

Recovery and Viability of Sulfur Cinquefoil Seeds From the Feces of Sheep and Goats

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Abstract

Targeted grazing by sheep or goats is a potentially useful tool for suppressing the noxious weed sulfur cinquefoil (*Potentilla recta* L.). However, possible transmission of weed seeds by grazing livestock is a serious ecological concern that must be addressed in any targeted grazing prescription. We investigated the effect of sheep and goat digestion on the viability of sulfur cinquefoil seeds collected from live plants growing on a foothill rangeland site in southwestern Montana. Eight sheep and eight goats (all wethers) were each gavaged with 5 000 sulfur cinquefoil seeds. Four animals of each species received immature seeds, and four animals received mature seeds. All animals were fed ground grass hay in excess daily, and intake averaged 2.0% body weight · d⁻¹. Total fecal collection began immediately after gavaging and continued for 7 consecutive days. Once each day, all identifiable sulfur cinquefoil seeds were recovered and counted from fecal subsamples. Seed viability before gavaging averaged 36% for immature seeds and 76% for mature seeds. Sheep and goats excreted similar numbers of viable seeds. Almost all (98%) of the viable seeds recovered from sheep and goats were excreted during Day 1 and Day 2 after gavaging. No viable seeds were recovered from either sheep or goats after Day 3. Our estimates of sulfur cinquefoil seed excretion and viability in sheep and goat feces are likely inflated compared with targeted grazing animals because gavaging with seeds bypassed mastication. Grazing livestock that consume sulfur cinquefoil seeds should be kept in a corral for at least 3 d to prevent transferring viable seeds to uninfested areas.

Key Words: endozoochory, *Potentilla recta*, prescribed grazing, targeted grazing, weed seeds

INTRODUCTION

Sulfur cinquefoil (*Potentilla recta* L.) is an exotic perennial forb that flourishes in a variety of soil types and climates (Rice 1999). It is currently classified as “noxious” in five western states (USDA-NRCS 2011) because of its ability to invade healthy, undisturbed rangeland and replace native species (Rice 1999). The plant reproduces only by seed, but each plant can produce thousands of seeds per year (Dwire et al. 2006; Frost and Mosley 2012), and individual plants can live as long as 20 yr (Perkins et al. 2006). Sulfur cinquefoil can quickly dominate disturbed areas such as roadsides, clear cuts, and abandoned fields and can dominate large areas of rangeland (Rice 1999). For example, one sulfur cinquefoil infestation in south-central Montana expanded to 3 000 ha before it was treated with herbicide (J. Mosley, personal observation). Once established, the plant is very difficult to control. There are no approved biological control agents (Duncan et al. 2004), and herbicides have provided mixed results (Lesica and Martin 2003). Current recommendations suggest that sulfur cinquefoil management should focus on suppressing seed production and preventing the introduction of seed into uninfested areas (Dwire et al. 2006; Perkins et al. 2006).

Previous research has demonstrated that defoliation of sulfur cinquefoil at the flower stage or later can reduce seed

production (Frost and Mosley 2012). Targeted livestock grazing is a potential way to defoliate sulfur cinquefoil on rangeland. Targeted grazing is the application of a species of livestock at a particular timing, intensity, and duration to accomplish specific vegetation management goals (Launchbaugh and Walker 2006). However, grazing livestock that consume viable seed may disseminate it in other areas, thereby contributing to weed expansion (Bartuszevige and Endress 2008).

Elk (*Cervus elaphus*), deer (*Odocoileus hemionus*), and cattle (*Bos taurus*) have all been observed eating the fruits of sulfur cinquefoil during autumn (Parks et al. 2008), and sheep (*Ovis aries*) and goats (*Capra hircus*) have been observed grazing both the foliage and the fruits (R. Frost, personal observation, and B. Olson, personal communication, April 2006). Viable seeds of sulfur cinquefoil have been recovered from white-tailed deer (*Odocoileus virginianus*) feces (Bartuszevige and Endress 2008), but it is unknown if livestock consuming the seeds of sulfur cinquefoil can further spread the plant through endozoochory. Livestock and wildlife are capable of passing viable seeds of other noxious weeds, including leafy spurge (*Euphorbia esula* L.; Lacey et al. 1992; Olson et al. 1997; Wald et al. 2005), spotted knapweed (*Centaurea stoebe* L.; Wallander et al. 1995), jointed goatgrass (*Triticum aestivum* L.; Lyon et al. 1992), thistle spp. (*Onopordum illyricum* L., *Carduus nutans* L., and *Silybum marianum* [L.] Gaertn.; Holst and Allan 1996), and perennial pepperweed (*Lepidium latifolium* L.; Carpinelli et al. 2005). Consequently, Hogan and Phillips (2011) contend that transmission of weed seed by livestock is a serious ecological concern that should be addressed at local, national, and global levels.

