University of Nevada, Reno

Simulated Ruminant Digestion Reduces Germination of Some Native Great Basin Species and Cheatgrass

&

Virtual Fences Successfully Contain Cattle Over a Wide Range of Stocking Densities and at Stubble Heights Below Common Riparian Management Targets

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Abstract

This thesis reports the results of two projects related to the effective management of rangelands. Feeding seeds to cattle to be spread in feces has long been suggested, but the survival of seeds through the digestive tract varies widely and is species-dependent. I studied germination of seven species commonly used for restoration in the Great Basin, and cheatgrass, an invasive annual grass, after exposure to simulated ruminant digestion. Increasing rumen residence time decreased germination rates of all species tested. Previous research indicates that most seeds recovered in the feces of cattle are recovered 24-48 hours after ingestion. Of the species tested, only crested wheatgrass and squirreltail maintained appreciable levels of germination after 48 hours of digestion. These species may be suitable for spread by cattle. The viability of cheatgrass seed declined precipitously after 24 hours of digestion, indicating that cattle likely do not substantially contribute to the spread of cheatgrass through seed consumption.

Virtual fences are an emerging animal management technology that use audio cues followed by a mild electrical pulse instead of physical barriers to contain animals. Virtual fences have long been conceptualized as a tool to help land managers achieve livestock production or land management goals, yet little research has focused on figuring out what factors influence virtual fence performance. We evaluated the effect of stocking density, the quantity of forage inside the paddock and the difference between the quantities of forage inside and outside the paddock on the effectiveness of a commercially available virtual fencing system. We tested the virtual fencing system at stocking densities from 5-20 animals/acre and measured stubble height as a proxy for the quantity of forage inside the paddock and the difference between the quantities of forage inside and outside the paddock. The predictability and controllability of the electrical pulse have been identified as key components of animal welfare associated with virtual fences, so we also evaluated the effect of stocking density, forage quantity, and the difference in forage quantities inside and outside the paddock on predictability and controllability. We found that neither stocking density, forage quantity, nor the difference in forage quantity inside and outside the paddock influenced the effectiveness of virtual fences or the predictability and controllability of the electrical pulse. This implies that virtual fences are likely to be reliable tools for livestock management in productive settings and for stocking densities up to 20 animals/acre and when stubble heights are at or below common management targets.

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I want to thank the Bureau of Land Management, U.S. Fish & Wildlife Service Partners for Fish and Wildlife Program, the Nevada Agricultural Experiment Station, UNR Extension, and the Nevada Agricultural Foundation for their generous support of my research projects, as well as the Bureau of Land Management, U.S. Forest Service, Vence Corp., Maggie Creek Ranch and Cottonwood Ranch for their in-kind contributions, without which this project would not have been possible.

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Preface: A Tale of Two Projects

The original plan was that this thesis would end up describing a single cohesive project. Under that plan, I would have developed a seed coating that would protect seeds from damage during digestion, and the protein supplement fed to the cattle grazing cured cheatgrass in the fall and winter would have included these coated seeds. I would have managed these cattle with virtual fences. It soon became apparent that this was not a realistic plan. The development of a seed coating could not be completed on a timeline conducive to a field trial where results would take six months or more to develop while also giving me time to analyze the data, write a thesis, and generally complete my degree in a timely manner. Additionally, neither of the areas where we could experiment with virtual fences on the ranches with which my advisors and I collaborated had significant acreage dominated by cheatgrass. Ecologically this was a very good thing, but it was unhelpful for answering my initial research questions. Therefore, I took a piece from each project to create a new plan described in the two chapters of this thesis, which is focused on the potential effects of ruminant digestion on seed germination and the extent to which stock density affects virtual fence performance.

There is substantial interest from both federal and state natural resource management agencies and other rangeland stakeholders in both virtual fences and fecal seeding. There are a multitude of potential applications for using virtual fences to manage grazing cattle, including but not limited to the targeted grazing of cheatgrass, creation of grazed fuel breaks, exclusion of animals from recently burned areas, more efficient use of large grazing allotments in rugged terrain, and intensive riparian grazing. Seeds could be included in the diet of cattle grazing cured cheatgrass to restore degraded landscapes or provided to cows when typical restoration approaches are cost prohibitive or otherwise logistically unfeasible. I anticipated that ruminant digestion would decrease germination rates and that a protective seed coating would allow more species to be fecally seeded. When this thesis was written, seed coating development and testing was progressing with a collaborative group of researchers and a Ph.D. student at UNR and Brigham Young University.

Finally, the two chapters of this thesis were both written as manuscripts to be submitted to an academic journal. The manuscripts will include coauthors when submitted, and I therefore used plural first-person pronouns throughout this thesis.

General Introduction

Invasive annual grasses are a large and growing problem in the western United States. Cheatgrass (*Bromus tectorum* L.) is now highly abundant on over 210,000 km^2 of the Great Basin, putting vast areas at high risk of wildfire (Bradley et al. 2018). Cheatgrass invasion increases the amount and continuity of fine fuels, and invaded sites experience fires that are larger, more uniform, more frequent, and earlier in the season than non-invaded sites (Balch et al. 2013; Whisenant 1990). After fires, cheatgrass often becomes the dominant species on the site, and recovery of the former plant community composition and structure is unlikely, particularly in hotter and drier sites such as those found extensively throughout the Great Basin (Chambers et al. 2007; Davies et al. 2012; Knapp 1996; Taylor et al. 2014).

The influence of invasive annual grasses on fire regimes has created substantial interest in fire prevention and restoration technologies from both state and federal natural resource management agencies along with livestock producers. Targeted grazing of cured cheatgrass by cattle in the fall and winter has been demonstrated to effectively reduce fuels and control cheatgrass (Schmelzer et al. 2014), but grazing alone does little to reestablish desirable vegetation on sites lacking desirable species. Feeding seeds to animals to be dispersed in their feces has long been suggested as a way to revegetate degraded landscapes (Lehrer and Tisdale 1956). Cattle grazing cured cheatgrass are often provided with a protein supplement, which would be a convenient route by which to introduce the seeds of desirable species into their diet, but the survival of seeds through the digestive tract varies widely and appears to be species-dependent (Gardener et al.

1993). However, the ability of many of the species that are widely used for restoration in the Great Basin to survive digestion is unknown. The ability for cattle to spread seed and restore degraded landscapes would be a powerful tool to increase the impact of targeted grazing.

Targeted grazing often requires animals to be held at high stocking densities for short periods of time in frequently moved paddocks, a trait it shares with management-intensive grazing in riparian systems (Bailey et al. 2019; Shawver et al. 2020). Frequent paddock moves require a degree of flexibility that the relative permanence of conventional fencing is generally incapable of accommodating, at least without the prohibitively high cost of building a multitude of individual paddocks. This need for management flexibility has created significant interest in virtual fences, which have been the subject of ongoing research and development since at least the late 1980s and are now commercially available.

Virtual fences use the association between a benign audio warning cue and a mild electrical pulse to prevent animals from crossing boundaries as opposed to a physical barrier such as barbed wire. Virtual boundaries can be placed anywhere on the landscape with little more than a few keystrokes and mouse clicks and can be moved just as easily, which makes them perfectly suited to targeted or management-intensive grazing operations. However, the capabilities of virtual fences for the intensive management required for targeted or riparian grazing remain mostly unknown. Because technological capabilities determine potential management use cases, it is essential to evaluate the performance of virtual fences in these scenarios before widespread implementation proceeds.

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Chapter 1: Simulated Ruminant Digestion Reduces Germination of Some Native Great Basin Species and Cheatgrass

Abstract

Many plant species produce seeds that are eaten and spread by animals. Because substantial portions of the Great Basin are infested with cheatgrass (*Bromus tectorum* L.), there is interest in ways to reestablish desirable vegetation in cheatgrass dominated rangelands. Cattle grazing cheatgrass in the fall are typically fed supplemental protein, which provides a route to introduce seeds of desirable species into their diet. In order to evaluate the effect of rumen digestion on germination of species commonly used for restoration, we performed *in vitro* incubations using bovine rumen fluid followed by a germination trial on seeds of crested wheatgrass (*Agropyron cristatum* [L.] Gaertn. x *A. desertorum* [Fisch. ex Link] J.A. Schultes), Indian ricegrass (*Achnatherum hymenoides* [Roemer & J.A. Schultes] Barkworth), Snake River wheatgrass (*Elymus wawawaiensis* J. Carlson & Barkworth), squirreltail (*Elymus elymoides* [Raf.] Swezey), gooseberryleaf globemallow (*Sphaeralcea grossulariifolia* [Hood. & Arn.] Rydb.), yarrow (*Achillea millefolium* L.), and Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young). Because livestock eat cheatgrass this species was also included. Germination decreased as incubation lengths increased for all species. Previous research indicates that peak rates of seed recovery in cattle feces occurs 24-48 hours after ingestion. Crested wheatgrass and squirreltail maintained high germination after 48 hours and are good candidates for fecal seeding. Yarrow and Snake River wheatgrass had high germination at 24 hours but low germination at 48 hours.

Germination of Wyoming big sagebrush reached 0% within 24 hours of incubation, and Indian ricegrass and gooseberryleaf globemallow had low germination regardless of incubation length. Cheatgrass germination was slightly reduced by 24 hours, but was reduced to 2% after 48 hours. We conclude that the spread of cheatgrass resulting from seeds consumed by cattle is possible, but unlikely, particularly given slower ruminal passage rates of low quality forages such as cured cheatgrass.

Introduction

Invasive annual grasses are a large and growing problem in the western United States. Cheatgrass (*Bromus tectorum* L.) is now highly abundant on over 210,000 km^2 of the Great Basin, putting vast areas at high risk of wildfire (Bradley et al. 2018). Cheatgrass invasion increases the amount and continuity of fine fuels, and invaded sites experience fires that are larger, more uniform, more frequent, and earlier in the season than non-invaded sites (Balch et al. 2013; Whisenant 1990). After fires, cheatgrass often becomes the dominant species on the site, and recovery of the former plant community is unlikely, particularly in hotter and drier sites such as those found extensively throughout the Great Basin (Chambers et al. 2007; Davies et al. 2012; Knapp 1996; Taylor et al. 2014).

The control of cheatgrass and the restoration of burned cheatgrass-dominated rangelands to native vegetation are both topics of extensive and ongoing research. Conventional restoration reseeding practices have a low success rate (James et al. 2011), creating significant interest in technologies or approaches that could improve success (Madsen et al. 2016). Fall and winter grazing has been demonstrated to effectively control cheatgrass (Schmelzer et al. 2014), however, grazing alone does nothing to reestablish preferred vegetation on rangelands lacking desirable species. Cheatgrass is widely regarded as poor quality forage once it has cured, and cattle are often fed a protein supplement to increase the digestibility and palatability of cured cheatgrass as well as maintain desired levels of animal performance (Heldt et al. 1999; DelCurto et al. 2000).

