OnLine Journal of Biological Sciences, 6 (1): 23-27, 2006
ISSN 1608-4217

Recovery and Germination of Grass Seeds Ingested by Cattle

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Abstract: Seeds of bluebunch wheatgrass [*Psuedoroegneria spicata (*Pursh) A. Love] and Sandberg bluegrass (*Poa secunda* Presl.) were fed to Holstein heifers in different amounts to investigate the effects of seed feeding level and seed size on the recovery and germinability of passed seed. Animals were fed 60,000, 30,000,15,000, and 7,500 seeds of each species. Passed seeds were recovered from dungcollected daily over a 4-day period and tested for germinability. In general, recovery of the larger-seeded bluebunch wheatgrass and the smaller-seeded Sandberg bluegrass declined as seed feeding levels decreased from 60,000 to 7,500 seeds per animal, and as time after seed ingestion increased from 1 to 4 days. Total seed recovery over the 4-day period was greater for bluebunch wheatgrass at the 60,000 seed feeding level, similar for both species at the 30,000 seed feeding level, and greater for Sandberg bluegrass at the 15,000 and 7,500 seed feeding levels.Germinability of bluebunch wheatgrass seeds decreased with each additional day in the digestive tract, while germinability of Sandberg bluegrass seeds remained constant or increased with time. Germinability of both species tended to increase as seed feeding levels decreased from 60,000 to 15,000 seeds per animal. Sandberg bluegrass seeds had greater germinability than bluebunch wheatgrass at all seed feeding levels and collection dates. Recovered seeds had significantly lower germinability than noningested seeds for both species. Results showed that livestock have the potential for dispersing enough germinable seeds on degraded rangelands.

Key words: Seed passage, seed recovery; germinability, seed dissemination, sandberg bluegrass, bluebunch wheatgrass

INTRODUCTION

Livestock are being considered as dispersal agents for spreading seeds of desirable plant species on degraded rangelands and can be a feasible option for revegetating overgrazed rangelands especially in arid and semi-arid regions[4, 11, 16]. For example, Ocumpaugh[20] compared fecal seeding with broadcast seeding and found that seedling emergence and establishment of switchgrass were better for fecal seeding. Therefore, several studies have investigated the recovery and germinability of seeds that have passed through ruminant digestive tracts [7, 19, 29, 21, 18, 26, 27, 6, 25, 3, 12, 22, 14, 15, 16, 9]. In general, the findings from these studies indicate that: 1) larger seeds have a greater probability of being damaged by mastication than smaller seeds; 2) larger seeds tend to pass through animals more slowly than small seeds, and elongated seeds tend to pass more slowly than round seeds; and 3) depending on retention time in the digestive tract, seeds with soft seed coats usually lose their germinability/viability more easily than seeds with hard seed coats.

In the majority of these studies, animals were fed one specified amount of seed of selected plant species. Little information is available concerning the effects of feeding different amounts of seed on the recovery and germinability of passed seed. Since the amount of seed that is fed to animals affects the cost and feasibility of this alternative revegetation strategy, more research is needed to determine the appropriate amount of seed that must be fed to animals to provide enough germinable seeds for successful seedling establishment in deposited dung. Seeds of two perennial, cool-season grasses -- bluebunch wheatgrass [*Psuedoroegneria spicata* (Pursh) A. Love] and Sandberg bluegrass (*Poa secunda* Presl.) -- were fed to cattle in different amounts to investigate the effects of seed feeding level and seed size on the recovery and germinability of passed seed.

MATERIALS AND METHODS

Plant species: Bluebunch wheatgrass and Sandberg bluegrass were selected for this experiment based on their desirability as revegetation species and differences in their seed size. Both species are adapted to low rainfall and most soil textures, and provideexcellent spring forage for livestock and a variety of wildlife species[28]. Bluebunch wheatgrass has a taller bunchgrass growth habit and a deeper root system than Sandberg bluegrass[28]. The numbers of seeds per 1-g mass are about 308 for Bluebunch wheatgrass and 2037 for Sandberg bluegrass[17]. Seeds of both species are

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approximately the same diameter (1-2 mm); however, the length of bluebunch wheatgrass seeds (8-9 mm) is twice that of Sandberg bluegrass seeds (3-4 mm) and seeds of neither species require pretreatment for germination[10 30].

