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Research Article

Goat Digestion Leads to Low Survival and Viability of Common Buckthorn (*Rhamnus cathartica*) Seeds

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ABSTRACT

The use of goat browsing for invasive plant management is growing in the United States, but many questions remain about the efficacy of goat browsing for invasive plant control. One common concern of land managers and other stakeholders is whether goats can spread invasive plants through endozoochory (seed dispersal via ingestion and excretion in feces). We evaluated this possibility using common buckthorn (*Rhamnus cathartica*), an invasive shrub for which goats are often employed as a control method. Goats were fed buckthorn berries, and their feces were collected and examined at 24 hr, 48 hr, and 72 hr post-ingestion for intact seeds that survived gut passage. A low proportion of buckthorn seeds (2%) made it through the goat digestive system intact. Of these, only 11% remained viable, compared to 63% viability of control seeds. We conclude that consumption of buckthorn fruits by goats effectively destroys seeds, indicating low risk of dispersal via gut passage. To put these results in context, and provide more guidance for land managers, we additionally reviewed literature investigating seed recovery following ingestion by goats. Based on a synthetic analysis across 28 plant species, we found that seeds >4 mm long were unlikely to be recovered from feces intact, while smaller seeds posed higher dispersal risk.

Index terms: browsing management; endozoochory; gut passage; invasive plant management; ruminants; seed viability

INTRODUCTION

Billions of dollars are spent annually to control invasive plants in the United States (Pimentel et al. 2005). There are a wide variety of potential control strategies used to manage invasive plants, but there is considerable uncertainty about the effectiveness of different approaches (Rohal et al. 2018). This uncertainty is particularly true for less commonly used methods, such as animal browsing or grazing (Shapero et al. 2018). The use of livestock to control invasive plants has received far less research attention than other control methods, such as herbicide application or cutting (Kettenring and Adams 2011). Given that use of livestock to control invasive species appears to be on the rise (Derner et al. 2017), the gap between knowledge and implementation is growing, necessitating more research on effectiveness and best management practices.

Goats are a popular choice for livestock-based invasive plant management, particularly in the United States. They are often utilized to control woody plants due to their browsing feeding behavior, but goats have also been used to control herbaceous plants such as leafy spurge (*Euphorbia esula*) and sulphur cinquefoil (*Potentilla recta*) (Hart 2001; Frost et al. 2013). In Minnesota, USA, goats are often employed by a variety of private, city, regional, and state land managers to address dense stands of the invasive shrub common buckthorn (*Rhamnus cathartica*, hereafter buckthorn) (J. Langeslag, pers. comm.).

Goats readily consume buckthorn fruits while browsing, which is perceived by land managers and members of the public as a potential dispersal risk if seeds survive goat digestion (K. Marchetto pers. obs.). Goat digestion takes between one and three days from ingestion to defecation (Mancilla-Leytón et al. 2011), so dispersal both within and between sites is possible. While several studies have examined the potential for seeds to survive goat digestion, results vary widely between plant species (e.g., Lacey et al. 1992; Mancilla-Leytón et al. 2011; Frost et al. 2013). Based on six plant species, Grande et al. (2016) found that this variation may be due to differences in seed volume and hardness.

Our goal was to evaluate the risk of buckthorn dispersal by goats via endozoochory (seed dispersal via ingestion and excretion in feces). To pose a dispersal risk, seeds in buckthorn fruits must pass through the goat digestive system both physically intact and viable. Thus, our objectives were to estimate (1) the proportion of buckthorn seeds recoverable from goat feces after fruit ingestion, and (2) the viability of consumed seeds relative to undigested control seeds. We also sought to contextualize our buckthorn findings, and enable broader guidance regarding risk of goat dispersal of invasive plants, by synthesizing results from previous gut passage studies on other species and testing for relationships between fruit/seed traits and seed recovery.

MATERIALS AND METHODS

Common buckthorn (*Rhamnus cathartica*), a shrub or small tree invasive in North America, has 0.5–1 cm diameter fruits, containing 1–5 seeds that are typically 5.1 × 3.2 × 3.0 mm (Qaderi et al. 2009).