Fecal reseeding of degraded landscapes by feeding seeds to grazing livestock has been suggested for decades (Lehrer and Tisdale 1956), and a large number of studies have shown viable seed in the feces of animals, but these studies include results from several invasive weeds (Lacey et al. 1992; Lehrer and Tisdale 1956; Simao Neto et al. 1987; Wallander et al. 1995). Seeds deposited in a fecal pat occupy a microsite with enhanced organic matter content, fertility, and water holding capacity which should enhance seedling establishment and survival (Ocumpaugh et al. 1996). Unfortunately, seed mortality during digestion often limits the potential success of this strategy, with limited exceptions that are primarily legumes with high degrees of hardseededness, a dormancy mechanism common in legumes (Gardener et al. 1993a, b). However, fecal seeding shows promise for revegetating degraded rangelands for species that survive digestion (Shinderman and Call 2001), and the protein supplement provided to cattle grazing cured cheatgrass would provide a convenient route by which to introduce seeds into their diet.

Recovery of seed fed to cattle generally peaks 24-48 hours after being fed (Burton and Andrews 1948; Doucette et al. 2001). Seeds can be recovered after as little as 12 hours or as long as 10 days after ingestion, however, very few seeds are generally recovered after four days (Burton and Andrews 1948; Doucette et al. 2001; Gardener et al. 1993a). Based on estimates of transit time through sections of the digestive tract (Wylie et al.

2000), seeds excreted in feces 24-48 h after ingestion will spend approximately 60-80% of their total residence time in the rumen. Survival in the digestive tract varies widely among species. Viable seeds have been recovered in feces up to 10 days after ingestion, while other species do not germinate at all after only six hours (Gardener et al. 1993b; Wallander et al. 1995), but germination rates typically have a negative relationship with residence time in the digestive tract (Gardener et al. 1993b; Hogan and Phillips 2011). For some species, germination rates may initially be increased by digestion before being reduced (Gardener et al. 1993b), a biological phenomenon known as hormesis. Seeds are exposed to multiple digestive processes in the ruminant digestive tract, but a majority of the damage to seeds occurs in the rumen. After in vitro simulation of digestion in the rumen and abomasum on four legume species, Simao Neto and Jones (1987) observed that at least 73% of the total reduction in germination was due to damage in the rumen. Damage in the rumen exceeded 89% of the total observed reduction of germination for three of the species they tested.

The objective of this study was to evaluate how residence time in the rumen influences the germination rates of species commonly used for restoration in the Great Basin and cheatgrass. Species selected for this study were bunchgrasses ‘Hycrest’ crested wheatgrass (*Agropyron cristatum* [L.] Gaertn. x *A. desertorum* [Fisch. ex Link] J.A. Schultes), ‘Nezpar’ Indian ricegrass (*Achnatherum hymenoides* [Roemer & J.A. Schultes] Barkworth), Snake River wheatgrass (*Elymus wawawaiensis* J. Carlson & Barkworth), and squirreltail (*Elymus elymoides* [Raf.] Swezey), along with perennial forbs, gooseberryleaf globemallow (*Sphaeralcea grossulariifolia* [Hood. & Arn.] Rydb.) and yarrow (*Achillea millefolium* L.), a shrub, Wyoming big sagebrush (*Artemisia tridentata*

Nutt. ssp. *wyomingensis* Beetle & Young), and cheatgrass, which we included because one recent study found that feral horses may spread cheatgrass (King et al. 2019).

Methods

Seed Sources

Seed samples of most species were purchased from a commercial supplier (Great Basin Seed, Ephraim, UT). Wyoming big sagebrush and cheatgrass were hand collected. Wyoming big sagebrush was collected in Humboldt county, NV, 60 km NE of Winnemucca, and cheatgrass was collected in Washoe county, NV, 23 km NW of Reno. Both collections were cleaned to remove as much inert particulate matter as possible. Seeds were stored at 5^{O} C from the time of purchase or collection until the study took place.

In vitro Incubations

The *in vitro* fermentation technique was previously described in detail (Tedeschi et al. 2009). Rumen fluid was collected in a pre-warmed, insulated thermos from four cannulated steers fed an ad libitum mix of alfalfa and grass hay. All steers were healthy in the weeks leading up to and at the time of rumen fluid collection. After collection, rumen fluid was filtered through six layers of cheese cloth to remove particulates and mixed with dH_2O and in vitro buffering media (Goering and Van Soest 1970) in the standard ratio of one part dH_2O, seven parts in vitro buffering media, and two parts filtered rumen fluid, with continuous CO_2 flushing throughout the entire process.

Each of the 160 mL serum bottles was flushed with CO_2 to create an anaerobic atmosphere after 1.00 g of seed was weighed into the bottle. Bottles were crimp sealed with lightly greased rubber stoppers. Each bottle was injected with 100 mL of the rumen fluid-media solution using a strict anaerobic technique. Pressure was equalized by inserting a needle into the rubber stoppers for approximately five seconds. Bottles were promptly placed into a 39.0 ^{O}C shaking incubator rotating at 95 RPM, and then incubated for 12, 24, 48, or 96 hours, according to the randomly assigned treatment. There were four replicate bottles of each species-time combination.

Germination Test

Seeds were removed from rumen fluid-media solution and rinsed with dH_2O to remove residue. A subsample of either 100 or 150 seeds was removed for testing germination, along with four replicate untreated samples of 150 seeds. Subsamples were obtained by individually picking seeds out of the entire sample, with no intentional selection or avoidance of particular seeds or seed characteristics. Fifty seeds were placed in a petri dish on top of three layers of paper towels that had been fully wetted and then drained under gravity for several seconds. This resulted in either two or three petri dishes per species-time combination. Petri dishes were sealed with parafilm to prevent dehydration and randomly placed in a growth chamber at 22 ^{O}C with a 16 h/8 h light/dark cycle, conditions close to the optimum range for germination of at least several species in the study (Buman and Abernethy 1988; Young et al. 2003). After 14 days, petri dishes were removed from the growth chamber, opened, and germinated seeds were counted and removed. Petri dishes were then resealed and placed back in the growth chamber for an additional seven days, when they were removed, opened, and counted again. Seeds were counted as germinated when there was both radical extension and shoot growth.

Data Analysis

Nonlinear regression with sigmoidal dose-response models is often used to model the response of an organism to a stressor, with the log-logistic model being the most common (Van der Vliet and Ritz 2013). Three sigmoidal models, the log-logistic model and two asymmetric extensions, were fit to the data using the package ‘drc’ (Ritz et al. 2015) in R version 4.1.0 (R Core Team 2021). The best fitting model function was selected using AIC and compared to a simple linear model with no effect of incubation time with a likelihood ratio test to test for effect of incubation time.

Log-logistic model

The log-logistic model is defined by the equation

$$f(x, b, d, e) = \frac{d}{1 + \exp(b(\log(x) - \log(e)))}$$

where x is the length of *in vitro* incubation, b is a slope parameter, d is the upper asymptote, reflecting the germination rate when incubation length is 0, and e is the inflection point of the function and corresponds to the length of incubation required to reduce germination by 50%.

Asymmetric log-logistic model

The asymmetric log-logistic model is defined by the equation

$$f(x, b, d, e, f) = \frac{d}{(1 + \exp(b(\log(x) - \log(e))))^{f}}$$

where x, b, and d, are all as described in the log-logistic model, e is no longer directly interpretable, and f is an asymmetry parameter. When $f > 1$, the descent from the upper asymptote is more gradual and the approach to zero more rapid when compared to the log-logistic model, and when $f < 1$, the descent from the upper asymptote is more rapid and the approach to zero is more gradual than the log-logistic model. Note that in the special case of $f = 1$ the model is equivalent to the log-logistic model.

Brain-Cousens hormesis model

The Brain-Cousens hormesis model was developed to model situations where low exposure of an organism to a stressor stimulates response (Brain and Cousens 1989), and is defined by the equation

$$f(x, b, d, e, f) = \frac{d + fx}{1 + \exp(b(\log(x) - \log(e)))}$$

where x, b, and d are all as described in the log-logistic model, e is not directly interpretable, and f is an asymmetry parameter reflecting the degree of hormesis. $f > 0$ is required for hormesis (Nweke and Ogbonna 2017), so in cases when the model fit resulted in an estimate of $f < 0$, estimation of f was constrained to be ≥ 0 and the model was refit. Note that in the special case of $f = 0$, the model is equivalent to the log-logistic model.

Results

In vitro incubation reduced germination for all species ($p = 0.02$ for Indian ricegrass, < 0.0001 for all other species; Table 1). The germination rates of two species, yarrow and cheatgrass, were enhanced by short incubation times (Fig. 1). Germination rates for all species after 24 h and 48 h of simulated digestion are shown in Table 2.

Table 1: Selected model and results of the likelihood ratio test (LRT) of the selected model and a simple linear model with a slope of zero, showing no effect of digestion length on germination of seeds of seven species commonly used for restoration in the Great Basin and cheatgrass, following *in vitro* simulation of rumen digestion after a 21-day germination period.

Species	Selected Model	LRT	
		X^2	*p*-value
Crested wheatgrass	Asymmetric log-logistic	848.9	< 0.0001
Indian ricegrass	Log-logistic	7.6	0.02
Snake River wheatgrass	Asymmetric log-logistic	890.0	< 0.0001
Squirreltail	Asymmetric log-logistic	1037.7	< 0.0001
Gooseberryleaf globemallow	Log-logistic	40.6	< 0.0001
Yarrow	Brain-Cousens hormesis	917.8	< 0.0001
Wyoming big sagebrush	Log-logistic	808.7	< 0.0001
Cheatgrass	Brain-Cousens hormesis	1801.9	< 0.0001

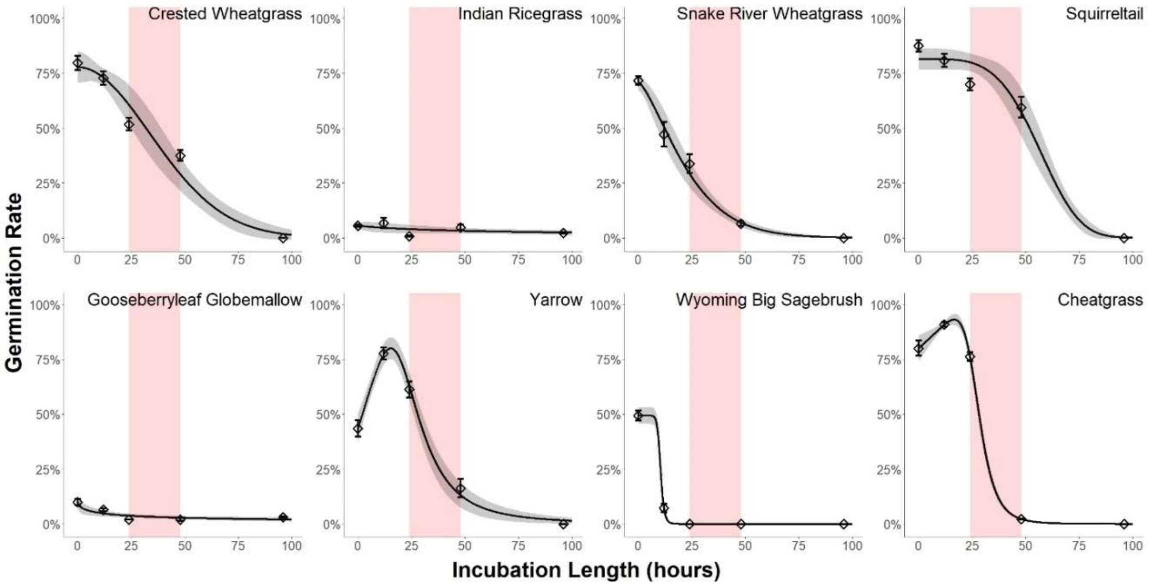

Figure 1: Germination rates of seeds from seven species commonly used for restoration in the Great Basin and cheatgrass after *in vitro* simulation of rumen digestion and a 21-day germination period. Error bars are ± one standard error. Nonlinear regression lines are shown with 95% confidence interval. The region highlighted in red represents the time after ingestion when most seeds are recovered (Doucette et al. 2001).

