



# Endozoochorous seed dispersal potential of five major southern U.S. weeds

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## Research Article

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## Abstract

Cattle (*Bos* spp.) grazing on weed-mixed forage biomass may potentially spread weed seeds, leading to plant invasions across pasturelands. Understanding the possibility and intensity of this spread is crucial for developing effective weed control methods in grazed areas. This research undertook an in vitro experiment to evaluate the germination and survival of five dominant weed species in the southern United States [Palmer amaranth (*Amaranthus palmeri* S. Watson), yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.], johnsongrass [*Sorghum halepense* (L.) Pers.], field bindweed (*Convolvulus arvensis* L.) and pitted morningglory (*Ipomoea lacunosa* L.)] upon incubation in rumen fluid for eight time periods (0, 4, 8, 12, 24, 48, 72, and 96 h). For the 96-h treatment, a full Tilley and Terry procedure was applied after 48 h for stopping fermentation, followed by incubation for another 48 h simulating abomasum digestion. Seed germination, upon incubation, varied significantly among weed species, with *I. lacunosa* reaching zero germination after only 24 h of incubation, whereas *A. palmeri* and *S. halepense* retained up to 3% germination even after 96 h of incubation. The hard seed coats of *A. palmeri* and *S. halepense* likely made them highly resistant, whereas the *I. lacunosa* seed coat became easily permeable and ruptured under rumen fluid incubation. This suggests that cattle grazing can selectively affect seed distribution and invasiveness of weeds in grazed grasslands and rangelands, including the designated invasive and noxious weed species. As grazing is a significant component in animal husbandry, a major economic sector in the U.S. South, our research provides important insights into the potential role of grazing as a dispersal mechanism for some of the troublesome arable weeds in the United States. The results offer opportunities for devising customized feeding and grazing practices combined with timely removal of weeds in grazeable lands at the pre-flowering stage for effective containment of weeds.

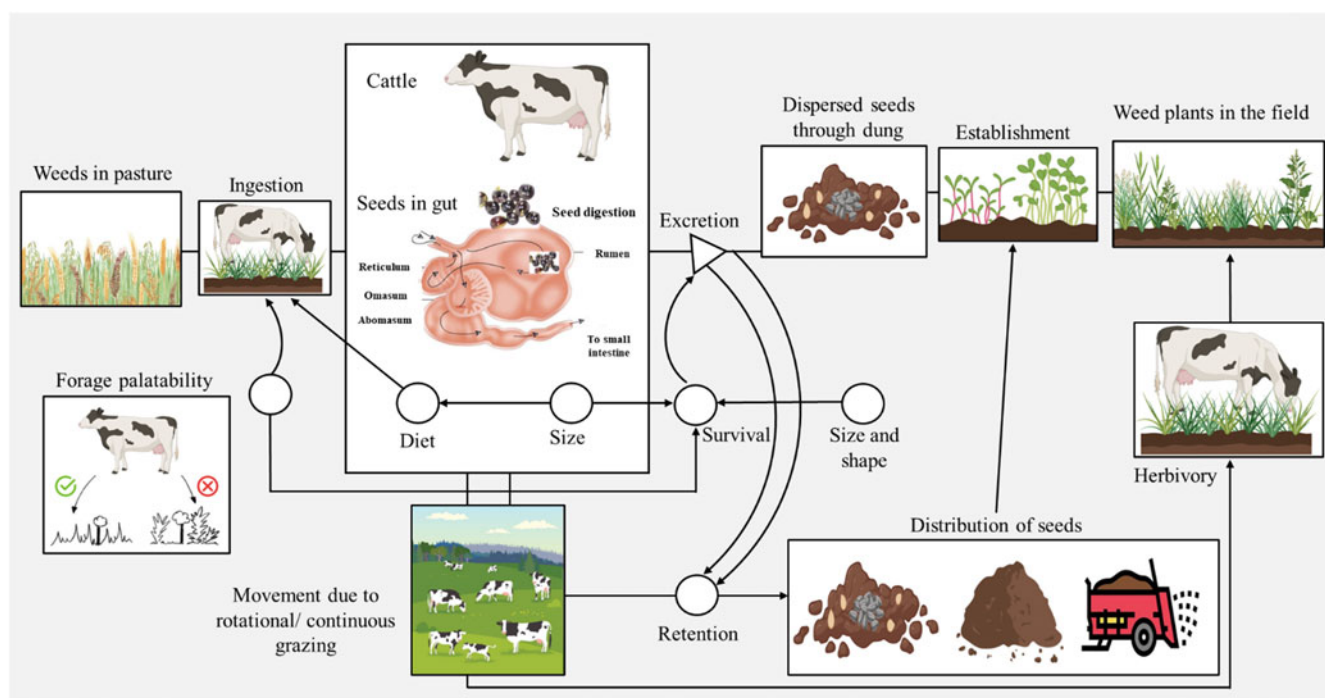
## Introduction

Success of invasive weeds can be attributed to several factors, including their adaptation for rapid dispersal, prolific seeding ability, rapid growth rates, and adaptability to diverse environmental conditions (Baker 1965). Adaptive trait diversity and phenotypic plasticity can facilitate survival and establishment in new areas, thereby aiding in rapid spread (Clements and Jones 2021a). Reports of rapid evolution and spread of herbicide resistance in dominant weeds and invasive species further complicate the issue (Clements and Jones 2021b; Norsworthy et al. 2012), due to the need of alternative herbicide chemistries or nonchemical weed control tools (Peterson et al. 2018; Swinton and Deynze 2017). As invasive weeds are prolific seed producers, the selection pressure from herbicides will have greater chances of selecting for the resistant survivors among a large number of treated plants (Bagavathiannan and Davis 2018). This phenomenon is particularly evident in Palmer amaranth (*Amaranthus palmeri* S. Watson) and johnsongrass [*Sorghum halepense* (L.) Pers.], which have shown rapid adaptation to herbicide applications and diverse dispersal routes, resulting in widespread infestations that threaten both agricultural productivity and biodiversity (Adkins and Shabbir 2014; Nakka et al. 2017; Peterson 1999).

Weed invasion through rapid dispersal complicates management efforts and increases weed control costs (Tataridas et al. 2022). Weed dispersal occurs through movement of contaminated soil, water currents, wind dispersal, animals, human activities such as agriculture and landscaping, and the transportation of goods and vehicles (Benvenuti 2007; Thill and Mallory-Smith 1997). One major strategy is to prevent dispersal of resistant weed populations (Bagavathiannan and Davis 2018; Beckie and Harker 2017), as the spread of resistance genes is a

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**Figure 1.** Flowchart illustrating the endozoochorous seed dispersal by cattle (modified from Mouissie et al. 2005). Boxes represent quantitative units, circles denote seed parameters, and triangles signify the spatial units essential for seed dispersal.

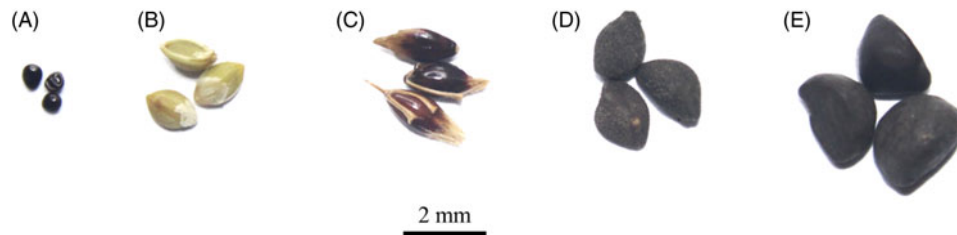
more significant source of new infestations than the selection of new resistant cases on-site (Heap and Duke 2018).

