineral fraction remained associated with target material after a single iteration of this procedure. Individual samples were therefore only processed once.

We recovered target seeds with the traditional net-skimming methodology by repeatedly skimming a fine-meshed aquarium net across the surface of the flotation solution (Fig. 3). Net-skimming was executed in the top chamber of the same device used for the valve method to ensure both methods were performed in identically-shaped containers. Skimming continued until we had collected all visible target seeds. As with the valve method, we washed the

decanted material into a sieve using a gentle stream of water and rinsed away fine particles. Target material was then transferred to Petri dishes with a spoon and dried at 60° C for 24 hours in preparation for analysis. Individual samples were processed by this procedure once.

We selected cheatgrass, *Bromus tectorum*; Indian ricegrass, *Achnatherum hymenoides*; and bottlebrush squirreltail; *Elymus elymoides,* as target seeds based on their varied relative densities. All seeds used in our experiments were milled. We determined the relative density of each species by dividing its weight (obtained after drying at 110° C for 4 hours) by its volume. Volume was estimated by measuring water displacement upon submerging samples in a 10 mL graduated cylinder. We used a rod with a rubber disk fixed to its tip to push seeds completely underwater and subsequently subtracted the volume of the disk and submerged portion of the rod from our estimates of seed volume. We estimated relative density to be 1.159, 1.146, and 0.886 for *Bromus, Achnatherum* and *Elymus* respectively.

We compared the performance of the valve method to net-skimming by using each methodology to decant a flotation solution containing a known quantity of soil and a known quantity of the variously dense seeds mentioned above. We used the recipe described by Malone (1967) to prepare the flotation solution; 75 g of magnesium sulfate (Epsom salts), 30 g of sodium hexametaphosphate (Calgon®), and 15 g of sodium bicarbonate (baking soda) added to 600 mL of tap water. We used 200 g of field-collected soil (collected at a sagebrush-dominated site in Rush Valley, Utah, USA; 40° 16' 48.189" N, 112° 15' 24.525" W) sifted with a 500 µm-mesh sieve to remove any and all target seeds naturally occurring therein. We added 50 milled seeds of each species (*Bromus, Achnatherum,* and *Elymus*) to this known quantity of soil (150 seeds total). Twenty replicates of the mixture resulting from the combination of flotation solution, soil, and seeds were then processed by each method. We prepared decanted material for analysis as

previously described. Once dried, target seeds were collected using tweezers, identified to species, and enumerated.

We ascertained the performance of each decantation method in terms of its effectiveness, rapidity, efficiency, and tendency to yield biased results based on the relative densities of target seeds. We determined effectiveness by calculating the proportion of seeds recovered from the known sample. We measured both the overall effectiveness (out of 150 seeds) and the species-specific effectiveness (out of 50 seeds for each species) of each method. We determined rapidity by timing (in seconds) how long each method took to decant a sample (decantation time). In the case of the valve method, decantation time was measured from the time the valve was first opened (Fig. 2b) to the time the target contents were washed from the device to the sieve (Fig. 2c). For the net-skimming method, decantation time was counted from the time of the first pass of the net across the surface of the flotation solution to the time the target contents were washed from the net to the sieve (Fig. 3). Decantation time did not include the time required to prepare or enumerate a given sample since these steps were identical for both methods. We reported efficiency as the mean number of seeds decanted per second by each method. We intended this measure be an intuitive appraisal of overall economy. Bias was evaluated by analyzing the interaction between the relative density of a target seed and its tendency to be recovered by each method (species-specific effectiveness).