Table 2: Germination rates (mean ± standard error) of seven species commonly used for restoration in the Great Basin and cheatgrass after 0, 24, and 48 h of simulated rumen digestion and a 21-day germination period.

Species	Simulated Rumen Digestion Length					
	0 h		24 h		48 h	
	Germination	SE	Germination	SE	Germination	SE
Crested wheatgrass	80%	3.3	52%	2.8	38%	2.4
Indian ricegrass	6%	0.6	1%	0.3	5%	1.3
Snake River wheatgrass	72%	1.9	34%	4.3	7%	1.2
Squirreltail	88%	2.6	70%	2.7	60%	4.7
Gooseberryleaf globemallow	10%	1.4	2%	0.0	2%	0.7
Yarrow	44%	3.8	61%	3.7	16%	4.3
Wyoming big sagebrush	49%	2.2	0%	0.0	0%	0.0
Cheatgrass	80%	3.5	76%	2.0	2%	0.2

Discussion

Except for yarrow and cheatgrass, which both exhibited pronounced hormesis, germination rates were reduced for all species as incubation time increased, but the rate of decline depended on the species. These findings are consistent with findings from a wide variety of species from across the world (Burton and Andrews 1948; Gardener et al. 1993b; Lacey et al. 1992; Simao Neto and Jones 1987; Whitacre and Call 2006).

When seeds are fed to cattle, recovery in feces is highest 24-48 h after ingestion (Burton and Andrews 1948; Doucette et al. 2001), however, cattle in most studies examining the passage of seeds were fed a diet of alfalfa and grass hay or straw. The ruminal passage rate is slower for lower quality forages than it is for higher quality forages such as alfalfa (Kammes and Allen 2012), although provision of a protein supplement does increase rate of passage and decrease retention time for animals grazing low quality forages (Caton et al. 1988). It is likely that the lower quality diet of animals grazing cured cheatgrass will result in seeds being retained in the rumen and digestive tract longer than previous studies have reported. However, passage of feedstuffs or ingested seeds from the rumen and through the digestive tract is a complicated and dynamic process that may substantially influence interpretation of the results of the current study. Examination of the passage rates of seeds when animals are fed cheatgrass-based diets should be a priority for future research related to the potential for using fecal seeding to restore cheatgrass-invaded rangelands.

Diet is not the only factor influencing rate of passage. Gardner and colleagues (1993a) found that, for legumes, larger seeds with high specific gravities passed through the digestive tract faster than small seeds or seeds with low specific gravities (range of sizes 1.0-8.4 mm x 0.8-6.0 mm, specific gravity 0.91-1.36). Using stepwise regression, they demonstrated that specific gravity explained 49.3% and seed size explained 11.2% of the observed difference between the rates of passage of legumes fed to cattle. Rates of passage did not differ among the three grass species for which they calculated rate of passage (range of sizes 2.0-5.5 mm x 0.5-2.9 mm, specific gravity 0.88-1.27) (Gardener et al. 1993a). Many of the legumes they tested have seeds that are larger and rounder than most grass seeds, both of the species they tested and that we used in this study. The long, narrow grass seeds may remain suspended in the rumen longer and not be passed from the rumen as quickly as large, round legume seeds. This indicates that diet is not the only determinant of the passage rate of seeds through the digestive tract. Seed characteristics may also influence residence time in the rumen and may need to be examined on a species-by-species basis.

Even though seed recovery rates are highest between 24 and 48 h after feeding (Burton and Andrews 1948; Doucette et al. 2001), germination after 48 h of simulated digestion is probably more representative of seed mortality likely to be incurred when seeds are fed to cows grazing cured cheatgrass because of the slower passage rates of low quality diets. Germination rates of squirreltail and crested wheatgrass had high germination rates after 48 h of (60% and 38%, respectively) and germination of these two species was markedly higher than the rest of the species studied. Squirreltail and crested wheatgrass are likely good candidates for fecal seeding. Germination of Snake River wheatgrass and yarrow was low after 48 h (7% and 16%, respectively). These species are likely poorly suited to fecal seeding by cattle, although much higher germination after only 24 h may mean that these species could be spread by cattle through fecal seeding in some circumstances. That being said, higher seed mortality during digestion means fewer established plants per unit of seed ingested. Species with moderate-low survival rates will reduce the efficiency of revegetating landscapes in this manner and take more time to achieve site management goals. Sagebrush seed was quickly killed during digestion, and Indian ricegrass and gooseberryleaf globemallow both had germination rates below 10% regardless of incubation length.

Both Indian ricegrass and gooseberryleaf globemallow are known to exhibit extensive seed dormancy that can limit successful establishment. Indian ricegrass exhibits both mechanical and physiological dormancy, whereas dormancy in gooseberryleaf globemallow is primarily moderated by an impermeable seed coat (Jones 1990; Kildisheva et al. 2011; Smith and Kratsch 2009). Scarification may be used to reduce dormancy and enhance germination in both species (Jones 1990; Kildisheva et al. 2011; Smith and Kratsch 2009). The results presented here indicate that exposure to the rumen environment does not provide the scarification necessary to break dormancy and enhance the germination of these species. It is unknown whether the low germination rates we observed were caused by continued dormancy, seed mortality caused by digestion, or a combination of the two. However, germination of both species is enhanced by treatment with sulfuric acid (McDonald Jr. and Kahn. 1977; Roth et al. 1987). Assuming that dormant seeds are not killed in the rumen, the exposure of seeds to acid in the abomasum may enhance the germination of these species. Alternatively, dormancy could have been broken if the seeds were exposed to the same real-world conditions that normally end their dormancy, and actual seed viability may have been much higher than we observed.

If rumen digestion does not break their dormancy, cattle may still be able to fecally spread these species, assuming managers are willing to wait for their dormancy to break, but further research is needed to determine if this is possible.

Seed dormancy mechanisms are extremely diverse, with individual species often having multiple mechanisms regulating dormancy (Adkins et al. 2002; Farley et al. 2013; Taylor 2005), and it is mostly unknown which dormancy mechanisms and/or combinations would be affected by digestion or may provide any protection from damage during digestion. Although there is evidence that hardseededness in legumes provides protection from digestion, the magnitude of this effect was still dependent on species (Gardener et al. 1993a). The potential for survival or enhancement of germination in species that exhibit extensive dormancy will likely depend on species-specific dormancy characteristics.

The dispersal of weed seeds by livestock becomes a concern once feeds or pasture contain weed inflorescences or mature seed (Hogan and Phillips 2011). To limit the spread of weeds by livestock, it is recommended to confine livestock or withhold weed seed-containing feedstuffs until seeds have either been passed or killed in the digestive system (Hogan and Phillips 2011). A minimum of three, and preferably seven, days of confinement or withdrawal from contaminated feeds was recommended as a general rule in a review by Hogan and Phillips (2011), and a five-day confinement period has also been recommended for sheep and goats grazing leafy spurge (Lacey et al. 1992). In the spring, prior to seed production, there are no mature seeds on cheatgrass plants and seed dispersal by cattle should not occur. Cheatgrass seeds mature in late spring or early summer, approximately when plants take on their characteristic red-purple color, and drop shortly thereafter (Klemmedson and Smith 1964; Hulbert 1955). However, seeds require a period of dry after-ripening to break dormancy, which happens by the fall (Meyer et al. 1997). Following after-ripening, seeds can germinate in the fall or following spring, although a small number of seeds carry over in the seedbank to germinate in future years (Smith et al. 2008). During the inflorescence stage and until seed dispersal, cheatgrass florets have barbed lemmas and awns (Hulbert 1955), which can cause injury to livestock. Because of this, the palatability of cheatgrass is low between the inflorescence stage and seed maturity and dispersal, and the consumption of cheatgrass seed by cattle is low (Mealor et al. 2013). However, individual cheatgrass plants can produce more than one hundred seeds (Chambers et al. 2007), and it is likely that cattle grazing cheatgrass in the fall and winter will consume some cheatgrass seed. This, combined with the fact that some seed is likely to be passed through the digestive tract in under 48 h, indicates that it may be possible for cattle to spread cheatgrass by consuming seeds. That being said, low seed consumption, combined with a precipitous decline in germination after 24 hours in the rumen indicates that, although the spread of consumed cheatgrass by cattle may be possible, it is unlikely that cattle substantially contribute to the spread of cheatgrass by consuming seeds, particularly given slow passage rates of low quality forages such as cured cheatgrass. Additionally, rapid declines in cheatgrass germination after 24 h of rumen digestion may indicate that producers may not need to confine animals after grazing mature cheatgrass for as long as they would if cattle had been grazing other weed species.

Seed coatings have been extensively used to overcome a wide variety of environmental limitations to seedling establishment and restoration success (Madsen et al. 2016). With further research, it may be possible to develop a seed coating that will protect seeds from damage during their passage through the digestive tract, allowing virtually any species to be coated for fecal seeding. This would provide land managers with a powerful tool for landscape restoration and livestock producers a tool for pasture improvement. However, the quantity of seed consumed will limit the rate of spread for both coated and uncoated seed. The success of fecal seeding may take longer to become evident than is typical for (re)seeding projects.

Implications

Digestion by cattle reduced seed viability for all species studied, but the rate of decline depended on the species, and both yarrow and cheatgrass had an initial increase in germination before germination rates declined after longer incubation. However, both crested wheatgrass and squirreltail may be well suited for dispersal by cattle through fecal seeding. Protein supplement fed to cattle grazing cured cheatgrass provides a convenient route by which to introduce seeds into their diet, but there are remaining questions that have the potential to determine the real-world feasibility of the strategy. Consumption of seeds in protein supplement and damage by mastication, the passage rate of cheatgrass-based diets, the ability for seeds to germinate and establish in the fecal pat, and the number of seeds that must be consumed for effective dissemination will all influence if and in what circumstances fecal seeding is a useful restoration tool. These questions likely cannot be adequately addressed in the lab and will require field studies in rangeland ecosystems. The development of a seed coating that would protect seeds from damage in the digestive tract would likely allow a greater diversity of species to be fecally seeded and would also be a fruitful subject for future research.

