Survival of the Tall Fescue Endophyte in the Digestive Tract of Cattle and Horses

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ABSTRACT

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The endophytic fungus of tall fescue, *Acremonium coenophialum*, is naturally disseminated only by infected seed. Grazing livestock could serve as potential vectors of this fungus, and stockmen could unwittingly encourage its spread by inappropriate movement of animals between pastures. To test this possibility, a series of experiments were conducted in which cattle were fed a single dose or were allowed to graze ad libitum. Viable endophyte-infected (EI) seeds were recovered from the feces. Recovery of live endophyte reached a maximum of 2.68 EI seeds per gram dry weight (gdw) of feces at 22 hr after feeding a single dose. The last viable endophyte was recovered at 38 hr after feeding, and the last viable seed was recovered at 84 hr. When allowed to graze fescue with EI seed ad libitum, cattle feces contained about 1 EI seed per gdw while feeding and continued to pass decreasing amounts of EI seed for 72 hr after removal from the infected pasture. Horses allowed to graze ad libitum passed 0.37 EI seed per gdw. Both cattle and horses were effective vectors of the endophyte and may be a significant source of infection when they are allowed to graze seed and moved to endophyte-free pastures without a period of quarantine. A 3-day quarantine period should be sufficient to prevent the spread of EI seed.

Additional keywords: fescue toxicosis, *Festuca arundinacea*

Tall fescue (*Festuca arundinacea* Schreb.) has been shown to be extensively infected by an endophytic fungus (*Acremonium coenophialum* Morgan-Jones & W. Gams) that causes a range of health problems in grazing livestock, known collectively as fescue toxicosis (6,7). Livestock producers have taken precautions to plant uninfected seed in establishing new pastures and have gone to great expense to kill established stands of endophyte-infected (EI) fescue to replant with uninfected seed. This is a practical solution to the problem because the fungus does not sporulate in vivo and, to date, is known to be disseminated only by hyphae in EI seed that grow endophytically into young plants as the seeds germinate. In the absence of a source of infected seed, a pasture should remain uninfected for the life of the stand.

Siegel et al (7) reported that livestock were a potential source of reinfestation and demonstrated passage of infected seed when introduced into a steer via rumen cannula. Melouk et al (5) observed passage of viable sclerotia of *Sclerotinia minor* Jagger when cattle were fed infested peanut hay. Similar dissemination mechanisms have been observed for *S. sclerotiorum* (Lib.) de Bary in sheep (1) and *Sclerotium rolfsii* Sacc. in sheep and cattle (4).

We began to suspect that cattle might be a potential source of endophyte introduction when several pastures at the Alabama Agricultural Experiment Station exhibited inexplicable foci of endophyte infestation after years of being uninfected. Station personnel move cattle between pastures of varying infestation without a period of quarantine.

A series of experiments were designed to determine the chronology of passage of EI seed through cattle and to quantitate the numbers of EI seed in feces of cattle and horses under field conditions.

MATERIALS AND METHODS

Experiment 1. In this preliminary experiment, our objectives were to determine the rate of passage of fescue seed through the digestive system and to determine the effects of passage on viability of the seed and fungal endophyte. A single 200-kg steer was placed in a metabolism stall to monitor feed intake and fecal output. For 7 days, the steer was fed (twice daily) a meal consisting of 4.5 kg of chopped bermudagrass hay to which had been added 375 g of EI seed that had been killed by autoclaving. This was done to condition the animal to eating seed so that the treatment meal would not be refused, promote the evacuation of any live fescue seed from the animal, and establish a consistent rate of feed movement through the animal. On day 8, the steer was fed a single treatment meal containing 188 g of live EI seed mixed with the hay. For the remainder of the experiment, the previous diet was resumed.