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To prevent the spread of noxious weeds, confining livestock in a corral is recommended as a best management practice before moving livestock to areas not infested with noxious weeds (Kott et al. 2006). It is important to know the length of time animals should be corralled in order to minimize the amount of time, labor, and feedstuffs required before returning animals to rangeland grazing. Furthermore, sulfur cinquefoil produces flowers indeterminately with new buds opening throughout the growing season, so any prolonged grazing during the flower stage could result in sheep or goats consuming seed at any stage of development. It is prudent to test these different stages of seed ripeness for viability postconsumption by livestock (Olson and Wallander 2002). Our objective was to determine the recovery and viability of sulfur cinquefoil seeds collected during the late-flower and seedset phenological stages from the feces of sheep and goats following oral gavage.

MATERIALS AND METHODS

Yearling wether crossbred (Spanish×Boer) goats and Targhee sheep (eight of each species) were used in this study. All treatments were approved by the Montana State University Animal Care and Use Protocol number AA-039. Animals were placed in individual metabolism stalls 7 d before the beginning of the trial and fitted with fecal collection bags for 2 d before the study began to familiarize the animals with the research protocol. Once daily, during the acclimation period and throughout the fecal collection period, animals were fed ground grass hay in excess (9.4% crude protein; 64% neutral detergent fiber; dry matter basis). Mean body weight for sheep and goats was 47 ± 1.2 kg and 32 ± 1.3 kg, respectively. Mean daily hay intake of sheep and goats was 1.0 ± 0.1 kg·d⁻¹ and 0.6 ± 0.1 kg·d⁻¹ (as fed basis), respectively, which equates to 2.0% of body weight.

Treatments

Sulfur cinquefoil seeds were collected from infested foothill rangeland near Bozeman, Montana. Seeds were collected at two developmental stages: immature seeds were collected during the late-flower phenological stage when approximately 80% of reproductive buds had already flowered and the remaining 20% were in full bloom. Seeds were collected on 17 July 2008 before fruits had begun to dehisce. Mature seeds were collected when fruits were beginning to dehisce (30 July 2008), and all reproductive buds were postbloom. Individual immature seeds weighed approximately 0.05 mg, averaged 0.9 mm in length, and were light brown in color with no visible white “netting.” Individual mature seeds weighed approximately 0.2 mg, averaged 1.1 mm in length, and were dark brown in color, with hardened seed coats and clearly visible white “netting” (Rice 1991). The seeds of sulfur cinquefoil are flat, comma shaped, and slightly rough when the netting has developed (Rice 1991). Collected seeds were purified by air-density separation, hand counted, and stored in a seed closet at ~38°C from July until October when administered to the animals. Viability of the collected seed was determined by testing three subsamples of 20 seeds each from both developmental stages (immature and mature) using the tetrazolium (TZ) test (Grabe 1970). Seeds were imbibed overnight on

water-soaked blotters, cut to allow TZ to penetrate, and soaked in a 1% TZ solution for 4–6 h. Seeds were evaluated according to Association of Official Seed Analysts (AOSA) standards for the Rosaceae family (AOSA 2010). Each animal was orally gavaged with 5000 sulfur cinquefoil seeds suspended in approximately 240 mL of water, with four goats and four sheep receiving immature seeds, and four goats and four sheep receiving mature seeds. For comparison, Wallander and colleagues (1995) fed 5000 spotted knapweed seeds to individual sheep. Other seed recovery studies have fed between 1500 and 25000 seeds of different plant species to individual animals (Simao Neto et al. 1987; Lacey et al. 1992; Holst and Allen 1996; Manzano et al. 2005).

Data Collection

Total fecal collection began immediately after gavaging and continued for 7 consecutive days. Fecal collection bags were emptied once daily at 0800 hours, and bags were re-fitted. Individual wet fecal output was recorded daily. Mean daily fecal output (dry matter basis) was 479 ± 11.4 g for sheep and 289 ± 4.1 g for goats. After thoroughly mixing fecal material within each collection bag, a 20-g sample was collected from each bag and dried at 100°C for 24 h to determine moisture content. An additional subsample (10% of each animal's total fecal output [wet weight]) was collected from each animal and examined for sulfur cinquefoil seeds. Large clumps of feces were gently separated, and the fecal material was washed over a 425-micron sieve (#40) until water ran clear. Fecal samples were then dried for 36 h at 40°C (Wallander et al. 1995). Dried samples were sifted with a Ro-Tapp machine to remove fines (<425 microns, #40 sieve) and excessively large particles (>850 microns, #20 sieve). Air density separation was used to further remove fines and concentrate the sulfur cinquefoil seeds into a smaller amount of dried fecal material. The remaining particles were hand-sorted under a ×10 magnifying lamp. All identifiable sulfur cinquefoil seeds were extracted, counted, and tested for viability using the TZ test. The number of viable sulfur cinquefoil seeds excreted by animals was calculated as: number of recovered seeds (corrected for total fecal output) × percent viability of recovered seeds.

