

Invasive Annual Grasses at Micro- and Macro- Scales:
Endophytes, Social-Ecological Systems, and Community-Based Observing Networks

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Abstract

Biological invasions by exotic plants have economic impacts and control costs in the United States on the order of tens of billions of dollars per year. Some of the most significant plant invaders have transformed landscapes and social-ecological systems, and yet mechanisms of invasion and management strategies are often incompletely understood due to mismatches in scale between social and ecological processes. The studies presented here include both micro-scale studies of the ecological effects of fungal endophyte infection on invasive grass productivity and community structure and the adaptation of a social-ecological systems science tool to the study and management of invasive plants at the regional scale. The first set of experiments discussed focus on the effects of *Sordaria fimicola* on the fecundity and growth characteristics of *Bromus tectorum*, and the results are interpreted in the context of the Habitat-Adapted Symbiosis Hypothesis. *S. fimicola* differentially affects the growth and fecundity of *B. tectorum* depending on site characteristics and timing of inoculation, suggesting that future research into the biocontrol potential of the dung fungus may be warranted. The second set of experiments assess the range of symbiotic relationships between new invader *Ventenata dubia*, competitors *B. tectorum* and *Pseudoroegneria spicata*, and a set of *Fusarium spp.* endophytes and pathogens isolated from *B. tectorum* and *V. dubia*. The results suggest limited support for the hypothesis that *V. dubia* is benefitting from symbiosis with *Fusarium spp.* that can simultaneously act as pathogens of its competitors in the invaded range, and that this may contribute to the annual grass's ability to invade both *B. tectorum* monocultures and remnant Palouse Prairie remnants. The final section focuses on the potential use of Community Based Observing Networks (CBONs) for monitoring environmental and social factors associated with mechanisms and impacts of plant invasion.

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Dedication

I'd like to dedicate this work to my wife, Alyssa N. S. Griffith, my parents, James and Lynda Griffith, my brothers, nephews, nieces, and the many friends and family members without whom I would not have been able to complete this dissertation. And a final thank you to Shiloh, who was an inspiration and a friend of great worth.

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Preface

The three papers presented here may, on the first read, seem wildly disparate in their scope, focus, style, and subject matter. The first two focus on the effects on plant growth and fecundity of specific fungal endophytes, and the third makes an argument for adapting a community observing tool used in the Arctic for use in the monitoring of invasive species in other social-ecological contexts. What ties all three together is that they are concerned, at least indirectly, with biotic invasion by exotic plants. The first chapter, titled “Expectations for habitat-adapted symbiosis may depend on site productivity,” examines the effects of a coprophilous fungal endophyte, *Sordaria fimicola*, on the growth and fecundity of the invasive annual grass *Bromus tectorum* across a productivity gradient. The habitat-adapted symbiosis hypothesis is concerned with one particular type of context dependency in the effects of endophyte infection, and we demonstrate that there is a need for a broader, more inclusive theory that will account for the differential effects of endophytism on invasive plants in different environments. Chapter 2, “Apparent competition in grasses may be driven by endophytes with cryptic effects on emergence and early growth,” is also concerned with invasive grasses, *B. tectorum* and new invader of the Pacific Northwest *Ventenata dubia*, and their symbiotic relationships with fungi. We show that endophytes from genus *Fusarium* isolated from Palouse prairie samples of *B. tectorum* and *V. dubia* can have pathogenic and negative cryptic effects on *B. tectorum* and native grass *Pseudoroegneria spicata*, while they can also have positive effects on *V. dubia* emergence and seedling growth. This may help to partially explain how *V. dubia*, a winter annual grass that produces very little aboveground biomass, is able to effectively compete with much more robust native perennials and the many times more productive invader *B. tectorum*. In the third piece, we present reasons why it makes good sense, from both management and research perspectives, to use the community-based observing network (CBON) developed for use in Arctic social-ecological systems as a model for more effective monitoring of invasive species presence and treatment outcomes. One of the persistent problems in the fight against invasive plants is the difficulty in tracking where new populations are established at a fine enough detail to effectively understand or treat infestations; we suggest that CBONS provide a method for partnering with local communities that will increase the resolution and coverage of observations while providing

high-quality, verifiable data, and that this can only reduce costs and increase effectiveness of tracking and responding to plant invasions.