Endozoochory, dispersal of seeds via animal digestive systems (Figure 1), is a key seed dispersal method in integrated crop-livestock systems where ruminants act as dispersing agents that can facilitate the spread of herbicide-resistant weeds (Ciontu et al. 2020; Schupp et al. 2010; Viero et al. 2018). Seeds can be transported long distances and deposited in viable form through animal fecal materials (Gardener et al. 1993; Guo et al. 2017). Pleasant and Schlather (1994) reported that 1 kg of cattle (*Bos* spp.) manure could contain up to 42 viable seeds of field bindweed (*Convolvulus arvensis* L.), with about 20% of ingested seeds surviving digestion and manure processing. Endozoochory can provide seed scarification in the animal gut or allow microbial degradation of the seed coat that can stimulate germination (Lima et al. 2015; Traveset and Verdu 2002) or provide a nutrient-rich environment that supports weed seedling establishment (Milotić and Hoffmann 2016; Traveset et al. 2001). Benvenuti (2007) warned that although endozoochory accounts for 1.5% of seed dispersal, the economic losses can be potentially higher in case of herbicide-resistant weed biotypes because of greater difficulty in controlling them.

The effectiveness of endozoochorous seed dispersal can be influenced by the digestive physiology of the dispersing cattle, seed traits, and environmental conditions (Gilhaus et al. 2017; Karimi et al. 2020). Variations in seed retention time among herbivores impact seed viability and germination, while seed characteristics determine survival and establishment success (Xu et al. 2022). The digestive process can variably affect seed viability, with some seeds experiencing reduced germination rates (Aper et al. 2014; Gselman and Brus 2023). Studies show that seeds with hard coats maintain high viability after digestion, with survival rates of 28% to 31% for certain legumes (Deminicis et al. 2020).

In the southern United States, several dominant weed species, including *A. palmeri*, *S. halepense*, and *C. arvensis*, have significant ecological and economic impacts on agricultural systems (Webster and Nichols 2012). *Amaranthus palmeri* has emerged as one of the most problematic weeds due to its rapid growth, high reproductive capacity, and ability to develop resistance to multiple herbicide classes (León and van der Laat 2021). Similarly, *S. halepense* can reduce crop yields in cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], and corn (*Zea mays* L.) up to 90%, 88%, and 80%, respectively (Klein and Smith 2021). *Convolvulus arvensis* is a perennial weed with an extensive root system that competes aggressively with crops, potentially reducing yields by up to 100% (Boldt et al. 1998). These weeds not only disrupt agricultural systems but also alter local ecosystems by out-competing native flora, thereby reducing biodiversity (Webster and Nichols 2012). Seeds of these weeds can disperse spatially to long distances through waterways, animals, wind, and agricultural activities like tillage or animal ingestion, contributing to their widespread distribution and genetic diversity (Maity et al. 2022; Martín et al. 2015; Rudi et al. 2018; Sosnoskie et al. 2020; Yu et al. 2021).

While research has explored the role of cattle in seed dispersal of some forbs and woody plants (Brown and Archer 1988; Chuong et al. 2016), the specific role they play in dispersing dominant U.S. southern weeds remains relatively understudied. As cattle grazing often overlaps with areas heavily infested with these invasive plants, understanding the endozoochorous dispersal potential of these weeds may have significant implications for weed management strategies in the region. We hypothesized that these weeds would survive digestion through cattle gut, thereby facilitating their dispersal and establishment in new areas. Therefore, this study aimed to analyze the effects of rumen fluid incubation on germination, viability, and seedling vigor of common weed species



**Figure 2.** Seeds of five weed species that underwent the rumen digestion experiment, arranged in the order of increasing seed size. From left to right: (A) *Amaranthus palmeri*, (B) *Setaria pumila*, (C) *Sorghum halepense*, (D) *Convolvulus arvensis*, and (E) *Ipomoea lacunosa*.

with contrasting seed traits found in pastures, thereby providing insights into the ecological consequences of endozoochorous seed dispersal.

## Materials and Methods

### Plant Material

Weed species that are known as common and troublesome in U.S. southern pastures were used in the experiment: *A. palmeri*, *C. arvensis*, pitted morningglory (*Ipomoea lacunosa* L.), yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.], and *S. halepense* (Figure 2). These species are prevalent in various agricultural settings and exhibit aggressive growth patterns that can outcompete desirable forage species, leading to reduced pasture productivity and quality (Culpepper 2006; Sarangi and Jhala 2018). The seeds of *A. palmeri* and *S. halepense* were collected in 2022 from research farms at College Station, TX, USA, and placed in permeable paper bags and stored under laboratory conditions at  $20 \pm 2$  C in the dark until commencement of the experiment. The seeds of the other three species (*C. arvensis*, *I. lacunosa*, and *S. pumila*) were obtained from Azlin Seed Service, Leland, MS, USA. A preliminary viability test was conducted to ensure adequate seed viability before the experiment. This involved using 20 seeds per species with three replications, subjected to a tetrazolium test at a 0.1% concentration. An in vitro lab experiment was conducted in 2023 and 2024 at Auburn University's Department of Animal Sciences, Upchurch Hall, Auburn, AL, USA. Each experiment was repeated three times.

### Rumen Fluid Extraction and Preparation

Simulated rumen digestion involved exposure of seeds to rumen fluid containing micro-organisms to undergo fermentation at a pH of about 6.5 for 0 to 96 h, followed by exposure to acidic conditions (pH 3.5) for 48 h, as found in the abomasum (true stomach) (Ocumpaugh and Swakon 1993). The rumen fluid (solid and liquid fractions; approximately 1,000 ml) used in the experiment was collected from two heifers with rumen fistula fitted with a cannula and maintained on an all-forage diet. At sampling, the cannula was opened, the rumen contents were stirred, and rumen fluid was transferred into prewarmed (39 C) thermos containers to retain fluid temperature. The containers were completely filled with rumen fluid to exclude oxygen and transported to the laboratory. On arrival at the laboratory, rumen inoculum was strained through two layers of cheesecloth to separate liquid phase (rumen fluid) from solid phase.

### Digestion Experiment/In Vitro Digestion

Fermentation was carried out in the laboratory using a Daisy II incubator (Ankom Technology, Macedon, NY, USA) according to the general procedure of Tilley and Terry (1963). About 300 to 400

seeds per Erlenmeyer flask marked with the species, replication number, and duration of incubation (0, 4, 8, 12, 24, 48, 72, and 96 h) were added with 40 ml of McDougall's buffer (McDougall 1948) under CO<sub>2</sub> to maintain anaerobic conditions. Then, 10 ml of strained rumen inoculum was added to this solution and stirred. Samples were stoppered with fitted airlocks and incubated in a Thermo Scientific Precision Shaking Water Bath (Waltham, MA USA) at 39 C for their prescribed incubation period. For the 96-h samples (those used to simulate total tract digestion), 50 ml of pepsin solution was added to stop fermentation after 48 h, and the flasks were incubated for an additional 48 h. The flasks were taken out of the water bath at the decided time, and seeds were placed on a filter paper for drying overnight.

