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Endozoochory by Goats of Two Invasive Weeds with Contrasted Propagule Traits

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Abstract: Invasive plants have very important ecological and socioeconomic impacts. Producing and dispersing many viable seeds are key plant functional traits for invaders. Ungulate grazing plays an important role in the endozoochorous seed dispersal within grasslands and rangelands. Grazing can be applied as a practical and economical control method for plant invasions. We analyzed the effects of seed passage through the goat digestive system on the germination and viability for Sorghum halepense and Malva parviflora, common invasive species with contrasted propagules and seed traits. Both studied species produced seeds able to survive, in a small percentages (c. 0.80–1.70%), after being eaten by goats. Most of the seeds (c. 40–55%) of both species were retrieved between 24–48 h after ingestion. Goat passage provoked a decrease (> 60%) in the germination percentage and seed viability of S. halepense that was higher with longer gut retention times. In M. parviflora, the goat gut passage did not break its primary physical dormancy, since no retrieved seed germinated with similar viability as the uneaten seeds (c. 90%). In view of our results, goat grazing can be applied as a useful method to control S. halepense and M. parviflora invasions. Goats should be kept in corrals for at least 4 days after grazing to prevent transferring viable seeds to uninfected areas.

Keywords: alien species; germination; gut passage; herbivory; Mediterranean climate; seed viability

1. Introduction

In a context of global environmental change, ecological perturbations caused by invasive plants are reducing biodiversity, altering ecosystem functions and causing considerable economic and human health impacts [1]. Many plant species with agronomic potential may become invasive. In addition, many weedy species may become a serious threat to agricultural productivity. Consequently, integrated management plans should be applied to avoid the dispersal of certain alien plant species from crop fields and grasslands to wild areas and vice versa [2].

With the aim of fighting biological invasions, it is useful to identify invasion syndromes as a combination of alien species traits and characteristics of the recipient ecosystem, which collectively result in predictable dynamics and impacts [3]. In this sense, producing many viable seeds that are
dispersed effectively to set persistent soil seed banks with broad germination requirements are key plant functional traits related to the spread and abundance of invaders [4,5].

The dispersal of propagules provides several advantages to new recruits such as reducing intra-specific competition between the seedlings and mother plants [6], decreasing seed predation that may be high near parent plants (escape hypothesis) [7], placing seeds into safe and disturbed sites (including dung) suitable for germination and establishment [8,9], and increasing genetic diversity [10]. In this context, animals actively dispersing seeds via ingestion (endozoochorous dispersers) can enhance the fitness of host plants by moving the seeds to new environments (colonization hypothesis) [6]. However, endozoochory interactions are not always positive for host plants since some seeds are killed during gut passage, depending on the interacting animal and plant species and abiotic factors involved [11,12]. Therefore, it is important to know the effectiveness of the seed dispersal of alien species by different animals to predict and fight invasions. The effectiveness of endozoochory is determined by the quantity and quality of dispersed seeds [13].

Herbivores dispersing seeds is a normal process in rangelands and grasslands. Many plant species in these ecosystems have co-evolved with grazers [14]. The most important grazers in many rangelands and grasslands around the world are ungulates, which shape plant traits and plant community composition through seed digestion and dispersion [15–17]. In fact, grazing ungulates can be used to introduce desirable species into degraded grasslands [18,19]. Moreover, grazing can be applied as a practical and economical control method for plant invasions. This is especially important with increasing environmental concerns and the elevated costs of chemical and mechanical control methods [20,21]. Both wild and domestic ruminant species, such as sheep, goat and cattle, play important roles in endozoochorous seed dispersal [22–24]. However, goats have advantages over other biological control methods of invasive species, such as improving the cycling of nutrients sequestered in weeds and converting them into saleable products [25], the germination of seeds after passing over the digestive tracts of goats has received relatively little attention. Therefore, more studies are needed to improve our knowledge on the effects of seed traits on their survival after being digested by goats.