We utilized an analysis of variance (ANOVA) and paired *t*-tests in Program R (R Development Core Team, 2009) to analyze our data. Species-specific effectiveness and bias were simultaneously evaluated using an AVOVA, which incorporated species-specific effectiveness, method (valve vs. net-skimming), and relative density of target seeds as factors. We assumed that effectiveness would remain relatively constant across all seed species

regardless of relative density for a non-biased method (i.e., no significant interaction between effectiveness and relative density). By contrast, we assumed the effectiveness of a biased method would significantly vary with the relative densities of target seeds (i.e., a significant interaction between effectiveness and relative density). Due to its species-specific nature, we were unable to incorporate total effectiveness (out of 150 seeds), rapidity, and efficiency in this ANOVA since decantation time (used to calculate both rapidity and efficiency) was only determined for each sample as a whole. We therefore used paired *t*-tests in Program R (R Development Core Team, 2009) to compare total effectiveness (out of 150 seeds), rapidity, and efficiency between methods. All data used in all analyses were square-root transformed for normality; we did not perform logit transformations as doing so would require division by zero (the valve method proved 100% effective on several samples).

RESULTS

The valve method performed more effectively, rapidly, and efficiently than traditional net-skimming while exhibiting less relative density-related bias. In terms of effectiveness, the valve method averaged 98% seed recovery (146.95 ± 0.56 SE of 150) per sample compared to 92% recovery (137.95 ± 1.55 SE of 150) per sample for traditional net-skimming ($P < 0.01$; Fig 4a). For species-specific effectiveness, the valve method recovered 94.6% of *Bromus* (47.3 ± 0.21 SE of 50) and 99.4% of *Achnatherum* (49.7 ± 0.13 SE of 50) seeds per sample compared to 93.1% (46.55 ± 0.68 SE of 50) and 84.9% (42.45 ± 0.88 SE of 50) respectively for net-skimming ($P < 0.01$ for both species; Fig. 5). The valve and net-skimming methods recovered *Elymus* seeds equally well, with both methods recouping over 97% (49.95 ± 0.05 SE vs. 48.95 ± 0.49 SE respectively; $P = 0.81$) of seeds per sample. The valve method decanted samples 4.27 times faster than net skimming ($P < 0.001$) with an average decantation time of 68 ± 1.90 seconds per

sample compared to 289 ± 12.03 seconds per sample, respectively (Fig. 4b). Accordingly, the valve-method was 4.43 times more efficient than net-skimming, recovering an average of 2.19 ± 0.05 seeds per second compared to 0.49 ± 0.02 seeds per second ($P < 0.001$; Fig 4c).

Density-related bias was significantly less for the valve method than for the traditional method ($P = 0.002$; Fig. 5). Specifically, the valve method recovered less *Bromus* seed (relative density = 1.159) than either *Achnatherum* (relative density = 1.146) or *Elymus* (relative density = 0.886; $P = 0.04$ and 0.02 respectively), but was equally effective for both *Achnatherum* and *Elymus* ($P = 0.99$; Fig 5). By contrast, traditional net-skimming was differentially effective for all 3 species; effectiveness decreased as the relative density of target seeds increased ($P < 0.05$ for all pair-wise relationships).

DISCUSSION

The valve method outperformed traditional net-skimming in terms of effectiveness, rapidity, and efficiency while exhibiting less bias associated with relative density of seeds. We posit that the valve method decants suspended seeds more effectively than net-skimming. Since the traditional method operates primarily at the surface of the solution, its effectiveness hinges on the ability of the solution to raise all target seeds to the surface where the skimming action of the net will be most concentrated. By contrast, the device used to perform the valve method does not physically operate on target seeds themselves but rather flushes mineral debris out the bottom, retaining the majority of the solution and associated target material. This action allows the valve method to recover not only seeds raised to the surface, but also those merely suspended in the solution. This ability may allow the valve method to recover a greater proportion of seeds across a broader range of relative densities than possible with the traditional method. Our data corroborate this claim. Since *Elymus* was the least dense seed (relative density = 0.886

compared to 1.159 and 1.146 for *Bromus* and *Achnatherum* respectively), it is not surprising that the valve and net-skimming methods recovered this species equally well (we expect chemical flotation to be relatively more successful at raising light seeds entirely to the surface than dense ones; Fig. 5). However, the effectiveness of traditional net-skimming decreased as the relative density of the target material increased such that effectiveness significantly differed for each species (Fig. 5). In contrast, the valve method was equally effective for *Achnatherum* and *Elymus* (Fig. 5). Although the valve method was relatively less effective for *Bromus*, it still proved more effective than net-skimming, as it did for all species (Fig. 5). Although the new method may still be susceptible to recovery biases associated with decanting target material with differential relative densities, our data suggest use of the valve method significantly reduces bias over the traditional method.

