EFFECTS OF A SPECIFIC *SORDARIA FIMICOLA* STRAIN ON FECUNDITY AND HERBIVORY OF *BROMUS TECTORUM*

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ABSTRACT

Cheatgrass (*Bromus tectorum*), an annual grass native to Eurasia, has a long history of invasion throughout North America. In much of the western United States, cheatgrass has the ability to dominate at a massive scale, resulting in entire landscapes existing in monoculture conditions. Cheatgrass also alters fire regimes, resulting in increased wildfires in dominated areas, making it a weed of significant concern throughout the intermountain west. Adding to the problem, the cost of available control methods, as well as their infeasibility in large scale invasions, makes successful control of cheatgrass a daunting task.

Recent discoveries in the field of endophytic fungi have yielded a large number of previously unsuspected relationships between cheatgrass and a wide array of fungal species. While many of these relationships are symbiotic, others appear neutral, and several fungal species appear to exert a negative effect on cheatgrass fitness, when present. In particular, *S. fimicola*, when present in cheatgrass endophytically, appears to negatively affect biomass production and fecundity. While traditionally considered a coprophilous species, the discovery of *S. fimicola* living endophytically indicates a seldom studied transition between two distinct lifestyles is taking place.

The objective of this research is to examine this transition, and evaluate the potential of *S. fimicola* as a potential biological control for cheatgrass, using grazing herbivores (sheep) as a vector for spreading the fungus into new areas. Results from field experiments showed significant impacts on both biomass and fecundity resulting from endophytic infection by *S. fimicola*. In experiments utilizing sheep, we determined that *S. fimicola* reliably transits the digestive tract and appears in dung when it is ingested with plant material, as well as when the fungus is cultured under laboratory conditions and artificially introduced to the sheep via esophogeal tube.

Transitioning between coprophilous and endophytic lifestyles by *S. fimicola* proved to be highly sporadic. This may provide a significant impediment to the use of the fungus as a means of biological control, as well as demanding further research into the conditions required to facilitate this poorly understood transition.

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CHAPTER 1 - INTRODUCTION AND BACKGROUND

Cheatgrass (*Bromus tectorum*) has become one of the most prolific and persistent invasive plant species in western North America since its introduction late in the 19th century (Young and Clements 2009). In the Great Basin and northwestern regions of the United States, cheatgrass can dominate entire landscapes, altering fire regimes, reducing biodiversity, and replacing bunchgrass and shrubland ecosystems (Knapp 1996, Mack 1981). While substantial efforts have been made to mitigate the spread of cheatgrass using chemical or mechanical means, most have met with failure, or proven too costly to implement over large landscapes (Young and Clements 2009, DiTomaso 2000). Targeted grazing, primarily by sheep, have met with some success in reducing the spread of cheatgrass. However, cheatgrass is highly adaptive, and the successful implementation of such grazing programs requires significant managerial expertise (Mosley 1996). Recent research has also questioned this practice, pointing to increased grazing intensity by cattle as a means of increasing, rather than mitigating, the spread of cheatgrass (Resner et al. 2013).

Cheatgrass is capable of rapidly producing prodigious quantities of seed, that can survive in the soil for up to 5 years (Young and Clements 2009). Additionally, cheatgrass responds rapidly to available moisture, often maturing and producing seeds just as native bunchgrasses initiate spring growth (Young and Clements

2009). These traits, coupled with the infeasibility of traditional plant control methods at a landscape scale, has created demand for methods to reduce cheatgrass using less traditional approaches.

Over the past decade, studies of endophytic fungi have revealed previously unsuspected relationships between these fungi and their plant hosts. Early research concerning grass-based endophytes focused largely on the mutualistic relationships between select species and their plant hosts (Saikkonen et al. 1998). Of particular interest were endophytic species capable of producing chemical compounds (e.g. alkaloids) that reduce herbivory via toxic effects imparted on the grazing animal (Schulz et al. 2002; Cheplick and Clay 1988; Clay 1988). More recent research has revealed that most plants host a diverse array of endophytes, and endophyte/host relationships exist on a continuum from mutual to antagonistic (Rodriguez et al. 2009; Shipunov et al. 2008; Paszkowski 2006; Saikkonen et al. 1998). The discovery of endophytic fungi that may exert negative effects on their host species raises the possibility of altering plant communities via the promotion of specific fungi.

Research Objectives

Recent research conducted at the University of Idaho to assay endophytic fungi in cheatgrass (Baynes 2011) isolated a strain of *Sordaria fimicola* (labeled CID323)

that appears to reduce cheatgrass fecundity. The strain appears to colonize cheatgrass endophytically, resulting in reduced fecundity and biomass. In light of this previously unobserved response, it is possible that successful dispersion of endophytic *Sordaria* into previously uninfected grassland communities could offer a viable method to biologically limit the invasive potential of cheatgrass.

This research focuses on the practicality of such an application for the CID323 strain of *S. fimicola* to limit cheatgrass growth and expansion. The objectives of this study were to assess the effect of endophytic *S. fimicola* infection on cheatgrass growth and fecundity, and to investigate whether any forage attributes altered by an infection would result in selective preference for or avoidance of cheatgrass in grazing herbivores. Additionally, this research examines the ability of *S. fimicola* to predictably transit a ruminant digestive tract under both natural and artificially introduced conditions, and explores the environmental conditions necessary for *S. fimicola* to make the transition between coprophilous and endophytic lifestyles.

Cheatgrass

Cheatgrass is an introduced annual grass that, over the last century, has become widely distributed on range and pasture lands throughout North America. Observations vary, but it has been estimated that, by the mid 1990s, 1.3 million hectares of public land in the Great Basin were dominated by cheatgrass, and

another 31 million hectares were infested to some degree (Pellant and Hall 1994).Though invasive throughout its introduced range, cheatgrass is of particular concern in the Great Basin and other intermountain regions of the western United States, where it can rapidly come to dominate and replace native plant communities (Young and Clements 2009; DiTomaso 2000; Mack 1981).

Cheatgrass was brought to the United States from Eurasia late in the 19th century, likely as a component of ship's ballast, and as a contaminant of crop seed (Mack 1981). Once established, cheatgrass spread rapidly, and by the 1920's, was ubiquitous across western rangelands (Knapp 1996; Mack 1981). As cheatgrass spread across the west, its invasive potential became readily apparent. Young and Clements (2009) observed that cheatgrass does not historically compete effectively with well-established native vegetation, but it is highly opportunistic and readily colonizes bare or degraded areas. This capability, coupled with the heavy grazing practices of the late 19th and early 20th centuries (Knapp 1996), provided cheatgrass with a foothold across the west, and a pathway to eventual dominance across much of the Great Basin region.

Once established in an area, cheatgrass is highly competitive. Prodigious seed production and a high degree of phenotypic plasticity allows the species to persist and expand, often to the exclusion of other plant species (Young and Clements

2009). Cheatgrass is a winter annual that typically germinates in the fall, and resumes growth in early spring. The onset of spring growth typically precedes that of perennial forage grasses, often reducing available moisture for these species.

When it is in its vegetative stage, cheatgrass is palatable to herbivores, and constitutes an important forage source for both livestock and wildlife (Young and Clements 2009). Cheatgrass matures rapidly and, under normal conditions, is dormant and relatively unpalatable by early summer (Pellant 1996).

The standing dormant material of cheatgrass is an excellent source of fine fuels for wildfires, and cheatgrass dominated rangelands are often characterized by drastically reduced fire intervals compared to native bunchgrass ecosystems (Whisenant 1990). This increased fire frequency results in more bare ground, which in turn can be readily colonized by cheatgrass. Much of the invasive capability observed in cheatgrass is attributed to this continual cycle and the resulting truncation of the otherwise normal successional progression to a bunchgrass-sagebrush ecosystem exhibited on native ranges (Young and Clements 2009).

Within the Great Basin, cheatgrass is a near perfect invader. It readily colonizes degraded areas, reproduces rapidly, adapts readily to changing conditions, and

alters site conditions to those more suitable for its own prevalence (Young and Clements 2009). Efforts to mitigate the spread of cheatgrass have met with minimal success (Young and Clements 2009; DiTomaso 2000). Chemical controls exist that can selectively reduce cheatgrass with relatively little harm to native species (Pellant 1996), but can be prohibitively expensive for land managers, and difficult to implement at a landscape scale. Attempts to introduce perennial grass species, primarily crested wheatgrass (*Agropyron cristatum*), to outcompete cheatgrass have met with occasional success, but are costly, not feasible in rough terrain, and ultimately rely on the introduction of non-native species (DiTomaso 2000). The use of targeted grazing by domestic herbivores has shown some success (Mosley 1996). When timed properly, selective grazing by sheep has reduced cheatgrass abundance, and allowed native perennials to reclaim invaded ranges (Diamond et al. 2012; Mosley 1996). While beneficial, grazing can often prove insufficient when used as a sole means of control (Reisner et al. 2013). Cheatgrass is a prodigious producer of seed. Past studies on rangelands in the Great Basin have shown its capability to produce in excess of 17,000 seeds per square meter (Pellant 1996). This level of production, coupled with the ability of those seeds to persist in the soil for up to five years, mean that properly timed grazing must occur over multiple growing seasons. An economically feasible and effective method of reducing and controlling the spread of cheatgrass is sorely needed.