If what binds these papers together is a shared, secondary focus on plant invaders (and invasive annual grasses, in particular), what differentiates them is the different scales involved in each study. The work in the second chapter describing some of the interactions of *Fusarium* strains and several host grasses is concerned with what could reasonably be described as the smallest scale relevant to plant communities: the individual field. While the experiments we set up took place primarily in the greenhouse, the original collections of plant material was from a very small plot of a very specific place: a remnant Palouse Prairie fragment, in which the native plant assemblage is actively being invaded by the winter annual grasses *B. tectorum* and *V. dubia*. The fungal isolates of interest were isolated from the first two nodes of samples of these grasses, and their relevance to the community composition was hypothesized based on an observation by an undergraduate researcher: the *B. tectorum* plants which were infected by *Fusarium* isolates were shorter and less productive than their conspecifics, and this pattern did not hold for *V. dubia* plants, which seemed to be unaffected by infection. This led to the effort to determine whether infection by any of the *Fusarium* strains isolated from the invasive grasses could affect growth differentially, or even directly change competitive outcome between *V. dubia*, *B. tectorum*, and native grass *P. spicata*. This study was inspired by close observation of a single plot, and is part of an effort to determine why such a weedy, seemingly insignificant grass like *V. dubia* can displace much more ‘impressive’ competitors in the invaded range. The experiments with *S. fimicola* and *B. tectorum*, on the other hand, grew out of a broader set of explorations of the microbiome of *B. tectorum* by members of the University of Idaho Plant Symbiosis Laboratory that has spanned many years, several states, and many, many populations of the persistent and ubiquitous invader of sagebrush steppe habitat in western North America. The experiments reported here explore the hypothesis that endophytes can have different effects on their host under different environmental conditions, so the design was to test effects of infection by *S. fimicola* along a productivity gradient at different sites in the same general ecosystem (a large research ranch in the Bitterroot Valley of Montana, in this case). In this case, the effects observed may have relevance at broader scales, and we hope that further work on this subject will contribute to a better understanding of how plant-fungal symbioses are dependent on environmental context.

The third chapter is the obvious outlier, in that it addresses the social and legal frameworks for dealing with invasive species. “The potential use of Community-Based Observing Networks (CBONs) in monitoring and managing plant invasions in arid environments of the American West” is an attempt to address plant invasion at scales where complex policy and management mosaics make collaborative, concerted control efforts difficult to organize. As anyone that works with invasive species quickly realizes, exotic organisms are not often deterred by local, regional, or even international borders, but such borders have great significance in how invasions are managed (or not managed). Two of the difficulties inherent in both researching and managing invasive species are in accurately determining the extent of establishment and spread in the novel range and with monitoring the outcomes of control or policy implementations. The reason is fairly straightforward: plant invasions may start with local establishment, but often spread so quickly and widely across the landscape that they are difficult to control and terribly expensive to monitor. While there are methods, such as remote sensing or habitat suitability mapping, that may have utility for monitoring invasive plants now or in the future, we think that a tool developed for use in remote Arctic environments is a good candidate for adapting to the monitoring of invasive plant species. CBONs are structured as equal partnerships between communities with a vested interest in having access to environmental data and research scientists and managers primarily interested in high quality, high frequency data acquisition. The detection and monitoring of invasive plant species seems an excellent candidate for building a CBON outside of the Arctic for a few reasons: there are existing communities in the arid West with a vested interest in monitoring invasive plants, including ranchers, hay and seed producers, and indigenous peoples; large areas of the Intermountain West and the Northern Rockies are difficult to access and expensive for university or agency teams to monitor; and there are both scientific and management needs for longer-term monitoring at larger spatial scales than is generally supported by typical research grants. There are also pre-existing networks of managers and community members in the Cooperative Weed Management Areas throughout the west; these networks, perhaps in combination with county and state extension weed specialists, are in a prime position to identify and organize key community members who would be willing and competent to work with scientists as partners in a CBON organized around invasive plants.