### Evaluation of Seed Germination and Viability

To evaluate seed germination and viability, germination tests were performed using a completely randomized design (CRD) with three replications. For each replication, 50 seeds of each weed species were placed in petri dishes (United Scientific (Libertyville, Illinois, USA) Supplies K1004-J Petri Dish, 90-mm diameter, 15-mm height, polystyrene (Libertyville, Illinois, USA)) that had two disks of germination paper (Whatman 114, 90 mm (Sanford, ME, USA); and Zenpore ST001-90, 90 mm (Fotan, NT, Hong Kong)) and 10 ml of distilled water. Petri dishes were placed in a growth chamber (Incubator-I-36NL, Percival Scientific (Perry, Iowa, USA)) set to a diurnal light cycle of 16 h and a temperature of 25 C. Germination was counted daily based on radicle protrusion (2 mm) for 21 d, and total cumulative germination was recorded. The final germination percentage, the time taken for the first seed to germinate, and the mean time to germination (MTG) were calculated. MTG is an index of seed germination speed, determined using the formula:  $MTG = \sum (n \times d) / N$ , where  $n$  represents the number of seeds that germinated on day  $d$ , and  $N$  is the total number of seeds that germinated in the treatment. The tetrazolium test was used to determine viable seeds. For this, nongerminated seeds were first pricked or scratched to break any hard seed coats and then sectioned to expose the embryo and soaked overnight in 2,3,5-triphenyl tetrazolium chloride solution (0.1%) in distilled water with pH 7 at 20 C in darkness. After 48 h, seeds whose embryos had stained red and had firm flesh were classified as viable. Nongerminated seeds with complete embryo coloring were considered to be dormant seeds (Maity et al. 2021).

### Membrane Integrity Test

For the membrane integrity test, 20 seeds of small-seeded weeds and 10 seeds of large-seeded weeds were selected and cleaned. These seeds were then soaked in 50 ml of deionized water at room temperature (approximately 25 C) for 24 h to allow for electrolyte leakage (Powell 1986). The electrical conductivity of the soaking



solution was measured using a Thermo-Scientific Orion Versastar Pro EC meter, calibrated with instrument-specific standard solutions before each measurement. Conductivity readings, recorded in microsiemens per centimeter ( $\mu\text{S cm}^{-1}$ ), indicated the extent of electrolyte leakage: high values suggested poor membrane integrity and lower seed vigor, while low values indicated good membrane integrity and higher seed vigor. Each sample was tested in triplicate to ensure reproducibility, and the average conductivity value was calculated for each seed sample.

### Seedling Vigor

Seedling vigor was assessed by measuring seedling length and dry weight. At the end of the 21-d germination test, the length of five random seedlings were measured from the base to the tip of the longest leaf to evaluate growth performance. Subsequently, these seedlings were placed for drying in an oven at 65 °C for 48 h. After drying, the five seedlings were weighed to determine their dry biomass. The seedling vigor was measured using two indices: vigor index I (percent germination by plant length) and vigor index II (percent germination by total dry biomass) (Abdul-Baki and Anderson 1973).

### Statistical Analysis

Seed germination and viability were expressed as percentages. All treatment combinations were treated as a CRD with four replicates and were repeated three times. Treatments comprised all combinations of five weed species with eight incubation times (digestion experiment; four replications). Data were analyzed in RStudio using Levene's test for homogeneity of three runs. Data from the three experimental runs were pooled, as there was no significant difference ( $P > 0.05$ ) among the runs. ANOVA was performed on seed germination, seed viability, seed membrane integrity, and seedling vigor data using JMP PRO v. 16 (SAS Institute, Cary, NC, USA). Means were separated using Tukey's honest significant difference (HSD) test at a significance level of  $\alpha = 0.05$ . Results are presented as estimated means  $\pm$  SE.

The time to reduce seed germination by 50% ( $G_{50}$ ) was estimated using a three-parameter log-logistic regression model with the DRC package in RStudio (Knezevic et al. 2007; Ritz et al. 2015) applied to cumulative seed germination data collected over 21 d. The following three-parameter regression model was fit (Equation 1):

$$y = \frac{d}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad [1]$$

In this equation,  $y$  is the response variable (i.e., seed germination [%]);  $x$  is the incubation period in rumen fluid;  $d$  is the upper limit;  $b$  is the relative slope around  $e$ ; and  $e$  is  $G_{50}$ , which is the incubation period in rumen fluid required for 50% reduction in germination.

## Results and Discussion

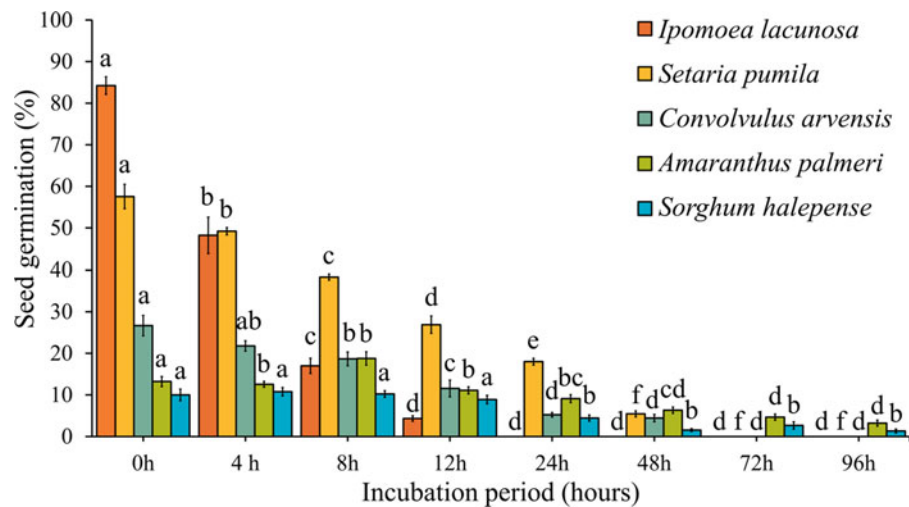
### Seed Germination Traits

In natural conditions, the majority of seeds are recovered from cattle within the first 24 to 48 h, with recovery continuing up to 72 h and beyond (Gardener et al. 1993; Xu et al. 2022). We observed significant effects of rumen fluid digestion on weed seed germination among the weed species in this study, as seed

germination rate progressively declined with increasing duration of incubation (Figure 3). *Ipomoea lacunosa* seeds were highly affected by rumen fluid incubation, starting with a high germination percentage of 85% at 0 h that fell dramatically to 0% by 24 h of incubation and beyond. This pattern is consistent with previous research, which suggests that prolonged exposure to rumen fluid can adversely affect needle grass (*Stipa aliena* Keng) seed germination, potentially due to microbial activity and changes in pH levels that inhibit germination (Chen et al. 2012). Blackshaw and Rode (1991) reported that downy brome (*Bromus tectorum* L.) and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] seeds had 0% germination after 24 h of rumen fluid incubation. *Convolvulus arvensis* and *S. pumila* showed a severe and continuous decline in germination rates as the incubation period increased. Germination percentages dropped from 27% at 0 h to 4% at 48 h for *C. arvensis*, and from 58% at 0 h to 5% at 48 h for *S. pumila*, with no germination observed beyond this period. Similar findings were reported by Blackshaw and Rode (1991) for green foxtail [*Setaria viridis* (L.) P. Beauv.], with seed germination decreasing from 88% to 14% after 24 h of rumen fluid incubation. In all three species (*C. arvensis*, *I. lacunosa*, and *S. pumila*), there was no germination beyond 48 h of incubation. Reduced seed germination can be attributed to the degradation of the embryo due to digestive enzymes in animal gastrointestinal systems. Additionally, microbial attachment in the rumen can further inhibit germination (Obara et al. 2009). The high microorganism content and acidity of rumen fluids can lead to a loss of seed germinability, particularly in seeds with higher seed coat permeability and thin coats (Horiguchi et al. 2008).

*Amaranthus palmeri* and *S. halepense* exhibited a relatively stable germination pattern, although there was a decline in germination over the incubation periods (Figure 3). Notably, these two species were still germinating even after 96 h of incubation, with *A. palmeri* and *S. halepense* showing germination rates of 3% and 1%, respectively. Additionally, after 8 h of rumen fluid incubation, *A. palmeri* seed germination increased to 19% from 13% in control, likely due to the acidic environment affecting the seed coat's integrity and permeability, similar to the effects of acid scarification, as observed in common lambsquarters (*Chenopodium album* L.) (Aper et al. 2014; Buhler and Hoffman 1999).