The spread of cheatgrass by cattle consuming seeds is unlikely but may be possible, and the rapid rate of seed mortality after 24 h means that the period of confinement or feed withdrawal can likely be shorter for cheatgrass than for other weed species. However, it should be noted that the same unknowns surrounding seed damage during mastication, the passage rate of cheatgrass-based diets, and the ability of seeds to germinate and survive in the fecal pat also apply to cheatgrass. Further study in field settings may be warranted.

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Chapter 2: Virtual Fences Successfully Contain Cattle Over a Wide Range of Stocking Densities and at Stubble Heights Below Common Riparian Management Targets

Abstract

Virtual fencing is an emerging animal management technology that uses audio cues followed by a mild electrical pulse instead of physical barriers to contain animals. Virtual fencing has long been conceptualized as a tool to help land managers achieve livestock production or land management goals, yet little research has focused on factors that influence virtual fence performance. We evaluated the effect of stocking density, the quantity of forage inside the paddock and the difference between the quantities of forage inside and outside the paddock on the effectiveness of a commercially available virtual fencing system. We tested the virtual fencing system at stocking densities from 5-20 animals/acre and measured stubble height as a proxy for the quantity of forage inside the paddock and the difference between the quantities of forage inside and outside the paddock. The predictability and controllability of the electrical pulse have been identified as key components of animal welfare associated with virtual fences, so we also evaluated the effect of stocking density, stubble height, and the difference in stubble heights inside and outside the paddock on predictability and controllability. We found that neither stocking density, forage quantity, nor the difference in forage quantity inside and outside the paddock influenced the effectiveness of virtual fences or the predictability and controllability of the electrical pulse. This implies that virtual fences are likely to be reliable tools for livestock management in productive settings and for stocking densities

up to 20 animals/acre even when stubble heights are at or below common management targets.

Introduction

Effective management of livestock distribution is a persistent challenge for livestock producers and land managers alike (Launchbaugh and Howery 2005), but provides substantial opportunities for positive outcomes when done well (Creamer et al. 2020). Following its invention in the 1870s, the use of barbed wire to fence pastures quickly became a widely used and effective tool to manage livestock distribution (Hayter 1939), and today fences are still often used to successfully meet certain livestock distribution objectives (e.g. keeping livestock in grazing allotments and pastures and protecting springs or other sensitive resources). However, fences have numerous disadvantages, including adverse impacts on wildlife populations (Jakes et al. 2018), high cost (Edwards et al. 2012), and structural permanence leading to a lack of management flexibility once built. There is significant interest in alternative technologies such as virtual fences for livestock management from not only livestock producers, but also federal and state natural resource management agencies and a variety of other rangeland stakeholders.

Whereas conventional fences employ a physical barrier to restrict animal movement, virtual fences rely on associative learning between an audio cue and an aversive, but not harmful, electrical pulse to deter animals from crossing boundaries created by the user through an online user interface (Umstatter 2011). An animal-borne device, usually a GPS collar, delivers the audio and electrical cues to the animal as it approaches or attempts to cross the boundary. Virtual fencing research and development has been ongoing since at least the late 1980s (Fay et al. 1989). Recent technological advances have allowed for rapid progress in the last 5-10 years, and virtual fencing systems are now commercially available.

The creation of virtual fence boundaries through a central user interface allows for a high degree of flexibility; boundaries can be created anywhere on the landscape with just a few mouse clicks, and moved just as easily. This flexibility has created interest for using virtual fences as a tool to implement targeted or management-intensive grazing, where animals are often held at high stocking densities for short periods of time in frequently moved paddocks (Bailey et al. 2019; Shawver et al. 2020).

Evaluating Virtual Fence Effectiveness

Since the beginning, virtual fence research has focused on management outcomes (e.g. Fay et al. 1989), and virtual fencing has been considered as a tool to achieve those outcomes. Previous studies have assessed the effectiveness of virtual fencing systems using the percentage of reported GPS locations that are inside the paddock (Boyd et al. 2022; Campbell et al. 2020), which may provide insight into livestock habitat use and may be useful to assess whether management goals have been met in some circumstances. While this approach is appropriate for certain applications and questions, it has several disadvantages for broad applicability, particularly as virtual fencing studies are implemented at ranch scales and over longer periods of time, where routine management activities or seasonal resource changes (e.g. drying of spring water sources fed by snowmelt) may alter livestock habitat use. While it is rather simple to make a methodological note that the collaborating livestock producer gathered animals which had escaped the boundary and returned them to the paddock every four days, for example, it is much more difficult to quantify the impact of such management actions on the results in statistical analyses. It will be more difficult still to compare the results obtained in one study to those of another study where cattle may only have been returned to the paddock weekly. Worse yet, ranches may not run on consistent schedules, and it is improbable that escaped animals will be returned to the paddock consistently during the course of a weeks- or months-long study. There are also many non-fence methods used to alter livestock distribution and habitat use (e.g. strategic placement of supplement and/or water) (Creamer et al. 2020) that could further skew results, depending on their implementation. Additionally, virtual fences operate as one-way gates, meaning that an animal receives the audio and electrical cues as it leaves the paddock, but can freely reenter at any time without receiving either cue. Designing the virtual paddock so that the only nearby water source is inside the paddock will require that escaped animals re-enter and get recaptured by the virtual fence in order to drink. However, it may not always be possible to ensure that there is only a single water source and that it is located inside the paddock, and cattle may be less likely to return if they can access all necessary resources outside the paddock. The likelihood that differences in livestock management practices or resource availability have the potential to influence location-based estimates of virtual fence effectiveness indicate that other measures of effectiveness should be considered and investigated.

A metric that is less sensitive to management actions, the spatial pattern of habitat use, or the location of available resources would have broader applicability than simply reporting the percentage of the time livestock were inside the designated paddock. Expressing effectiveness as whether or not an animal is successfully held inside the paddock or escapes through the boundary each time the animal interacts with the boundary and receives at least the audio cue would be less sensitive to the factors mentioned above. While it makes sense to combine virtual fences with other sound management strategies in order to achieve grazing management objectives, their combination may result in location-based performance estimates that may not be applicable to other operations with different resources or when implemented with different management strategies. Thus, being able to more accurately determine the effectiveness of the virtual fence relative to other management actions becomes more important.

When virtual fences are consistently successful at turning animals around and keeping them from escaping through the boundary, interaction-based and GPS-based effectiveness measures will be highly correlated because there will be few, if any animals escaping and spending time outside the paddock. However, as interaction-based measures of effectiveness decline, GPS-based measures exhibit greater potential variability due to differences in management or resource availability and distribution both inside and outside the paddock (Fig. 1). Location-based measures of actual habitat use may be informative about the achievement of some management goals and the resultant ecological outcomes, but care should be taken before equating quantitative estimates of management outcomes with the virtual fence successfully turning animals around and preventing escapes on a consistent basis.

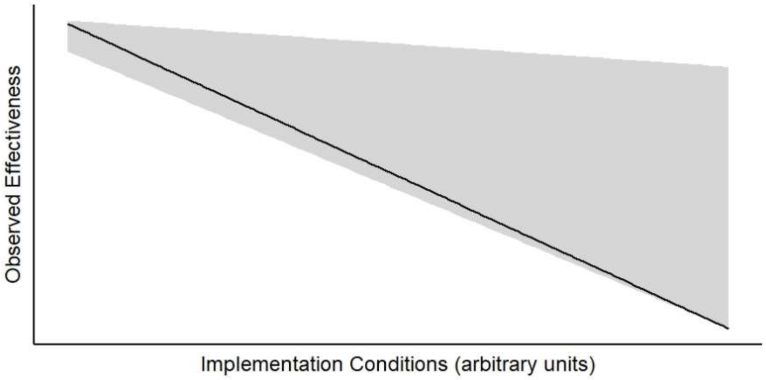

Figure 1: Proposed conceptual relationship between observed virtual fence effectiveness (y-axis) when comparing an interaction-based metric evaluating whether an animal was or was not successfully kept inside the paddock by the virtual fence (solid black line) with metrics based on realized land use calculated from GPS locations (gray shaded region). When the interaction-based metric is high, GPS-based effectiveness measures are also high, but as effectiveness declines, GPS-based measures exhibit increased potential variability due to management or resource distribution related issues (referred to as Implementation Conditions on the x-axis).

Evaluating Welfare Outcomes from Virtual Fences: Predictability and Controllability of the Electrical Pulse

The welfare impacts of using an electrical pulse as negative reinforcement to deter animals from crossing the boundary has been of interest since the start of virtual fencing research, particularly in some European markets (Umstatter 2011). The electrical pulse has been demonstrated to be no more stressful than standard animal restraint and management procedures (Kearton et al. 2019), but a recent framework for evaluating the welfare outcomes associated with virtual fences proposed that it was the ability of animals to predict and control the receipt of the electrical pulse that determined stress and welfare outcomes, not the electrical pulse itself (Lee et al. 2018). The purpose of the audio cue is to provide this predictability by allowing animals to stop or turn and retreat from the boundary in order to avoid the electrical pulse (Lee et al. 2009). This study is the first to attempt to quantify and evaluate the predictability and controllability of virtual fences.

Real-world environments provide animals with a multitude of cues which they use to inform their behavior (Launchbaugh and Howery 2005). Cues may signal the imminent occurrence of one or several possible outcomes and change in salience and relevance for behavior according to how reliably they predict future events (Esber and Haselgrove 2011). Therefore, the key to predictability is not simply that the cue chronologically precedes the pulse, per se, but that the association formed between the cue and the pulse is such that the cue tells the animal something about the near future. Some of the studies foundational to the framework for evaluating welfare outcomes of virtual fences were lab studies of rats. Conditions during lab trials with rats may be controlled tightly enough so that stating that the cue always precedes the pulse may be equivalent to animals accurately predicting the near future, and some researchers have chosen to define predictability in this way (Lee and Campbell 2021). However, field trials of virtual fences do not take place in a tightly controlled environment, and this linguistic choice of definition shifts emphasis away from the knowledge and its behavioral implications. An alternative definition of predictability in a virtual fence context would be that the animal knows that the audio cue means that it has reached a boundary and will receive the electrical pulse if it keeps moving forward. The animal must alter its behavior (i.e. stop forward motion and/or turn and retreat from the boundary) if it is to avoid the electrical pulse (or terminate its continued delivery). The ability of an animal to do so has been defined as controllability (Lee and Campbell 2021).