Ultimately, the potential for building and strengthening collaborative and cooperative research and management efforts are what make CBONs a critical tool for applying outside the Arctic. They have been used effectively in collecting high quality, but hard to gather, data on a range of variables from sea ice extent to population density and health characteristics of sea mammals. The focus on researcher-community relationships and on examining linked social and ecological variables has situated CBONs as an important technique in applied social-ecological systems (SES) science, and this leads to an additional reason why they should be useful in studying and managing invasive plants. Traditionally, the study of invasive plants seems to have segregated into disciplinary tracks: ecologists have studied biological drivers of invasion, including what makes some systems more invasible than others, and what characteristics make some plants better at invading novel ranges; social science researchers and economists have almost exclusively focused on economic impacts and changes to perception of value. It seems that an entire range of linked social-ecological phenomena have largely been left unexplored in much of the invasion ecology and biology literature. How does land-use change affect plant invasion at different scales from plot to regional? How do cross-scalar interactions between individual managers, producer groups, and policy makers affect the distribution and spread of invasive species? How do jurisdictional boundaries between local, state, and federal, or between public and private lands contribute to management success or failure? There are any number of research questions about invasive species from an SES perspective that are yet to be asked, and a tool such as the CBON are ideal for collecting linked, interoperable social and ecological data at appropriate scales. There are also purely ecological questions of interest that have come out of our own work on the microbiome of invasive grasses that could benefit from data collected by CBONs. While we have shown, for example, that infection by some *Fusarium* strains is likely to confer some advantage to *V. dubia* in some environments, we have very little data about how common it is for *V. dubia* and *Fusarium* species to co-exist. *Fusarium* is not universally distributed, and is often thought to be most concentrated in agricultural soils. While it would be extraordinarily expensive for a university lab to conduct a comprehensive survey of rhizosphere fungi in the invaded range of this grass, it would be a much different prospect for an organized network of trained community members to collect soil and plant samples along with relevant environmental data. Such an organized, trained network (CBONs are

characterized by recruited and trained, rather than strictly volunteer, participants) could provide data of a type and quality allowing for novel hypotheses to be tested at costs likely much lower than the traditional single-lab exercise.

The final reason for the breadth of the topics covered here is that these papers reflect an ongoing evolution in collaborative research and cooperative agreements. The initial research reported here came out of work with academic and agency researchers, supported by funds from the US Forest Service. Much of that work has focused on fungal symbioses with *B. tectorum* across its range in the western United States, and benefited greatly from support and collaboration with skilled ecologists at MPG Ranch in the Bitterroot Valley of Montana. The experiments with *V. dubia* grew out of collaborations with a University of Idaho NSF-funded Research Experiences for Undergraduate program, including excellent undergraduate researchers and colleagues from the College of Agriculture. Part of this research included the opportunity to attend stakeholder meetings and extension events where it became very clear that many of the producers and land-owners on the Palouse are seriously concerned about the environmental and economic impacts of *V. dubia*. Some of the *V. dubia* work and all of the CBONs research was supported by the Mountain Social Ecological Observatory Network (MtnSEON) and the Center for Resilient Communities, and interaction with SES scientists and management professionals largely led to exploration of CBONs as a tool for collaborative invasive plant research and management. As such, this dissertation is a product of a sustained interdisciplinary effort and many complex collaborations, and we hope that the work described here will continue to provide ongoing scientific and management benefits.

Chapter 1: Expectations for habitat-adapted symbiosis may depend on site productivity

Abstract

According to the generalized habitat-adapted symbiosis hypothesis, the most positive effects of symbiosis are expected in the most stressful, or least productive, sites for a plant host. Stress varies with site variables but also varies during the life cycle of a plant, with winter annuals experiencing the most stress after fall emergence. For phenotypically plastic winter annuals like *Bromus tectorum*, seed and vegetative productivity can thus vary tremendously from small plants with a few seeds to large, multi-tillered plants bearing thousands of seeds each. Symbionts of *B. tectorum* include many dung fungi. One of these, *Sordaria fimicola*, has been shown to have significant effects on growth and fecundity of *B. tectorum*. These effects have varied from positive to negative, thus making this symbiont an appropriate one to test the generalized habitat-adapted symbiosis hypothesis in sites expected to vary in stress or productivity. Sites along a productivity gradient were selected within the MPG Ranch in western Montana, and inoculum of *S. fimicola* was applied as a spray in fall or spring treatments. We hypothesized that site and seasonal stress are additive, and that the effects of *S. fimicola* inoculation would be most positive in the most stressful or least productive site and more positive after fall application (before the most stressful stage in the host's life cycle) than after spring application. Site stress varied considerably: un-inoculated control plants of the most stressful site were more than 9 times smaller and 7 times less fecund than those of the least stressful site. Site, treatments, and their interaction explained approximately 60% of the variation in vegetative biomass and fecundity of *B. tectorum*. As predicted by the generalized habitat-adapted symbiosis hypothesis, the most positive effects on biomass and seed number (+48.96%; $p < 0.0007$ and +39.04%; $p < 0.0001$, respectively) were observed in the most stressful site after fall application of *S. fimicola*. Fall applications had more positive effects than spring applications in the two most stressful sites, again as expected. The results from the least stressful site, on the other hand, were contrary to expectations: the fall application had a highly negative effect on biomass and seed number (-38.50%; $p < 0.0001$ and -25.59%; $p < 0.0002$, respectively); the spring application had a positive effect on biomass and seed number (+18.05%; $p < 0.0216$ and +18.14%; $p < 0.032$, respectively). *Synthesis*. These findings are preliminary and will need to be confirmed by studies with larger gradients. However, they do suggest that the habitat-adapted symbiosis

hypothesis does not apply to differences in seasonal stress experienced by winter annuals in more productive sites.