Statistical Analysis

Response variables included: 1) number of immature and mature sulfur cinquefoil seeds recovered from sheep and goats and 2) number of viable immature and mature seeds recovered from sheep and goats. Analysis of variance (ANOVA) in the Mixed procedure of SAS (version 9.2; SAS Institute, Inc, Cary, NC) was used to compare treatment means and their interactions. Individual animals were random effects, animal species and seed stages were fixed effects, and differences were considered significant at $P \leq 0.05$. Day-to-day differences during the 7-d fecal collection period were analyzed with repeated measures ANOVA, with day as the repeated measure and the covariance structure defined as AR(1).

RESULTS

Total Seeds

More immature seeds were recovered from sheep than goats (26% vs. 17%, respectively, $P=0.03$). Recovery of mature

Table 1. Total number of seeds recovered each day from the feces of individual sheep and goats (\pm SE) during 7 consecutive days following gavaging with either 5 000 immature or 5 000 mature sulfur cinquefoil seeds per animal.

Seed stage	Species ¹	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Cumulative
Immature	Goats	112.4 (43.8) a ² A ³	621.1 (101.0) bA	64.3 (26.7) aA	19.5 (4.1) aA	10.8 (0.9) aA	0 cA	0 cA	828 (116.9) A
	Sheep	431.4 (101.4) aA	703.7 (81.3) aA	104.8 (12.6) aA	20.0 (13.5) bA	27.44 (13.1) bA	12.5 (4.8) bB	5.0 (5.0) bA	1305 (152.5) B
Mature	Goats	741.9 (111.5) aA	1138.3 (110.5) aA	123.0 (17.0) bA	6.7 (3.4) cA	3.3 (3.4) cdA	0 dA	0 dA	2013 (236.2) A
	Sheep	1486.6 (284.6) aB	930.9 (225.5) aA	84.9 (29.0) bA	20.0 (10.8) cA	12.5 (4.8) cA	7.5 (2.5) cA	0 dA	2542 (336.8) A

¹N=4 animals of each species assigned to each seed maturity stage.

²Means in the same row through Day 7 followed by the same lowercase letter are not different ($P > 0.05$).

³Means in the same column within the same seed stage (immature or mature) followed by the same uppercase letter are not different ($P > 0.05$).

seeds, however, did not differ between sheep and goats, averaging 46% recovery (Table 1). As expected, more mature seeds were recovered than immature seeds, and this was true for both sheep and goats (Table 1).

Recovery of immature seeds peaked on Day 2 for both sheep and goats (Table 1). Recovery of mature seeds also peaked on Day 2 for goats, but recovery of mature seeds peaked on Day 1 for sheep (Table 1). Overall, 92% of all recovered seeds were recovered within 2 d after gavaging. No seeds of either maturity stage were recovered from goats after Day 5. No mature seeds were recovered from sheep after Day 6, but immature seeds were recovered from sheep through Day 7.

Viable Seeds

Neither the cumulative number of immature ($P=0.19$) nor mature ($P=0.11$) viable seeds recovered differed between sheep and goats (Table 2). Most (91%) of the viable seeds that were recovered were mature seeds.

Almost all (98%) of the viable seeds that were recovered from sheep and goats were excreted during Day 1 and Day 2 (Table 2). No viable sulfur cinquefoil seeds were recovered from either sheep or goats after Day 3.

Sulfur cinquefoil seed viability before gavaging averaged 36% (± 1.7 SE) for immature seeds and 76% (± 4.5 SE) for mature seeds. Passage through the digestive tract of sheep or goats reduced the viability of both immature and mature seeds. Viability of excreted seeds averaged 3% for immature seeds and 27% for mature seeds.

DISCUSSION

Overall, 39% of gavaged sulfur cinquefoil seeds were recovered from sheep, whereas 28% were recovered from goats. These

recovery rates were 1.4- to 2.3-fold greater than spotted knapweed (Wallander et al. 1995), leafy spurge (Lacey et al. 1992), and small-flowered mallow (*Malva parviflora* L.; Michael et al. 2006). Greater recovery rates in our study most likely resulted from gavaging the seeds, rather than allowing the sheep and goats to eat the seeds and damage them via mastication (Miller 1995). Also, the small size of sulfur cinquefoil seeds likely enhanced their passage. Smaller seeds fed to sheep generally have higher recovery rates than larger seeds (Russi et al. 1992).