While it may be difficult to separate learning and motivation in some circumstances (e.g., did the animal receive electrical pulses because it was unable to avoid them, or because it judged resources outside the paddock as worth the risk?), much of behavior stems from learning (Launchbaugh and Howery 2005). It is therefore reasonable to expect that behavior reflects learning to a large degree and to use behavior as an assessment of learning. As will be shown below, the different combinations of predictability and controllability are likely to result in the delivery of different numbers of electrical pulses to animals. Lee and Campbell (2021) suggested that the number of audio cues and electrical pulses might be used to assess predictability and controllability of virtual fences, but the study described in this thesis is the first to implement this approach.

During an interaction that is both predictable and controllable, it is reasonable to expect that an animal would approach the boundary, receive the audio cue and then stop or turn and retreat from the boundary, avoiding the electrical pulse entirely. However, during an interaction that is controllable but not predictable, the animal would receive the audio cue but not know what it meant, and would continue forward until it received the electrical pulse, at which time it would stop and/or turn and retreat from the boundary in order to stop delivery of the electrical pulse after receiving a single, or perhaps very few, electrical pulses.

During uncontrollable interactions an animal will proceed through the audio cue zone and enter the electrical pulse zone, where it would stay until it eventually finds a way out, either back into the paddock or by crossing the boundary and exiting the paddock. This is likely to result in the animal receiving many electrical pulses. Note that while it may be possible to conceptually differentiate predictable-uncontrollable and unpredictable-uncontrollable interactions, these two types of interactions may be difficult to empirically differentiate because animals are likely to receive a large number of electrical pulses regardless of whether the pulses were predictable or not. The different types of interactions with respect to predictability and controllability are shown in Figure 2.

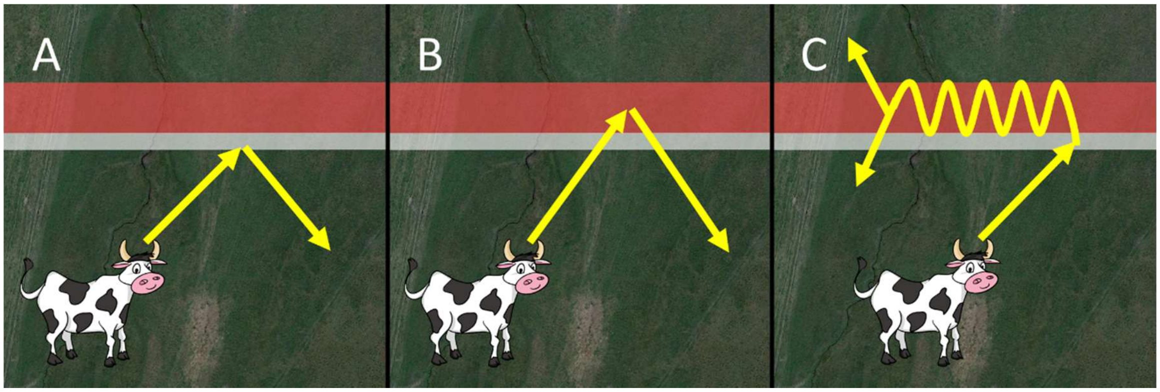

Figure 2: Conceptualized types of animal interactions with virtual fence boundaries depicting different combinations of predictability and controllability. The region shaded in white is the audio cue zone, and the region in red is the electrical pulse zone. (A) A predictable-controllable interaction. An animal would approach the boundary, receive the audio cue and then stop or turn and retreat from the boundary, avoiding the electrical pulse entirely. (B) An unpredictable-controllable interaction. An animal would approach the boundary and receive the audio cue but not know what it meant, and would continue forward until it received the electrical pulse, at which time it would stop and/or turn and retreat from the boundary in order to stop delivery of the electrical pulse after receiving a single, or perhaps very few, electrical pulses. (C) An uncontrollable interaction. An animal would pass through the audio cue zone and enters the electrical pulse zone, where it would stay until it eventually exits the zone.

Study Objectives

Most research conducted on virtual fencing to date uses very small groups of animals (usually 20 head or fewer) at stocking densities around 1-2 animals per acre (Campbell et al. 2017; Campbell et al. 2019; Campbell et al. 2020; Keshavarzi et al. 2020), and little is known about the capabilities of virtual fences to contain cattle at higher stocking densities which may be used in intensive or targeted grazing applications. Additionally, virtual fences have proven effective at keeping animals from approaching a food reward in experimental settings with trial periods lasting several minutes (Ranches et al. 2021), but ranch-scale implementation conditions are unlikely to be similar to experimental conditions. It is unknown how much forage is necessary to prevent cattle from crossing the boundary in search of better feeding sites over time periods representative of potential implementation conditions. Stocking density is known to influence diet selection (Brunsvig et al. 2017), so as stocking density changes, an animal's perception of resource availability and its corresponding motivation to seek resources outside the boundary may also change. As stocking density increases, cattle may break through the virtual boundaries to access other resources outside the paddock or for personal space and comfort. Furthermore, the consumption of forage inside the paddock creates a difference in the amount of forage available inside relative to outside the paddock. As this difference grows, so will its visual prominence. Because cattle use vision to locate feed (Howery et al. 2000), the magnitude of this difference may influence the probability that cattle will break through the virtual boundary to access resources on the other side. The influence of stocking density on diet selection (Brunsvig et al. 2017) may also mean that the difference in forage quantity needed to attract animals across the boundary changes with stocking density.

In order to make the most effective use of virtual fences as a management tool, it is critical to know their capabilities. The purpose of this study was to evaluate how stocking density influences virtual fence effectiveness and the predictability and controllability of the electrical pulse, as well as determine the extent to which the quantity of available forage or the difference in the amount of available forage between the inside and outside of the paddock affects the relationship between stock density and the effectiveness, predictability, or controllability of virtual fences. We tested the performance of a virtual fencing system across a range of stocking densities while also measuring stubble heights inside and outside the paddock to estimate the amount of forage inside and outside the paddock. This study was conducted using a herd of 162 yearling heifers in an intensive riparian grazing setting.

Methods

Study Site

The study was conducted during July and August 2021 in two adjacent subirrigated pastures at the Lamoille Unit of Maggie Creek Ranch, approximately 25 km southeast of Elko, Nevada (40.75 N, 115.48 W). During most years, both pastures are flood irrigated, however, this study took place during a drought and neither pasture was irrigated. One pasture was 21 ha and the other was 25 ha. Both pastures were dominated by Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire), meadow foxtail (*Alopecurus pratensis* L.), Nebraska sedge (*Carex* *nebrascensis* Dewey), Baltic rush (*Juncus arcticus* Willd.), and analogue sedge (*Carex simulata* Mack.). Both pastures were flat and at an elevation of 1750 m.

Virtual Fencing System

The CattleRider virtual fencing system (Vence Corp., San Diego, CA) was used for this study. The system consisted of individual collars worn by each animal, a solar powered radio base station, and HerdManager, the online central user interface. Virtual boundaries consisted of an audio zone and an electrical pulse zone. When approaching the boundary, animals first encountered the audio zone and received the audio cue only. If the animal continued forward through the audio zone they encountered the electrical pulse zone and received both the audio cue and electrical pulse. Boundaries functioned as one-way gates, meaning that cattle received both the audio cue and electrical pulse when trying to leave, but could freely reenter the paddock without receiving either the audio cue or electrical pulse. Animals that reentered the paddock received both the audio cue and electrical pulse if they attempted to leave again. The virtual boundaries could be programmed to stay in one location for a defined period of time or to move gradually. Gradual movement was accomplished by expanding the electrical pulse zone across multiple time intervals. We specified both the distance of expansion and the time interval. Herd Manager allowed us to set boundary locations, widths of the audio and electrical pulse zones, and times when boundaries activated, disabled, or moved. These settings were communicated over a cellular network to the base station, which in turn transmitted the settings to the individual collars. Collars delivered the audio cues with a built-in speaker. The collars used in this study delivered electrical pulses to the animals with two blunt metal electrodes spaced 5 cm apart on the back of the collar. When an animal entered the audio zone, it received a 0.5 s tone followed by a 1.5 s pause. This repeated until the animal either left the audio zone or proceeded into the electrical pulse zone, at which point the collar delivered a 0.5 s, 800 V pulse, followed by a 1 s tone and then a 3.5 s pause. Unless the animal exited the electrical pulse zone, this cycle repeated 20 times, at which point the collar temporarily stopped delivery of both audio cues and electrical pulses for 3 min. If the animal remained in the electrical pulse zone after the 3 min. pause in pulse delivery, the collar resumed delivery of both the audio cue and electrical pulse. If the animal had not left the electrical pulse zone after four such cycles, the collar disabled and stopped delivery of both the audio cue and electrical pulse until we re-enabled the collar in HerdManager. Collars sent a message as soon as an animal started interacting with either the audio or electrical pulse zone and every 70 s thereafter until the animal had left both the audio and electrical pulse zones. Hourly status update messages were also sent by the collars. These messages were proprietary in nature but included a non-spatial indicator of whether the animal was inside or outside of the boundary. GPS location messages were sent every 10 minutes. Collars transmitted all data to the base station, ultimately to be uploaded to the cloud and accessed through HerdManager. Location data were assumed to have a 3-5 m accuracy (Todd Parker, Vence Corp., personal communication).

Collars did occasionally invert, leaving the electrodes facing away from the animal and unable to deliver the electrical pulse to it. Collars could also un-invert, resuming the correct orientation. Collar inversion may have been caused by animals rubbing on each other or other objects (e.g. fence posts, water troughs). Often collars that had inverted during the study were identifiable when collars were removed following the study due to twisted straps, but it was not possible to determine when a collar became inverted, if or when it un-inverted, whether a collar inverted/un-inverted once or multiple times, or guarantee that collars without twisted straps never inverted during the course of the study. The issue of collar inversion has since been fixed in a new version of the collars released by Vence Corp. after this study was conducted.

Cattle and Virtual Fence Training

A herd of 162 yearling Angus heifers was outfitted with CattleRider collars using a squeeze chute with a head catch. Their necks were individually measured and collars were fit approximately 2.5 cm larger than the neck measurement. After being outfitted with collars, the heifers were exposed to a 4-day virtual fence training period as a herd. The training protocol was created under advisement of staff at Vence Corp and conducted in the 21 ha pasture. On the first day of training, a 5 m wide electrical pulse zone was created on the inside of the north, east, and west sides of the barbed wire perimeter fence of the pasture. On the second day of training, this electrical pulse zone was expanded to 15 m. A 5 m wide audio zone was added to the 15 m electrical pulse zone on the third training day. On the fourth and final day of training, the audio and electrical pulse zone widths were held constant and the north boundary was moved to exclude access to the northernmost part of the pasture where heifers spend a disproportionate amount of time every year (Travis Whitely, manager, Lamoille Unit, Maggie Creek Ranch, personal communication). The study commenced six days after the completion of training.