Keywords: *Bromus tectorum*, *Sordaria fimicola*, coprophilous fungi, endophyte, habitat-adapted symbiosis, symbiosis

1.1 Introduction

Endophytic fungi, those that infect plants but do not cause disease symptoms, are ubiquitous in plants (Arnold *et al.* 2000). They have been isolated from above and below ground tissues, including roots, shoots, and reproductive structures, and live within their hosts (although they may sporulate externally upon host senescence). Many symbiotic lifestyles, including mutualism, commensalism, and parasitism, and different functional roles have been described for particular endophytes, and several theories regarding endophyte effects on host ecology have been generalized to large groups of endophytic fungi (Rodriguez *et al.* 2009). With increasing interest in describing endophytes, their evolution, and their functional roles in host fitness and community structure, two general views of endophytes have emerged over the last thirty to forty years. One view emphasizes those relationships which fungal endophytes form with their hosts and can be described as mutualistic, while the other emphasizes fungal relationships with hosts along the entire ‘endophytic continuum’ from mutualistic to pathogenic.

Some non-clavicipitaceous endophytes which form systemic infections of roots and above-ground tissues are hypothesized by Rodriguez *et al.* (2009) to provide increased stress tolerance to plants in high-stress habitats and, more generally, positive fitness effects to their hosts. They describe this type of relationship as ‘habitat-adapted symbiosis,’ and propose that it is critical in allowing many plant species to adapt to stressful habitats (Rodriguez *et al.* 2008). Examples of these complex symbioses involving plants, fungi, and viruses have been demonstrated to increase heat tolerance in all symbionts (Marquez *et al.* 2007). Infections of *Bromus tectorum* by isolates from the *Morchella elata* clade have been found to increase thermotolerance of seeds, increase biomass, and increase fecundity of host plants (Baynes *et al.* 2012a). Increased fitness in stressful environments from infection by fungal endophytes has been shown for *Oryza sativa* in saline environments (*Fusarium culmorum* (FcRed1)), for

several host plants in drought conditions (multiple *Colletotrichum spp.*), and for *Solanum lycopersicum* in agricultural fields with high incidents of disease (*Colletotrichum magna*) [Rodriguez, Redman, & Henson 2004; Rodriguez & Redman 2008; Redman *et al.* 2011]. Some authors have gone so far as to suggest that endophyte-conferred stress tolerance is critical to plant establishment in stressful habitat and explains the relative lack of adaptation by plants to those habitats (since, presumably, such plant populations rely on symbionts to provide resistance to environmental stressors) [Rodriguez *et al.* 2008; Rodriguez & Redman 2008]. A generalized form of the habitat-adapted symbiosis hypothesis (HASH) can be stated as: the positive effects of endophyte-plant symbiosis are expected to be greatest in the most stressful environmental conditions a plant experiences in its lifecycle and range.

Stress for plants varies not only along environmental gradients, but also during the life cycle of a plant. Winter annual grasses, such as *Bromus japonica* and *Bromus tectorum*, experience greatest stress during the winter after fall germination (Baskin & Baskin 1981; Mack & Pyke 1984). This is likely to mean that *when* a plant is infected by an endophyte (or any other type of fungal symbiont) is significant in terms of fitness effects and outcome, although most of the literature relating to this area is focused on order of assembly rather than lifecycle of hosts. Studies have shown that the order of assembly of microbial communities can have significant effects on both hosts and symbionts; for example, varying the order of microbial community assembly can alter endophyte-pathogen interactions in hosts from inhibitory to facilitative (Adame-Álvarez, Mendiola-Soto, & Heil 2014). The order of arrival of endophytes has been shown to affect the microbial community structure in some host plants (such as *Zea mays*), since fungal-fungal interactions are significant as well as fungal-host interactions (Pan & May 2009). Since endophyte communities are not always determined by host species, in the sense that they can vary greatly between individuals within populations, order of arrival can play a significant role in structuring endophyte communities within plant populations as well as individuals (Cannon & Simmons 2002). Since numerous studies have shown that endophytes, from vertically transmitted, clavicipitaceous symbionts of grasses to leaf endophytes of tropical hardwoods, have significant effects on host responses to the environment, and also that the timing of microbiome assembly can impact host and symbiont fitness, it is reasonable to assume that varying the timing of endophyte infection in a plant's lifecycle could have significant effects on plant host fitness.