Animals and feeding: The seed passage experiment was carried out with Eight Holstein heifers (age 10 ± 0.3 months, weight 250 ± 12 kg; mean ± SE) at the Utah State University Caine Dairy Farm. Animals were kept in individual pens for feces collection. All animals were fed a grass hay diet that did not include seeds of either bluebunch wheatgrass or Sandberg bluegrass. Daily dry matter intake was 3% of live body weight for each animal, and feed was equally divided between feeding times at 0800 hours and 1800 hours. Animals had continuous access to water during the experiment. Seed feeding treatments (on a per animal basis) included 60,000, 30,000, 15,000, and 7,500 seeds of each grass species. Animals were randomly selected and fed foreach treatment. Seeds of each grass species were mixed with calf feed that did not contain seeds of any species used in this study and offered to animals before the early morning ration of grass hay. Animals did not consume the total amount of seed offered for any species at any seed feeding level, and the proportion of unconsumed seeds varied from 4 to 8% for bluebunch wheatgrass and from 2 to 8% for Sandberg bluegrass. Unconsumed seeds were collected and quantified, and the values were used to determine the actual number of seeds ingested by animals at each seed feeding level.

Seed passage and recovery: After seed ingestion, a 1 kg fecal sample was taken from the rectum of each animal every 24 h for 4 days. The samples were washed over a series of sieves and dried to recover seeds for seed passage estimates and germination tests. This method provides a more accurate, point-in-time measurement of seed recovery than collecting subsamples from total fecal output at 24-h intervals, which has been done in most other seed passage and recovery studies. Undamaged recovered seeds (lemma and palea not injured in outward appearance) were separated by hand (using a 10X magnifying lens). Seed recovery was expressed as the actual number of undamaged seeds recovered. After the fourth day of collecting feces containing passed seeds, animals were given an additional 3 days to ensure that no seeds remained in the digestive tract before feeding the next seed treatment.

Germinability of recovered seeds: Depending on the availability of undamaged passed seeds, one to four replications of 25 undamaged seeds from each fecal sample were placed on moistened filter paper in Petri dishes to estimate germinability. Petri dishes were placed in a controlled environment chamber with a night / day temperature regime of 10 / 20 °C.

After passing through the cattle digestive tract, seeds of both species had significantly lower germinability than unpassed seeds. For bluebunch wheatgrass, the mean germination percentage for recovered seeds from all seed feeding treatments was 10%, compared to 69% for unpassed seeds. Similarly, mean germination percentages for recovered and noningested seeds of Sandberg and a 12-h photoperiod. The number of germinated seeds was counted every day over a 21-day period. Grass seeds were considered germinated when the coleoptile had emerged and the radicle had elongated to 5 mm[8]. To compare the germination percentages of passed seeds with unpassed seeds for the same plant species, four replications of 25 unpassed seeds for each plant species were placed in Petri dishes in the same controlled environment chamber and at the same time.

Experimental design and analysis: The experiment was arranged in a three-way factorial, randomized, split-plot design. The three factors were plant species (bluebunch wheatgrass and Sandberg bluegrass), seed feeding levels (60,000, 30,000, 15,000, and 7,500 seeds per animal), and seed collection dates (four days) and eight animals were replications. The numbers of seeds in 1-kg fecal samples were log-transformed prior to using analysis of variance[31]. Means were compared using Fisher's LSD ($P<0.05$). Germination analyses of undamaged passed seeds and control seeds were analyzed using a binomial chi-square test[31].

RESULTS AND DISCUSSION

The actual numbers of seeds recovered from 1-kg fecal samples were used to decide which seed feeding level and seed collection day were the best for dungpat deposition in the field after animals consumed seeds. The numbers of undamaged bluebunch wheatgrass and Sandberg bluegrass seeds recovered from 1 kg dung samples declined as seed feeding levels decreased from 60,000 to 7,500 seeds per animal, and as time after seed ingestion increased from 1 to 4 days (Table 1). Significant reductions in the numbers of recovered seeds for both species were observed by the third day after seed ingestion for all seed feeding levels except for the 7,500 seed feeding level for Sandberg bluegrass, which did not show a significant reduction until the fourth day after seed ingestion. During the first and second days after seed ingestion, seed recovery was similar for both species at all seed feeding levels except the 7,500 seed feeding level, where seed recovery on the second day was significantly higher for Sandberg bluegrass than for bluebunch wheatgrass. More seeds of Sandberg bluegrass were recovered at all seed feeding levels during the third and fourth days after seed

Table 1: Numbers of seeds recovered from 1-kg fecal samples after cattle ing ested seeds of bluebunch wheatgrass and Sandberg bluegrass.