Endophytic Fungi

The invasive potential of cheatgrass, and the ability of its seeds to withstand fire may be enhanced by mutualistic relationships with endophytic fungi (Baynes et al. 2012). Endophytic fungi are those species that spend all or part of their life cycle colonizing and inhabiting plant tissue (Wilson 1995). Endophytes are also defined as asymptomatic with regard to the host plant, and fungal species that cause visible pathogenic symptoms to their host species are not typically classified as endophytes (Wilson 1995). Some endophytes appear to be generalists regarding the plants they inhabit, while others seem to display a high degree of host specificity (Saikkonen et al. 1998). Endophytes may form symbiotic relationships with their hosts, and result in associations ranging from mutualistic to antagonistic (Saikkonen et al. 1998; Clay 1996; Wilson 1995). The endophytic classification encompasses a diverse array of fungal species, and it is generally agreed that all plants in natural settings carry an endemic population of fungal endophytes, the composition of which varies widely (Rodriguez et al. 2009; Saikkonen et al. 1998). It has been suggested that the actual number of endophytic fungal species is currently inestimable (Hyde et al. 2009).

For the past three decades, the majority of research concerning endophytic fungi in herbaceous plants has focused on mutualistic relationships between endophytes and their host plants (Saikkonen et al. 1998; Wilson 1995). In the

case of grass endophytes, research has focused heavily on relationships called 'Defense Mutualisms', wherein the fungal component provides some means of reducing herbivory of the host plant (Clay 1988). Bacon et al. (1977) demonstrated that the toxic effects commonly exhibited by cattle grazing tall fescue (*Festuca arundinacea*) resulted from alkaloid compounds produced by the endophytic fungus *Acremonium coenophialum*. Though scientists have been aware of endophytic fungi since the late 19th century (Rodriguez et al. 2009), this experiment by Bacon and colleagues (1977) established the first causal link between an endophytic fungi and a pathogenic effect on an herbivore. Similar relationship between perennial ryegrass (*Lolium perenne*) and the endophyte *Neotyphodium lolii* was shown to be responsible for the negative neurological effects commonly displayed by animals grazing this species (Fletcher and Harvey 1981). Subsequent research utilizing both tall fescue and perennial ryegrass showed that these fungal associations often led to an increase in competitive ability of the host, even against uninfected members of the same plant species (Marks et al. 1991). Considerable research has further illustrated the mutualistic effects of endophytes that function to reduce herbivory in their host species (Schulz et al. 2002; Clay 1988; Cheplick and Clay 1988). These mutualistic relationships may also function to mitigate abiotic stresses. For example, experiments conducted with grasses found in coastal and geothermal areas

identified endophytes that conferred increased salinity and heat tolerance to their host species (Rodriguez et al. 2008).

While mutualistic relationships between endophytic fungi and plants have been studied extensively, studies concerning pathogenic relationships between an endophyte and its host plant are comparatively rare (Saikkonen et al. 1998). Much research has focused on the negative effects of mutualistic endophytes to hosts in moisture or nutrient limited soil conditions, where the maintenance costs borne by the host plant often proves detrimental to overall fitness (Lehtonen et al. 2005; Cheplick 2004; Cheplick et al. 1989,1997, 2000). However, the effects outlined in these studies result from the biological costs of otherwise mutualistic fungi rather than from an actual antagonistic relationship with the endophyte. Other studies have shown that endophytes in a grass species may increase susceptibility to other types of pathogenic fungi (Wali et al. 2006; Newsham et al. 1994). More direct antagonistic relationships between an endophyte and its plant host have also been examined, including negative impacts on rate of photosynthesis (Costa Pinto et al. 2000), and reduced reproductive potential (Newcombe et al. 2009; Schardl et al. 2004; White 1988).

In a study of the relationship between forage grasses and the fungal endophytes then classified as *Epichloe typhina*, White (1988) stratified the observed

relationships into three categories. In Type 1 interactions, *E. typhina* targeted inflorescences on the host plant, effectively blocking the plant's sexual reproduction in favor of its own. Interactions classified as Type 2 were those that had both mutual and antagonistic relationships occurring on the same plant. Type 3 relationships consisted of mutualistic relationships wherein the endophyte produced toxins that reduced herbivory of the plant.

Recent studies have revealed a much higher endophytic diversity in most plants than previously thought, as well as a less stratified, more continual picture of plant endophyte interactions (Paszkowski 2006; Saikkonen et al. 1998; White 1988). For example, Shipunov et al. (2008) observed 92 distinct haplotypes of endophytic fungi occupying various populations of spotted knapweed (*Centaurea stoebe*). Subsequent experiments concerning the interactions of several of these endophytes yielded a broad spectrum of mutual, neutral and antagonistic interactions (Newcombe et al. 2009).

Coprophilous Fungi, and the link to Endophytes

Coprophilous fungi spend all or a portion of their life cycle dwelling within the deposited feces of animals. These fungi are thought to play a significant ecological role in the biological breakdown of dung material, and in providing food for coprophageous insects (Richardson 2001; Angel and Wicklow 1975). The

ability of fecal material to retain moisture and provide nutrients makes it an exceptional substrate for fungal growth (Herrera et al. 2009; Garrett 1951), and studies have revealed highly diverse populations of coprophilous fungi in a variety of substrates (Richardson 2001; Wicklow et al. 1980; Parker 1979; Angel and Wicklow 1975). It remains unclear whether species diversity of coprophilous fungi populations are more closely governed by geographical location, or by the specific characteristics of feces generated by various mammalian hosts. Differences in fungal species composition have been shown to exist in the fungal populations resident in feces originating from animals of the same species occupying different habitat types (Nyberg and Persson 2002); as well as feces from different species of animals occupying the same habitat (Herrera et al. 2011; Richardson 2001; Parker 1979; Angel and Wicklow 1975), or when hand fed an identical diet (Angel and Wicklow 1980).

In a study of coprophilous fungi inhabiting a Colorado grassland, Angel and Wicklow (1975) noted a high degree of fungal diversity among mammalian species inhabiting a short grass prairie. Feces from four animals were examined; pronghorn, cattle, rabbits, and small mammals. Researchers also observed that several of the fungal species were specific to one animal species, or one method of digestion (e.g. ruminants vs. cecal fermentation). The experiments conducted by Parker (1979) displayed a similar degree of association between fungal species

and a particular fecal substrate. That study involved fecal samples taken from three domestic species (horse, cattle, and sheep), and two wild herbivores (deer and rabbits). Of the 41 fungal species observed, 20 showed no association to animal species or digestion type. The remaining 21 displayed associations based on digestive type and herbivore species.

Angel and Wicklow (1980) examined the link between digestive system and the resulting fungal community by removing differences in habitat and diet selection. A single sheep and a single rabbit were fed a common diet of alfalfa. Of 21 total species isolated from fecal material, 12 displayed a significant difference in frequency between the two species. This indicates that digestive morphology of the animal affects the resulting fungal community.

The reasons for this variety of coprophilous fungal communities among different animal species occupying the same habitat are not clearly understood. Richardson (2001) and Angel and Wicklow (1975) observed a high degree of variability in the Carbon to Nitrogen (C/N) ratio across the dung of various species. Richardson (2001) also noted that several other chemical aspects of dung were similar across species, and theorized that the differences in the C/N ratio could account for differences in fungal colonization. It is also clear that competition between fungal species plays a role in final community composition. Brewer et al.

(1972) observed that a particular coprophilous fungus, *Sporormia minima,* was able to continue growth in the near anaerobic conditions provided by a sheep rumen. Brewer theorized that this ability to thrive and grow while other species merely survive and persist during their passage through the animal gut could give *S. minima* a competitive advantage once the dung was deposited.

Other competitive interactions among species of coprophilous fungi have also been observed. For example, *Podospora pleiospora* growing in rabbit dung was shown to increase its competitive advantage by producing metabolites that contained antifungal properties, impairing the fitness of other fungal community members (Weber et al. 2005).

Until very recently, nearly all assays of coprophilous fungal communities have been conducted using laboratory culturing methods. It has been posited that these methods leave knowledge gaps in the interactions of coprophilous species, resulting from lag times in culturing, or the inability to culture certain species under laboratory conditions. To address this possible deficit, Herrera et al. (2011) utilized molecular DNA analysis methods to characterize the fungal populations from cattle, bison, pronghorn, and prairie dogs. The fungal populations in the dung of bison and prairie dogs grazing the same site were compositionally similar,

indicating that fungal community composition may be more closely associated with habitat than with herbivore species or digestive morphology.

It has previously been noted that normally coprophilous fungal species may also be endophytic, or that the same fungus may have both endophytic and coprophilous stages within their life cycle (Sanchez Marquez 2012; Porras-Alfero 2008; Petrini 1986). Petrini, however, regarded such occurrences as incidental and of little consequence. Porras-Alfero et al. (2008) noted the presence of normally coprophilous fungal species living endophytically in the roots of blue grama grass (*Boutela gracilis*). They theorized that, in arid ecosystems, this association may play a significant role in seedling establishment, noting that the occurrence of fungi with coprophilous and endophytic stages within a single life cycle warranted further investigation. To our knowledge, studies have never been undertaken that examine this transition from a coprophilous to an endophytic lifestyle, and little has been done to examine the effect that these fungi have on the plant species in which they dwell.

Sordaria fimicola

This knowledge gap with regard to fungal behavior is of particular note in the case of *S. fimicola*. Most members of the *Sordaria* family, including *S. fimicola*, have a long history of use in scientific studies ranging from cell meiosis (Engh et al. 2010),

to gene recombination (Saleem et al. 2001). Additionally, *S. fimicola* has long been used as a model for classroom experiments observing the 'crossing over' phenomenon associated with cell meiosis (Madrazo and Hounshell 1979).

While the genetic and reproductive behavior of *S. fimicola* are well documented, studies concerning it's ecology are comparatively rare. Petrini's passing observation of fungi transitioning between coprophilous and endophytic lifestyles did include members of the *Sordaria* family (Petrini 1986). However, as mentioned earlier, this was regarded as of little consequence. An extensive review of available literature failed to reveal any previous research related to how and why such transitions occur, or the environmental conditions required to facilitate them.