In order to test the generalized form of HASH and explore the interaction between habitat type and timing of endophyte infection on host fitness responses, we conducted a field experiment using a strain of *Sordaria fimicola* (CID 323), a fungus often isolated as an endophyte, and *Bromus tectorum*, a common invasive annual grass in the Intermountain West region of the United States. Three different sites, with distinct habitat types and different levels of *B. tectorum* productivity, were chosen at a location in the Bitterroot Valley, MT, and sites were divided into plots which were treated as controls, fall inoculation with *S. fimicola* spore suspension, or spring inoculation of *S. fimicola* spore suspension. The sites were characterized as lying on a continuum from most stressful habitat to least stressful habitat (ranked according to control-plot productivity of *B. tectorum*). Given the winter annual lifecycle of *B. tectorum*, and the higher levels of stress indicated by seedling mortality in overwintering rosettes, it is reasonable to assume that site and seasonal stress are cumulative: overwintering seedlings in low-productivity sites (characterized as “most stressful” in our study) experience the most stress, and those seedlings which survived winter at high-productivity sites (characterized as “least stressful” in our study) experience the lowest stress. Aboveground biomass (from crown to seed heads) was collected and the number of seeds per plant was counted approximately a week before expected seed shatter the following summer. We hypothesized that if *S. fimicola* is a habitat-adapted symbiont of *B. tectorum*, the positive effects on host fitness of endophyte colonization would be greatest in more stressed populations (low-productivity, fall inoculation) of *B. tectorum* and would be lesser with increasing productivity and spring inoculation.

1.2 Materials and Methods

Bromus tectorum, or cheatgrass, has been an invasive species with serious economic impact in the American West for more than a century (Piemeisel 1938; Mack 2011). Research into endophytes of *B. tectorum* has demonstrated mutualistic relationships with some fungi conferring greater thermotolerance and increased growth and fecundity (Baynes *et al.* 2012a). *B. tectorum* has also been shown to provide a microcosm in which a fungivorous nematode can ‘farm’ an endophytic fungus without causing positive or negative effects for the plant host (Baynes *et al.* 2012b). In previous work (submitted), we have shown

that some endophytes normally identified as dung fungi can have negative effects on *B. tectorum* biomass and fecundity in both greenhouse and field experiments.

Sordaria fimicola is most often described as a coprophilous fungus associated with herbivore dung, and is generally described as having a cosmopolitan distribution (Haberle *et al.* 2015). A strain of *S. fimicola* isolated from *B. tectorum* at a low-productivity site near Moscow, ID has been shown to reduce biomass and fecundity of cheatgrass when applied in field and greenhouse experiments (submitted for publication). In other experiments, we have demonstrated that *S. fimicola* can form extensive infection in hosts, can infect hosts through a spray application of spore suspensions to shoot and leaves or through direct mycelial contact with roots, and is best described as having multiple lifestyles (endophytic, saprophytic, and coprophilous). Multiple symbiotic lifestyles for *S. fimicola* is supported by other studies: it has been isolated as an endophyte from maize, in which it can act as a weak pathogen (Chambers & De Wet 1987), and has been shown to increase biomass and reduce disease effects in wheat and rye-grass (Dewan *et al.* 1987).

1.2.1 Isolation and culturing of *S. fimicola* (CID 323)

The Moscow strain of *S. fimicola*, designated as CID 323, was isolated from *B. tectorum* plants collected on Moscow Mountain (off of W Twin Rd, 46°48'45.7"N 116°55'11.0"W) at approximately 1,280m elevation at a site with minimal slope, an aspect of ~300, on the roadside in a mixed conifer stand where *B. tectorum* is estimated at < 5% cover. Endophytic *S. fimicola* was isolated from surface sterilized plant tissue incubated on Potato Dextrose Agar (PDA) using methods described in Baynes *et al.* (2012a). Cultures were maintained by subculturing onto PDA from reference cultures stored at 4°C. Each plate of *S. fimicola* was examined microscopically (for appropriately formed and colored asci and ascospores) when producing perithecia to ensure that cultures were viable. Cultures with malformed or discolored perithecia, asci, or ascospores were discarded.