The aim of this study was to analyze the effects of seed passage through the goat digestive system on the germination and viability of two common invasive herbaceous species with contrasted propagules and seed traits. With this aim, we recorded the number of retrieved seeds, their viability and their germination characteristics for Sorghum halepense (L.) Pers. (Johnsongrass; Fam. Poaceae) and Malva parviflora L. (Little Mallow; Fam. Malvaceae). To our knowledge, no study has analyzed endozoochory effects on the seeds of S. halepense. Previous studies have described that M. parviflora seeds need abrasion to break their physical dormancy [26,27]. Due to the characteristics of the propagules of studied species, we hypothesized that few seeds of both species would survive passing through the goat’s gut, but experiencing different effects, since a higher number of larger M. parviflora seeds would be crushed during chewing than those of S. halepense that, instead, would show lower viability since they are less protected against gastric acids and digestion enzymes.

2. Material and Methods

2.1. Study Species

Sorghum halepense is native from the Mediterranean Basin and Asia, eastwards to India. It is cultivated for fodder in most warm regions. S. halepense is invading the American Continent, Africa and Australia, and has been included in the ten most dangerous weed species globally [28]. S. halepense is a rhizomatous perennial C-4 grass with a hybrid origin that grows erected up to 1.5 m high with 10–55 cm long panicles bearing racemes of 1–5 spikelet pairs. S. halepense presents fruits with coriaceous coat that are dispersed protected with bracts forming spikelets (4–5 mm long). Its dispersal unit is the spikelet containing one uniseeded cariopsis [29,30].
**Malva parviflora** is native to Europe, North Africa, and temperate Asia, and it is invasive along the American Continent, Australia and New Zealand [31], where it acts as a noxious weed with allelopathic effects on crops and no-till farming systems [32–34]. *M. parviflora* is an annual broad-leaved herb with a single-long taproot and oblate to discoid schizocarp fruits, usually depressed in the center. Its fruits have indehiscent and 8–12 laterally compressed mericarps (c. 9 mm long), drying brown and acting as dispersal units. *M. parviflora* produces one reniform to spherical seed per mericarp with a hard and impermeable coat [35,36].

### 2.2. Fruit Collection and Seed Characteristics

The fruits of *M. parviflora* and *S. halepense* were randomly collected in a rangeland located in South Valley University farm (Egypt, 26°11′00.6″ N—32°44′46.2″ E) from 20 different adult plants of each species in February 2018. Both species are common and largely consumed by goats in this location (Abbas, personal observation). Taxonomic identification was made following [30,37,38]. The collected fruits were stored under dry and dark conditions at +20 °C until the beginning of the experiment in March 2018. The number of seeds per fruit was counted in 50 fruits of *M. parviflora*. Each fruit of *M. parviflora* contained an average of 10 seeds. Each fruit of *S. halepense* contained just 1 seed. The seeds of each plant species were measured (length, width and height) using an electronic Vernier caliper and weighted (balance Radwag AS 220/C/2, Poland) before (n = 35 seeds per species) and after retrieval (all recovered seeds).

### 2.3. Seeds Retrieved after Gut Passage

Five female adult domestic goats (*Capra aegagrus hircus* L.) of similar weight (c. 35 kg) and age (2 years old) were fed and individually housed at the Teaching and Experimental Farm of the Faculty of Veterinary Medicine at the University of South Valley (Egypt, 26°11′8.8″ N—32°43′56.4″ E). At the beginning of the experiment, 1000 spikelets of *S. halepense* and 100 fruits of *M. parviflora* (c. 1000 seeds of each species by goat) were offered mixed with roughness of flour for almost an hour until they were fully consumed by each goat. Afterwards, the goats were fed with lucerne hay and sunflower pellets, and they had unrestricted access to water. All the dung pellets produced by each goat were collected every 24 h for 5 days (0–24, 24–48, 48–72, 72–96 and 96–120 h after ingestion) [16]. No seeds were recovered after these intervals. The dung pellets were dried at +25 °C for 72 h in a bell jar with silica gel to avoid seed fermentation and damage. For each goat and time interval, all dung pellets were crushed manually one day after defecation and the number of seeds of each species was counted.

### 2.4. Seed Germination after Gut Passage

The germination of the seeds recovered after gut passage was compared to the germination of seeds that were not eaten. Seeds of each species eaten by different goats and retrieved during the same time interval were mixed together for the germination experiment. Due to the low number of seeds recovered in some time intervals, the germination was only evaluated in periods with greater recovery. Retrieved seeds of *S. halepense* were classified in: (i) seeds retrieved between 0–24 h after digestion; and (ii) between 24 and 48 h after digestion. In the case of *M. parviflora*, germination was evaluated only for the seeds retrieved between 24 and 48 h after ingestion; very few seeds were retrieved during the other time intervals. A control treatment with seeds that were not eaten was included for each species. Uneaten seeds were stored until the germination experiment in dry and dark conditions at +20 °C, simulating environmental conditions when seeds are buried in the soil during the dry season.