Research related to the effects of endophytic *Sordaria* on plant tissue are also rare, and often reach contradictory results. In a study of *S. fimicola* dwelling endophytically in maize tissue, Sneh et al. (1987) observed significant reductions in dry matter accumulation, root length, and seedling vigor in maize plants infected with *S. fimicola*, in comparison to uninfected counterparts. Infection rates were highly sporadic, however, and while they occurred under field conditions, researchers were unable to reproduce them in a laboratory.

Conversely, Dewan et al. (1994) observed a beneficial effect on growth in both wheat and rye grass resulting from *S. fimicola* infection. Additionally, plants of both species, when infected by *S. fimicola*, displayed a significant reduction in mortality resulting from the pathogenic fungi *Gaeumannomyces graminis*.

While it is clear that endophytic infection by *S. fimicola* can have an effect on various attributes of the plant host, the mechanisms behind these effects, whether resulting from competition between fungi or direct effects on the host plant, are poorly understood and warrant further investigation.

CHAPTER 2 -MATERIALS AND METHODS

This project is designed to examine the effects of *S. fimicola* on cheatgrass and the ability of a grazing herbivore (i.e., sheep) to transfer the fungus into previously uninfected cheatgrass communities. We conducted 7 experiments.[1]

Experiments 1 and 2 examined hypothesized differences between cheatgrass plants containing endophytic Sordaria, and their uninfected counterparts. Experiment 1 examined differences in biomass and seed production between the two groups. Experiment 2 explored the possibility of differences in nutritional forage value for herbivores.

[1] All methods involving sheep were approved by the University of Idaho Animal Care and Use Committee (Protocol #2011-7)

Experiment 3 tested the hypothesis that *S. fimicola*, as a dung obligate species, would elicit preferential grazing of the host plant by a grazer. In this experiment we presented with both infected and uninfected material to sheep and measured intake to indicate selective preference or aversion towards *S. fimicola* bearing cheatgrass.

Experiments 4 and 5 examined the ability of *S. fimicola* to transit a ruminant digestive tract. Additionally, Experiment 5 examined the time required for transit, as well as the length of time Sordaria would remain in the gut, and appear in dung, following an artificial dosing.

Experiment 6 placed sheep on cultivated stands of cheatgrass, and examined the ability of grazing livestock to carry *S. fimicola* from infected to uninfected cheatgrass populations.

Experiment 7 examined the final stage of the hypothesized *S. fimicola* life cycle, the transition from a dung fungus back to an endophytic lifestyle. Additionally, given the moisture requirements of previous experiments (Baynes 2011), Experiment 7 examined the level of moisture on the plant surface necessary for the transition to take place.

Experiment 1 - Biomass and Seed Comparisons

Plant biomass, and average seed production were assessed to examine the effects of infection of cheatgrass with the CID323 strain of *S. fimicola* at the plant level. A stand of cheatgrass, 600 square meters in size, was cultivated near Moscow, ID (46.73° N latitude; 117.00° W longitude), using seeds gathered during previous experiments, from various regions of Idaho.

Half of the stand was infected, via leaf inoculation, with *S. fimicola.* Fruiting bodies, collected from laboratory cultures, were suspended in 18 liters of sterile water. This mixture was applied to the plot with a handheld sprayer. Application occurred on 15-May-2011, beginning at 0900. On the same day, the remainder of the stand was sprayed, in a similar way, with sterile water only. The infected portion of the stand was separated from the uninfected portion by a 1 m buffer zone in which no spraying occurred. The presence of endophytic *S. fimicola* in cheatgrass plants on the treated portion of the site was confirmed using leaf tissue samples collected from both the treated and untreated portions of the site on 05-Jun-2011, and again on 01-Jul-2011. All samples were surface sterilized with 50% ethanol, rinsed with sterile water, and plated on 55-mm filter paper. *S. fimicola* presence or absence was assessed visually over the following 14 days.

Biomass samples (N=100) were collected on 30-Jun-2011 when cheatgrass was in the early seed development phenological stage. Samples were collected at randomly selected intervals along a 30-m transect placed down the long axis of the infected and uninfected treatment plots, 2 m from the 1-m buffer strip. Each collection point represented a randomly selected centimeter distance (0 to 30 m) from the origin of the transect, and a random distance (0 to 2 m) left or right of the transect line. The plant closest to the collection point was clipped to ground level and placed in a paper bag. Samples were dried for 48 hours in a forced air dryer at 43°C. Later, the samples were dried for 24 hours at 50°C in a forced air oven and weighed to determine average biomass for each plant. The weights of plants belonging to treatment and control gorups were compared using an independent T-test.

Once plants in the field plots achieved seed maturity (11-Aug-2011), 40 plants were collected from the treatment and control plots to assess seed production. The sampling procedure was identical to that described above for biomass production, with the exception of the collection of 40 samples instead of 100. After drying, each plant was stripped of its seeds, and seeds were weighed. The mean seed production per plant in the treatment and control plots were compared and evaluated for significance using an independent T-test.

Experiment 2 - Comparison of Forage Quality Attributes

Forage quality assessment were conducted using samples (approximately 100 g) of *S. fimicola* positive (S^+) and *S. fimicola* negative (S^-) material from 10 plants clipped to ground level from within each treatment. These samples were collected daily for 8 days beginning on 23-June-2011, while cheatgrass was in the flowering and early seed production stages. Within 30 minutes following collection, samples were heated to 70° C in a microwave oven, to curtail metabolic activity (Popp et al. 1996). Samples were then dried at 50° C in a forced air oven for 48 hours, and stored for later analysis. Samples were submitted to Dairy One Laboratories, Ithaca, NY, for forage analysis. Analyses conducted by Dairy One included assays for crude protein (CP), as well as acid and neutral detergent fiber content (ADF and NDF). Analyses were conducted using traditional chemical methods. Crude Protein was assessed using a Leco FP-528 Nitrogen/Protein analyzer. To assess acid and neutral detergent fiber content, samples were digested, in a solution appropriate to each fiber type, in an ANKOM A200 Digestion Unit.

Experiment 3 - Preference Trial

Preferential selection or avoidance of *S. fimicola* infected cheatgrass compared to uninfected cheatgrass was examined using 6 yearling Suffolk ewes provided by the University of Idaho Palouse Research, Education, and Extension Center near Moscow, ID. Sheep were housed in a communal pen with *ad libitum* access to

water and locally grown grass hay, which was comprised primarily of tall fescue (*Festuca arundinaceae*). Hay was removed at 2000 each evening. The preference trial lasted 12 days and consisted of 2 phases: a protocol conditioning phase, (Day 1 to 4) followed by preference testing (Day 5 to 12). The protocol conditioning phase familiarized the animals with the preference trial procedure using daily treatments over a 4 day period. At 0800 each morning, sheep were placed in individual pens, and each sheep was offered 100 g. each of smooth brome (*Bromus inermis*), and meadow foxtail (*Alopecuris pratensis*), both of which were familiar to the sheep subjects. All feed samples were gathered from nearby pastures and material was hand chopped into segments <4 cm in length. All feeds were collected daily, between 0700 and 0900, and offered in a fresh state. Feeds were offered in 14x14x16 cm plastic containers placed adjacent to one another. Containers were removed when 90% of one feed, by visual estimate, had been consumed from one container, or when one hour had passed. Preference was indicated by consumption of one grass at a rate greater than 60% of the total amount of both grasses offered. Avoidance was ascribed to samples that constituted consumption of 40% or less of the total amount of grass offered.

Daily preference trials were conducted over 8 days beginning 23-Jun-2011. The presentation protocol used in the cheatgrass trial was identical to the protocol used in the pre-conditioning phase of the experiment. Cheatgrass samples were

collected from the same *S. fimicola* infected and control stands described in Experiment 1. For the first 5 days, each sheep received 100 g each of infected (S^+) and uninfected (S^-) cheatgrass. On days 6 and 7 sheep received 150 g each of S^+ and S^- cheatgrass and, on the final day, 200 g of S^+ and S^- cheatgrass. Preference or avoidance was measured as the daily intake of S^+ cheatgrass, expressed as a percentage of total daily consumption of cheatgrass (S^+ and S^-). Preference was indicated by consumption of one grass at a rate greater than 60% of the total amount of both grasses offered. Avoidance was ascribed to samples that constituted consumption of 40% or less of the total amount of grass offered.

The percent consumption of S^+ cheatgrass for all ewes was evaluated in a repeated measures analysis of variance with daily tests as the repeated variable (Prism Version 5.04 software, GraphPad 2010). An arcsine square root transformation was performed to normalize the percentage data (Ahrens et al. 1990).

Experiment 4 - Fecal Examination

Two trials were conducted to assess the passage of *S. fimicola* through the sheep digestive tract. In the first trial, 8 yearling ewes were placed in adjacent individual 1.5 x 1.5 m pens for 48 hours beginning 30-Jun-2011. Each sheep was fed a randomly assigned diet of 500 g of S^+ or S^- cheatgrass (4 sheep to each

treatment). Cheatgrass was hand collected, and chopped to fragments <4cm in length before presentation. Feeding occurred twice daily. Fecal samples from each sheep were collected, via rectal palpation, 24 and 48 hours following the initial feeding of cheatgrass.

All samples were incubated in a sealed plastic container with a moist paper towel. Presence of *S. fimicola* was assessed by placing a 0.5 g portion of each sample on a sterile towel saturated with deionized water. Each sample was then placed in a sterile petri dish and covered with 55-mm sterile filter paper. A sterile filter paper disc was then placed over the 55-mm paper filter. Samples were visually assessed for presence of *S. fimicola* fruiting bodies on the filter paper 10 days following their placement.