1.2.2 Inoculation of *B. tectorum* with *S. fimicola* in the field

S. fimicola inoculum was produced by incubating cultures on PDA at ambient laboratory conditions (20°C with a 10:14 light:dark photoperiod) for 28 days. Petri dishes with mature perithecia were scraped into sterile deionized water (SDW) and shaken to form a suspension of spores and mycelium. In total, 14 plates of mature *S. fimicola* were suspended

in 5 liters of SDW. Final concentration of spores and mycelial fragments were estimated using a hemocytometer at ~1.05 million fungal units per mL of SDW.

Field plots were established at three different sites (see **Table 1** for site descriptions) on MPG Ranch land in the Bitterroot Valley, MT. Sites were chosen to reflect a gradient of *B. tectorum* productivity, as evidenced by land-manager observations of percent cover and relative fecundity in the growing seasons preceding the experiment. The inverse of *B. tectorum* productivity is taken to be stress for the plant; as such, the sites are considered ranked along a gradient from most stressful to least stressful for *B. tectorum* growth. The least stressful site is largely dominated by cheatgrass, very close to flat with a southerly aspect, the lowest elevation, and was a former fenced pasture, so the soil is expected to be higher in organic matter than the other two sites. The moderately stressful site is more westerly (SSW), steeper, and has greater plant diversity, but less direct sunlight; it also has a greater percentage of bryophytes, lichens, and gravel as ground cover. The most stressful site was the steepest, with a similar aspect to the moderate site; has the highest percentage of gravel and bare ground; has significant woody debris and plant litter; and has a greater diversity of grasses (including perennials) than the other two sites. Each site was divided into 9 sub-plots of 3x5m, with 1m strips separating sub-plots, and treatments assigned to subplots so that each row and column contained each treatment level (control, spring application, and fall application). In early December 2012, after germination and emergence of *B. tectorum* was confirmed at the field sites, and there was sufficient precipitation (primarily light snow) to ensure constant leaf moisture, *S. fimicola* inoculum was applied to the three fall application sub-plots at each site at a rate of approximately 0.5L per sub-plot with a backpack sprayer.

In March of 2013, after snowmelt and during a period of continuous light rain, *S. fimicola* inoculum was applied to a further 3 sub-plots of each site at a rate of approximately 0.5L per sub-plot with a backpack sprayer (with inoculum prepared in the same manner and quantities as described above). The final 3 sub-plots of each of the three sites are designated as control (treated with SDW rather than *S. fimicola*).

Table 1.1. Site descriptions

Site	Aspect (°)	Elevation (m)	Slope (%)	Plant Species (>5%)	Cover Estimates	Habitat-type	Expected Cheatgrass Productivity
<i>Least Stressful</i>	170	1113	2	<i>Bromus tectorum</i> (50%) <i>Sisymbrium altissimum</i> (5%)	bare ground (5%) litter (40%)	annual grass monoculture	Most productive
<i>Moderately Stressful</i>	205	1200	11	<i>Poa bulbosa</i> (18%) <i>Hesperostipa comata</i> (17%) <i>Poa secunda</i> (7%) <i>Euphorbia esula</i> (6%) <i>Bromus tectorum</i> (5%) <i>Centaurea stoebe</i> (5%)	bare ground (2%) litter (39%) moss/lichen (53%) gravel (3%)	shrubland	Moderately productive
<i>Most Stressful</i>	204	1177	15	<i>Bromus tectorum</i> (41%) <i>Hesperostipa comata</i> (32%) <i>Pseudoroegneria spicata</i> (6%) <i>Thinopyrum intermedium</i> (6%)	bare ground (9%) litter (60%) moss/lichen (4%) gravel (12%)	non-irrigated grass plantation	Least productive

1.2.3 Collection and processing of *B. tectorum* from the field

Beginning in early summer, weekly observations of plots were made to monitor progress towards seed ripening and plant senescence in *B. tectorum*. When plants had dried sufficiently that little additional growth was expected, but before seed shatter, 5cm x 10cm cotton bags were tied around at least 100 inflorescences per sub-plot to collect the seeds. We left the plants to dry in the field, and then harvested all plots in the first week of August. With seed collection bags attached, plants were pulled from the ground and placed in paper envelopes. Despite the use of seed collection bags, for some plants seed shatter occurred earlier than expected, and all seeds were not collected.

