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Spotted knapweed seed viability after passing through sheep and mule deer

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Abstract

Spotted knapweed (*Centaurea maculosa* Lam.), an introduced perennial plant, has invaded large areas of rangeland in the northwestern United States. Grazing animals may disseminate the weed by transporting seeds in their digestive system and depositing them in their feces. In this study percent viability and emergence of spotted knapweed seeds that passed through mule deer (*Odocoileus hemionus hemionus*) and sheep (*Ovis aries*) were determined. Percent viability included seeds that germinated and seeds that tested positive with tetrazolium. In the first trial, we pulse dosed 3 mule deer and 4 ewes with 5,000 spotted knapweed seeds each. Seed recovered from manure collected daily for 10 days after dosing was tested for percent viability. We recovered 11% of the knapweed seeds from the 3 mule deer, and 4% from the sheep. Based on high variability in (0 to 26%) percent viability of recovered seed, we thought that our drying the manure at 50°C may have killed some of the spotted knapweed embryos. To determine if drying at 50°C affected viability, we pulse dosed 4 rams with 5,000 spotted knapweed seeds each in a second trial. One subsample of manure was washed the same day to recover seeds and then dried at 35°C, a second subsample was dried at 50°C, washed, and then dried at 35°C. We recovered 17% of the spotted knapweed seeds from the 4 rams. No viable seeds were recovered from manure heated at 50°C, and no viable seeds were recovered more than 2 days after dosing. Percent viability of seeds recovered from manure dried at 35°C ranged from 0 to 22%. In both trials, percent viability of recovered seeds was lower compared with seeds that did not pass through animals. Sheep and mule deer can ingest, transport, and disseminate viable seeds of spotted knapweed in their feces.

Key Words: *Centaurea maculosa,* weed, seed, rangeland, dispersal

Spotted knapweed (*Centaurea maculosa* Lam.) is a perennial plant introduced from Europe that has infested over 2.9 million ha in 9 states and 2 Canadian provinces in western North America (Lacey 1989). Although this noxious weed can be controlled with chemicals, widespread use of herbicides may be undesirable or infeasible over vast areas of native rangeland (Griffith and Lacey 1991). Weed scientists and land managers now recommend an integrated weed management approach, which includes grazing when and where appropriate to control noxious range weeds. Grazing weeds with livestock is appealing because it provides weed control and income to the landowner.

Seeds of many forage plants are consumed by grazing animals. While seeds can be destroyed by mastication and digestion (Atkeson et al. 1934, Thill et al. 1986), some pass through grazing animals. Using livestock to introduce desirable forage seed to new areas, or areas inaccessible to conventional seeding equipment has been recommended (Dore and Raymond 1942, Archer and Pyke 1991, Gardener et al. 1993), however, dispersing weed seed with livestock would be undesirable (Harmon and Keim 1934, Heady 1954, Lehrer and Tisdale 1956, Piggin 1978, Lacey et al. 1992).

Our objective was to determine if sheep (*Ovis aries*) and mule deer (*Odocoileus hemionus hemionus*) could disseminate spotted knapweed seed. Specifically we determined 1) seed passage, 2) percent viability of spotted knapweed seed after passing through digestive tracts, and 3) emergence of passed seed.

Materials and Methods

Trial 1

We collected mature spotted knapweed seeds at an infested site near Bozeman, Mont. Ten lots of 1,000 seeds were weighed to determine the mean weight of 5,000 seeds (8.38 g). Eight lots of 8.38 g of seed were placed into vials. Four tame mule deer and 4 sheep were penned individually at Utah State University. One deer was unable to adjust to the small pen and was removed from the study. Animals were fed 70 g rolled barley and alfalfa pellets ad libitum daily during the trial, beginning 7 days before they were pulse dosed with 8.38 g of spotted knapweed seeds mixed with the barley. A small amount of molasses was added to bind the knapweed seeds to the barley. Each animal was observed to ensure that all spotted knapweed seeds were consumed. Manure was collected from each animal, and oven-dried at 50°C daily for 10 days after dosing. After drying, manure samples were trans-

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ported to Montana State University. Some samples contained enough moisture to support active fungal growth when the samples arrived in Bozeman, thus all samples were redried at 50°C for 24 to 48 hours.