Days after ingestion	Seed feeding level (x1000)							
	60	30	15	7.5	60	30	15	7.5
	Bluebunch wheatgrass				Sandberg bluegrass			
1	176a	71b	39bc	25cg	136a	80ab	49be	23g
2	123ad	50bf	22c	7h	72de	34cf	40ce	42ceg
3	25b	8d	6d	1ef	29bg	16dg	21gh	33g
4	5c	3ce	1ef	0f	22bh	10d	15dh	12dh

Note: Means with different letters are significantly different in each column and each row for the same species, and between species at the same day same seed feeding level as determined by the LSD test (P<0.05)

Table 2: Percent germination of bluebunch wheatgrass and Sandberg bluegrass seeds recovered from cattle dung 1 to 4 days after seed ingestion.

Days after ingestion	Bluebunch wheatgrass	Sandberg bluegrass
1	14a	21d
2	6b	21d
3	4c	28e
4	—†	22de

Note: Means with different letters are significantly different in each row and each column as determined by the binomial chi-square test (P<0.05)

Table 3: Percent germination of bluebunch wheatgrass and Sandberg bluegrass seeds recovered from cattle dung after feeding cattle different amounts of seed.

Seed feeding level	Bluebunch wheatgrass	Sandberg bluegrass
60,000	10c	21b
30,000	10c	27a
15,000	15b	31a
7,500	14bc	19b

Note: Means with different letters are significantly different in each row and each column as determined by the binomial chi-square test (P<0.05) † Not enough seeds recovered for germination test.

ingestion, and seed numbers were significantly higher than those for bluebunch wheatgrass on the fourth day.

After passing through the cattle digestive tract, seeds of both species had significantly lower germinability than unpassed seeds. For bluebunch wheatgrass, the mean germination percentage for recovered seeds from all seed feeding treatments was 10%, compared to 69% for unpassed seeds. Similarly, mean germination percentages for recovered and noningested seeds of Sandberg bluegrass were 23 and 44%, respectively.

Seed retention time in the digestive tract had different effects on the germinability of bluebunch wheatgrass and Sandberg bluegrass seeds (Table 2). Germinability of bluebunch wheatgrass seeds decreased significantly with each additional day in the digestive tract. In contrast, germinability of Sandberg bluegrass seeds remained constant or increased with time, with a significantly higher germination percentage 3 days after seed ingestion. Sandberg bluegrass seeds had significantly greater germinability than bluebunch wheatgrass at all seed collection dates.

In general, seed germinability for both species tended to increase as seed feeding level decreased from 60,000 to 7,500 seeds per animal (Table 3). Germinability was significantly higher for bluebunch wheatgrass at the 15,000 seed feeding level, and for Sandberg bluegrass at the 30,000 and 15,000 seed feeding levels. Sandberg bluegrass had significantly higher germinability than bluebunch wheatgrass at all seed feeding levels except for the 7,500 seed feeding level.

Only one other study [18] has examined the effects of different seed feeding levels on seed recovery in a ruminant animal. Jones and Simao Neto[18] fed sheep 12,000, 18,000, and 24,000 seeds of Bahia grass [*Paspalum notatum* Fluegge (Bogdan)] and carpet grass (*Axonopus affinis* Chase), and found that level of seed intake did not have a significant influence on percentage recovery for either species over a 5-day period. However, seed recovery was expressed as percentage instead of actual recovered seed numbers in their study.

Several other studies have investigated the recovery of ingested grass seeds in ruminants[18, 26, 6; 3, 13, 15]; however, only one seed feeding level was used for each grass species. Gökbulak[15] fed bisons 60,000 seeds of three cool-season, perennial grass species and two forb species, including bluebunch wheatgrass, and found that percent seed recovery peaked 2 days after seed ingestion and declined to near zero 5 days after seed ingestion. A similar pattern was observed when steers were fed 60,000 seeds of alkali sacaton [*Sporobolus airoides* (Torr.) Torr.] and 21,000 seeds of blue panicgrass (*Panicum antidotale* Retz.)[6] and 60,000 seeds of five cool-season, perennial grass species[3]. Only three of eight tropical and subtropical grasses survived passage through cattle digestive tracts, and seed recovery was greatest for them between 24 and 36 h after seed ingestion[12]. It is difficult to directly compare seed recovery data from these studies with data from this study because: 1) sheep and cattle have different-sized mouthparts, resulting in different levels of mastication damage, and different-sized orifices in their digestive tract, which can influence the flow of different-sized seeds[24, 26]; 2) animal diets were different and diet quality can significantly influence seed passage rate[18]; 3) percent recovery of seeds in other studies was determined on a daily total fecal output basis, while number of seeds recovered in this study was determined from 1-kg rectal samples at the end of each collection day; 4) seed feeding levels for different grass species varied from 12,000 to 555,600 seeds per animal across the studies; and 5) seed sizes for different grass species varied considerably across the studies. Even though Sandberg bluegrass seeds are half the size of bluebunch wheatgrass seeds, recovery did not fit the accepted pattern of smaller seeds passing more rapidly than larger seeds through ruminant digestive tracts[26]. Similar numbers of seeds were recovered for both species at most seed feeding levels during the first and second days after seed ingestion. More seeds of Sandberg bluegrass were recovered at most seed feeding levels during the third and fourth days after seed ingestion. This recovery pattern could be an expression of differential chewing damage associated with rumination rather than ingestion[23]. It is also possible that seeds the size of bluebunch wheatgrass (8-9 mm long and 1-2 mm diameter) were not large enough to be restricted by the reticulo-omasal orifice in cattle, as was observed for a similar-sized seed of a tropical grass species[12]. Seeds as large as those of sideoats grama [*Boutelous curtipendula* (Michx.) Torr.] have been shown to hinder seed recovery from cattle digestive tracts[6]. Since damaged seeds were likely to have has negligible germination percentages (C. Call, Ustah State University, personal communication), only undamaged seeds were tested for germination in this study. Also, germiantion tests were carried out in the laboratory rather than by observing seedling emergence in dungpats, beacuse crust formation in dungpats[2] and location of seeds within the dungpats[27, 14, 16] can affect seedling emergence in dungpats.