The harvested plants were dried for 72 hours at 60°C. Seeds that remained attached to the plant were removed and roots were clipped just below the point of attachment of the lowest tiller. Aboveground biomass of plant tissue excluding seeds was recorded to 0.001 g, and these are the weights used in the analysis below. With plants that had not shattered prior to attachment of seed collection bags, the number of seeds were counted and seed mass was recorded to 0.001 g. When shatter had occurred prior to collection, the number of spikelets with glumes attached was counted and recorded as a proxy for seed number. We counted any seeds present and recorded seed mass to 0.001 g. Where estimates were not possible due to

plant damage or other inability to discern seed counts, seed number was coded as missing (and not analyzed).

1.2.4 Statistical analysis

Aboveground biomass (not including seed heads) and seed number per plant were each log-transformed (in order to remove heteroskedasticity). Total number of plants analyzed from the experiment was $N=2,627$, and the experiment was mildly unbalanced (due to damaged plants being discarded), with sub-plot average $n=97$ and a range from 92 to 106. A model [$\log(y) = \alpha x_1 + \beta x_2 + \gamma x_1 x_2 + \varepsilon$; where y =dependent variable, x_1 =site, and x_2 =treatment] was used to produce both regression and ANOVA analyses. ANOVA was used to determine significance of site, treatment, and the interaction. Regression was used to determine the proportion of variability that the model was able to explain. Differences in treatment means within sites were analyzed using Tukey's HSD. All calculations were computed in R 2.14.0 (R Development Core Team, 2013).

1.3 Results

The interaction of site and treatment was significant for both aboveground biomass ($F=32.9074$; $p<0.001$) and number of seeds per plant ($F=42.218$; $p<0.001$) when the dependent variables were log transformed. The manager observations about expected productivity of *B. tectorum* were confirmed by the control (non-inoculated) biomass and seed-production numbers (i.e., most stressful produced the lowest average biomass and seed number per plant, and the least stressful site exceeded that average by approximately an order of magnitude). Tukey's HSD indicates that: the fall treatment at the most stressful site had a positive, significantly different effect (+48.96% biomass) compared to control for the same site ($p<0.001$); the fall treatment at the least stressful site had a strong negative, significantly different effect (-38.50% biomass) compared to control for the same site ($p<0.001$); and the spring treatment at the least stressful site had a positive, significantly different effect (+18.05% biomass) compared to control for the same site ($p<0.022$). The magnitude of these effects can be seen clearly in the plotted "site X treatment" mean biomass with standard errors in **Figure 1.1**. The results for number of seeds per plant show that all treatments except spring at the moderately stressful site were significantly different from the respective site controls: fall treatment at the moderately stressful site was positive compared to control

(+25.05%) and significant ($p=0.001$); the fall treatment at the most stressful site was positive compared to control (+39.04%) and significant ($p<0.001$); the spring treatment at the most stressful site was negative compared to control (-18.08%) and significant ($p=0.006$); the fall treatment at the least stressful site was negative compared to control (-25.59%) and significant ($p<0.001$); and the spring treatment at the least stressful site was positive compared to control (+18.14%) and significant ($p=0.032$). The “site X treatment” means and standard errors for number of seeds per plant are shown in **Figure 1.2**. The mean weights, number of seeds, and differences from control plots are summarized for all sites in **Table 1.2**.

The model fits for the regression were fairly good for both $\log(\text{weight})$ ($F=430.1$ on 8 and 2618 df ; $p<0.001$; Adjusted R-squared of 0.57) and $\log(\text{number})$ ($F=536.6$ on 8 and 2618 df ; $p<0.001$; Adjusted R-squared of 0.62), considering the model only makes use of two categorical independent variables and their interaction. The factor ‘site’ captures only very broad differences in site characteristics, and the *B. tectorum* sub-populations within sites are likely to be closely related and not expected to react differentially to endophyte treatments. There are obvious differences in the mean biomass and seeds produced per plant between sites, with the plants from the control plots at the least stressful site being more than 9 times larger and producing ~7.5 times as many seeds, on average, as plants from the control plot at the most stressful site. It is worth noting that the sign of the effects for both biomass and number of seeds are the same in the moderately stressful and least stressful sites with both treatments, but are different at the most stressful site (with a marginally positive but non-significant effect on biomass from fall inoculation, but a significant negative effect on number of seeds in the spring treatment). The pattern is the same when using biomass or seed number as a dependent variable. At the site that can be characterized as least stressful for *B. tectorum*, the fall treatment with *S. fimicola* decreased both aboveground biomass and number of seeds per plant and the spring treatment increased both aboveground biomass and number of seeds per plant. As for the two higher stress sites, the magnitudes of positive effects across all results were highest at the lowest stress site after fall inoculation, with significant increases in biomass (most stressful, fall) and seed number (moderately stressful, fall; most stressful, fall) as well as a significant decrease in seed number (most stressful, spring). This indicates that while *B. tectorum* populations in both ‘high stress’ and ‘low stress’ sites had significant interactions with *S. fimicola*, those effects were not consistent