Each manure sample was thoroughly mixed, weighed and divided in half. One half was passed through a roller mill to crush pellets, washed through #10 (2 mm) and #30 (0.58 mm) sieves, and dried at 37°C. Spotted knapweed seeds were hand collected, counted and compared with unfed seeds for percent viability.

Two small subsamples (approximately 35 g) were taken from the second half of each manure sample and weighed. One subsample was placed on the surface of moist potting soil in a small flat. The second subsample was lightly crushed with a mortar and pestle, to simulate decomposing manure, before it was placed on potting soil. Ten controls were prepared at the same time, each with 40 unfed seeds placed on moist potting soil. All flats were randomly located in a greenhouse and misted daily for 5 weeks. Seedlings that emerged were counted and removed. To encourage additional germination, these flats were then placed in a vernalization chamber and moist stratified (4°C, 12 hours light/dark) for 10 weeks. After stratification, flats were returned to the greenhouse and misted daily for 10 weeks. Seedlings that emerged were counted and removed.

Trial 2

Results from Trial 1 indicated that oven drying at 50°C may have affected the viability of some of the spotted knapweed embryos. A second trial was conducted to determine the effect of oven drying at 50°C on percent viability of spotted knapweed seeds after dosing and then passing through sheep. Spotted knapweed seeds were collected from the same area as for Trial 1 near Bozeman, Mont. All herbaceous material was removed and 10 lots of 500 seeds were weighed to estimate the weight of 5,000 seeds (8.68 g). Four lots of this weight were separated.

Four 2-year old Rambouillet rams were penned individually at the O.O. Thomas Nutrition Center at Montana State University. Animals were fed 2.5 kg of alfalfa pellets and 70 g cracked barley daily during the trial, beginning 1 week before they were pulse dosed with 8.68 g of spotted knapweed seeds bound with molasses to the barley. After dosing, manure was collected daily for the next 10 days. The manure was separated into 4 subsamples and weighed daily. One subsample was washed over sieves as described above, and immediately oven-dried at 35°C. The second subsample was first oven-dried at 50°C, washed over sieves, and then oven-dried at 35°C. Seeds recovered from the first 2 subsamples were tested for percent viability. The third subsample was oven dried and reweighed to determine moisture content of the fresh manure. To determine seedling emergence, the fourth subsample was placed directly on moist potting soil in a greenhouse and misted daily for 1 month. Five controls were established at the same time as these subsamples. In each control, 40 spotted knapweed seeds were placed directly on moist potting soil and misted daily. In this trial, no seedlings emerged from the manure samples after 1 month so the experiment was terminated.

Percent Viability

Seeds were soaked in 10% (v/v) chlorine bleach solution for 10 minutes and rinsed 3 times with distilled water before testing for percent viability. Unfed seeds served as a control and were tested at the same time. From 1 to 100 seeds were tested depending on the number of seeds recovered. When possible seeds recovered daily from each subsample were divided into 5 groups to estimate variance of percent viability. We placed up to 20 seeds on moistened blotter paper in petri plates. Petri plates were placed in an uncovered box in a greenhouse with day and night temperatures of 21°C and 13°C, respectively. Distilled water was added as needed. Germinated seeds (radicles $\geq$ 5 mm) were counted every other day and removed. A 2 week germination period was followed by 1 month in cold moist storage (4°C) and a second 2 week germination test in the greenhouse. After the second germination test, seeds that did not germinate were tested for viability using a 0.1% unbuffered tetrazolium solution (Grabe 1970). Percent viability included seeds that germinated and seeds that tested positive with tetrazolium.