The germinability of grass seed generally decreases with length of time in the ruminant digestive tract. The glumes and/or palea and lemma surrounding the caryopsis in many grass species are not as resistant to microbial fermentation, and hydolytic and enzymatic activity, as is the hard seed coat of many legume species[13]. Fairly abrupt decreases in the germination percentage of bluebunch wheatgrass with time in cattle digestive tracts have been observed for several cool-season grasses, including bluebunch wheatgrass in other studies as well[3, 15]. Very gradual decreases in percent germination with time in cattle, sheep, and goat digestive systems were reported for several tropical and subtropical grasses[18, 26]. Increases in percent germination with time in cattle digestive tracts, similar to those for Sandberg bluegrass in this study, have been noted for alkali sacaton, a small-seeded, warm-season grass[6]. Increases in germinability as seed intake level declines, such as those observed for both species in this study, have not been reported elsewhere. Jones and Simao Neto[18] indicated that level of seed intake (12,000, 18,000, and 24,000 seeds per sheep) had negligible influence on the germinability of Bahia grass and carpet grass. Changes in percent germination of bluebunch wheatgrass and Sandberg bluegrass seeds more likely resulted from the length of time seeds were in the digestive tract rather than from differences in seed feeding levels. Also, numbers of recoverable seeds for germination tests were low in most treatments except for the 60,000 and 30,000 seed feeding levels the first and second days after seed ingestion.

CONCLUSION

The amount of dung voided in an excretion event by Holstein steers in this experiment ranged from 0.8-2.5 kg (personal observation). Using bluebunchwheatgrass as an example, the actual number of undamaged seeds in a cattle dungpat 1 day after seed ingestion (time of maximum seed recovery) could range from 140-440 seeds in the 60,000 seed feeding level to 20-62 seeds in the 7,500 seed feeding level. Three days after seed ingestion, actual seed numbers for the same feeding levels drop to 20-62 and 0.8-2.5 seeds per cattle dungpat, respectively. When these seed numbers are multiplied by the mean germination percentage (10%) for recovered bluebunch wheatgrass seeds, it becomes evident that only 14-44 germinable seeds might be deposited in a dungpat 1 day after a cow has ingested 60,000 seeds while consuming a fairly high-quality grass hay diet. Fewer germinable seeds will be deposited in dungpats from lower seed feeding levels and longer digestion periods.

Germination and seedling establishment in these dungpats will be strongly influenced by plant characteristics, and by site conditions, including temperature, available moisture, dungpat crust formation, competition from existing vegetation, and granivory and herbivory[1, 5]. Having 14-44 germinable bluebunch wheatgrass seeds in a dungpat may be sufficient to ensure the establishment of one or more seedlings by the end of a growing season with favorable conditions. When similar Holstein steers were fed 60,000 seeds of Hycrest crested wheatgrass, dungpats contained an average of 68 germinable seeds[5]. During a growing season with above-normal precipitation and below-normal temperatures, about eight Hycrest seedlings established in each dungpat on bare soil, while one to three seedlings established in each dungpat placed in existing perennial vegetation.

Acknowledgments: I thank Dr. C. Call, Department of Forest, Range, and Wildlife Sciences at Utah State University, for his valuable comments and financial support. Without his help I would not be able to conduct this research. Also, I thank Donal V. Sisson and Susan Durham for their great help in experimental design and statistics.

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