A 7-day fecal collection period was begun 10 hr after consumption of the live EI seed. Sampling intervals were as follows: every 2 hr from 10 to 48 hr, every 4 hr from 48 to 96 hr, and every 6 hr from 96 to 168 hr. Total fecal output for each interval was collected and weighed and a single subsample was taken. All fecal samples were air-dried to approximately 5% moisture on a greenhouse bench at ambient temperature (30 ± 5 C), and 10 × 4 g replicates were planted in 15-cm plastic pots and covered with 1 cm of potting soil. Pots were watered regularly and maintained under favorable conditions for germination of the seed. Fecal percent dry matter was calculated by comparing fecal weights before and after drying. Emergence data were recorded at 8 wk after planting, and all plants were harvested and examined microscopically for endophyte in basal leaf sheath tissue (6). As a control, nonfed live EI seeds of the same seed lot were planted and examined in the same manner.

Experiment 2. This experiment was designed to measure the passage of EI seed under field conditions. Five steers weighing approximately 300 kg each were placed on an EI fescue pasture that contained viable seed. This field historically had been a source of viable endophyte and was monitored for seed development. Steers were placed on this pasture and allowed to graze ad libitum for 1 wk to obtain maximum voluntary seed ingestion. Fecal collection began immediately after removal of steers from the field and continued for 6 days. Animals were placed in individual stalls with concrete floors for 1 hr each morning, after which they were moved to an uninfected fescue pasture that had been carefully mowed to prevent seed formation. No attempt was made to monitor total fecal output. Instead, a single daily sample was taken. Drying, planting, and plant data were as for experiment 1.

Experiment 3. Five mares were placed on an EI field bearing viable seed and allowed to graze ad libitum for 1 wk as in the cattle experiment. Single samples were collected in the field. Mares were not removed from the pasture, so no attempt was made to measure the chronology of seed passage for horses.

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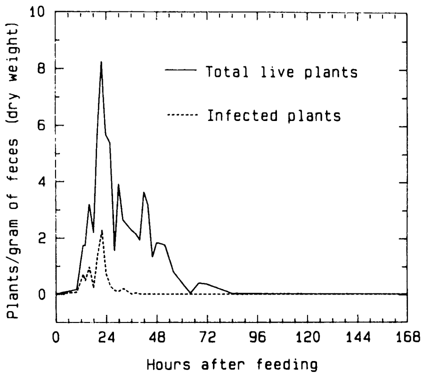

Fig. 1. Recovery of viable seed of tall fescue (*Festuca arundinacea*) and associated endophyte (*Acremonium coenophialum*) from feces of a steer fed a single meal of infected fescue seed.

Table 1. Recovery of live seed of tall fescue (*Festuca arundinaceae*) and associated endophytic fungus (*Acremonium coenophialum*) from feces of five steers allowed to graze ad libitum

Days after feeding	Plants per gram of feces[a]			Mean percent infection	Infected plants per gram
	Mean	Max	Min		
0[b]	1.13	1.40	0.82	85	0.96
1	0.98	1.70	0.43	56	0.55
2	0.20	0.39	0.10	35	0.07
3	0.03	0.17	0.00	5	0.002
4	0.00	0.00	0.00	0	0.00
5	0.00	0.00	0.00	0	0.00
6	0.00	0.00	0.00	0	0.00

[a] All weights expressed as grams dry weight.
[b] Sample taken immediately after removal from infected pasture.

Table 2. Recovery of live seed of tall fescue (*Festuca arundinaceae*) and associated endophyte (*Acremonium coenophialum*) from feces of five horses grazing ad libitum[a]

Horse ID	Plants per gram of feces[b]	Percent infection	Infected plants per gram of feces
1	0.38	88	0.33
2	0.61	89	0.54
3	0.39	100	0.39
4	0.13	100	0.13
5	0.70	67	0.47
Mean	0.44	88.80	0.37

[a] One-time sample of five mares at maximum voluntary seed ingestion.
[b] All weights expressed as grams dry weight. Mean of three subsamples per animal.

Instead, potential contamination with endophyte at maximum voluntary seed intake at a point in time was evaluated. Sample handling and all other procedures were as described earlier.