Gut Passage Experiment

Approximately 2000 buckthorn fruits were collected from a single population in River Falls, Wisconsin, USA, in October

2018 and refrigerated for 2–4 wk before being used in gut passage experiments. Two gut passage trials were performed, 15 d apart. During the first trial, goats were each fed 250 buckthorn fruits mixed with grain. In the second trial, the number of fruits was increased to 300, for a total of 1650 fruits fed. Whole fruits were used, rather than de-pulped seeds, to mimic natural consumption behavior. Three female goats with a history of buckthorn consumption were used in both gut passage experiments. Two of the goats were American LaMancha purebreds (68 kg and 56 kg, both 2.5 y old), and one was an American LaMancha/ Oberhasli mix (45 kg, 1.5 y old). Prior to the initial experiment, they had not consumed buckthorn fruits for several weeks. The goats were fitted with fabric fecal collection bags, which they wore for 24 hr prior to the experiment to ensure they were comfortable with the bags and behaving normally. Fecal collection bags were emptied at 24, 48, and 72 hr after fruit ingestion. We chose to collect feces until 72 hr after ingestion because other studies have shown that few viable seeds are recovered after this point (Mancilla-Leytón et al. 2011, 2012; Frost et al. 2013). Between the two experimental replicates, we collected a total of 18 fecal samples. The experimental procedure was reviewed and approved under University of Minnesota IACUC protocol 1802-35546A.

Feces were dried in a 40 °C oven prior to processing (Wallander et al. 1995; Frost et al. 2013) to a constant mass. The total dry mass of feces from each goat at each collection time (24, 48, and 72 hr post-ingestion) was recorded and 85 g from each collection was subsampled for analysis. Samples were well mixed prior to subsampling so that subsamples would be representative. The subsamples represent an average of 17% ± 1% SE of the total dry mass of each sample. Each subsample was rehydrated, and then pellets were manually broken and searched for seeds. Hereafter, seeds refer to filled seeds that appeared to contain an embryo rather than being a flat seed coat. The number of seeds present in each sample was estimated by extrapolating the number of seeds recovered in the subsample to the total dry mass of feces produced. Outcomes were analyzed as a success/fail process, where successes constituted counts of recovered seeds and failures constituted counts of unrecovered seeds (Crawley 2007). The effect of collection time point on successes/failures was analyzed using binomial generalized linear mixed effects models (GLMM), with goat and trial as random-error intercepts, using the *lme4* package (Bates et al. 2015) in R (R Development Core Team 2019).

Concurrently, three sets of 100 fruits from the same collection were manually de-pulped to serve as controls. The number of filled seeds was recorded for each set of 100 fruits. A subsample of these seeds was dried in a 40 °C oven to account for possible effects on viability of drying under elevated temperature.

Seed Viability Testing

Gut-passaged seeds and control seeds dried at 40 °C were tested for viability using a tetrazolium (TZ) assay (Patil and Dadlani 2009). Seeds were soaked in a 1:5 bleach–water solution for 15 min, then rinsed five times. Seeds were then placed on damp paper in covered petri dishes for 24 hr to rehydrate them and increase respiratory activity. The coat of each seed was slit with a razor blade, and seeds were soaked in a 1% tetrazolium solution overnight in a 37 °C incubator. Seeds were then rinsed three times, cut in half, and visually examined for color change indicative of respiration. These seeds were compared to 15 sets of 10 randomly sampled control seeds (150 seeds total). A Fisher's exact test was used to test for differences between the counts of viable and nonviable seeds from gut-passaged and control seeds (Sokal and Rohlf 1995).

Meta-Regression Analysis

We examined the scientific literature to more broadly explore the effects of goat passage on seed viability. We searched Google Scholar for "goats" and "seeds", with or without additionally including "proportion recovered", and identified studies in which goats were fed a known number of seeds or fruits, and the feces were collected and searched for intact seeds. Results for a total of 28 plant species derived from nine papers plus the current study were identified and used in this analysis (Lacey et al. 1992; Holst and Allan 1996; Smit and Rethman 1996; Mancilla-Leytón et al. 2011; Mancilla-Leytón et al. 2012; Baraza and Fernández-Osores 2013; Frost et al. 2013; Grande et al. 2013; Grande et al. 2016). The plant species represented a wide range of fruit types, including achenes, berries, capsules, caryopsis, drupes, and legume pods, with fruits ranging in size from 1 mm to 70 mm long and seed lengths from 1.1 mm to 8.3 mm long. For each plant species, we recorded numbers of seeds fed and recovered in experiments, fruit mass and length, and seed mass and length. Of the fruit and seed characteristics examined, seed length was the most predictive of seed recovery. The relationship between seed length and the proportion of seeds recovered after gut passage was examined using beta regression with a log link in the *betareg* package in R (Grün et al. 2012). Beta regression was used because, while all studies reported proportions of seeds recovered, not all studies reported actual numbers of recovered and destroyed seeds, information that would be needed for a binomial generalized linear model.