Statistical Analyses

In Trial 1, repeated measures analysis of variance was used to determine the effect of animal species (deer or sheep) and day after dosing on the number of seeds recovered in manure (SAS 1988). Each animal was considered an experimental unit ($n = 3$ and $n = 4$ for deer and sheep respectively). The number of recovered seeds was the dependent variable in the repeated measures analysis of variance. Number of recovered spotted knapweed seeds was calculated by multiplying the number of recovered seeds from each animal each day by 2 (50% of the manure was washed to recover seed). Percent viability and estimated number of viable seeds were not analyzed because as the trial proceeded 1-3 sheep did not pass seed, and therefore percent viability could not be tested, and because the estimated number of viable seeds was a derived variable.

After determining sample variances of seed recovered, the ratio of sheep and deer variances exceeded the critical value of the Fmax test, thus these data were transformed [log 10 (# seed recovered + 1)] (Sokal and Rohlf 1981). Nontransformed means and standard errors are presented in tables. Numbers of seeds recovered each day were compared with the number recovered on day 2 because it was the first day that seeds were recovered from all of the animals. Probability levels are presented in the results (Gill 1981).

In Trial 2, repeated measures analysis of variance was used to determine the effect of day after dosing on the number of seeds recovered in manure (SAS 1988). The number of recovered seed from the 2 washed subsamples of manure were multiplied by the ratio of the total weight of manure relative to the sum of the weights of the 2 washed subsamples of manure. Similar to Trial 1, each ram was an experimental unit, and the number of recovered seeds was the dependent variable in the repeated measures analysis of variance.

Results

Seed Passage

We recovered 11% of the 5,000 knapweed seeds from 3 mule deer and 4% from the 4 sheep by the end of Trial 1. By the fifth day after pulse dosing, 84% and 89% of the recovered seeds had passed through the deer and sheep, respectively (Table 1, 2). We did not recover any seeds from 2 of the deer on day 1. The number of seeds recovered from both species declined over the 10 day period after dosing ($P < 0.03$), however the deer were still pass-

Table 1. Number of spotted knapweed seeds recovered, percent viability, and estimated number of viable seeds recovered from manure of 3 mule deer 10 days after dosing in Trial 1. Percent viability of control seeds = 98% ± 1.22 (SE). Percent viability included germinated seeds and seeds that tested positive with tetrazolium. Estimated number of viable seeds recovered was derived by multiplying the number of seeds recovered by percent viability. Values following means represent ± 1 SE.

Day after ingestion	Seeds recovered	Viability	Estimated viable seeds
	(No.)	(%)	(No.)
1	161 ± 161	11.5[1]	18.5
2	134 ± 84	2.3 ± 2.3	3.1
3	89 ± 75	4.5 ± 2.9	4.0
4	38 ± 36	5.5 ± 5.5	2.1
5	27 ± 15	17.0 ± 0.8	4.6
6	11 ± 3	4.2 ± 4.2	0.5
7	6 ± 3	12.2 ± 6.2	0.7
8	14 ± 12	2.3 ± 2.3	0.3
9	19 ± 15	4.8 ± 4.8	0.9
10	35 ± 17	24.5 ± 12.8	8.5

[1]SEM was not calculated because seeds were recovered from only 1 animal.

Table 2. Number of spotted knapweed seeds recovered, percent viability, and estimated number of viable seeds recovered from manure of 4 sheep 10 days after dosing in Trial 1. Percent viability of control seeds = 98% ± 1.22 (SE). Percent viability included germinated seeds and seeds that tested positive with tetrazolium. Estimated number of viable seeds recovered was derived by multiplying the number of seeds recovered by percent viability. Values following means represent ± 1 SE.

Day after ingestion	Seeds recovered	Viability	Estimated viable seeds
	(No.)	(%)	(No.)
1	52 ± 20	14 ± 12	7.0
2	67 ± 36	0	0
3	29 ± 20	26 ± 15	7.4
4	16 ± 8	0.9 ± 0.9	0.1
5	13 ± 11	6 ± 6	0.7
6	7 ± 7	0	0
7	6 ± 5	2 ± 2	0.9
8	6 ± 4	0	0
9	2 ± 2	0	0
10	2 ± 1	0	0

ing numerous viable seed when the trial ended on day 10. Numbers of seeds recovered on day 4-10 were lower than the number of seeds recovered on day 2 ($P < 0.10$ for all comparisons).