All the seeds were disinfected by immersion in 1% sodium hypochlorite solution for 2 min and thoroughly rinsed with sterile distilled water (10 min) before the germination treatment [39]. Retrieved seeds with no evidence of apparent external damage, examined under a microscope, were subjected to germination. Four seeds per species and time interval were placed on filter paper in 9-cm Petri dishes (3–8 replicated dishes), and 5 mL of distilled water was added to each dish. The dishes were then wrapped with parafilm and placed in a germinator (Lab Line Instruments Inc, Melrose Park, ILL,
Illinois, USA) under a regime of 12 h/12 h of light (35 \mu mol m^{-2} s^{-1} of wave length between 400 and 700 nm)/darkness at +20 °C for 27 days, until no germination was recorded for 7 days. This temperature was chosen as the optimum temperature for seed germination for the two studied species [40,41]. The dishes were inspected daily and the germinated seeds were counted. A seed was considered to have germinated when its radicle emerged [42]. The final germination percentage, time to first germination (the time required for the first seed to germinate) and the mean time to germination (MTG; an index of seed germination speed calculated as: MTG = \Sigma (n \times d)/N, where n is the number of seeds germinated at day d, and N is the total number of seeds that germinated in the treatment) were calculated [16]. The viability of the ungerminated seeds was recorded using the tetrazolium test [43]. The overall effect of goat passage on seed germination of each species was calculated by multiplying the mean percentage of retrieved seeds by their mean germination percentage [16].

2.5. Data Analyses

SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all the statistical analyses. The deviations were calculated as the standard error of the mean (SE). A significance level of 0.05 was applied for every analysis. The data series were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Levene’s test. Differences in the number of retrieved seeds between the species and in the biometric characteristics and mass between uneaten and retrieved seeds were tested using a Student t test for independent samples. Differences in mean germination parameters and mean seed viability between uneaten and retrieved seeds at different time intervals were tested using one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (HSD) test as post hoc analysis.

3. Results

The percentage of retrieved seeds from the goat dung pellets tended to be higher for Sorghum halepense (1.70%), than for Malva parviflora (0.80%), without showing significant differences (t-test, \( p > 0.05 \)). Most of the seeds of S. halepense (75%) were retrieved between 0 and 48 h after ingestion. The majority of the seeds of M. parviflora (55%) were retrieved between 24 and 48 h after ingestion (Figure 1). Retrieved seeds of S. halepense were significantly longer than the uneaten seeds (t-test, \( t = 2.25, p < 0.05 \)). The retrieved seeds of M. parviflora were significantly taller and heavier than the uneaten seeds (t-test, \( t = 2.39, p < 0.05 \)) (Table 1).

![Figure 1](image-url). Percentage of the seeds for Sorghum halepense and Malva parviflora retrieved from the goats dung every 24 h after gut passage. Values are mean ± SE (n = 5). First letter indicates significant differences among treatments for the same species (GLM, \( p < 0.05 \)), and the asterisks indicate the differences between the two species for the same treatment (t-test, \( p < 0.05 \)).
Table 1. Morphological characteristics (mm) and mass (mg) for uneaten (control) and retrieved seeds by goats for *Malva parviflora* and *Sorghum halepense*. Values are mean ± SE (*n* = 30–35 seeds). Different letters in the same column indicate significant differences between uneaten and retrieved seeds (Student *t*-test, *p* ≤ 0.05).

|                  | Malva parviflora | Sorghum halepense |
|------------------|------------------|------------------|
|                  | Length           | Width            | Height           | Mass             | Length           | Width            | Height           | Mass             |
| Uneaten seeds    | 1.56 ± 0.02      | 1.72 ± 0.02      | 1.51 ± 0.03      | 6 ± 0            | 4.25 ± 0.08      | 2.23 ± 0.04      | 1.43 ± 0.03      | 16 ± 2           |
| Retrieved seeds  | 1.74 ± 0.05      | 1.74 ± 0.05      | 1.28 ± 0.06      | 13 ± 3           | 4.64 ± 0.14      | 2.29 ± 0.04      | 1.44 ± 0.03      | 13 ± 0           |