The proposed method may not address some of the problems inherent with flotation in general. For example, several authors have noted flotation methodologies tend to be biased towards large, easily visible species (Roberts 1981; Gross 1990). It is unknown whether the valve method perpetuates this generality. In addition, flotation procedures may not discriminate between viable and non-viable seeds (dead seeds may float just as well or better than viable ones; Gross 1981). It is unlikely that the valve method mitigates this complication.

The differential performance we observed between the valve method and net-skimming highlights the importance of decantation in the flotation procedure and underscores the necessity of specifying the method of decantation used in any study employing chemical flotation to analyze seed banks. Unfortunately, many authors have overlooked the importance of specifying the method of decantation utilized in their research (e.g., Malone 1967; Gross 1990; Price and Joyner 1997). Omission of these methods can compromise experimental replicability and trans-

study comparisons. For example, Malone (1967) reports chemical flotation to be approximately 100% effective whereas Gross (1990) abandons the same procedure after finding it inadequate. Neither author specifies how their samples were decanted. As a result, subsequent researchers may find it difficult to precisely replicate these experiments. Indeed, failure to specify the method of decantation may at least partially account for the variable accuracy some associate with flotation procedures in general (Roberts 1981; Gross 1990). Any future work relying on flotation to analyze seed banks should clearly describe how samples are decanted and should consider the valve method as a potential means of enhancing the efficiency of chemical flotation while decreasing recovery biases.

ACKNOWLEDGEMENTS

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LIST OF FIGURES

B can also be emptied into a sieve and searched for target material as a simple gauge of the device's effectiveness (not shown). The contents of B are typically discarded. This basic procedure can be modified to accommodate individual applications as needed.

Figure 3 – Schematic representation of our execution of the net-skimming method of decantation, performed in the same device used for the valve method (Fig. 1). With the valve (C) completely closed, the flotation solution (preparation not shown) is added to the top of the device (A), after which the soil sample is immediately but slowly added. Agitation is avoided. After 30 seconds, a fine-meshed aquarium net (D) is used to decant target material from the solution. Material collected in the net is washed unto a sieve, dried in a drying oven at 60° C for 24 hours (often in a separate container such as a Petri dish) and enumerated (not shown).

Figure 4 – Comparisons of performance between traditional net-skimming (Net) and our proposed method (Valve) ± SE. Performance is reported in terms of effectiveness (mean proportion of total seeds recovered per sample; a), rapidity (mean decantation time per sample; b), and efficiency (mean number of seeds recovered per second per sample; c). P-values determined using paired *t*-tests.

Figure 5 – Comparison of effectiveness (mean ± SE proportion of seeds recovered per sample) between traditional net-skimming (Net) and our proposed method (Valve) for each species used in our tests: *Bromus tectorum* (relative density = 1.16), *Achnatherum hymenoides* (relative density = 1.15), and *Elymus elymoides* (relative density = 0.886). Means sharing letters do not significantly differ ($P \geq 0.05$; P-values determined using an ANOVA).