The second trial was conducted beginning 17-Jul-2011, with the same eight sheep utilized in the initial fecal trial. The protocol of this trial was identical to the previous trial, with three exceptions. The amount of cheatgrass provided was increased from 500 g to 700 g at each feeding. Fecal samples were collected at 36 and 48 hours following initial cheatgrass consumption, with the inclusion of an additional sample taken before the initial feeding, to establish a baseline. Because palatable cheatgrass was unavailable, the final feeding on 18-Jul-2011 consisted of alfalfa pellets. It was assumed that passage rate would not allow the alfalfa to

have a significant effect on the fungal component of collected feces, as the final fecal collection occurred less than 12 hours following this feeding.

Experiment 5 - Transit of Sordaria Through a Ruminant Digestive Tract

During Experiment 4, attempts to record the passage of *S. fimicola* through the digestive tract of the sheep were confounded by the presence of *S. fimicola* and other fungi in dung samples collected prior to consumption of cheatgrass. The *S. fimicola* presumably originating from some component of sheep diets before the trial. Experiment 5 sought to remove such confounding variables, and determine whether cultured *S. fimicola* can successfully transit the sheep digestive tract.

Beginning on Day 0 (27-Feb-2012), four open ewes of the suffolk breed were placed in a 2 x 3 m indoor pen, with *ad libitum* access to water. On days 0-2, all sheep were fed a daily ration of 2.25 kg alfalfa pellets per sheep. All feed was heated, in a conventional oven, to 50° C for 30 minutes. Feed was heated to kill viable fungal material inherent in the feed. The alfalfa pellets were intended to function as a 'flushing' agent to remove fungi dwelling in the digestive tract of the sheep, an effect noted in Experiment 4. Beginning on day 3, sheep were switched to a daily diet of 2.25 kg beet pulp pellets, heated to 50° C for 30 minutes before feeding. *S. fimicola* was incubated, on potato dextrose agar, in 4 standard 100-mm petri dishes for 21 days at 20°C. Spores and mycelia from these plates were

combined and suspended in sterilized water at 20° C. At 0830 on days 7 and 8 of the experiment all sheep were dosed, via esophogeal tube, with 200 ml of this solution.

Prior to *S. fimicola* dosing, fecal samples were collected from each ewe, via rectal palpation, once daily. Following dosing, collection of samples occurred twice per day, until the end of the experiment on day 11. All fecal samples were incubated and assessed for presence of *S. fimicola* fruiting bodies in a manner consistent with Experiment 4.

Experiment 6 - Sheep as a Vector for the Spread of Sordaria

This experiment was conducted using the same cheatgrass plot established for experiments 1 through 5. Attempts were made to re-isolate *S. fimicola* from leaf samples collected at the site in early May 2012. In all cases, plants were shown to be free of preexisting *S. fimicola*. On 25-May-12, the eastern portion of the plot was inoculated with *S. fimicola* using fruiting bodies and mycelia collected from laboratory cultures, mixed with 4 liters sterile water. Application of this mixture was conducted using a household sprayer, and was in all respects identical to the inoculation procedure used in the previous experiments. Beginning at the south end of the plot, 5 pens were constructed. All pens were 4 m wide and 20 m in length, and spanned both the infected and uninfected portions of the site. A 1-m

wide buffer zone was left between each pen. Two additional 20 m by 4 m plots were marked off, and used as positive and negative controls (Figure 2.1).

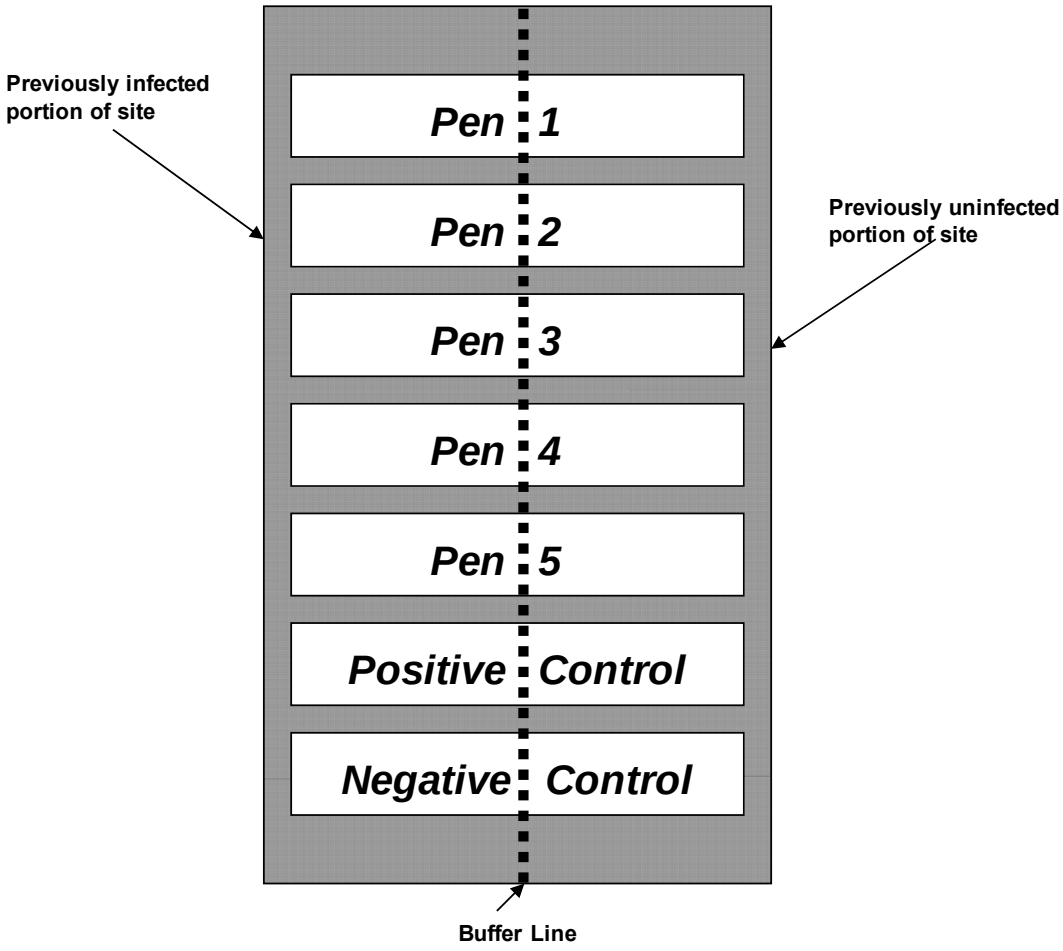

Figure 2.1: Diagram of pen construction for experiment 6. Pens 1-5 contained sheep for the duration of the experiment. The buffer line, (---) delineates the portion of the site that was infected with *S. fimicola* from the uninfected area.

Five adult Targhee ewes were selected for the experiment. Beginning on 18-Jun-12, all sheep were placed on a diet of alfalfa pellets, in an enclosed pen, away from other forages. On Day 1 of the experiment (20-Jun-12), pre-experiment fecal

samples were collected, via rectal palpation, and each sheep was placed individually into one of the five pens located at the cheatgrass site. Sheep were left in these pens for 6 days. Fecal samples were collected from each sheep, at 0700, on days 3, 5, and 6. Three 1-g samples of dung were analyzed from each subject for the presence of *S. fimicola* in a manner consistent with previous experiments. The remaining collected dung was commingled and 1kg of the resulting mixture was hand scattered each day over the uninfected portion of the positive control area of the site. At 1800 on day 6, sheep were removed, and the site was allowed to sit vacant until the following Spring.

On 22-Apr-2013, samples were collected to assess the presence or absence of endophytic *S. fimicola*. Six samples from each enclosure, 3 each from the previously infected and uninfected portions of each pen, were selected. Collection points were randomly distributed along a transect extending longitudinally down the center of each enclosure. At each collection point, a random number between +2 and -2 was assigned, and was used to determine the distance and direction of the sampling point, perpendicular to the transect. A total of 42 samples were collected.

From each plant sample, 5 leaf segments were cut > 1 cm. Each leaf sample was surface sterilized by immersion in 90% ethanol for 5 minutes, rinsed with sterile water, and plated on potato dextrose agar.

Experiment 7 - Endophytic infection of cheatgrass with dung borne S. fimicola

The purpose of this experiment was to observe endophytic infection of cheatgrass by *S. fimicola* grown from a sheep dung substrate, effectively completing the hypothesized life cycle, as well as to assess the moisture conditions required for the transition to occur.

Cheatgrass was sown in 40, 8 x 8 x 8 cm square pots, at a rate of 8 seeds per pot, using standard all purpose potting soil. All seeds were collected from the uninfected portion of the cultivated site used in previous experiments. Once plants reached the vegetative stage, all pots were thinned to 4 plants per pot.

S. fimicola bearing manure was generated using 3 Suffolk sheep. Beginning on 25-Jul-2012, sheep were placed on a diet of *ad libitum* alfalfa pellets for 48 hours. On 27-Jul-2012, sheep were placed on a diet of grass hay, and dosed with *S. fimicola*. As in previous experiments, sheep were dosed using *S. fimicola* fruiting bodies and mycelia, collected from laboratory cultures, and suspended in 200 ml

of sterilized water. This mixture was administered via esophogeal tube. Fecal material was collected, via rectal palpation, at 12 hour intervals from 28-Jul-2012 through 30-Jul-2012. Samples from each collection were plated on filter paper, as in Experiment 5, and all fecal material was then commingled.