Final germination percentage for uneaten seeds was five times greater for *S. halepense* than for *M. parviflora* (*t*-test: *t* = 13.85, *p* < 0.05) (Figure 2A). In *S. halepense*, the germination percentage for uneaten seeds (100 ± 0%) was significantly higher than for retrieved seeds. The seeds retrieved at 0–24 h after ingestion showed a 2-fold higher germination percentage than the seeds retrieved between 24 and 48 h (ANOVA, *F*$_{2,10}$ = 55.56, *p* < 0.0001). For *M. parviflora*, the germination of uneaten seeds was 20 ± 5%, and no seeds of *M. parviflora* germinated after ingestion by the goats (Figure 2A).

![Figure 2](image-url)  
**Figure 2.** Percentage of the final germination (A), the number of days to first germination (B), the mean time to germination (MTG) (C), and the viability of ungerminated seeds (D) for uneaten seeds (control) and retrieved seeds from goat’s dung after gut passage for *Sorghum halepense* and *Malva parviflora*. Values are mean ± SE (*n* = 3–8). First letter indicates significant differences among treatments for the same species (one-way GLM, *p* < 0.05), and the asterisks indicate the differences between the two species for the same treatment (*t* test, *p* < 0.05).
The time to first germination and the mean time for germination (MTG) for uneaten seeds of \textit{S. halepense} was similar to that of \textit{M. parviflora} (\textit{t}-test, \( p > 0.05 \)). In \textit{S. halepense}, the time to first germination tended to increase after goat digestion, ranging from 1 ± 0 days for the uneaten seeds to 4 ± 1 days for the retrieved seeds at 24–48 h after ingestion, but no significant differences were found (ANOVA, \( F_{2,8} = 2.364, p > 0.05 \)) (Figure 2B). The MTG for uneaten seeds tended to be lower for \textit{S. halepense} (1.0 ± 0.1 days) than for \textit{M. parviflora} (5.0 ± 1.0 days), but no significant differences were found (\textit{t}-test: \( t = 2.43, p > 0.05 \)). The highest MTG for \textit{S. halepense} was recorded for the retrieved seeds (4 ± 1 days) and the lowest was for the uneaten seeds (1 ± 0 day) (ANOVA, \( F_{2,8} = 2.12, p > 0.05 \)) (Figure 2C).

The viability of the \textit{S. halepense} seeds decreased from 100 ± 0% for the uneaten seeds to 14 ± 4% for the retrieved seeds between 24 and 48 h after ingestion, with the seed retrieved between 0 and 24 h after ingestion showing intermediate values (ANOVA, \( F_{2,10} = 21.45, p < 0.0001 \); HSD test, \( p < 0.001 \)) (Figure 2D). The uneaten seeds of \textit{M. parviflora} and those retrieved between 0 and 24 h and between 24 and 48 h after ingestion showed similar viability c. 90% (ANOVA, \( F_{2,6} = 2.14, p > 0.05 \)) (Figure 2D).

When considering gut passage and germination together (global effect), gut passage notably depressed the seed germination in \textit{S. halepense} from 100% for uneaten seeds to 0.35% for the retrieved seeds. The global effect of the gut passage for \textit{M. parviflora} seeds changed their germination from 20% for the uneaten seeds to 0% for the retrieved seeds.

4. Discussion

Our results show that targeted grazing by goats is a potentially useful method for controlling the invasion of herbaceous plants with agronomic use such as \textit{Sorghum halepense} and \textit{Malva parviflora}. However, goats may also help to the dispersal of weed seeds in their native and invasive ranges. Our results show that both studied species produced seeds that were able to survive, in a small percentage (c. 0.80–1.70%) after being ingested by goats.

Size and hardness are two important factors determining the probability of ingested seeds to survive chewing and rumination [44]. In this sense, seeds >4 mm long, such as those of \textit{M. parviflora}, were unlikely to be recovered from the feces of goats intact [45]. Thus, the lower survival of seeds of \textit{M. parviflora} compared to \textit{S. halepense} could be related to their bigger size. The seeds of \textit{S. halepense} and \textit{M. parviflora} could be effectively dispersed by endozoochory, since few seeds of both species survived through the digesting system of the goats [46]. In addition, most of the seeds (c. 50%) of both species were retrieved between 24 and 48 h after ingestion, coinciding with the retrieval times found in various shrub seeds consumed by goats [24]. The temporal pattern of defecation may favor seed dispersal distance, 30–90 km in transhumant herd [16]. Since few viable seeds of both studied species are able to be dispersed by goats, grazing livestock consuming their seeds in non-native areas should be kept in a corral for at least 4 days to prevent transferring viable seeds to uninfected areas as an invasion control strategy. Recovered seeds showed a bigger size than the uneaten seeds, probably due to hydration during gut passage that may favor germination in field conditions [39].