Fig. 1

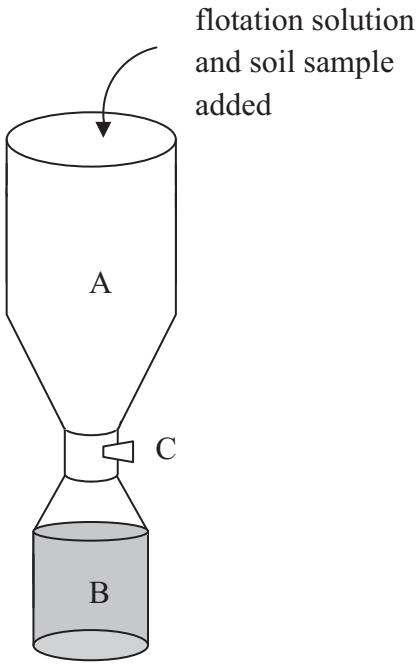


Fig. 2

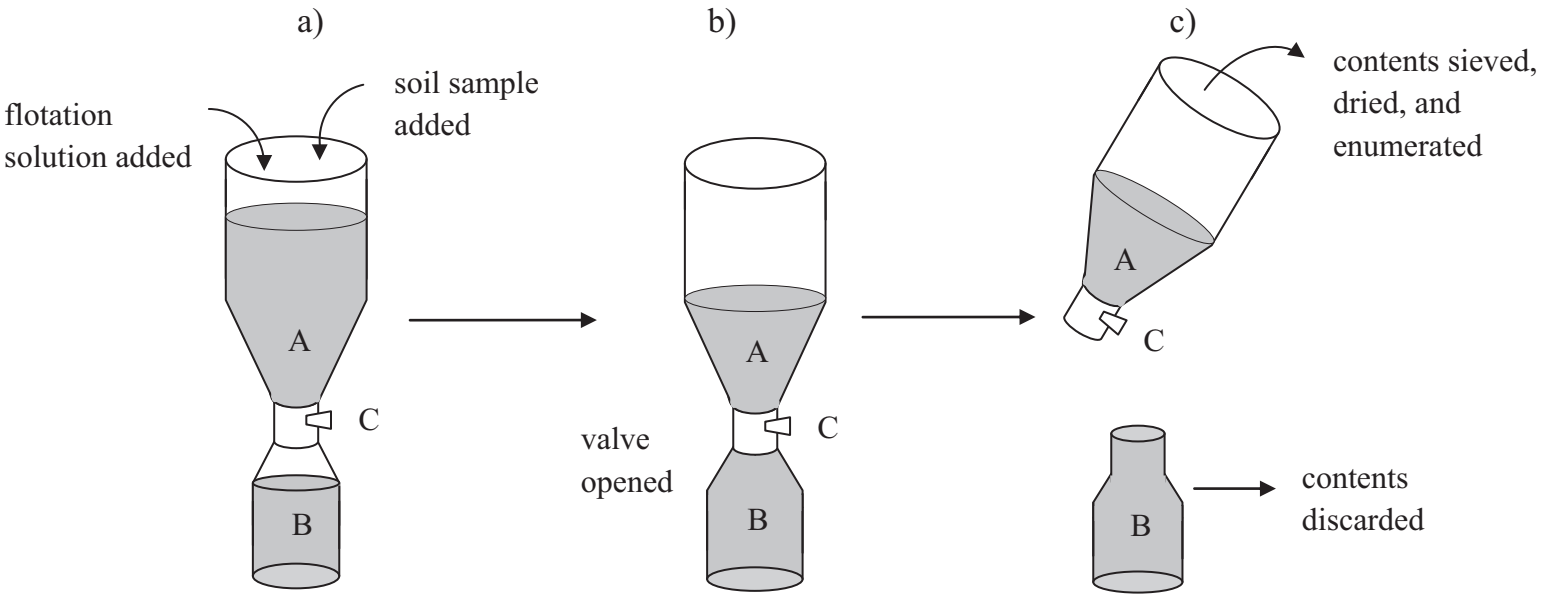

a)
b)
c)
flotation solution added
soil sample added
A
C
B
valve opened
contents sieved, dried, and enumerated
contents discarded