Seeds of *A. palmeri* are reported to survive digestive tracts of various birds (De Vlaming and Proctor 1968; Farmer et al. 2017) and ruminant animals (Blackshaw and Rode 1991; Yu et al. 2021). *Amaranthus palmeri* is an aggressive invader by nature due to several advantageous adaptive traits, including its seed morphology and diverse dispersal routes. Its high fecundity, tolerance to extreme temperatures and drought, and outcrossing nature due to dioecy leading to high genetic diversity, along with its ability to develop herbicide resistance, make it a formidable weed (Franssen et al. 2001; Ward et al. 2013). These traits, combined with its prolonged survival in rumen fluid, suggest a high potential for the spread of herbicide-resistant populations. Similarly, *S. halepense* is a persistent invasive species with high seed dormancy and can thrive more aggressively under stress conditions (Acciaresi and Guamet 2010; Ghersa et al. 1992). Seeds of *S. halepense* need fluctuating diurnal temperatures for seed dormancy release (Taylorson and McWorther 1969). Upon animal grazing, seeds of *S. halepense* showed enhanced germination when retrieved from fecal materials in 24 to 48 h, primarily due to loosening of external bracts from scarification processes in the gut passage (Abbas et al. 2020; Grande et al. 2016; Kaur and Soodan 2017). Moreover, *S. halepense* can hybridize with other *Sorghum* species,



**Figure 3.** Effects of different duration of rumen fluid incubation on seed germination of five weed species. Bars with different letters indicate significant difference among the individual species across durations at  $P < 0.05$ . Errors bars indicate standard error of the mean (SEM).

**Table 1.** Percent final germination, number of days to first germination, and the mean time to germination (MTG) of the weed seeds after various durations of incubation in rumen fluid (hours).

Species	Incubation period	Final germination percentage	Time to first germination	MTG
	h	%	d	
<i>Convolvulus arvensis</i>	0	27	3	5
	4	22	3	4
	8	19	3	4
	12	12	3	4
	24	5	3	4
	48	4	3	4
	72	0	0	0
	96	0	0	0
<i>Sorghum halepense</i>	0	10	5	8
	4	11	5	12
	8	10	5	9
	12	9	5	9
	24	4	5	9
	48	2	7	8
	72	3	7	12
	96	1	13	13
<i>Amaranthus palmeri</i>	0	13	3	7
	4	13	3	5
	8	19	3	5
	12	11	5	6
	24	9	5	7
	48	6	5	5
	72	5	5	6
	96	3	7	7
<i>Ipomoea lacunosa</i>	0	84	3	4
	4	48	3	4
	8	17	3	6
	12	4	3	6
	24	0	0	0
	48	0	0	0
	72	0	0	0
	96	0	0	0
<i>Setaria pumila</i>	0	58	5	8
	4	49	5	9
	8	38	5	9
	12	27	5	9
	24	18	5	9
	48	5	7	10
	72	0	0	0
	96	0	0	0

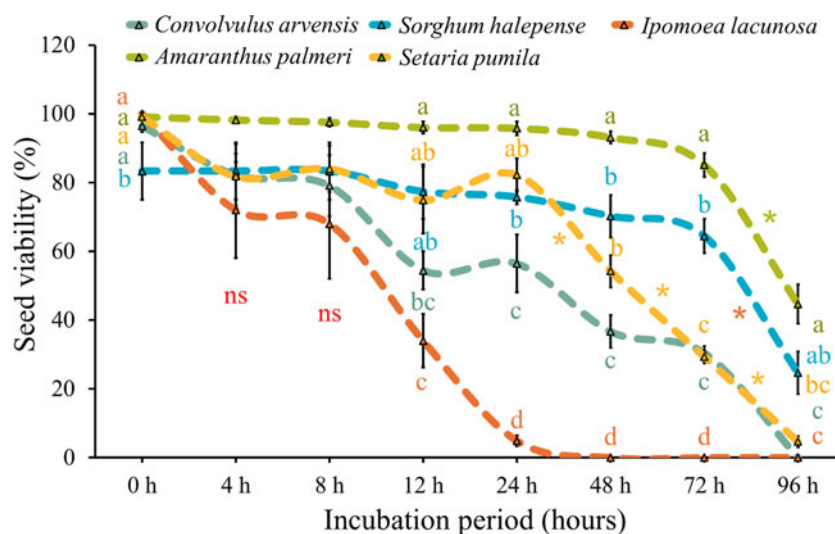
creating resilient perennial and rhizomatous hybrids that thrive in harsh environments and establish in new habitats (Klein and Smith 2021).

#### Days to First Germination, the MTG

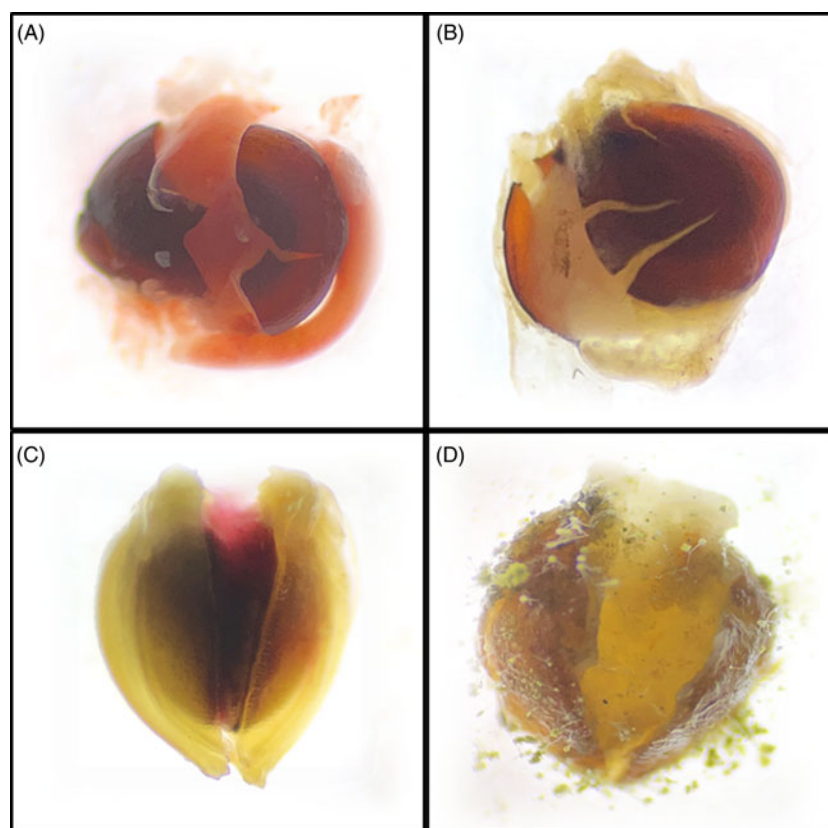
The time to first germination remained constant at 3 d for all incubation periods up to 48 h and 12 h, after which no germination was observed in *C. arvensis* and *I. lacunosa*, respectively (Table 1). In *A. palmeri*, the initial germination occurred rapidly within 3 d up to 8 h of incubation. This duration extended to 5 d up to 72 h and further increased to 7 d at 96 h of incubation. In *S. halepense*, the time to first germination was consistently 5 d for most periods of incubation, except for 48 and 72 h, where it increased to 7 d, and 96 h, where it significantly increased to 13 d. The decrease in speed of germination in *A. palmeri* and *S. halepense* may be due to genetic variability in seed coat impermeability, with more impermeable seeds surviving longer in rumen fluids and emerging more slowly in germination tests. This delay likely stems from solute leakage and membrane damage caused by exposure to rumen fluid disrupting osmotic balance and germination processes. Gama et al. (2021) reported that water deficits can significantly slow down germination rates, which may be a survival strategy in arid conditions. Early-germinated seeds may have a higher risk of dying from factors like desiccation, pathogens, or predators (Traveset et al. 2008) as observed in *I. lacunosa* and *C. arvensis* (Figure 3). Highly permeable seed coats in these species are likely digested by rumen fluids, suggesting that seed coat permeability plays a crucial role in seed survival.