All animal care and management procedures were approved by the University of Nevada, Reno Institutional Animal Care and Use Committee (protocol #21-02-1138).

Experimental Design and Procedure

Virtual paddock size was varied to create 11 different stocking densities ranging from 5-20 animals per acre. The highest stocking density in each pasture was tested first, followed by the rest of the paddocks in ascending order by stocking density (Fig. 3). This was done because the only reliable water sources were located in the southern part of each pasture and it was unknown if there would be sufficient forage remaining in the highest stock density paddock if it was the final run in each pasture. Additionally, observations suggest that when paddocks are made larger (i.e. stocking density lowered), grazing activity tends to be concentrated in the part of the paddock most recently added (Todd Parker, Vence Corp., personal communication). This was also observed in a study published after the present study took place (Aaser et al. 2022). That situation would have significant potential to cause observed stocking densities to deviate from planned paddock stocking densities, which would conflict with the objectives of this study.

Residual stubble height is a commonly used riparian management target (Clary and Leininger 2000), and is highly correlated to forage biomass (Lommasson and Jensen 1943). We therefore used stubble height as a proxy for forage quantity. Three stubble height measurements were taken before and after each run along four, north-south pace transects in each pasture: all herbaceous vegetation, Nebraska sedge, and Baltic rush. Nebraska sedge was chosen because it is a preferred forage species and is commonly used for riparian monitoring. Livestock generally avoid grazing Baltic rush when given a choice, so substantial consumption of Baltic rush may have indicated that cattle shift their diet instead of breaking through the virtual boundaries. However, preliminary analysis revealed that all three measurements were highly correlated, so Nebraska sedge stubble

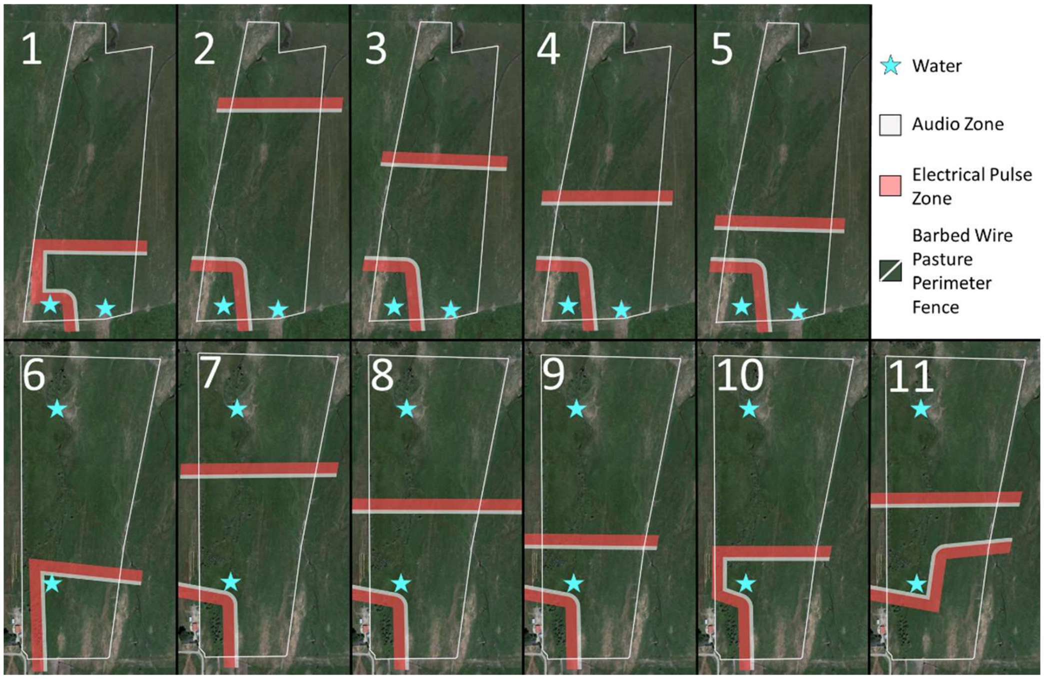

Figure 3: Study treatment layout for testing virtual fence effectiveness, predictability, and controllability as affected by stocking density and available forage in two irrigated pastures with barbed wire perimeter fences at the Lamoille Unit of Maggie Creek Ranch, near Elko, NV. The number in the top left corner of each panel indicates the order in which paddocks were used (run). A 21-ha pasture was used for runs 1-5, and the adjacent 25-ha pasture was used for runs 6-11. For every run after the first in each pasture, the boundary was moved to decrease the virtual paddock size. The region shaded in white is the audio cue zone, where animals (162 Angus yearling heifers) received only the audio cue after entry, and the region in red in the electrical pulse zone where animals received both the audio cue and electrical pulse after entry.

height was selected for analysis because it is regionally one of the most commonly used indicator species for riparian management. The stubble height in the 50 m immediately outside the northern edge of the paddock was also measured before each run. The stubble height measurements taken inside the paddock before and after each run were averaged to represent stubble height during the run. This average stubble height was subtracted from the stubble height measured outside the paddock to determine the difference in forage quantity between the inside and outside of the paddock (hereafter the difference in stubble heights).

For data collection purposes, each run started at 7:00 AM and continued until 6:00 PM the following day, resulting in a 35-hour run at each stocking density. Paddock boundaries activated at 8:30 PM on the day prior to the beginning of the run. To transition from one paddock to the next, boundaries were programmed to move 9 m every 3 min. by expanding the electrical pulse zone. The width of the audio zone did not change. Boundary movement was timed to finish at 6:45 AM, 15 min. prior to the start of the run. Except when boundaries were actively moving, all boundaries consisted of a 30 m wide electrical pulse zone and a 10 m wide audio zone. Any collars that had automatically disabled were re-enabled through HerdManager prior to the start of each run.

Data Processing and Analysis

Individual boundary interaction messages were grouped into interactions if they were sent less than four min. apart, as indicated by non-experimental preliminary data and preliminary analysis on study data (Appendix). Individual interaction messages were used to calculate the number of electrical pulses delivered during the course of an interaction. There was no reliable counter of the number of audio cues delivered during an interaction (Todd Parker, Vence Corp., personal communication), but we assumed that animals received only audio cues during interactions where zero electrical pulses were delivered.

Proprietary details of collar function would occasionally block delivery of the electrical pulse at times it would normally have been delivered (Todd Parker, Vence Corp. personal communication). Interactions where this happened were considered malfunctions and excluded from the analysis. Additionally, some animals would occasionally stay in the electrical pulse zone long enough trigger the automatic-disable safety mechanism in the collars. Interactions from these animals were included in the analysis up to and including the interaction when the collar disabled, but excluded thereafter.

An interaction was deemed to be successful if the animal remained inside the boundary after an interaction had finished. Whether an animal was inside or outside of the boundary following an interaction was determined one of three ways. First, an interaction was counted as successful if the first GPS location reported after the conclusion of the interaction showed the animal as inside the boundary. Second, the interaction was counted as successful if a collar status message showed the animal to be inside the boundary. Finally, if a new interaction began before either a GPS location message or a collar status message, this was also interpreted as a successful interaction because new interactions must begin from inside the boundary. The first of these indicators to happen following the conclusion of an interaction was used to define success for that interaction.

Individual interactions were classified as predictable and/or controllable according to the number of electrical pulses delivered during the interaction. Preliminary analysis revealed that only about 5% of interactions resulted in animals receiving five or more electrical pulses (Appendix). Four electrical pulses would be delivered over a 20 s timeframe (see Methods: Virtual Fencing System). This should give an animal adequate time to respond appropriately and exit the electrical pulse zone if it has learned to do so. We therefore classified interactions where animals received four or fewer electrical pulses as controllable and interactions where animals received five or more electrical pulses as uncontrollable. Interactions where no electrical pulses were delivered were classified as both predictable and controllable (Lee et al. 2018). Interactions with four or fewer electrical pulses were classified as unpredictable and controllable. Interactions with five or more electrical pulses were classified as uncontrollable. We did not classify uncontrollable interactions (Fig. 2 C) as either predictable or unpredictable because we did not believe it was possible to separate predictable-uncontrollable and unpredictable-uncontrollable interactions using the number of electrical pulses. Predictability and controllability across treatments were evaluated using the number of interactions classified as described above. The metrics used to calculate effectiveness, predictability, and controllability are shown in Table 1.

Logistic regression is often used for response variables that are proportions, as is the case with the virtual fence performance metrics described in Table 1. The amount of forage available to animals in this study was quantified using two different approaches. One approach (stubble height) considered only the amount of forage inside each virtual paddock. The other approach (difference in stubble heights) compared the amount of forage inside each virtual paddock to the amount of forage just outside each paddock. Therefore, two logistic regressions were developed for each performance attribute (effectiveness, predictability, and controllability). One regression looked at the effects of stock density and stubble height and the other looked at the effects of stock density and

Table 1: Metrics used to evaluate virtual fence effectiveness, predictability, and controllability in a study of virtual fence performance using the CattleRider virtual fencing system from Vence (San Diego, CA) on 162 yearling heifers. Metrics were calculated using the number of times animals interacted with the boundary. Interactions were classified as successful if the animal was held inside the boundary during the interaction, controllable if four or fewer electrical pulses were delivered during the interaction, and both predictable and controllable if no electrical pulses were delivered during the interaction. All controllable interactions include those that are predictable and those that are unpredictable.

Performance Attribute	Metric
Effectiveness	$\frac{\text{Successful Interactions}}{\text{Total Interactions}}$
Predictability	$\frac{\text{Interactions that are both Predictable and Controllable}}{\text{All Controllable Interactions}}$
Controllability	$\frac{\text{Controllable Interactions}}{\text{Total Interactions}}$

the difference in stubble heights. All regression models also included pasture because runs were blocked by pasture in the experimental design.

We recognized the small size of the dataset and the corresponding limited ability to fit large numbers of terms. We therefore selected potential interactions in an Akaike's Information Criterion framework while keeping all main effects (pasture, stocking density, and stubble height or the difference in stubble heights) in each model. Models containing all combinations of potential interactions were ranked using Quasi-AIC adjusted for small samples sizes (QAICc) due to overdispersion (Burnham and Anderson 2002; Lebreton et al. 1992). The model with the lowest QAICc was used for inference. All models were fit using the function 'glm' with the logit link and the quasibinomial family to account for overdispersion in R version 4.2.1 (R Core Team 2022).

Results

Stubble height ranged from 4.6 to 19.9 cm inside virtual paddocks across all runs and the difference in stubble heights ranged from 2.1 to 9.5 cm. Stubble height was always taller outside the paddock. Heifers interacted with the boundary a total of 16,232 times during the study, of which 15,534 interactions were controllable and 11,284 interactions were both predictable and controllable. Animals were successfully kept inside the boundary during 14,908 interactions. Over the course of the study, effectiveness, predictability, and controllability ranged from 78-96%, 59-80%, and 92-98%, respectively. The QAICc-best models included no interactions except for when controllability was modeled using pasture, stocking density, and stubble height.