A 4-g portion of the commingled dung was placed in the center of each pot. Pots were divided into two groups. Group 1, the control group, received moisture via soil only. Water sufficient to wet the soil was applied to each pot daily at a point sufficiently remote to all plants to ensure that leaf moistening did not occur. Group 2, the treatment group, in addition to soil moisture, received daily misting with water, sufficient to leave beaded moisture on all vegetative surfaces.

To assess for the presence of *S. fimicola* spores, a 50 mm plastic petri dish was lined with double sided tape and suspended above each pot using plastic markers to act as a trap for upward moving spores.

All plants were harvested on 17-Sep-2012. A 2-cm sample of the innermost leaf of the plant tiller was taken from each plant. These samples were surface sterilized with 50% ethanol, rinsed with sterile water, and plated on potato dextrose agar for later visual assessment of *S. fimicola* presence.

CHAPTER 3 - RESULTS AND DISCUSSION

Experiment 1 - Biomass and Seed Comparisons

The presence of endophytic *S. fimicola* affected the biomass and seed production of cheatgrass. Biomass of individual plants grown on the *Sordaria* infected site was 26.7% lower (1.31 g) than plants grown on the site not infected with *Sordaria* ($p<0.05$; Figure 3.1).

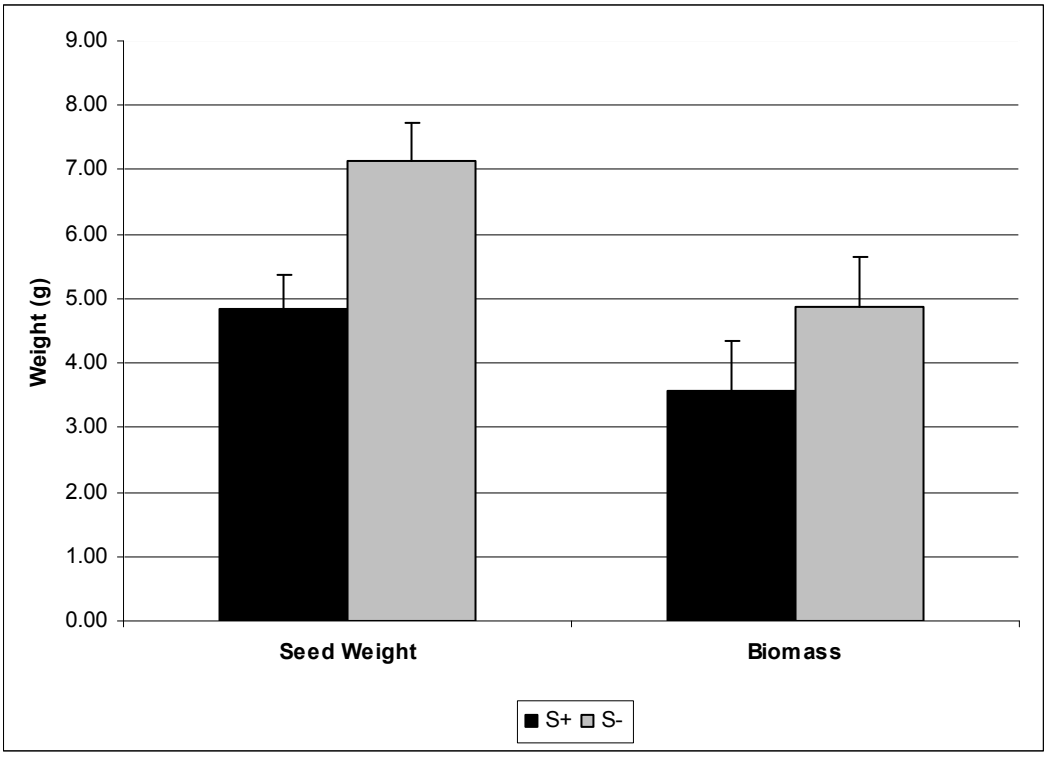

Figure 3.1: Mean biomass and seed production (by weight) of S+ and S- cheatgrass samples. Note that biomass and seed samples were taken at differing stages of the plant's life cycle, accounting for the higher seed weight relative to biomass.

The weight of seeds produced by *S. fimicola* infected (S+) plants was 32% lower (2.29 g) than that of uninfected (S-) plants ($p<.005$; Figure 3.1). Mean weight of an individual seed was .0035 g. Thus, estimated actual seed production, based upon seed weight, was 1021 seeds per plant among *S. fimicola* positive individuals, and 1395 seeds among *S. fimicola* negative plants. During the course of the experiments utilizing this site, this difference between *S. fimicola* infected and uninfected sites was revealed in biomass measurements and was also visibly apparent. It is unclear, however, whether this is a truly pathogenic effect, or the result of the biological cost of an otherwise mutualistic or neutral relationship between the fungus and the cheatgrass host. White (1988), in his characterization of fungal relationships, characterized a true pathogenic relationship as one where plant reproduction was directly interfered with by the infecting fungi. As the strain of *Sordaria* used in our experiments does not appear to inhabit the reproductive areas of the plant, this is not the case here.

It is also possible that the reduced growth and fecundity observed is a result of the metabolic cost borne by the cheatgrass plant in harboring *S. fimicola*. In a study of endophytes in perennial ryegrass (*Lolium perenne*), Cheplick et al. (1989) observed that normally mutualistic endophytic infections resulted in poorer performance on the part of infected plants vs. uninfected under nutrient limited conditions. Under those conditions, it was apparent that the otherwise beneficial

relationship with the endophyte was outweighed by the ecological cost of supporting it.

Competition between different fungi may also play a role in the observed result. Research into fungal communities, in particular coprophilous fungi, has often revealed that species composition in those communities can be subject to intense competition (Herrera et al. 2010; Weber et al. 2005; Brewer et al. 1972). Additionally, Dewan et al. (1994) observed reductions in wheat and ryegrass mortality resulting from *G. graminis* infection, when the plants were inoculated with *S. fimicola*. This is suggestive of a competitive relationship in which *S. fimicola* neutralized *G. graminis*, thus freeing the host plant from the pathogenic effect. It is possible that a similar relationship could yield the results observed here. Given the wide array of endophytic fungi identified in cheatgrass by Baynes (2011), introduced *S. fimicola* could be interacting with an otherwise mutualistic endophyte of another species, resulting in lower overall fitness of those plants relative to uninfected counterparts. Regardless of the mechanism however, given the deleterious effect on seed and biomass production observed, it remains likely that the successful introduction of endophytic *S. fimicola* could function to partially regulate species composition and dominance of cheatgrass in currently uninfected communities.

Experiment 2 - Comparison of Forage Quality Attributes

The forage analysis of *S. fimicola* infected (S+) and uninfected (S-) cheatgrass, grown under the same conditions, yielded no differences in crude protein or fiber content. Crude protein across both treatment and control averaged 11.5% with a standard error of 0.39, while the mean acid and neutral detergent fiber contents were 34.5% and 55%, with standard errors of 0.58 and 0.89, respectively (Table 3.1).

	Crude Protein		ADF		NDF		TDN	
	S+	S-	S+	S-	S+	S-	S+	S-
Mean	12.00	10.94	34.44	34.53	54.63	56.13	60.00	59.63
Standard Error	0.46	0.61	0.67	1.00	1.24	1.32	0.38	0.32
T Statistic	1.39		-0.07		-0.83		0.75	
P Value	0.19		0.94		0.42		0.46	

Table 3.1: Mean comparisons of forage attributes of Sordaria infected (S+) and uninfected (S-) cheatgrass.

Despite differences in seed and biomass production, *S. fimicola* infected cheatgrass did not differ significantly from uninfected counterparts with regard to its nutritive value to grazing mammals. Similar results have been recorded in ryegrass, where a comparison of endophyte infected versus uninfected counterparts yielded no difference in nutritional values, despite differences in biomass production (Kallenbach et al. 2003). Conversely, in a study of Sorghum (*Sorghum bicolor*) inoculated with two endophytes, *Glomus fasciculatum* and *Azospirillum brasilense*, researchers noted changes in plant physiology sufficient to alter its nutritional composition, particularly with regard to mineral content (Pacovsky 1989).

While our results do not indicate any significant nutrient compositional changes to cheatgrass resulting from *S. fimicola* infection, it is possible that differences in mineral content exist, as these were not assayed. From the standpoint of the grazing animal, this lack of difference in forage value conforms logically with the results of Experiment 3, explained below.

Experiment 3 - Preference Trial

Over the course of the conditioning phase, sheep expressed a significant preference for smooth brome over meadow foxtail (P=0.0094). Given a hypothesized mean of 50% brome consumption, the sheep exceeded this by 29% and 13% on days 3 and 4, respectively. During the preference trial phase, sheep readily consumed both S^+ and S^- cheatgrass. The data did not indicate a preference for or avoidance of, S^+ cheatgrass (P=0.7767; Figure 3.2).