#### Seed Viability

Overall, seed viability was significantly affected by rumen fluid incubation across the species (Figures 4 and 5). Although the initial germination percentage varied across the weed species, the initial viability was more than 95%, except for *C. arvensis* (89%). Similar to germination reduction, there was a gradual decrease in seed viability with increased duration of rumen fluid incubation. Simao Neto and Jones (1987) observed an inverse relationship between incubation time and seed viability in six pasture species (signal grass [*Urochloa decumbens* (Stapf) R. Webster; syn.: *Brachiaria decumbens* Stapf], common carpetgrass [*Axonopus fissifolius*



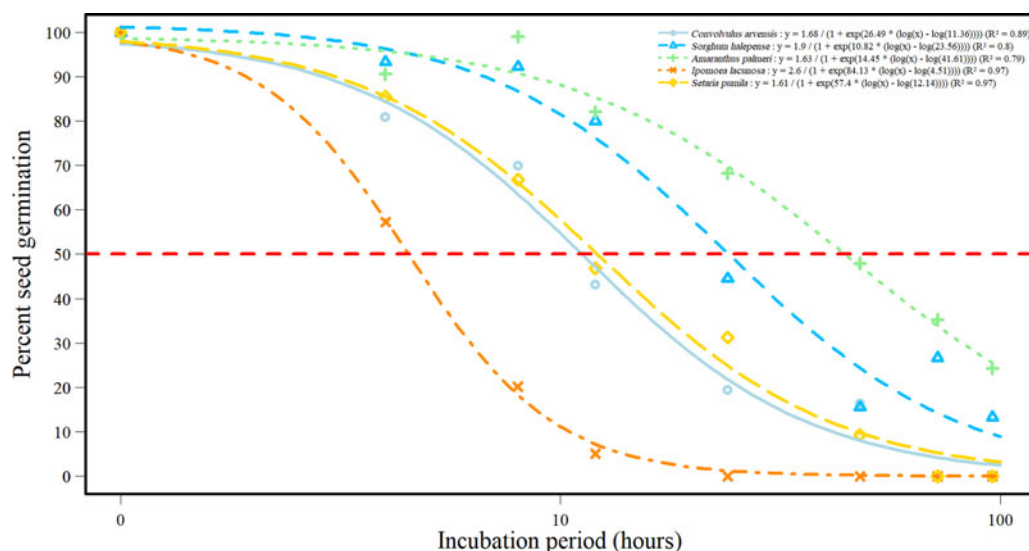
**Figure 4.** Effect of different duration of rumen fluid incubation on seed viability (germinated + dormant but viable seeds) of five weed species. Letters on the data points indicate significant difference ( $P < 0.05$ ) among the species within each observation timing. Asterisks (\*) indicate significant difference ( $P < 0.05$ ) between two consecutive observation timings for an individual species. Errors bars indicate standard error of the mean (SEM).



**Figure 5.** (A) Viable and (B) dead seed of *Amaranthus palmeri*; (C) viable and (D) dead seed of *Setaria pumila*, after a tetrazolium test under a Leica EZ4 Stereo Microscope at 35 $\times$  magnification.

(Raddi) Kuhlman; syn. *Axonopus affinis* Chase], perennial soybean [*Neonotonia wightii* (Wight & Arn.) Lackey], Kenya clover [*Trifolium semipilosum* Fresen.], cheesy-toes [*Stylosanthes hamata* (L.) Taubert] and shrubby stylo [*Stylosanthes scabra* Vog.]. However, this decline was not gradual for all species in our data. The viability of *I. lacunosa* seeds decreased sharply from 100% at 0 h to 34% by 24 h, and ultimately to 0% after 48 h. Similarly, the

viability of *C. arvensis* seeds dropped from 96% at 0 h to 54% by 24 h, and finally to 0% after 96 h; and *S. pumila* began with a viability of 99% and maintained viability above 80% up to 24 h, declining sharply to 5% by 96 h. Haidar et al. (2010) observed a similar trend, with a steep decrease in viability of *C. arvensis* seeds in the first 24 h of rumen fluid incubation from 80% at 0 h to 30% by 24 h. Similar observations were made in case of kochia [*Bassia*



**Figure 6.** Percent germination response curve for five weed species over the incubation period at 3 wk after treatment with rumen fluid. The horizontal red dashed line in the plot represents 50% of the relative germination percentage. Data normalized to 100% according to control.

*scoparia* (L.) A.J. Scott], for which viability sharply declined from 94% at 0 h to 15% after 24 h of incubation (Blackshaw and Rode 1991). This rapid loss of viability suggests that this species is highly sensitive to the conditions created by rumen fluid. *Ipomoea* spp. are highly sensitive to flood-induced anaerobic conditions, as high temperatures and humidity in rumen fluid triggered the water gap (a specialized area in the seed coat that facilitates the entry of water into the seed) to open, as seen in the case of rumen fluid (Jayasuriya et al. 2007; Siahmarguee et al. 2020) which inhibited their germination.

On the other hand, *A. palmeri* maintained relatively high viability throughout the incubation period, starting at 99% and decreasing to 47% by 96 h. Similarly, *S. halepense* started at 83% and decreased to 27% by 96 h. The slower decline in viability suggests that *A. palmeri* and *S. halepense* may have mechanisms like hard seed coats and high seed tannin content (Adjesiwor et al. 2024; Bagavathiannan and Norsworthy 2013; Burt 1954; Egley and Chandler 1978; Schutte et al. 2016) that allow them to withstand the inhibitory effects of rumen fluid longer than other species. Blackshaw and Rode (1991) found that seeds of redroot pigweed (*Amaranthus retroflexus* L.) and *C. album* retained viability rates of 45% and 52%, respectively, even after 24 h of rumen fluid incubation. This remnant viability suggests that even after being subjected to harsh conditions, such as digestion in the rumen, these seeds retain the post-excretion potential for germination upon onset of favorable environmental conditions. This adaptive strategy is critical for their survival and proliferation in competitive agricultural ecosystems.

#### Time to 50% Germination Reduction ( $G_{50}$ )

All species exhibited a significant reduction in seed germination compared with the control (Figure 6). *Amaranthus palmeri* and *S. halepense* demonstrated a gradual decrease in germination over time, with the greatest reduction occurring within the first 24 h. In contrast, *C. arvensis*, *I. lacunosa*, and *S. pumila* showed a more rapid decline in germination, reaching 50% within the first 12 h. The time required to reduce germination by 50% ( $G_{50}$ ) also differed significantly ( $P < 0.001$ ) among the species (Figure 6). The  $G_{50}$  values for individual weed species reflect their unique