When collars were removed following completion of the study, 12 collars were inverted and an additional 34 showed evidence of having been inverted at some point during the study. Eighteen animals had experiment-wise success rates of less than 80%. Of these animals, 11 had collars that were either inverted when collars were removed after the study was completed or had evidence of being inverted at some point during the study. The remaining seven animals had collars that did not show evidence of being inverted.

Effectiveness

The models with stubble height and the difference in stubble heights had estimated overdispersion parameters of 18.0 and 11.7, respectively. Virtual fences were less effective in one pasture (odds ratio = 0.499) when stubble height was used for analysis (P = 0.028), but not when the difference in stubble heights was used (P = 0.075). Stocking density did not affect virtual fence effectiveness regardless of whether stubble height or the difference in stubble heights was included in the analysis (P = 0.122 and P = 0.051, respectively). However, in the analysis that included the difference in stubble heights, the 95% confidence interval for stocking density did not include zero (Table 2). Neither stubble height nor the difference in stubble heights affected virtual fence effectiveness (P = 0.491 and P = 0.089, respectively).

Predictability

The models with stubble height and the difference in stubble heights had estimated overdispersion parameters of 26.3 and 18.0, respectively. The predictability of the electrical pulse did not differ between pastures in the analysis including stubble height or the difference in stubble heights (P = 0.458 and P = 0.872, respectively). Likewise, stocking density did not affect predictability in the analysis using stubble height (P = 0.879) or the analysis using the difference in stubble heights (P = 0.833). Neither stubble height nor the difference in stubble heights affected predictability (P = 0.529 and P = 0.092, respectively).

Controllability

When stubble height was included in the analysis for controllability, the QAICc-best model included pasture, stocking density, stubble height, and the pasture × stubble height interaction. In the analysis using the difference in stubble heights between inside and outside the paddock, the best model for controllability included only pasture, stocking density, and the difference in stubble heights. The models had estimated overdispersion parameters of 11.6 and 11.1, respectively.

When stubble height was used for analysis neither pasture nor the pasture × stubble height interaction affected the controllability of the electrical pulse ($P = 0.315$ and $P = 0.200$, respectively). There was also no difference between the pastures difference in stubble heights was used for analysis ($P = 0.730$). There was no effect of stocking density on controllability regardless of whether stubble height ($P = 0.739$) or the difference in stubble heights ($P = 0.605$) was included in the analysis. Finally, neither stubble height nor the difference in stubble heights affected controllability ($P = 0.693$ and $P = 0.127$, respectively).

Discussion

Virtual fences have long been conceptualized as a tool to help land managers achieve livestock production or land management goals. Because virtual fences must work if they are to be a useful management tool, it is critical to understand what factors influence their performance in conditions likely to be encountered as commercial adoption of this technology proceeds. This study was intentionally designed to begin answering these questions by focusing on the potential effects of stock density and the quantity of forage available inside, and just outside of virtual paddocks. We tested virtual fence performance across a wide range of stocking densities, far higher than those used in any previous studies. We had hypothesized that effectiveness would decline as stocking density increased, but our results do not support this. Our results indicate that stocking density, forage quantity, and the difference in forage quantity inside and outside the paddock do not influence virtual fence performance, and effectiveness and controllability were both consistently high, even when stubble heights were well below common management targets and stubble heights were taller outside the paddock. Additionally,

Table 2: Coefficient estimates of logistic regression models including pasture, stocking density, and stubble height or the difference in stubble heights inside and outside the paddock for virtual fence effectiveness, predictability, and controllability. Performance attributes were calculated using the number of effective, predictable, or controllable interactions from 11 runs using a virtual fence system on a herd of 162 yearling heifers in two irrigated pastures on a ranch in northeastern Nevada. D is stocking density, SH is stubble height, DSH is the difference in stubble heights, OR is odds ratio, LCL and UCL are the lower and upper limits of the 95% confidence interval, and P is the p-value.

Attribute	Model	Predictor	OR	Coefficient	LCL	UCL	P
Effectiveness	D, SH						
		Pasture	0.499	-0.695	-1.195	-0.208	0.028
		Density	1.052	0.051	-0.005	0.109	0.122
		Stubble Height	0.978	-0.022	-0.078	0.041	0.491
	D, DSH						
		Pasture	0.954	-0.472	-0.919	-0.023	0.075
		Density	1.054	0.052	0.009	0.096	0.051
		Difference	0.906	-0.098	-0.199	-0.003	0.089
Predictability	D, SH						
		Pasture	0.863	-0.148	-0.516	0.222	0.458
		Density	1.004	0.004	-0.041	0.048	0.879
		Stubble Height	0.986	-0.014	-0.053	0.028	0.529
	D, DSH						
		Pasture	1.030	0.030	-0.315	0.381	0.872
		Density	1.004	0.004	-0.032	0.039	0.833
		Difference	0.920	-0.084	-0.168	0.000	0.092
Controllability	D, SH						
		Pasture	1.093	0.886	-0.700	2.480	0.315
		Density	0.987	-0.013	-0.088	0.060	0.739
		Stubble Height	0.987	-0.013	-0.071	0.051	0.693
		Pasture × Stubble Height	0.886	-0.121	-0.284	0.047	0.200
	D, DSH						
		Pasture	1.120	0.114	-0.488	0.759	0.730
		Density	1.016	0.016	-0.042	0.074	0.605
		Difference	0.879	-0.129	-0.282	0.011	0.127

while lower than effectiveness and controllability, our results indicate that a large majority of virtual fence interactions were predictable. This indicates that virtual fences can likely be used across a broad range of conditions without declines in effectiveness or adverse welfare impacts.

The flexibility of virtual fencing makes it likely to be an excellent tool to manage targeted or intensive riparian grazing, where animals are typically held at high stocking densities in small, frequently moved paddocks. We tested virtual fences at stocking densities up to 20 animals/acre, far higher than other studies to date, and found that stocking density did not affect virtual fence effectiveness. However, our results were slightly ambiguous, as the confidence interval for stocking density in the analysis using the difference in stubble heights did not include zero. Further research, possibly over a larger range of stocking densities, is needed to provide a clear answer on the effect of stocking density. However, the estimated coefficient we report (Table 2) suggests a slight increase in effectiveness with increasing stocking density (roughly a 0.4% increase in effectiveness for each additional animal per acre, at the experiment-wise average effectiveness rate of 91.8%). This may be because virtual fence interactions are socially facilitated by the behavior and interactions of nearby animals (Keshavarzi et al. 2020); as stocking density increases animals are closer together and the likelihood of one animal influencing the interactions of other animals increases. Ultimately, between the direction and small size of the estimated effect, it is likely safe for producers to assume that virtual fence effectiveness will remain consistently high or improve slightly as stock densities increase from 5 to 20 animals/acre under conditions similar to those used in this study.

While virtual fences are effective at keeping animals from approaching a food reward over a period of several minutes (Campbell et al. 2018; Ranches et al. 2021), researchers conducting several field-based trials made sure that cattle had plenty of feed inside the paddock, sometimes providing supplemental hay or straw (e.g., Campbell et al. 2019a; Campbell et al. 2017; Campbell et al. 2020). Stubble heights in this study were as low as 4.6 cm, indicating that virtual fences are effective when stubble heights are well below commonly used riparian management targets. However, this study took place during a drought and we could only evaluate virtual fence performance across a limited range of stubble heights. It is possible that a relationship between virtual fence effectiveness and stubble height may appear across a larger range of stubble heights, and further research examining this issue may be warranted. Additionally, virtual fences remained effective even when stubble height was nearly 10 cm higher outside the paddock. This means that virtual fences will likely be good tools to manage riparian strip grazing or fuel break creation, where there may be an obvious difference in the amount of forage inside and outside the boundary. However, as with stubble heights inside the paddock, drought conditions limited the size of the difference in stubble heights that we were able to test.

That being said, it is important to note that our results are consistent with other studies which have shown that virtual fences are highly, but not entirely, effective at keeping animals inside the boundary (Boyd et al. 2022; Campbell et al. 2017). It makes sense to combine virtual fences with other sound livestock management strategies, and the achievement of grazing management objectives may depend on the whole suite of strategies used, not just the performance of virtual fences.

In order to prevent long term adverse animal welfare outcomes, animals must learn to predict and control the receipt of the electrical pulse (Lee et al. 2018). While previous studies that have examined predictability and controllability have used direct measures of stress responses (e.g., cortisol and body temperature) (Kearton et al. 2020; Kearton et al. 2019), Lee and Campbell (2021) suggested that the numbers of audio cues and electrical pulses delivered during an interaction could be used to evaluate predictability and controllability. This study was the first to attempt to assess the predictability and controllability of the electrical pulse in conditions that are likely similar to commercial applications. We found no relationships between stocking density, forage quantity, or the additional quantity of forage present outside of the paddock for either predictability or controllability. We found this unsurprising because predictability and controllability are established during learning (Lee et al. 2018; Ursin and Eriksen 2004), and cattle used in this study underwent a multi-day learning protocol prior to the beginning of the study.

During this study predictability ranged from 59-80% and controllability ranged from 92-98%. Controllability was consistently high, but predictability could potentially be increased by refinement in implementation or adjustment of the audio or electrical pulse zone widths. When possible, the placement of boundaries along visually prominent landscape features such as roads may increase predictability by more clearly communicating boundary location, but may result in animals associating the boundary location and electrical pulse with the visual marker rather than the audio cue (Umstatter et al. 2015). Reducing the width of the audio cue zone may increase predictability by strengthening the relationship between the audio cue and the electrical pulse. On the other hand, widening the audio cue zone may increase controllability by allowing animals a larger area in which to appropriately respond to the audio cue and avoid the electrical pulse entirely.

Further research should examine the question of what levels of predictability and controllability are necessary to prevent adverse long-term animal welfare outcomes. There has been no systematic study of learning protocols needed to appropriately train animals to respond to virtual fences. Research examining how animals learn to respond to virtual fences and producing training guidelines would be valuable as virtual fences begin to see widespread commercial adoption.

Foraging theory provides evidence that animals avoid novel foods or environments when resources are adequate, but will more readily try new strategies to meet physiological requirements when conditions are limiting (Provenza et al. 1998). A lack of relationships between stocking density, stubble height, or the difference in stubble heights and predictability and controllability indicates that neither competition for resources, limited forage (stubble heights under 5 cm), or larger quantities of forage outside the paddock were sufficient to justify the risk of the electrical pulse. Alternatively, one could argue that as resources became limiting cattle may have run through the electrical pulse zone quickly as an alternate strategy to control the number of electrical pulses received. However, this would manifest as decreased virtual fence effectiveness, which is not supported by the data.

The pasture estimated to have a lower effectiveness when stubble height was used in the analysis was adjacent to other pastures that held animals not involved in this study. Social interactions across the barbed wire fence that separated study and non-study animals, or curiosity about what non-study animals were doing in the adjacent pastures, may have contributed to the lower effectiveness.