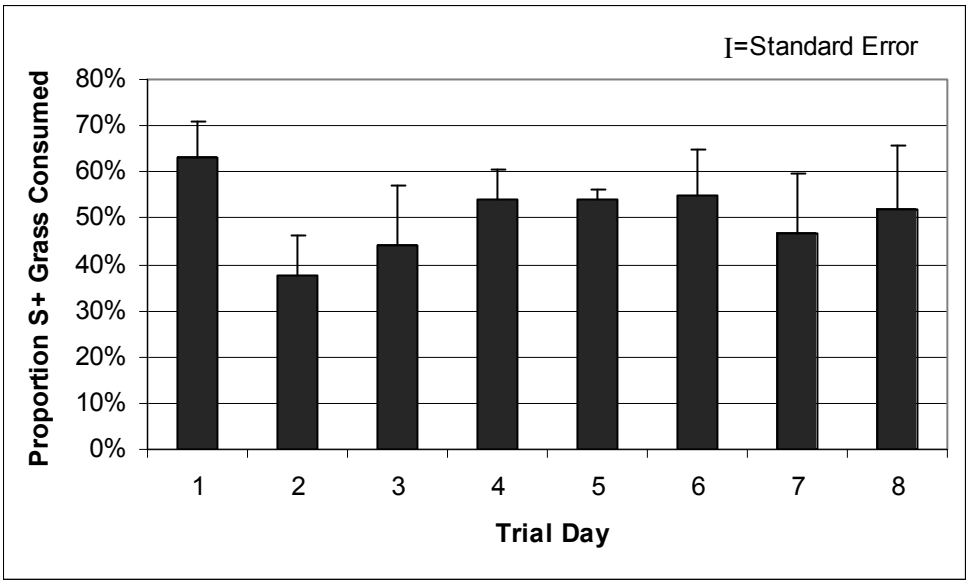

Figure 3.2: Mean daily consumption of Sordaria infected (S+) cheatgrass expressed as percent of total cheatgrass consumed when 6 sheep were presented with equal amounts of infected and uninfected cheatgrass.

Our original hypothesis predicted that herbivores would exhibit a preference for the CID323 strain of *S. fimicola*. Based on the presumed necessity of digestion for continuance of its life cycle, it was logical to theorize that the strain would have developed characteristics to increase the likelihood of herbivory. Newcombe et al. (2009), for example, identified a strain of endophytic *Fusarium* in *Centauria steobe* plants that, when present, increased the likelihood of herbivory by aphids.

However, the results of this experiment are consistent with the results of Experiment 2. Dietary selection in sheep is largely a response to the post ingestive consequences of consuming a particular feed. Whether the

consequences of ingestion are positive or negative, the animal associates that feedback with the feed in question, and adjusts it's diet accordingly (Ganguli et al. 2010; Ginane et al. 2009; Cooper et al. 1995). Given the lack of difference in nutritional values between infected and uninfected cheatgrass observed in Experiment 2, it is logical to conclude that the sheep had no differing post ingestive feedback upon which to base a preference.

While the hypothesized preference by sheep for S^+ cheatgrass was not apparent, this, in and of itself, does not present a challenge to the practical application of *S. fimicola* as a potential means to manage cheatgrass. The lack of preference extends both ways, and while no preference was exhibited for S+ cheatgrass, no preference against the material was observed. Rather, the sheep readily consumed cheatgrass from both treatment and control, regardless of infected status.

Experiment 4 - Fecal Examination

Culturing of fecal samples collected during both portions of Experiment 2 confirmed the presence of *S. fimicola* regardless of animal treatment, in several cases even before the trial had commenced, leading to inconclusive results. Specifically, baseline dung samples collected from 6 of the 8 sheep tested yielded significant levels of *S. fimicola* prior to any treatment that involved introducing *S.*

fimicola into the rumen. Following treatment, just 2 of the eight samples collected yielded measurable levels of *S. fimicola*.

While largely unsuccessful, the results of this experiment yielded information of great use in subsequent experiments. It was noted that the feeding of alfalfa pellets at the close of the trial resulted in a lack of any significant fungal component in the resulting fecal material within 24 hours post-ingestion. Alfalfa pellets were thus utilized, in subsequent experiments, as a purgative to remove preexisting fungi in the animal's digestive tract. This reduced the appearance of other fungi in the resulting dung, increasing our ability to visually assess the presence of *S. fimicola*.

The apparent presence of coprophilous *S. fimicola* prior to commencement of the trial is not altogether surprising. While little research has been conducted to examine what plant species may commonly host *S. fimicola*, studies of fungal communities in animal dung have commonly shown the presence of members of the *Sordaria* family, indicating a certain level of ubiquity within the coprophilous fungal community (Nyberg and Persson 2002; Richardson 2001; Wicklow et al. 1980; Parker 1979; Angel and Wicklow 1975).

Experiment 5 - Transit of Sordaria Through a Ruminant Digestive Tract

All sheep dosed with the *S. fimicola* suspension produced dung which was positive for *S. fimicola* between 24 and 72 hours following initial dosing on day 7 (Table 3.2). All four subjects produced dung positive for *S. fimicola* at some point following dosing. Following the feeding of alfalfa pellets, no subjects produced *S. fimicola* positive dung prior to dosing, indicating the success of this protocol in removing the fungal contaminants that confounded Experiment 4. No sheep produced dung positive for *S. fimicola* at 96 hours following the final dosing on day 8.

Hours (from initial dose)	Sheep Subject				
	A	B	C	D	
-168	-	-	-	-	
-144	-	-	-	-	
-120	-	-	-	-	
-96	-	-	-	-	
-72	-	-	-	-	
-48	-	-	-	-	
-24	-	-	-	-	
0	-	-	-	-	Dosing Occurred
24	-	-	+	-	
36	+	-	-	-	
48	na	-	na	-	
60	-	-	+	+	
72	-	+	+	-	
84	-	-	+	-	
96	na	-	+	-	
108	na	-	na	-	

Table 3.2: Analysis of Sordaria presence on plated sheep dung, 28 days post sampling. + indicates the presence of Sordaria (species confirmed by microscopic examination); - indicates absence; na indicates no sample collected.

The experiment indicates that *S. fimicola* transits the sheep gastrointestinal tract within 24-72 hours. Additionally, it will produce viable perithecia on the resulting

dung, under laboratory conditions (20°C, moist chambers, sealed in plastic bags), between 21 and 28 days post deposition. However, there is significant lack of consistency in the results between subjects. This may be the result of bacterial differences between the digestive tracts of individual animals. It may also be the result of inconsistent consumption by the subjects of beet pulp, which was not readily accepted as a novel feed, leading to inconsistent rates of digestion and fecal production.

While considerable research has been conducted concerning the influences that habitat and host species have on community composition among coprophilous fungi (Herrera et al. 2010; Richardson 2001; Parker 1979; Angel and Wicklow 1975), to our knowledge, studies have never addressed potential differences in the success of a specific fungi across individuals of the same species consuming the same diet. Similarly, little has been done to examine the persistence of *S. fimicola*, or any coprophilous fungi, in the digestive tract once the source has been removed from the diet. Yet this factor weighs heavily on the potential of these fungi as a form of biological control, if grazing animals are to be the vector for introduction into novel areas.

Experiment 6 - Sheep as a Vector for the Spread of Sordaria

Laboratory culturing of pre-grazing dung samples, conducted in Jul-2012, yielded no *Sordaria* growth across all subjects. Subsequent samples of cultured dung

from all subjects displayed positive *Sordaria* growth beginning on day 6, approximately 120 hours following introduction to the pens (Table 3.3). In the absence of another source, this indicates that *Sordaria* was present in the artificially infected portion of the cheatgrass stand, and that *Sordaria* laden dung was deposited within the enclosures.

	Subject				
Hours Post Introduction to Cheatgrass	**A**	**B**	**C**	**D**	**E**
0	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
48	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
96	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
120	+	+	+	+	-
	+	+	+	+	+
	+	+	+	+	+

Table 3.3: Analysis of Sordaria presence on plated sheep dung, 28 days post sampling, 3 repetitions per sample. + indicates the presence of Sordaria (species confirmed by microscopic examination); - indicates absence.

In all previous experiments, involving *S. fimicola* introduction via esophageal tube, *Sordaria* began appearing in the resulting dung 24-72 hours post ingestion. The reason for the delay in *Sordaria* appearance in this case is not immediately clear. In experiments 5 and 7, *S. fimicola* began appearing in dung within 72 hours following ingestion, despite a marked difference in the feeds used as substrates (beet pulp and grass hay). In light of this, it cannot readily be assumed that the

delay experienced in this experiment is the result of different passage rates of the materials.

The difference, however, may lie in how *S. fimicola* itself was introduced. To our knowledge, no research has ever been conducted examining the difference in passage rate of endophytic versus free floating fungi through a digestive tract. It may be significant that, in all other experiments involving introduction of *S. fimicola* to the ruminant tract, fungal spores and mycelia were introduced separate from the feed substrate used to generate dung. In this experiment, however, the fungus was introduced as an integrated portion of the consumed plant. It is possible that a higher degree of digestive breakdown was necessary to facilitate the release of *Sordaria* from within the intracellular space of the plant.

Cultured leaf samples, collected from the cheatgrass stand in Apr-2013, produced no *S. fimicola*, despite its known presence in the dung applied to the site during the previous year, as well as in approximately half of the cheatgrass plants on the site at that time. The method by which *S. fimicola* survives the winter months, and passes to the next generation of annual grass the following year, remains unclear. The implications of this are addressed to a greater degree in the results of experiment 7.

Experiment 7 - Endophytic infection of cheatgrass with dung borne Sordaria

On 31-Aug-2012, 30 days following dung application, a subsample of 10 spore traps from each treatment was examined for the presence of germinated *S. fimicola* spores. Plates were visually examined, using a 10x microscope, for spore presence. Of the plates examined, 3 from the treatment (water misting) group, and 4 from the control (soil moisture) group, contained expelled spores, indicating germination of *S. fimicola* from the dung.

Results following the final collection and plating of plant material on 17-Sep-2012 were inconclusive. Under laboratory conditions, the samples were overrun by other fungi and molds to a degree that made distinction of *S. fimicola* presence nearly impossible. Over all samples, only a single instance of endophytic infection by *S. fimicola* was observed.