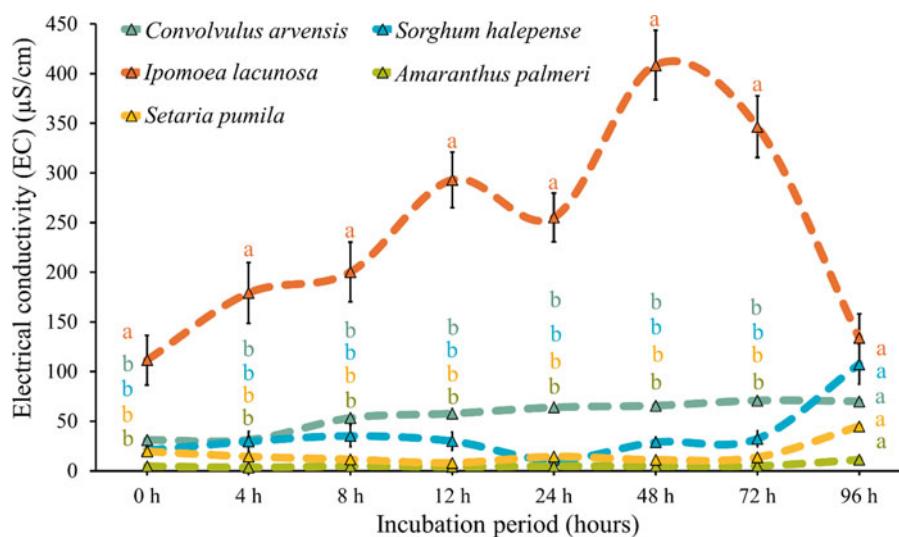
physiological and ecological adaptations to environmental conditions, particularly in response to rumen fluid treatment. The rapid  $G_{50}$  value for *I. lacunosa* of only 5 h indicates a high sensitivity to the inhibitory effects of rumen fluid. *Convolvulus arvensis* and *S. pumila*  $G_{50}$  values (11 and 12 h, respectively) suggest a moderate sensitivity to rumen fluid. The longer  $G_{50}$  for *A. palmeri* (42 h) and *S. halepense* (24 h) suggests a high level of resilience to the inhibitory effects of rumen fluid. The differences in  $G_{50}$  values indicate that different weed species exhibit varying degrees of sensitivity and resilience to rumen fluid treatment that ultimately dictate their establishment speed and success under natural conditions. The decline in germination and viability might be attributed to alterations in membrane and protein structures, as well as a rise in embryo mortality (Hendricks and Taylorson 1976). Haidar et al. (2010) found that seed germination and viability of various weed species decreases with longer incubation in the rumen due to microbial activity. Additionally, volatile fatty acids from fermentation can inhibit germination by altering osmotic potential, as noted by Cordeiro et al. (2022).

The  $R^2$  values for the fitted regression models indicate a strong relationship between incubation period and germination for all species, suggesting that rumen fluid treatment effectively inhibits seed germination in these weed species (Figure 6). The nonlinear model (Equation 1) provided a good fit to the germination data for all five weed species, as indicated by the high  $R^2$  values (0.79 to 0.97). However, the model's predictive accuracy varied among species, as reflected in the root mean-square error (RMSE) values (Figure 6). RMSE values were higher for *C. arvensis* (12.33), *S. halepense* (17.00), and *A. palmeri* (14.05), suggesting that the model's predictions for these species were less precise. In contrast, *I. lacunosa* and *S. pumila* had the lowest RMSE values (5.77 and 5.76, respectively), indicating a higher degree of model accuracy for these two species.

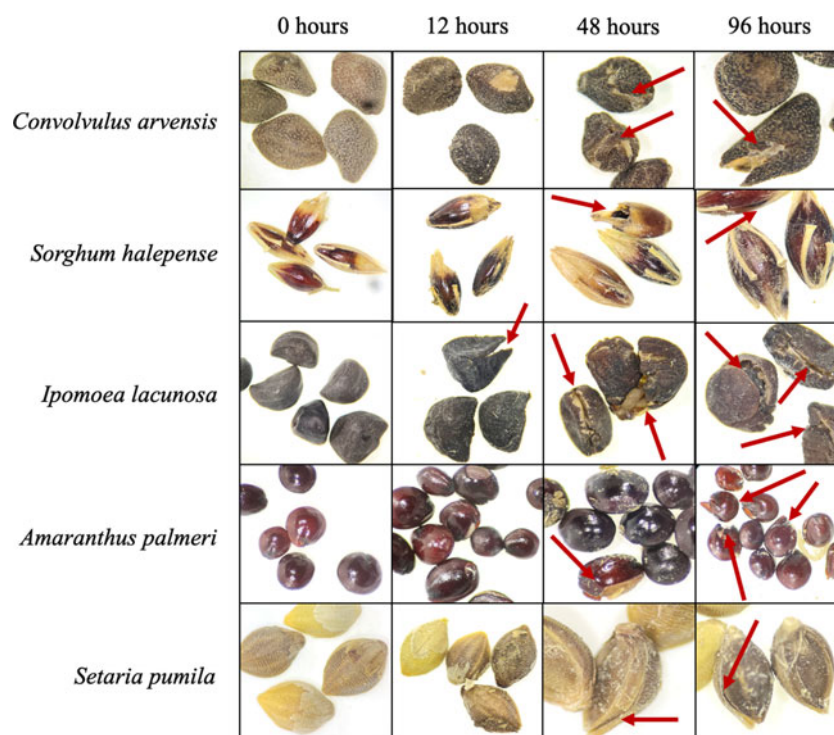
#### Seed Coat (Membrane) Integrity

Membrane integrity of seed coat was significantly affected by different periods of rumen fluid incubation among the weed species (Figures 7 and 8). The seed coats of *I. lacunosa* and





**Figure 7.** Membrane integrity test to examine damage to seed coat due to incubation in rumen fluid. Letters on the data points indicate significant difference ( $P < 0.05$ ) among the species within each observation timing. There was no significant difference between two consecutive observation timings for an individual species. Errors bars indicate standard error of the mean (SEM).

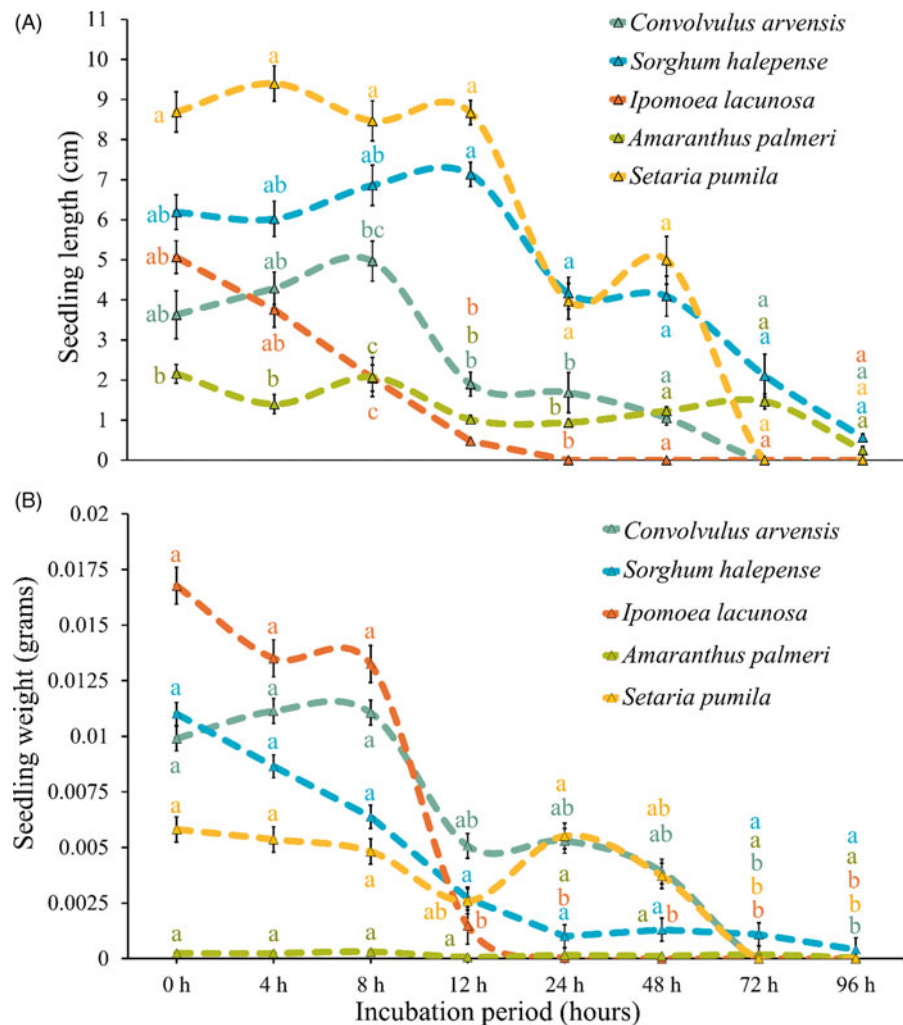


**Figure 8.** Seed morphology after incubation in rumen fluid at different time intervals under a Leica EZ4 Stereo Microscope at 35 $\times$  magnification. Red arrows show the damage and cracks on seed coats with increasing duration of rumen fluid incubation.