RESULTS

Each buckthorn fruit contained an average of 2.98 ± 0.04 SE seeds. Therefore, the estimated number of seeds fed to each goat in trials 1 and 2 were 745 ± 9 SE and 894 ± 11, respectively (for a total of approximately 4917 seeds fed).

Overall, only 2.2% ± 1.0% SE of seeds fed to goats were recovered after gut passage. No intact seeds were recovered 24 hr post-ingestion (Figure 1). There was no significant difference in the mean proportion of seeds recovered 48 hr and 72 hr post-ingestion: 1.0% ± 0.5% and 1.3% ± 0.6% SE, respectively ($p = 0.38$).

Only nine ingested seeds were recovered from the goat feces sub-samples and therefore available for testing. Of these nine recovered seeds, only one was found to be viable, making viability of gut-passaged seeds 11% (95% confidence interval [C.I.]: 1%–43%). In contrast, 63% of control seeds were viable (55%–71% C.I.), which was significantly higher than the gut-passaged seeds ($p = 0.003$).

We found a significant, negative, nonlinear relationship between seed length and recovery of seeds following gut passage in our meta-regression (Table 1, Figure 2, $p < 0.001$, pseudo-R^2

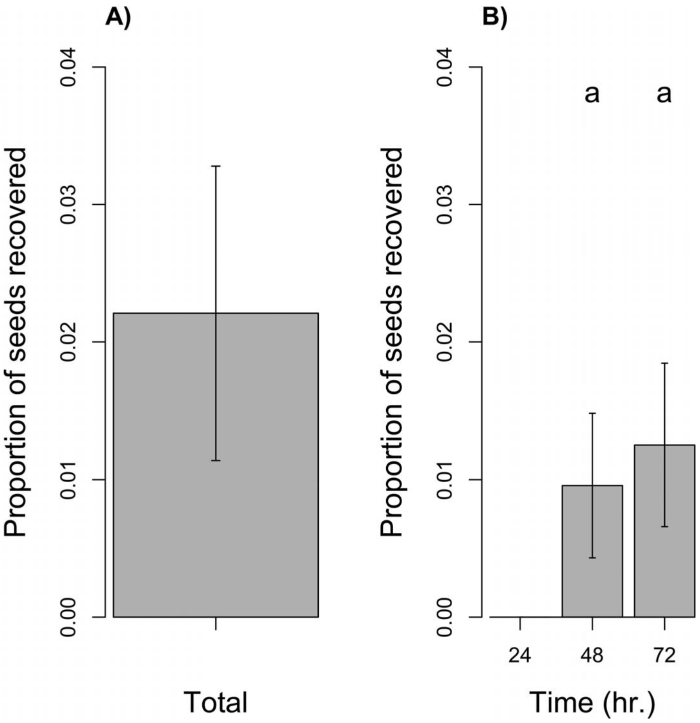

Figure 1.—Proportion of seeds recovered after gut passage (A) in total and (B) by sample collection time. Bars denote standard errors. Within a panel, bars with the same letter are not significantly different ($p > 0.05$). Data were analyzed as binomial, success/fail data but are represented here as proportions.

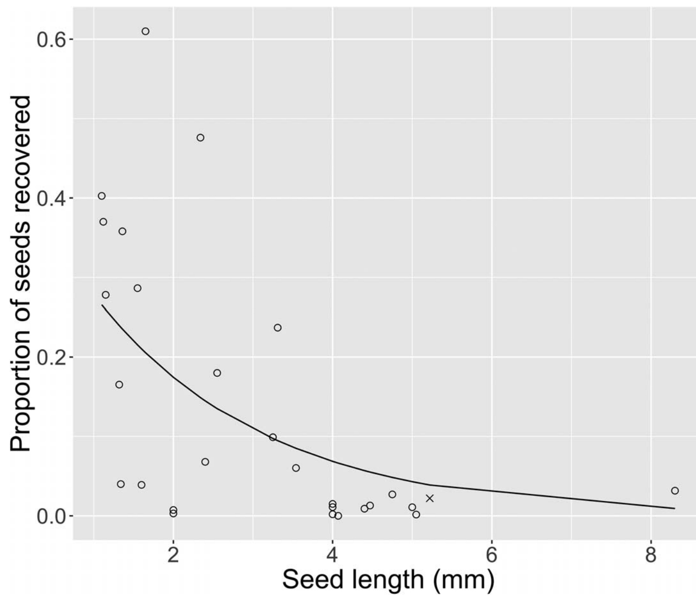

Figure 2.—Proportion of seeds recovered from goat feces by seed length for 28 plant species. Each symbol represents a different species, with the result for buckthorn from the present study shown as an x. Fitted line predicted from beta regression model.