While several of the plated samples gave the appearance of containing *S. fimicola*, only a single case of true endophytic infection was documented in the course of the experiment. While these results present obvious challenges to the practical application of *S. fimicola* as an agent of biological control, it does mirror earlier research utilizing the same fungus. Despite multiple cases of endophytic *S. fimicola* observed affecting the health of maize plants in the field, Sneh et al. (1987) was likewise unable to reproduce the phenomenon under laboratory

conditions. During the course of this research, however, endophytic *S. fimicola* was clearly documented under field conditions, as has been observed elsewhere (Sanchez Marquez et al. 2012; Porras-Alfero et al. 2008; Petrini 1986). Clearly further research is warranted to examine the transition to an endophytic lifestyle by otherwise coprophilous species, and the conditions that facilitate this occurrence in the natural world.

CHAPTER 4 - SUMMARY AND IMPLICATIONS

At the outset of these experiments, our original hypotheses centered around the potential of *Sordaria fimicola* as a practical means of biologically suppressing cheatgrass production , using grazing livestock as the vector for dispersal. The results of this research present a broader view into the lifecycle of S. fimicola, which has largely gone unexamined, despite the ubiquitous use of the species in various fields of study (Engh et al. 2010; Saleem et al. 2001; Madrazo and Hounshell 1979).

Cheatgrass Response

As observed in experiment 1, endophytic infection of cheatgrass with the CID323 strain of *S. fimicola* does appear to yield a significant negative effect on both biomass production and fecundity. These attributes are encouraging from the standpoint of the species' potential use as a biological control for cheatgrass. While methods vary, most attempts to reduce cheatgrass dominance via non-

chemical means have focused on reduction of the available seed bank, thus reducing dominance of the species on a site over time. Historically, this has been accomplished either by direct removal of the plant prior to seed production, as with targeted grazing, or by attempts to reduce the viability of banked seeds, prior to germination (Diamond et al. 2012; Young and Clements 2009; Meyer et al. 2008). Given the reductions in seed production observed as a result of *S. fimicola* infection, the species exhibits a strong potential to decrease the quantity of cheatgrass seeds available for germination in subsequent seasons. As with other methods of control, this reduction in banked seed would lead to a corresponding reduction in cheatgrass dominance at a given site over time.

Sheep as a vector to the spread Sordaria fimicola

The sheep utilized in these experiments did not appear to exhibit a preference either for or against cheatgrass infected with *S. fimicola.* Our original hypothesis proposed a preference for infected material, based upon the supposed need of *S. fimicola* to transit a digestive tract in order to complete its lifecycle. However, in light of the results of Experiment 2, which yielded no significant differences in forage composition between infected and uninfected cheatgrass, the lack of preference displayed by the sheep is logical. This lack of preference, however, does not preclude the use of sheep as a potential vector for the spread of *S. fimicola* into new areas.

To evaluate the practicality of spreading *S.* fimicola to new areas, it was necessary to examine methods of introducing the material to grazing animals, as well as the amount of time *S. fimicola* persists in the digestive tract following such introductions. During the course of our experiments, we introduced *S. fimicola* to the sheep digestive tract both artificially, and by allowing them to graze known infected sites. In all cases, we produced *S. fimicola* laden dung, capable of producing spores that could conceivably infect nearby grasses, including cheatgrass. We may conclude that, whether *S. fimicola* is introduced to sheep via grazing or artificial inoculation, they present a suitable vector to spread dung borne *S. fimicola*. In Experiment 5, which examined the persistence of *S. fimicola* in the digestive tract of sheep, retention appeared to be limited to 96 hours post introduction. However, owing to the reticence of sheep to consume a diet of beet pulp, further examination in this area may result in greater retention times, if a different base diet is utilized.

Lifecycle of Sordaria fimicola

Until recently, little attention has been given to the lifecycle portion of an otherwise coprophilous fungal species that occurs outside of dung. While earlier research noted occasional instances of transitioning between coprophilous and endophytic lifestyles, it was more commonly believed that the spores of most coprophilous

fungi simply rested on the surface of plant material, awaiting ingestion by an herbivore to continue the cycle (Petrini 1986). Under this model, the plant and fungi did not interact in any significant way. More recent research has disproven this model, showing that many traditionally coprophilous fungi spend portions of their lifecycle dwelling endophytically in a host plant, where a wide array of interactions with the plant host are possible (Porras-Alfero 2008).

Clearly, *S. fimicola* can be characterized as a fungus with both a coprophilous and endophytic growth form. However, actual observance of this transition from coprophilous to endophytic lifestyles has proven much more difficult than previously imagined. Throughout the course of these experiments, such infection did occur and was observed. However, infection rates were highly sporadic, and appear dependent on conditions that are not yet apparent. This difficulty is consistent with previous research examining endophytic *S. fimicola*, where repetition of observed phenomenon in the field has proved unrepeatable under laboratory conditions (Dewan et al. 1994; Sneh et al. 1987).

The difficulty in understanding the conditions required for this transition presents obvious challenges to the use of *S. fimicola* as a biological control agent for cheatgrass, or any plant. While the observed effects of *S. fimicola* infection in cheatgrass are encouraging, they are of little use if the fungi cannot be reliably

introduced to the plant. Further research into the ecology of *S. fimicola*, with particular attention to the environmental conditions necessary for a successful transition into an endophytic lifestyle is warranted.

LITERATURE CITED

Ahrens WH, Cox DJ, Budhwar G. 1990. Use of the Arcsine and Square Root Transformations for Subjectively Determined Percentage Data. Weed Science 38:452-458.

Angel K, Wicklow DT. 1975. Relationships between Coprophilous Fungi and Fecal Substrates in a Colorado Grassland. Mycologia 67:63-74.

Arnold AE, Engelbrecht BMJ. 2007. Fungal Endophytes Nearly Double Minimum Leaf Conductance in Seedlings of a Neotropical Tree Species. Journal of Tropical Ecology 23:369-372.

Bacon CW, Porter JK, Robbins JD, Luttrell ES. 1977. Epichloe typhina from toxic tall fescue grasses. Appl. Environ. Microbiol. 34:576-581.

Baynes MA. 2011. Endophytic fungal communities of *Bromus tectorum*: Mutualisms, community assemblages and implications for invasion. [Masters Thesis]. Moscow Idaho. University of Idaho. 2011. 118p.

Baynes M, Newcombe G, Dixon L, Castlebury L, O'Donnell K. 2012. A novel plant–fungal mutualism associated with fire. Fungal Biology 116:133-144.

Brewer D, Duncan JM, Safe S, Taylor A. 1972. Ovine ill-thrift in Nova Scotia. 4. The survival at low oxygen partial pressure of fungi isolated from the contents of the ovine rumen. Canadian Journal of Microbiology 18:1119-1128.

Brewer D, Taylor A. 1969. Aspergillus fumigatus and *Sporormia minima* Isolated from the Rumen of Sheep. Journal of General Microbiology 59:137 -139.

Brooks ML, D'Antonio CM, Richardson DM, Grace JB, Keeley JE, DiTomaso JM, Hobbs RJ, Pellant M, Pyke D. 2004. Effects of Invasive Alien Plants on Fire Regimes. BioScience 54:677.

Cheplick GP, Clay K, Marks S. 1989. Interactions between infection by endophytic fungi and nutrient limitation in the grasses L*olium perenne* and F*estuca arundinacea*. New Phytologist 111:89-97.

Cheplick GP, Clay K. 1988. Acquired chemical defences in grasses: the role of fungal endophytes. Oikos 52:309-318.

Cheplick GP, Perera A, Koulouris K. 2000. Effect of drought on the growth of *Lolium perenne* genotypes with and without fungal endophytes. Functional Ecology 14:657-667.

Cheplick GP. 1997. Effects of endophytic fungi on the phenotypic plasticity of *Lolium perenne* (Poaceae). American Journal of Botany 84:34-40.

Cheplick GP. 2004. Recovery from drought stress in *Lolium perenne* (Poaceae): are fungal endophytes detrimental? American Journal of Botany 91:1960 -1968.

Clay K. 1988. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. Ecology 69:10-16.

Clay K. 1996. Interactions among fungal endophytes, grasses and herbivores. Researches on Population Ecology 38:191-201.

Cooper SB, Nolan JV, Kyriazakis I. 1995. Diet selection in sheep: the role of the rumen environment in the selection of a diet from two feeds that differ in their energy density. British journal of nutrition 74:39-54.

Costa Pinto LSR, Azevedo JL, Pereira JO, Carneiro Vieira ML, Labate CA. 2000. Symptomless infection of banana and maize by endophytic fungi impairs photosynthetic efficiency. New Phytologist 147:609-615.

David L H. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research 105:1422-1432.

Davies KW. 2011. Plant community diversity and native plant abundance decline with increasing abundance of an exotic annual grass. Oecologia 167:481-491.

Dewan MM, Ghisalbertib EL, Rowland C, Sivasithamparam K. 1994. Reduction of symptoms of take-all of wheat and rye-grass seedlings by the soil-borne fungus *Sordaria fimicola*. Applied Soil Ecology 1(1):45–51.

Diamond JM, Call CA, Devoe N. 2012. Effects of targeted grazing and prescribed burning on community and seed dynamics of a Downy Brome (*Bromus tectorum*)–dominated landscape. Invasive Plant Science and Management 5(2):259–269.

DiTomaso JM. 2000. Invasive weeds in rangelands: Species, impacts, and management. Weed Science 48:255-265.

Edwards JE, Kingston-Smith AH, Jimenez HR, Huws SA, Skøt KP, Griffith GW, McEwan NR, Theodorou MK. 2008. Dynamics of initial colonization of nonconserved perennial ryegrass by anaerobic fungi in the bovine rumen. FEMS Microbiology Ecology 66:537-545.

Engh I, Nowrousian M, Kück U. 2010. *Sordaria* macrospora, a model organism to study fungal cellular development. European Journal of Cell Biology 89: 864–872.