*C. arvensis* were highly damaged after 48 h of incubation and were completely disintegrated at 96 h. The conductivity values for *C. arvensis* seeds increased from 31.09  $\mu\text{S cm}^{-1}$  at 0 h to a peak of 70.71  $\mu\text{S cm}^{-1}$  at 72 h, before slightly decreasing to 69.93  $\mu\text{S cm}^{-1}$  at 96 h. This gradual increase in conductivity suggests a decline in membrane integrity over time, indicating leakage of solutes from the seed, which ultimately diminishes seedling vigor. *Ipomoea lacunosa* displayed the highest conductivity values, starting at 111.50  $\mu\text{S cm}^{-1}$  and peaking at 408.73  $\mu\text{S cm}^{-1}$  at 48 h, before declining to 133.95  $\mu\text{S cm}^{-1}$

at 96 h. Declining value suggests that seeds were fully exhausted of ions and completely dead following 72 h of incubation. *Sorghum halepense* seeds had the least damage, even after 96 h of rumen fluid incubation. *Amaranthus palmeri* seeds were significantly damaged after 96 h of incubation, but plenty of seeds survived the rumen fluid. *Amaranthus palmeri* showed relatively low conductivity values, ranging from 4.62  $\mu\text{S cm}^{-1}$  at 0 h to 11.19  $\mu\text{S cm}^{-1}$  at 96 h. The low values indicate good membrane integrity and higher seed vigor, suggesting that this species is more resilient to stress conditions.





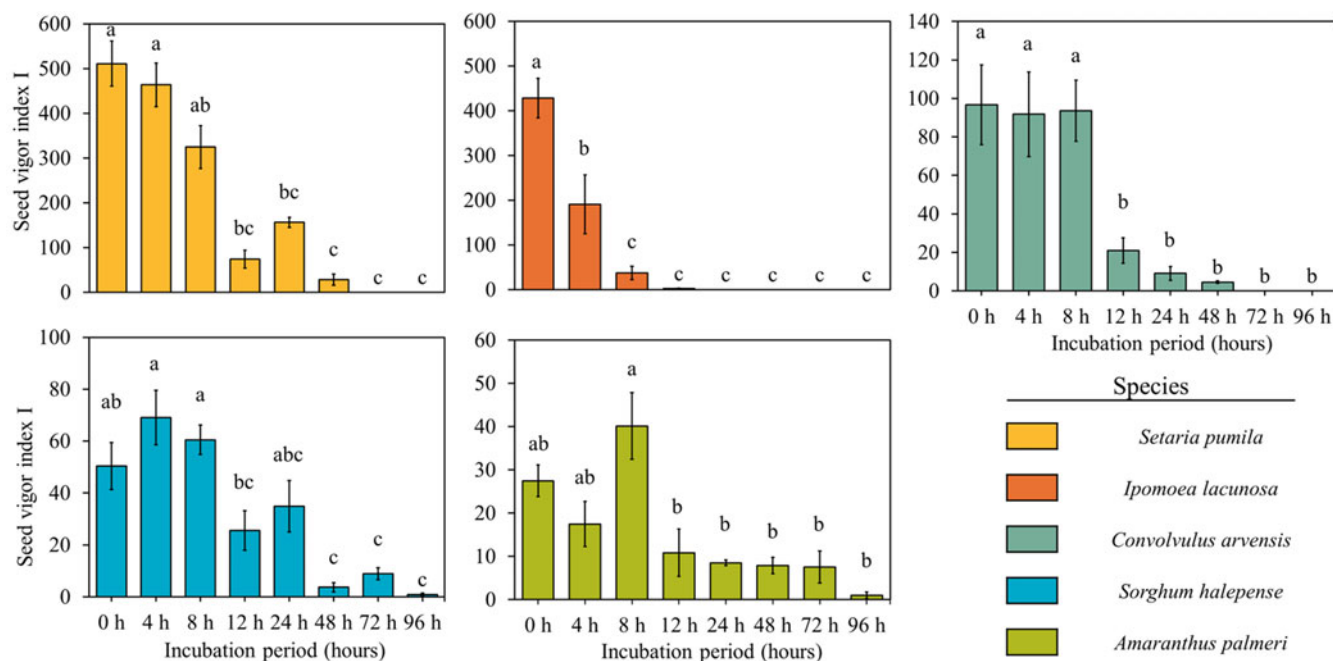
**Figure 9.** Effect of different durations of rumen fluid incubation on (A) seedling length and (B) seedling weight of five weed species. (A) Letters on the data points indicate significant difference ( $P < 0.05$ ) among the species within each observation timing. There was no significant difference between two consecutive observation timings for an individual species. (B) Letters on the data points indicate significant difference ( $P < 0.05$ ) between two consecutive observation timings for an individual species. There was no significant difference ( $P < 0.05$ ) among the species within each observation timing. Errors bars indicate standard error of the mean (SEM).

*Amaranthus palmeri*'s hard seed coat may have contributed to its survival in rumen fluid, unlike *I. lacunosa*'s larger seed size and quickly imbibed seed coat due to the opening of the water gap in rumen fluid. *Amaranthus* spp. seeds have a high lignin content (Calabrò et al. 2022) and peptides with antimicrobial activity (Lipkin et al. 2005), which primarily provides structural integrity and impermeability to seed coats. Additionally, lignin may protect seeds from microbial pathogens and various biotic and abiotic stresses (Tobimatsu et al. 2013). On the other hand, *S. halepense* relies on its substantial hemicellulose and cellulose content for protective functions. The hard, impermeable seed coat blocks rumen fluid from entering the seed and acts as a barrier against microbial enzymes, ensuring seed survival (Michael et al. 2006). The hard and impermeable seed coat of small-flowered mallow (*Malva parviflora* L.) helped it to survive rumen digestion (Michael et al. 2006). On the other hand, Olson and Wallander (2002)