Besides these common responses, the passage through the digestive tract of goats had different effects on the seed populations depending on the species. The fruit of \textit{S. halepense} is surrounded by accessory bracts along with persistent pedicels and a rachis segment. These structures protect and prepare the seed for dispersal through endozoochory [29]. Even so, the goat passage provoked a decrease (>60%) in the germination percentage and seed viability that was higher with longer gut retention times, as reported previously for other grasses [19,24]. Mortality generally increases with the length of time seeds remain in an animal’s digestive tract due to the damage to the embryo by acidic conditions and enzymes during digestion [47,48]. The bracts surrounding the fruit of \textit{S. halepense} confer it physical dormancy that is broken by scarification [49], which happened during gut passage in our study, since almost all the retrieved viable seeds germinated.

In \textit{M. parviflora}, goat gut passage did not break its primary physical dormancy, since no retrieved seed germinated, showing similar viability to the uneaten seeds (c. 90%). In this sense, seed
viability did not decrease with the duration of goat passage, probably due to their hard coat [27]. These results are in agreement with Michael et al. [41], who recorded that additional scarification after retrieval from sheep feces was essential to elicit germination in *M. parviflora*. Physical dormancy does not always break during gut passage and seeds may need other dormancy-breaking cues in the post-dispersal environment, such as seasonal temperatures or secondary dispersal [44]. In addition to that physical dormancy, *M. parviflora* also shows shallow physiological dormancy [32,33]. We recorded 20% germination for non-scarified uneaten seeds just one month after collection, indicating that some seeds of *M. parviflora* lacked any kind of deep physiological and physical dormancy, as reported previously [32,33]. In contrast, no seeds of *M. parviflora* germinated after gut passage, pointing that those seeds without hard cover (physical dormancy) were killed during digestion. Mechanical grinding, rumination, and exposure to digestive juices vary among animal species [50], which determines the total number of seeds germinating after gut passage [51]. Michael et al. [41] reported 20% of recovered intact seeds of *M. parviflora* from sheep feces, which is in agreement with *M. parviflora* seeds being highly tolerant to damage [49]. In contrast, we retrieved only c. 1% of ingested seeds of *M. parviflora*, indicating than goat grazing on *M. parviflora* is a much more effective weed management strategy than sheep grazing.

*S. halepense* has become resistant to different herbicides [52]. In this context, agronomic practices such as crop rotation and deep tillage are useful techniques to control the invasion of *S. halepense* [53]. In view of our results, these functional applications may be combined with grazing by goats to control both *S. halepense* and *M. parviflora* invasions using integrated weed management strategies. However, *S. halepense* is common in many grazing pastures, grazing on this grass should be watched since its leaves may contain high concentrations of cyanogenic glucoside that yield cyanide following their enzymatic breakdown and can cause poisoning [54]. The fragmentation of agricultural landscapes has led to a loss of functional connectivity and a change in seed dispersal processes. In rural areas, human-mediated dispersal vectors are prevalent and the weeds dispersed by humans are frequent [55]. In addition, birds and mammals also disperse many seeds of invasive alien species to short and long distances in rural landscapes [56,57]. In this context, improving our knowledge of weed seed dispersal, survival, and germination after gut passage is essential for the effective eco-compatible management of rural areas [58]. Our results show that goats can provide regulating services in agricultural landscapes by depleting the aerial seed bank of weeds such as *S. halepense* and *M. parviflora*, rather than facilitating plant recruitment by endozoochory. Since few seeds survived the digestion and were retrieved mostly during the first 48 h after ingestion by goats, a 4 day quarantine period for goats would be sufficient in order to reduce the risk of the internal transport of seed. In this sense, goats appear as a more effective method to control weed dispersion than sheep that transport many seeds adhered to the wool [59]. In addition, our results show that goats may be used for introducing native fodder species with hard or protected seeds in fallows while manuring.

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