Social interaction of study and non-study animals across barbed wire fences may also have contributed to the high overdispersion observed. Inverted collars may also have contributed to overdispersion.

Roughly 11% of the animals used in this study had experiment-wise success rates below 80%. However, these animals were disproportionately likely to have collars that displayed evidence of inversion when removed. About 24% of animals with collars that had evidence of inversion had success rates below 80%, compared to only 6% of animals with collars that did not have evidence of inversion. It is possible that animals with success rates below 80% did not successfully learn to use the virtual fencing system. That being said, the presence of inverted collars is a strong confounding factor, particularly because we could not guarantee that collars without evidence of inversion had not been inverted at some point during the study, and it was not possible to determine the cause of these low success rates from the results of this study. However, as several previous studies have observed variation among animals in how they learn and respond to virtual fences (Campbell et al. 2019; Campbell et al. 2020; Lee et al. 2009), future research addressing whether all animals can successfully learn to use virtual fences may be beneficial.

This study also resulted in a few observations of potential managerial importance. However, they were not the purpose of this study and no attempt was made to quantify or test these observations. Virtual fence studies with different boundary locations have primarily expanded the area to which animals have access (Campbell et al. 2017; Campbell et al. 2020). During this study, most boundary location changes reduced the area to which animals had access, and animals appeared to recognize and respect the new boundary location, which is consistent with the behavior observed in another study (Campbell et al. 2019a). This implies that virtual boundaries can likely be moved to either expand or restrict the area and resources to which animals have access without negatively impacting performance. Additionally, although gradually moving boundaries were suggested even before testing of commercial prototype virtual fences became widespread (Anderson et al. 2014), this study is, to our knowledge, the first that used gradually moving virtual boundaries. We moved the boundaries 9 m every 3 min. for 50 m to 200 m during the early morning hours of animal activity, which appeared to reliably push animals from one paddock to the next. Further research is needed to assess the capabilities and limitations of using dynamically moving boundaries to manage cattle.

Finally, we observed several instances where nearly the entire herd ran through the boundary as a group. These mass escapes were not included in the analysis because a majority were directly attributable to external factors (e.g. the ranch manager going out to check water or doctor an animal) and we believed that their inclusion would not be representative of virtual fence performance across implementation conditions. However, it may still be of managerial interest that a herd of yearling heifers may bolt across virtual boundaries when pressured or attracted by other management activities.

Implications

Virtual fences consistently performed well over the conditions studied. Stocking density did not affect or slightly enhanced virtual fence effectiveness as stocking densities increased up to 20 animals/acre. Likewise, virtual fences were effective even when stubble heights were well below common management targets or were taller outside the paddock. This indicates that virtual fences are likely to be a reliable tool for livestock producers to use to manage riparian grazing. There is also potential for virtual fences to be valuable tool for other grazing applications like targeted grazing or creating fuel breaks, where animals are often held at high stocking densities and the high levels of utilization which may be required to achieve management goals may create stubble heights similar to those tested in this study. Observationally, gradually moving boundaries worked well in this study, but this capability should be studied systematically.

Predictability and controllability were not affected by stocking density or the quantity of forage inside or outside of the paddock. Controllability was consistently high, but further research on training protocols or refinement in how virtual fences are used may be able to increase predictability. Our results indicate that virtual fences can likely be used to manage riparian grazing without causing animal welfare issues.

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Appendix: Virtual Fence Preliminary Analysis

Separating Interactions

Vence CattleRider collars send a message once an animal starts interacting with either the audio cue zone or the electrical pulse zone and then every 70 s thereafter as long as the animal remains in either zone. This means that there can be multiple messages during a continuous period of an animal interaction with the boundary. Before any analysis can be conducted on the interactions themselves, it is critical to determine which messages belong to the same interaction and which represent the beginning of a new interaction.

We approached this problem by plotting the number of interactions observed as messages included in progressively longer periods of time were grouped into a single interaction (Fig. A1). We defined the interaction break limit as the time after which a new message was considered to represent the start of a new interaction. There was a prominent spike in the rate of decline at 230 s in data from both the current study and non-experimental exploratory studies undertaken the year before the current study at the Lamoille Unit of Maggie Creek Ranch and the Cottonwood Ranch, both in Elko County, Nevada. This spike indicates that there are many messages sent 230 s apart by the collars that are getting grouped into the same interaction. Multiple messages with only a short time between them indicates an increased probability that the animal remains in close proximity to the boundary for an extended period, likely testing the boundary at multiple locations over a period of several minutes. We think that it makes sense to consider this as a single interaction, and therefore set the interaction break interval just above this spike, at 240 s (4 min.).

Defining Controllability

The number of audio and electrical pulses delivered during an interaction has been suggested as a way to assess predictability and controllability (Lee and Campbell 2021). Previous research indicates that cattle can achieve controllability by responding to the audio cue, avoiding the electrical pulse entirely (Campbell et al. 2019). Accounting for potential unpredictability, the number of electrical pulses delivered during the interaction can be used to separate controllable interactions from uncontrollable interactions. Controllable interactions should result in delivery of few electrical pulses (or zero for a predictable-controllable interaction) and uncontrollable interactions should result in delivery of many electrical pulses (see Evaluating Welfare Outcomes from Virtual Fences: Predictability and Controllability in the text). Preliminary exploration of data from this study indicates that roughly 95% of interactions had four or fewer electrical pulses, and only about 5% had five or more (Table A1). Four electrical pulses would be delivered over a 20 s timeframe (see Methods: Virtual Fencing System in the text). This should give an animal adequate time to respond appropriately and exit the electrical pulse zone if it has learned to do so. We therefore decided to classify interactions that resulted in the animal receiving five or more electrical pulses as uncontrollable.

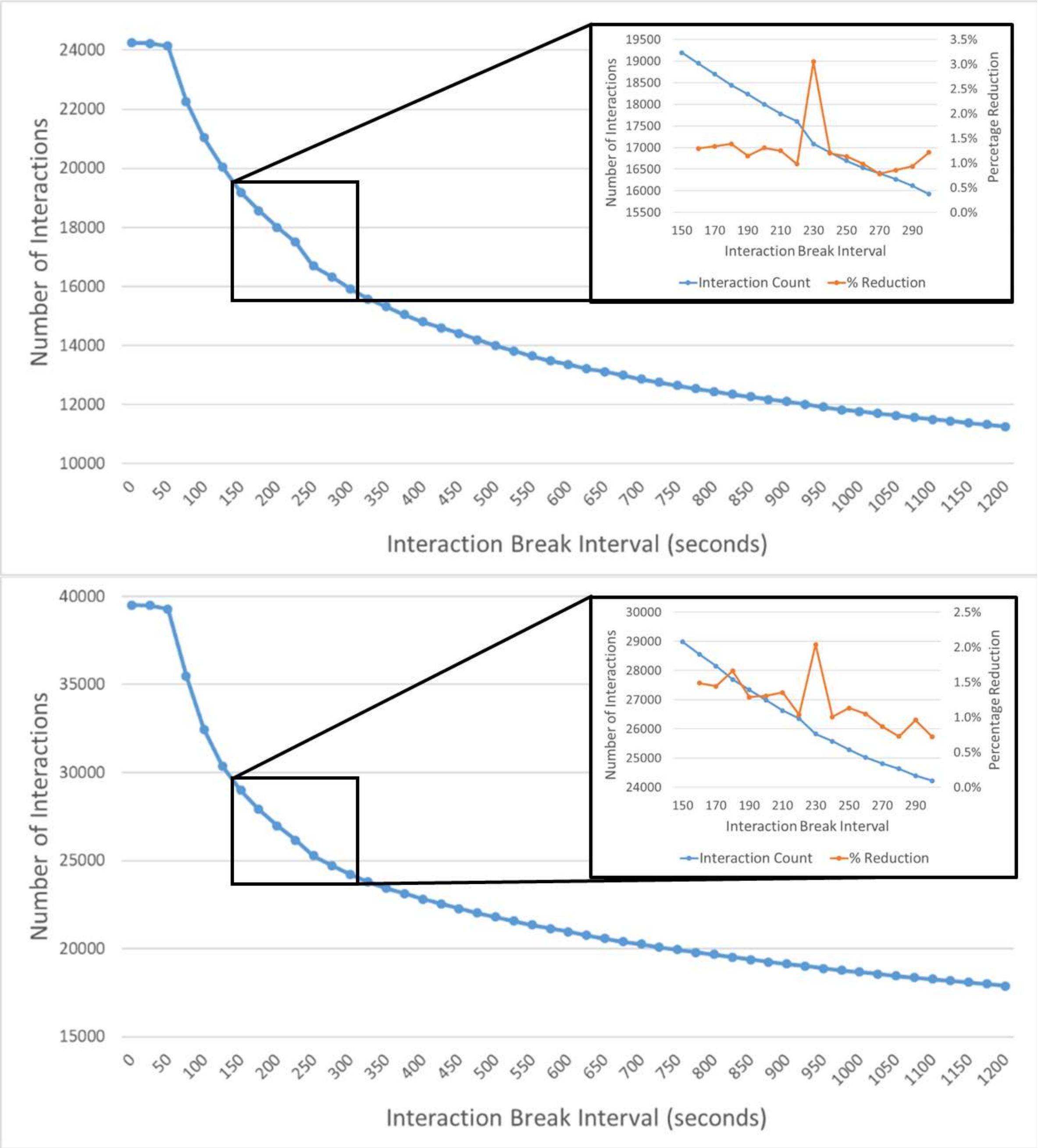

Figure A1: The total number of virtual fence interactions as the time which must elapse before a new interaction message is counted as an entirely new interaction (Interaction Break Interval) changes. Note the spike in the rate of decline (Percentage Reduction) at 230 seconds. Top: non-experimental preliminary data from the Lamoille Unit, Maggie Creek Ranch and the Cottonwood Ranch (Wells, Nevada, USA). Bottom: preliminary analysis of study data from a herd of 162 yearling heifers at the Lamoille Unit, Maggie Creek Ranch, during July and August 2021.

Table A1: The number of virtual fence interactions involving the delivery of different numbers of electrical pulses. This summary includes all interactions during which the collar was not auto-disabled (as described in Methods: Virtual Fencing System).

Electrical Pulses Delivered	Number of Interactions	Percentile
0	14366	68.35%
1	3665	85.79%
2	1088	90.97%
3	496	93.33%
4	330	94.90%
5	172	95.72%
6	116	96.27%
7	99	96.74%
8	73	97.09%
9	59	97.37%
10	46	97.59%
11	27	97.72%
12	28	97.85%
13	20	97.94%
14	20	98.04%
15	20	98.13%
16	15	98.21%
17	14	98.27%
18	20	98.37%
19	13	98.43%
20	19	98.52%
21	14	98.59%
22	12	98.64%
23	10	98.69%
24	11	98.74%
25	6	98.77%
>25	258	100.00%

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