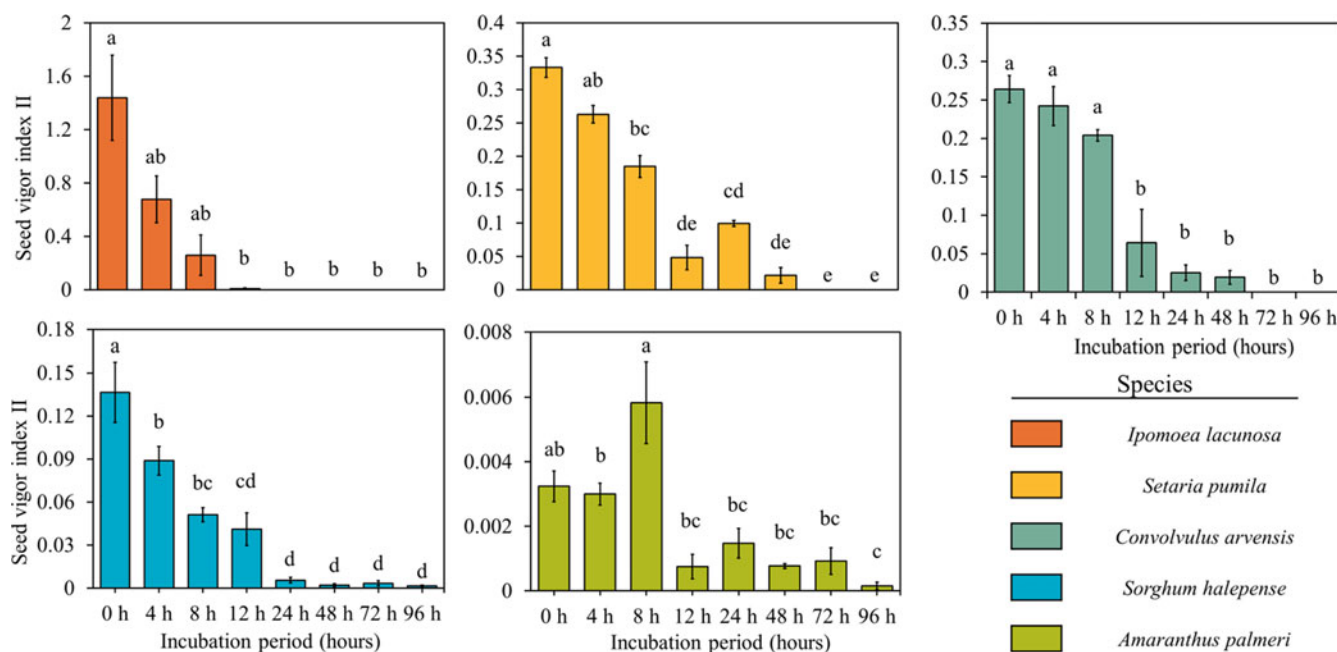
found that in some species, the seed coat softens over time in rumen fluid and becomes susceptible to microbial digestion.

### Seedling Vigor

Seedling length and weight, which control the seedling vigor indices, decreased significantly over incubation time in most of the weed species but were not much affected in the case of *A. palmeri* (Figure 9). Seedling vigor, which indicates the strength of a seed or seedling to thrive and establish a healthy plant, was significantly affected by rumen fluid, and there was a gradual decrease in seedling vigor with increased duration of incubation for all the species (Figures 10 and 11). The initial vigor index of *I. lacunosa* was high, but it decreased to 0 after 24 h of exposure to rumen fluid. Similarly, *S. pumila* began with the highest seedling vigor index among all the species, which decreased to 0 by 72 h. This rapid decline suggests that, despite their higher initial vigor, both species



**Figure 10.** Effect of different duration of rumen fluid incubation on seedling vigor index I of five weed species. Bars with different letters indicate significant difference ( $P < 0.05$ ) between two consecutive observation timings for an individual species. Errors bars indicate standard error of the mean (SEM).



**Figure 11.** Effect of different duration of rumen fluid incubation on seedling vigor index II of five weed species. Bars with different letters indicate significant difference ( $P < 0.05$ ) between two consecutive observation timings for an individual species. Errors bars indicate standard error of the mean (SEM).

are highly susceptible to the conditions imposed by rumen fluid. Although both species exhibited similar trends, *S. pumila* maintained its vigor for a longer duration. Similarly, the other three species showed lower initial values but sustained their vigor for a longer period. Although *A. palmeri* and *S. halepense* exhibited low initial vigor, they were able to maintain their vigor for the longest duration among all the species. Notably, *A. palmeri* and *S. halepense* retained substantial vigor even after 96 h of rumen fluid incubation, demonstrating their high resistance and

adaptability to stress conditions. While *A. palmeri* and *S. halepense* are resilient, prolonged exposure to rumen fluid significantly impairs their growth potential. Early seedling vigor, a key seed-quality attribute, ensures rapid, uniform germination and strong seedling establishment under various environmental conditions, with high-vigor seeds providing better resistance to stresses compared with low-vigor seeds (Bourgeois et al. 2019; Foolad et al. 2007). Rapid seedling growth, positioning *S. halepense* as the leading

species in terms of seedling size among dominant warm-season grasses, contributes significantly to its invasive success (Reichmann et al. 2016).

Our study showed that passage of weed seeds through the digestive tract of cattle reduces the viability of weed seeds. Consequently, cattle grazing on weeds can help in decreasing the number of seeds entering the weed seedbank, further aided by the mechanical damage caused by their teeth (Stanton et al. 2002). However, some seeds remain viable after digestion, depending on the weed species (Asaduzzaman et al. 2022; Wang et al. 2017). Weed seeds are generally less affected by ingestion by cattle compared with sheep (*Ovis* spp.) or birds (Farmer et al. 2017; Gardener et al. 1993; Haidar et al. 2010; Michael et al. 2006). Thus, viable weed seeds in cattle excreta can potentially lead to weed infestations in fields (Wilson et al. 2022).

In our study, *A. palmeri* and *S. halepense* seeds showed a high tolerance to rumen fluid compared with *I. lacunosa*, *C. arvensis*, and *S. pumila*. Specifically, *I. lacunosa* seeds were dead after 48 h of incubation, with no germination observed after 24 h. Seeds of *C. arvensis* and *S. pumila* were almost dead after 96 h of incubation, with no germination occurring after 72 h. *Amaranthus palmeri* is particularly concerning due to its prolific seed production, with a single female plant capable of producing up to 600,000 seeds (Keeley et al. 1987). Additionally, looking at the herbicide-resistance scenario in these two weeds (Heap 2024), there is a risk of these seeds being spread by cattle and a potential risk of herbicide resistance spread. Results indicated that, unlike soft seeds, some hard-coated seeds remained viable in the digestive system of cattle for more than 4 d. Use of manure compost piles by farmers in the U.S. Southeast might contribute unknowingly to the spread of weeds and, consequently, herbicide resistance. Consequently, farmers should avoid grazing cattle in areas infested with weeds like *A. palmeri* and *S. halepense*, especially after the formation of viable seeds. Cattle that have grazed in such infested fields should not be moved to new, uninfested areas, and their fresh manure should not be used on arable lands. This is crucial, because cattle can inadvertently transport viable weed seeds through their manure, which can lead to the establishment of these weeds in previously noninfested fields.

Effective grazing management is crucial for herders aiming to mitigate weed invasion while optimizing livestock productivity. Cattle can consume various weeds like *C. album*, Canada thistle [*Cirsium arvense* (L.) Scop.], *Setaria* spp., and *S. halepense*. Marten and Anderson (1975) observed that *A. retroflexus*, a species similar to *A. palmeri*, has forage nutritive value similar to high-quality alfalfa (*Medicago sativa* L.) when harvested at the vegetative stage. Strategic grazing timing can significantly affect weed phenology, particularly preventing flowering stages of certain weed species. Grazing before the flowering of summer annuals such as common ragweed can reduce local weed occurrence effectively; however, this has been shown to be context dependent and influenced by various factors throughout the growing season. Rotational grazing practices tailored to specific weed phenologies further enhance this effect by allowing herders to manage the grazing of different areas over time and avoid periods that favor weed reproduction.

Furthermore, the treatment of manure before field application can be an effective strategy to mitigate the risk of weed seed dispersal. Composting manure at high temperatures ( $\geq 60$  °C) can kill most of the viable weed seeds, thereby reducing the potential for these seeds to germinate and establish in agricultural fields

(Larney and Blackshaw 2003). High temperature in combination with organic acids reduces seed viability during composting (Liu et al. 2020). This practice is particularly relevant for farmers who rely on manure as a fertilizer, as it allows them to utilize this resource without inadvertently introducing weed seeds into their cropping systems.

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