Fletcher LR, Harvey IC. 1981. An association of a *Lolium* endophyte with ryegrass staggers. New Zealand Veterinary Journal 29:185-186.

Frost RA, Wilson LM, Launchbaugh KL, Hovde EM. 2008. Seasonal change in forage value of rangeland weeds in northern Idaho. Invasive Plant Science and Management 1:343-351.

Ganguli AC, Hale MB, Launchbaugh KL. 2010. Seasonal change in nutrient composition of spotted knapweed and preference by sheep. Small Ruminant Research 89:47–50.

Garrett SD. 1951. Ecological groups of soil fungi: A survey of substrate relationships. New Phytologist 50:149-166.

Ginane C, Baumont R, Duncan AJ. 2009. Diet learning through post-ingestive consequences in sheep: The case of starch and casein variously combined in the same foods. Animal 3:135-142.

GraphPad Prism version 5.04 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Herrera J, Poudel R, Khidir HH. 2010. Molecular characterization of coprophilous fungal communities reveals sequences related to root-associated fungal endophytes. Microbial Ecology 61:239-244.

Hierro JL, Maron JL, Callaway RM. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. Journal of Ecology 93:5-15.

Hyde KD, Bussaban B, Paulus B, Crous PW, Lee S, Mckenzie EHC, Photita W, Lumyong S. 2007. Diversity of saprobic microfungi. Biodiversity and Conservation 16:7-35.

Hyde KD, Jones E. 2002. Introduction to fungal succession. Fungal Succession:1–4.

Jones MD, Smith SE. 2004. Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? Canadian Journal of Botany 82:1089-1109.

Kallenbach RL, Bishop-Hurley GJ, Massie MD, Rottinghaus GE, West CP. 2003. Herbage Mass, Nutritive value, and ergovaline concentration of stockpiled tall fescue. Crop Science 43(3):1001.

Knapp PA. 1996. Cheatgrass (*Bromus tectorum*) dominance in the Great Basin Desert: History, persistence, and influences to human activities. Global Environmental Change 6:37-52.

Lewis AC. 1984. Plant Quality and grasshopper feeding: Effects of sunflower condition on preference and performance in *Melanoplus Differentialis*. Ecology 65:836-843.

Mack RN. 1981. Invasion of *Bromus tectorum L*. into western North America: An ecological chronicle. Agro-Ecosystems 7:145-165.

Marks S, Clay K, Cheplick GP. 1991. Effects of fungal endophytes on interspecific and intraspecific competition in the grasses *Festuca arundinacea* and *Lolium perenne*. Journal of Applied Ecology 28:194-204.

May EA. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal Biology Reviews 21:51-66.

Meyer SE, Nelson DL, Clement S, Beckstead J. 2008. Cheatgrass (*Bromus tectorum*) biocontrol using indigenous fungal pathogens. In: Kitchen SG,

Pendleton RL, Monaco TA, Vernon J (Comps). Proceedings- Shrublands Under Fire: Disturbance and Recovery in a Changing World (2006): 6-8.

Miller SL. 1995. Functional diversity in fungi. Canadian Journal of Botany 73:50-57.

Mosley JC. 1996. Prescribed sheep grazing to suppress cheatgrass: a review. Sheep and Goat Research Journal 12:74-80.

Newcombe G, Shipunov A, Eigenbrode S, Raghavendra AK, Ding H, Anderson CL, Menjivar R, Crawford M, Schwarzländer M. 2009. Endophytes influence protection and growth of an invasive plant. Communicative and Integrative Biology 2:29-31.

Newman JA, Harvey A, Orr RJ, Penning PD, Parsons AJ. 1994. Fasting affects intake behaviour and diet preference of grazing sheep. Animal Behaviour 47:185-193.

Newsham KK, Fitter AH, Watkinson AR. 1994. Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. Journal of Ecology 82:805-814.

Nyberg Å, Persson IL. 2002. Habitat differences of coprophilous fungi on moose dung. Mycological Research 106:1360-1366.

Pacovsky RS. 1989. Influence of inoculation with *Azospirillum brasilense* and *Glomus fasciculatum* on sorghum nutrition. In: Skinner FA, Boddey RM, Fendrik I, editors. Nitrogen Fixation with Non-Legumes. Developments in Plant and Soil Sciences. Springer Netherlands; pp. 235–239.

Parker AD. 1979. Associations between coprophilous ascomycetes and fecal substrates in Illinois. Mycologia 71:1206-1214.

Parsons AJ, Newman JA, Penning PD, Harvey A, Orr RJ. 1994. Diet preference of sheep: Effects of recent diet, physiological state and species abundance. Journal of Animal Ecology 63:465-478.

Paszkowski, U. 2006: Mutualism and parasitism: The yin and yang of plant symbioses. Current Opinion in Plant Biology 9 4: 364–370.

Pellant M, Hall C. 1994. Distribution of two exotic grasses on public lands in the Great Basin: Status in 1992. In: Monsen, Stephen B, Kitchen SG, compilers. 1994.

Proceedings--ecology and management of annual rangelands; 1992, May 18-22; Boise, ID, Gen. Tech. Rep. INT-GTR-313. Ogden, UT: United States Department of Agriculture, Forest Service, Intermountain Research Station.:109-112.

Pellant M. 1996. Cheatgrass: the invader that won the west. Unpublished report. On file with: US Department of Agriculture, Forest Service.

Popp M, Lied W, Meyer AJ, Richter A, Schiller P, Schwitte H. 1996. Sample preservation for determination of organic compounds: microwave versus freeze-drying. Journal of Experimental Botany 47:1469.

Petrini, O. 1986. Taxonomy of endophytic fungi of aerial plant tissues. - In: Microbiology of the Phyllosphere. (eds.): N. Fokkema & J. Van Der Heuvel, Cambridge University Press: 175-187.

Porras-Alfaro A, Herrera J, Sinsabaugh RL, Odenbach KJ, Lowrey T, Natvig DO. 2008. Novel root fungal consortium associated with a dominant desert grass. Applied and Environmental Microbiology 74(9):2805–2813.

Reisner MD, Grace JB, Pyke DA, Doescher PS. 2013. Conditions favouring *Bromus tectorum* dominance of endangered sagebrush steppe ecosystems. Journal of Applied Ecology 50(4): 1039-1049

Richardson DM, Allsopp N, D'antonio CM, Milton SJ, Rejmánek M. 2000. Plant invasions — the role of mutualisms. Biological Reviews 75:65-93.

Richardson MJ. 2001. Diversity and occurrence of coprophilous fungi. Mycological Research 105:387–402.

Richardson MJ. 2002. The coprophilous succession. Fungal Diversity 10:101–111.

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS. 2008. Stress tolerance in plants via habitat-adapted symbiosis. The ISME Journal 2:404-416.

Richardson MJ. 1972. Coprophilous ascomycetes on different dung types. Transactions of the British Mycological Society 58:37-48.

Rodriguez RJ, White JF, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. New Phytologist 182:314-330.

Saikkonen K, Faeth SH, Helander M, Sullivan TJ. 1998. Fungal endophytes: a continuum of interactions with host plants. Annual Review of Ecology and Systematics 29:319-343.

Saleem M, Lamb BC, Nevo E. 1994. Inherited differences in crossing over and gene conversion frequencies between wild strains of *Sordaria fimicola* from "Evolution Canyon." Genetics 159(4):1573–1593

Sánchez Márquez S, Bills GF, Herrero N, Zabalgogeazcoa Í. 2012. Non-systemic fungal endophytes of grasses. Fungal Ecology 5(3):289–297.

Schardl CL, Leuchtmann A, Spiering MJ. 2004. Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology 55:315-340.

Schulz B, Boyle C, Draeger S, RÃ¶mmert A-K, Krohn K. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 106:996-1004.

Sharrow SH, Mosher WD. 1982. Sheep as a biological control agent for tansy ragwort. Journal of Range Management 35:480-482.

Shipunov A, Newcombe G, Raghavendra AKH, Anderson CL. 2008. Hidden diversity of endophytic fungi in an invasive plant. American Journal of Botany 95:1096 -1108.

Sneh B, Salomon D, Pozniak D. Isolation of *Sordaria fimicola* from maize stalks. 1987. Journal of Phytopathology 120(4):369–371.

Villalba JJ, Provenza FD. 1999. Effects of food structure and nutritional quality and animal nutritional state on intake behaviour and food preferences of sheep. Applied Animal Behaviour Science 63:145-163.

Wäli PR, Helander M, Nissinen O, Saikkonen K. 2006. Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). Canadian Journal of Botany 84:1043-1051.

Weber R, Meffert A, Anke H, Sterner O. 2005. Production of sordarin and related metabolites by the coprophilous fungus *Podospora pleiospora* in submerged culture and in its natural substrate. Mycological Research 109:619-626.

Whisenant S. 1990. Changing fire frequencies on Idaho's Snake River Plains: ecological and management implications. In: McArthur ED, Romney EM, Smith

SD, Tueller PT, compilers. Proceedings--Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management; 1989 April 5-7; Las Vegas, NV. Gen. Tech. Rep. INT-276. Ogden, UT: United States Department of Agriculture, Forest Service, Intermountain Research Station.:4-10.

White JF. 1988. Endophyte-host associations in forage grasses. XI. A proposal concerning origin and evolution. Mycologia:442–446.

Wicklow DT, Angel K, Lussenhop J. 1980. Fungal community expression in lagomorph versus ruminant feces. Mycologia 72:1015-1021.

Wilson D. 1995. Endophyte: The evolution of a term, and clarification of its use and definition. Oikos 73:274-276.

Young JA, Clements CD. Cheatgrass: Fire and Forage on the Range. Reno, NV: University of Nevada Press. 2009. 368 p.