We recovered 17% of the 5,000 spotted knapweed seeds in Trial 2. By the fifth day after dosing, we had recovered 99.5% of the seeds that passed (Table 3). Recovery of seeds decreased over time after dosing ($P < 0.001$).

Percent Viability

In Trial 1, percent viability of seeds recovered from mule deer (2–25%) and sheep (0–26%) manure was much lower than of the control seeds (98%). We recovered viable seeds from deer manure each day for the 10 day period after dosing. Viable seeds were not recovered from sheep after day 7. In Trial 2, seeds recovered from the manure dried at 50°C did not germinate and did not test positive with tetrazolium. Embryos recovered from seed in manure dried at this temperature were brown, and had a rubber-like texture when they were placed in tetrazolium. Embryos of control seeds were white with a firm texture.

In Trial 2, percent viability of seeds recovered from sheep manure dried at 35°C (0–22%) was lower compared with seeds which were not fed to sheep (88%). Although some seeds were still being recovered on day 10, seeds recovered after day 2 were not viable. Cotyledons from 4 seeds recovered 1 day after dosing developed, but their radicles did not, and thus they were classified as not viable. With tetrazolium, some cotyledons stained red whereas the associated radicle did not stain red, indicating that the radicle was dead.

Emergence

Few seedlings emerged from the manure placed on moist potting soil in Trial 1. From the manure of 1 deer, 5 and 10 spotted knapweed seedlings emerged from uncrushed and crushed manure, respectively. From another deer, 5 knapweed seedlings emerged from crushed manure, while only 1 seedling emerged from uncrushed manure. No seedlings emerged from sheep manure. With unfed seed, 92.7% (±1.83 S.E.) of the seeds established from soil.

No spotted knapweed plants emerged from any of the manure samples during the 1 month test period in Trial 2. With unfed seed, 42% (±3.90 S.E.) of the seeds established from soil.

Discussion

In both trials, over 84% of the excreted knapweed seeds were recovered within 5 days of dosing. With jointed goatgrass (*Aegilops cylindrica* Host), the number of joints recovered from the rumen decreases approximately 80% 48 hours after being fed to cattle (Lyon et al. 1992). The recovery of leafy spurge (*Euphorbia esula* L.) seeds in manure of sheep and goats also decreases over time (Lacey et al. 1992). Nonetheless, we were still recovering viable spotted knapweed seed in the deer manure when we ended Trial 1 after 10 days. Janzen (1981) found seeds of the guanacaste tree (*Enterolobium cyclocarpum* (Jacq.) Griseb.) in horse manure 70 days after the seeds were ingested.

Morphophysiological differences between sheep and deer may result in different residence times of seeds within the rumen. Hofmann (1989) characterized sheep as animals that consume grass and roughage, that graze for relatively long periods, and that ruminate for long periods during which forage is repeatedly chewed. Long ruminating periods may enhance seed destruction. Hofmann (1989) described mule deer as intermediate feeders, animals that mix grass and roughage with more concentrated feed represented by forbs and shrubs. By consuming less roughage, mule deer should ruminate less and thus pass more seed, which they did. However, they were continuing to pass seed 10 days after dosing, indicating that some of the seed may have been temporarily caught in folds within the reticulo-rumen, and are then released at sporadic intervals. Despite this long residence time, percent viability of seeds that were excreted in the latter days of the trial was no lower than percent viability of seeds that had passed through the gastrointestinal tract during the first three days.

Table 3. Number of spotted knapweed seeds recovered, percent viability, and estimated number of viable seeds recovered from washed subsamples of manure from 4 rams 10 days after dosing in Trial 2. Percent viability of control seeds = 88% ± 6.2 (SE). Percent viability included germinated seeds and seeds that tested positive with tetrazolium. Estimated number of viable seeds recovered was derived by multiplying the number of seeds recovered by percent viability. Values following means represent ± 1 SE.