RESULTS

Experiment 1. Viable seeds were recovered from 10 to 84 hr after the treatment meal, with the mean maximum recovery of 8.25 live seeds per gdw at 22 hr (Fig. 1). Viable endophyte also reached a peak of 2.68 EI seeds per gdw at 22 hr after feeding but declined more rapidly. The last viable endophyte was recovered at 38 hr after feeding. Based on seed weight and germination tests, we estimate that the treatment meal contained 83,083 viable seeds. By extrapolation based on plants germinating from feces, we were able to account for the passage of 9,672 seeds or 11.64% of the treatment meal. By extrapolation, we recovered 1,142 viable EI seeds from the treatment meal, or 11.8% of recovered viable seeds were EI. Nonfed controls were 98% EI.

Experiment 2. Steers grazing ad libitum had a maximum of 0.96 EI seed per gdw of feces (Table 1), considerably less than the maximum of 2.68 EI seeds per gdw observed in the single-dose experiment. As would be predicted, both viable seed and viable endophyte declined after removal from the seed source, with the last viable seed and viable endophyte recovered on the third day after removal. As in the previous experiment, endophyte viability was more sensitive to digestive tract conditions than was seed viability. Endophyte viability declined to 5% by the third day, whereas nonfed controls were 98% infected in this test. Using total fecal output data from the previous experiment (1,948 gdw of feces per day), we estimate that a single steer grazing EI fescue with seed can pass 3,080 viable EI fescue seeds in the 3 days after it is removed from the source of infestation.

Experiment 3. Fecal seed content for horses grazing ad libitum was 0.44 viable seed per gdw and 0.37 EI seed per gdw (Table 2). This single observation would correspond to the data for day 0 sample for cattle, which was 1.13 and 0.96 seed per gdw, respectively. The lower seed content of horse feces was not attributable to grazing behavior because all horses were observed to eat seed of the fescue during the course of the experiment.

DISCUSSION

These data clearly demonstrate that cattle and horses are capable of transmitting the endophyte of tall fescue into uninfected pastures when animals are allowed to graze pastures or eat hay or other feed containing viable EI seed, animals are moved directly to NI pastures with no quarantine period, and favorable conditions allow the seed in the feces to germinate and form mature plants. Our steer data suggest that a quarantine period of 3 days in which EI seed are removed from the diet would be sufficient to prevent infestation of uninfected pastures. Conditions in the digestive tract of the steer had obvious deleterious effects on viability of the seed and endophyte, with the endophyte being more susceptible.

Although horses are less efficient vectors of the endophyte, they also are a potential source of infection. We were surprised to find fewer live seeds in feces of horses and can only speculate that chewing must be more efficient in the horse or some other digestive phenomenon must be the cause. Because we did not measure the chronology of passage in the mares, we can offer no firm recommendation of a quarantine period.

The kinetics of digestion in ruminants has been extensively investigated, and factors affecting the mean retention time (MRT) of digesta have been described (3). Increased feed intake, higher fiber, pregnancy, lactation, and lower ambient temperature all reduce MRT in ruminants, whereas the age of the animal and feed particle size appear to have variable or no effects on MRT. Although conditions that decrease MRT would theoretically decrease the quarantine period required to prevent the passage of EI seed, our primary considerations are fungal and seed viability, both of which decrease with prolonged retention times. This would suggest that the recommended 3-day quarantine period is adequate even when quarantine conditions might tend to increase MRT.

Finally, there is the matter of plant survival, which is not addressed by these experiments. Brown and Archer (2) studied the dispersal of mesquite (*Prosonis glandulosa* Torr.) in cattle dung and observed 16% more seedlings emerging from dunged areas than those without dung. More relevant, perhaps, was their observation that two grasses, *Dichanthelium oligosanthes* (Schult.) Gould and *Cenchrus incertus* M. A. Curtis were present only in gaps created by dung, apparently carried in the feces and nurtured by the gap in the canopy and the stimulation of the fertility from the dung. Our planting of the feces in potting soil certainly represents an optimization of seedling habitats, but we believe many of the seedlings would survive under favorable field conditions.

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