= 0.07). The predictive model can be expressed as:

$$e^{(-0.8126-0.4665^{*}(\text{seed length in mm}))} = \text{proportion of seeds recovered after gut passage}$$

Species with seeds ≥4 mm long had low proportions of recoverable seed, including buckthorn in the present study (mean length of 5.22 mm). For species with seeds shorter than 4 mm, there was considerably more variation in the proportion of seeds recovered, with recovery rates as high as 61% or as low as 0.7% (Figure 2).

DISCUSSION

We found that consumption of buckthorn fruits by goats effectively destroyed their seeds, indicating low risk of goats dispersing buckthorn between sites. Based on values derived from the meta-regression, seed destruction may be a general pattern when goats eat plant species with long seeds (≥4 mm). In contrast, species with shorter seeds exhibited more potential for endozoochory by goats but high variability, with 0.7% to 61% recovery depending on species. Some of this variation may be due to differences in seed hardness or aspect ratio (proportional relationship between seed width and height; Grande et al. 2016). In cases where goats are used to manage small-seeded species, the destruction of ≥40% of seeds is still beneficial *within* sites. However, to prevent between-site dispersal of invasive plants with small seeds, managers should consider not browsing plants after invasive species' seed has set (Frost et al. 2013). Alternatively, goats could be corralled for a few days away from fruiting plants to allow complete passage before movement to a new site (Kott et al. 2006; Frost et al. 2013).

Table 1.—Beta regression model for the relationship between seed length and the proportion of seeds recovered from goat feces after gut passage. Data represent 28 species from 9 primary literature papers and the current study. ** $p < 0.01$, *** $p < 0.001$

	Estimate	Std. Error	z	p	
Intercept	−0.81	0.31	−2.63	0.009	**
Seed length	−0.47	0.11	−4.38	1.17×10^{-5}	***

Due to logistical constraints leading to relatively low sample sizes (e.g., three goats and subsampling of fecal collections), there is uncertainty in our estimates of percent recovery and seed viability. While the variability in the estimates of percent seed recovery was not high, there is additional uncertainty associated with non-detection of seeds at many of the sub-sampling time points. An observation of 0 seeds in a subsample does not mean that there were no seeds in the full sample (Chik et al. 2018), but we lacked the information that would be necessary to estimate detection probabilities from our data. An alternative approach is to determine how sensitive our data are to such non-detects, or false negatives. If, for example, we found one seed in all of the subsamples for which no seeds were detected, then our estimate of the percentage of seeds recovered would increase to 4.4%. This would be a substantial change, but not so different in terms of its implications for spread risk. In addition, our observed recovery rate of 2.2% is consistent with expectations from our meta-regression analysis (Figure 2), given the size of buckthorn seeds. There is also considerable uncertainty in our seed viability estimate due to low recovery of testable seeds from feces, but this uncertainty is easier to quantify statistically. The 95% confidence interval for this parameter is quite wide (1%–43%) since we were only able to recover nine seeds for testing. Even if the true viability rate is at the high end of our confidence interval (i.e., 43%), we would still expect $<$1% of ingested seeds to survive goat gut passage in a viable condition.

While the use of goats for invasive species control is on the rise, other animals, such as sheep and cattle, are also commonly used to control undesirable vegetation. Sheep and goats have similar effectiveness in destroying seeds across plant species (Lacey et al. 1992; Smit and Rethman 1996; Frost et al. 2013), whereas cattle usually destroy the fewest seeds via digestion (Neto et al. 1987; Alvarez et al. 2017).

In conclusion, goats are unlikely to disperse buckthorn seeds via endozoochory, because seeds are both unlikely to emerge intact in goat feces and the seeds that do emerge are likely to have low viability. Further research should be conducted to evaluate whether our finding, that species with seeds $>$4 mm long are unlikely to be dispersed in goat feces, can be used as a general rule of thumb. For invasive species with smaller seeds, care should be taken to prevent dispersal between sites.

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