Day after ingestion	Seeds recovered	Viability	Estimated viable seeds
	(No.)	(%)	(No.)
1	430 ± 46	22 ± 2	93
2	282 ± 27	0.3 ± 0.3	0.9
3	91 ± 19	0	0
4	26 ± 5	0	0
5	9 ± 3	0	0
6	1 ± 1	0	0
7	0	0	0
8	1 ± 1	0	0
9	1 ± 1	0	0
10	1 ± 1	0	0

Percent Viability

Initially, we thought that the viability of recovered seed from Trial 1 had been reduced by drying the manure samples at 50°C. Thus, in the second trial we compared oven drying at 50°C with drying at 35°C. Drying manure at 50°C in Trial 2 killed embryos of spotted knapweed seed. Embryos of these seeds were darker than embryos of unfed, unheated seed. However, viability of seed recovered from manure dried at 50°C in Trial 1 was similar to the viability of seed recovered from manure dried at 35°C in Trial 2. Apparently, drying at 50°C in Trial 1 had minimal effect on viability whereas it had a significant effect in Trial 2. These different responses could be attributed to water content of the manure, the length of time that the manure was stored before complete drying thereby allowing imbibition, or a combination of these factors.

Davis (1990) showed that seeds of spotted knapweed are fully imbibed after 13 hours and germinate within 18 hours. While dry seeds withstand heating to 50°C, imbibition and initiation of germination may be interrupted at high temperatures (Bradbeer 1988). The dark color of the embryos of recovered seeds in Trial 2 may have been due to the enzymes or proteins that were destroyed when the manure was heated at 50°C.

Viable seeds were recovered in deer manure 10 days after dosing, but few were recovered from sheep manure after 3 days. Workers collecting the manure observed that most of the deer feces were pellets whereas the sheep feces were patties. This indicates that deer feces were drier than sheep feces, therefore less moisture was available for knapweed seed imbibition. Oven drying may not kill unimbibed, or incompletely imbibed seeds.

Spotted knapweed seeds were not viable after 7 days inside the gastrointestinal system of sheep in Trial 1, whereas they were not viable after only 2 days in Trial 2. Blackshaw and Rode (1991) found that weed seeds are able to survive for a short period of time in the rumen but viability drops off rapidly. The period of exposure in the rumen varies for different weed species. They suggested that there may be a lag before rumen fluids degrade the embryo; however, this would not explain the viability of seed recovered from deer throughout the 10 day trial.

Emergence

In Trial 1, the manure was dried and stored for several months before it was placed on potting soil to test for emergence, which may have induced dormancy in the seed (Bradbeer 1988). More seedlings established from mule deer manure compared with sheep manure, which agrees with the greater number of seeds that passed through deer.

The lack of seedling emergence from manure placed on potting soil in Trial 2 may have been partly due to the plywood floors in the pens since urine was mixed with fecal material, which does not normally occur in the field. Excessive urine in the manure may have affected the emergence of spotted knapweed seedlings. Overall, emergence from these trials may not represent field conditions. By misting the flats daily in a greenhouse that only varied 8°C, we did not replicate moisture or temperature conditions that can fluctuate widely in the field, which can enhance germination (Bradbeer 1988).

Conclusions

Viable spotted knapweed seeds passed through the digestive systems of sheep and deer. Sheep manure contained viable spotted knapweed seeds up to 7 days after dosing in Trial 1. In the second trial, viable spotted knapweed seeds were found for only 2 days after dosing. From mule deer, we continued to recover viable seeds from their manure 10 days after dosing. Viability of seeds was reduced, but not eliminated, by passing through sheep and mule deer. Although few spotted knapweed seeds emerged from manure in the greenhouse, we believe that sheep and especially mule deer are likely to transport viable seeds of spotted knapweed and thus disseminate weed seeds. Managers cannot control the movements of deer, but can control the movements of sheep. Based on our 2 trials, we recommend that sheep be confined for at least 7 days after grazing a spotted knapweed infested area to allow viable seed to pass.

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