Fig. 3

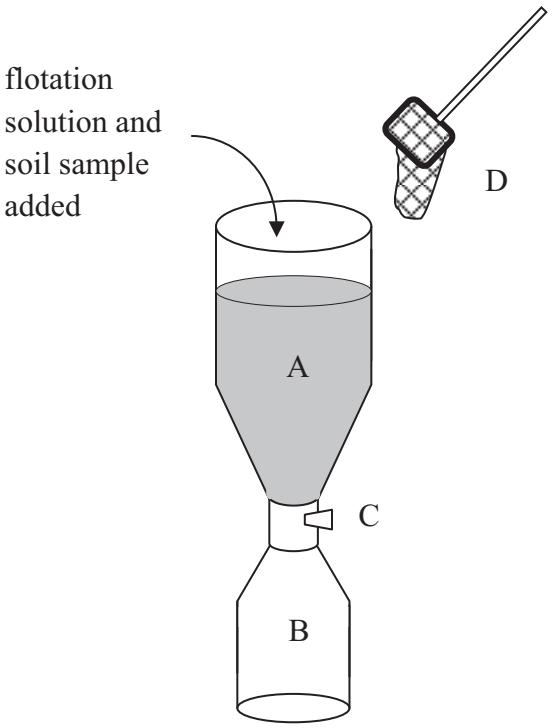


Fig. 4

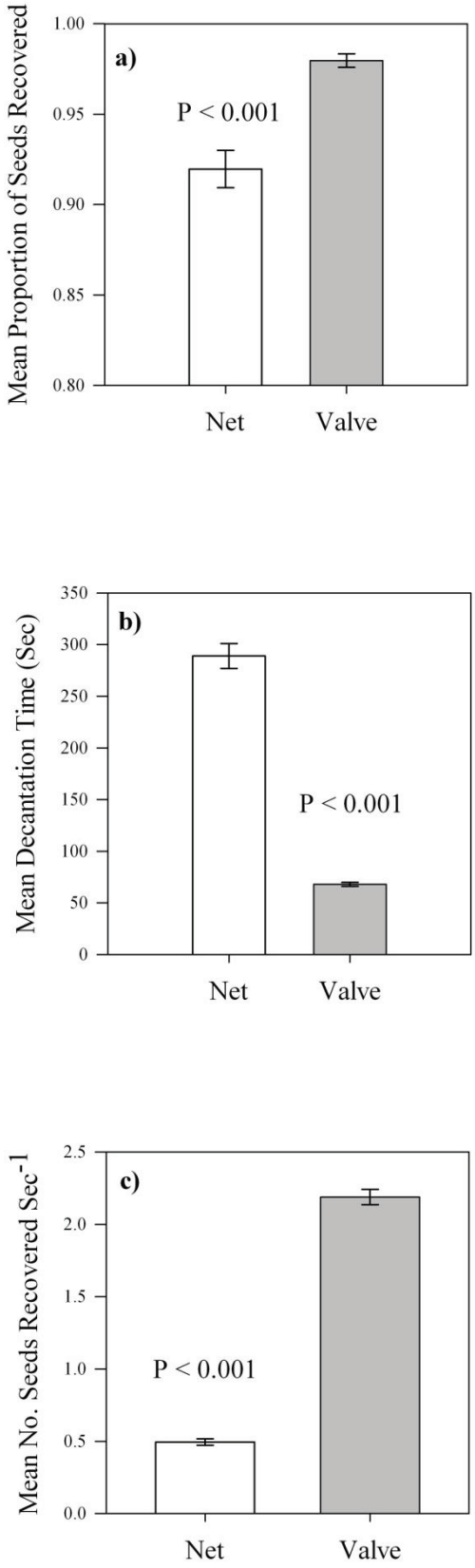
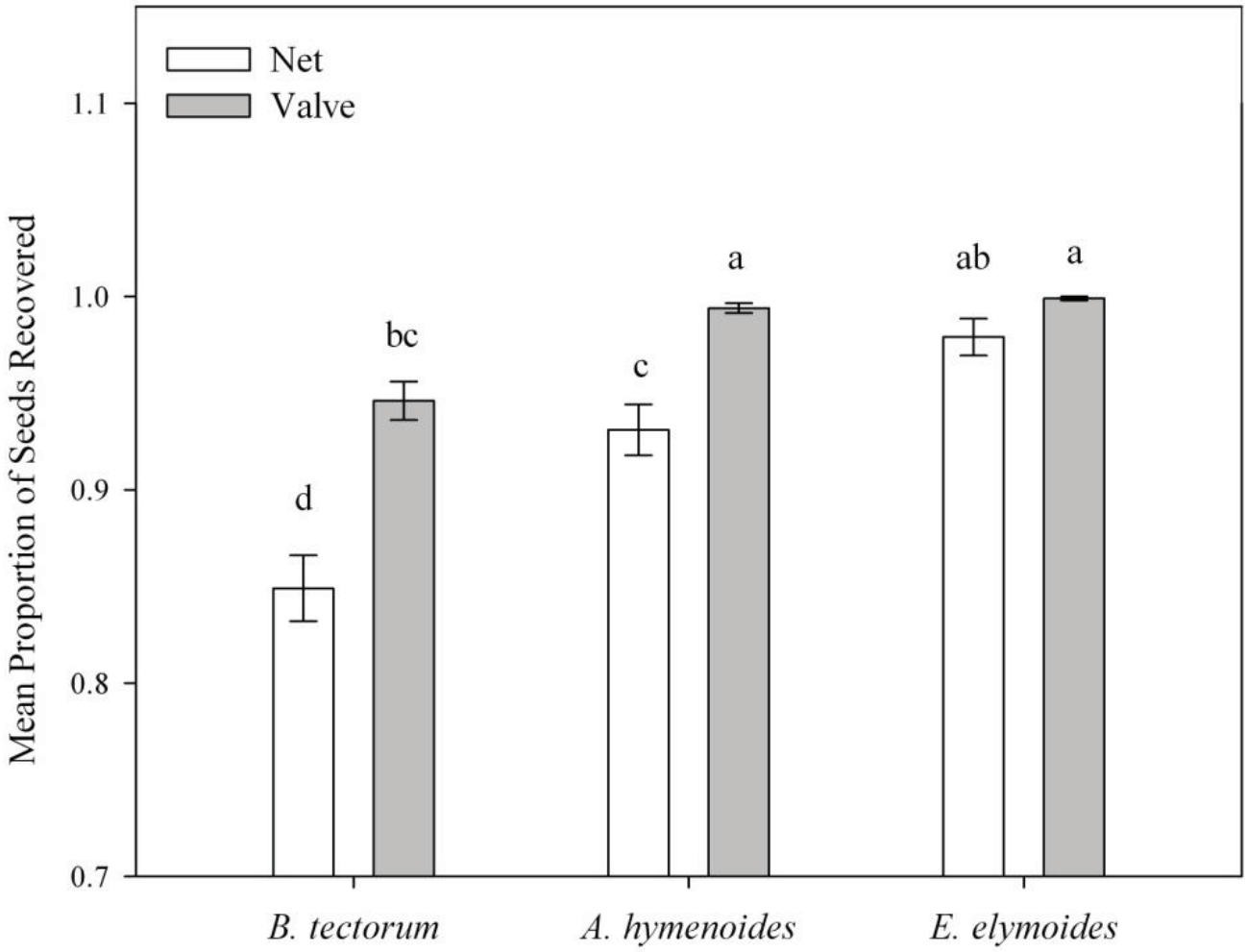

a)
Mean Proportion of Seeds Recovered
1.00
0.95
0.90
0.85
0.80
P < 0.001
Net
Valve
b)
Mean Decantation Time (Sec)
350
300
250
200
150
100
50
0
P < 0.001
Net
Valve
c)
Mean No. Seeds Recovered Sec-1
2.5
2.0
1.5
1.0
0.5
0.0
P < 0.001
Net
Valve

Fig. 5


Net
Valve
Mean Proportion of Seeds Recovered
1.1
1.0
0.9
0.8
0.7
d
bc
c
a
ab
a
B. tectorum
A. hymenoides
E. elymoides