Sheep and goats in our study excreted equivalent numbers of viable sulfur cinquefoil seeds. In Australia, Simao Neto et al. (1987) also documented that sheep and goats excreted similar amounts of viable seed when fed a mixture of six tropical pasture plant species. However, Lacey et al. (1992) in Montana reported that sheep excreted fewer viable leafy spurge seeds than goats. Sheep and goats usually excrete fewer viable seeds than either cattle or horses (Harmon and Keim 1934; Simao Neto et al. 1987), but not for all plant species (Cosyns et al. 2005).

Excretion pattern of viable sulfur cinquefoil seeds did not differ between sheep and goats. Almost all viable seeds excreted by sheep or goats were excreted by Day 2, and all were excreted by Day 3. Our results compare favorably with studies of sheep excreting viable or germinable seeds of spotted knapweed (Wallander et al. 1995), leafy spurge (Lacey et al. 1992), and Patterson's curse (*Echium planigineum* L.; Piggitt 1978). In contrast, Olson and Wallander (2002) recovered viable leafy spurge seeds from sheep through Day 4, and Lacey et al. (1992) recovered germinable leafy spurge seeds from goats through Day 4. Most viable halogeton (*Halogeton glomeratus* [M. Bieb] C.A. Mey.) seeds fed to sheep were excreted by Day 4, but a few were not excreted

Table 2. Viable seeds recovered each day from the feces of individual sheep and goats (\pm SE) during 7 consecutive days following gavaging with either 5 000 immature or 5 000 mature sulfur cinquefoil seeds per animal.

Seed stage	Species ¹	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Cumulative
Immature	Goats	52.7 (37.7) a ² A ³	67.4 (2.9) aA	13.1 (6.2) bA	0 cA	0 cA	0 cA	0 cA	133 (36.1) A
	Sheep	101.8 (32.0) aA	66.2 (4.7) aA	4.9 (4.9) bA	0 bA	0 bA	0 bA	0 bA	173 (31.4) A
Mature	Goats	593.1 (130.5) aA	567.7 (40.9) aA	37.1 (4.2) bA	0 cA	0 cA	0 cA	0 cA	1198 (158.7) A
	Sheep	1206.1 (296.5) aA	527.1 (159.8) aA	12.2 (7.7) bA	0 cA	0 cA	0 cA	0 cA	1745 (323.5) A

¹N=4 animals of each species assigned to each seed maturity stage.

²Means in the same row through Day 7 followed by the same lowercase letter are not different ($P > 0.05$).

³Means in the same column within the same seed stage (immature or mature) followed by the same uppercase letter are not different ($P > 0.05$).

until Day 9 (Lehrer and Tisdale 1956). Passage of forb seeds from cattle are typically as rapid as for sheep and goats. For example, no seeds of cicer milkvetch (*Astragalus cicer* L.) were recovered from cattle after Day 3 (Willms et al. 1995), and 99% of gooseberry globemallow (*Sphaeralcea grossularifolia* [Hook. & Arn.] Rydb.) seeds were recovered from cattle by Day 3 (Whitacre and Call 2006).

Exposure to the digestive tract of sheep or goats reduced the viability of sulfur cinquefoil seeds. Therefore, even when sheep or goats excrete sulfur cinquefoil seeds on the landscape, the number of viable seeds added to the soil seedbank will be less when grazed than without sheep or goat grazing. This effect will be greater for immature seeds because digestion decreased the viability of immature seeds more so than mature seeds (92% decrease vs. 64% decrease, respectively). The hardened seed coat of mature seeds limits digestive impacts (Gardner et al. 1993). Finally, our estimates of sulfur cinquefoil seed excretion and viability in sheep and goat feces are likely inflated compared with targeted grazing animals because gavaging with seeds bypassed mastication.

MANAGEMENT IMPLICATIONS

Targeted (or prescribed) grazing by sheep or goats is a promising tool for reducing the number of viable sulfur cinquefoil seeds added to the soil seedbank. However, some viable seed is capable of surviving the digestive system of sheep and goats. Sulfur cinquefoil plants in the preflower phenological stage have not yet produced viable seeds. Therefore, livestock that prescriptively graze sulfur cinquefoil during this stage do not need to be quarantined before moving to a new area. Sulfur cinquefoil plants in the early-flowering stage also have not yet produced viable seeds. However, a sulfur cinquefoil infestation characterized to be in the flowering stage likely contains some sulfur cinquefoil plants that are more phenologically advanced into the seedset stage. Moreover, sulfur cinquefoil plants produce flowers indeterminate so viable seeds may be present in fruits of primary shoots while secondary and tertiary shoots are still flowering. Therefore, sheep or goats that prescriptively graze sulfur cinquefoil infestations during flowering or later phenological stages should remain in a corral for at least 3 d to allow any viable seeds to be excreted before moving the livestock to a new area.

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