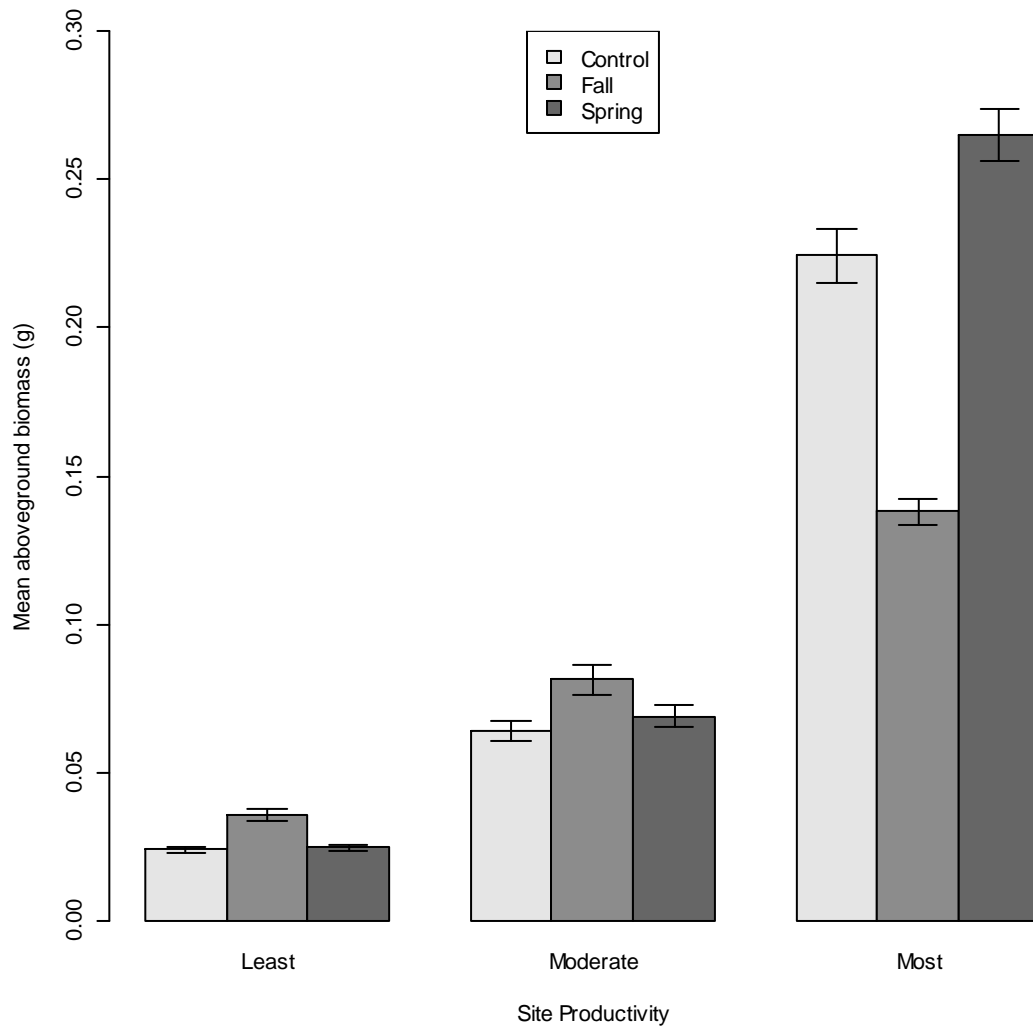


Figure 1.1: Effects of *S. fimicola* (CID323) on *B. tectorum* biomass (bars indicate means and whiskers indicate SEM) at different sites (ranked from least to most productive) and time of inoculation (Fall=More Stressful; Spring=Less Stressful)

across timing (i.e., fall inoculation produces different effects on host fitness than spring inoculations), and the effects were not consistent across sites (signs of interactions are opposite for ‘high stress-’ interactions and ‘low stress-’ interactions for all significant effects).

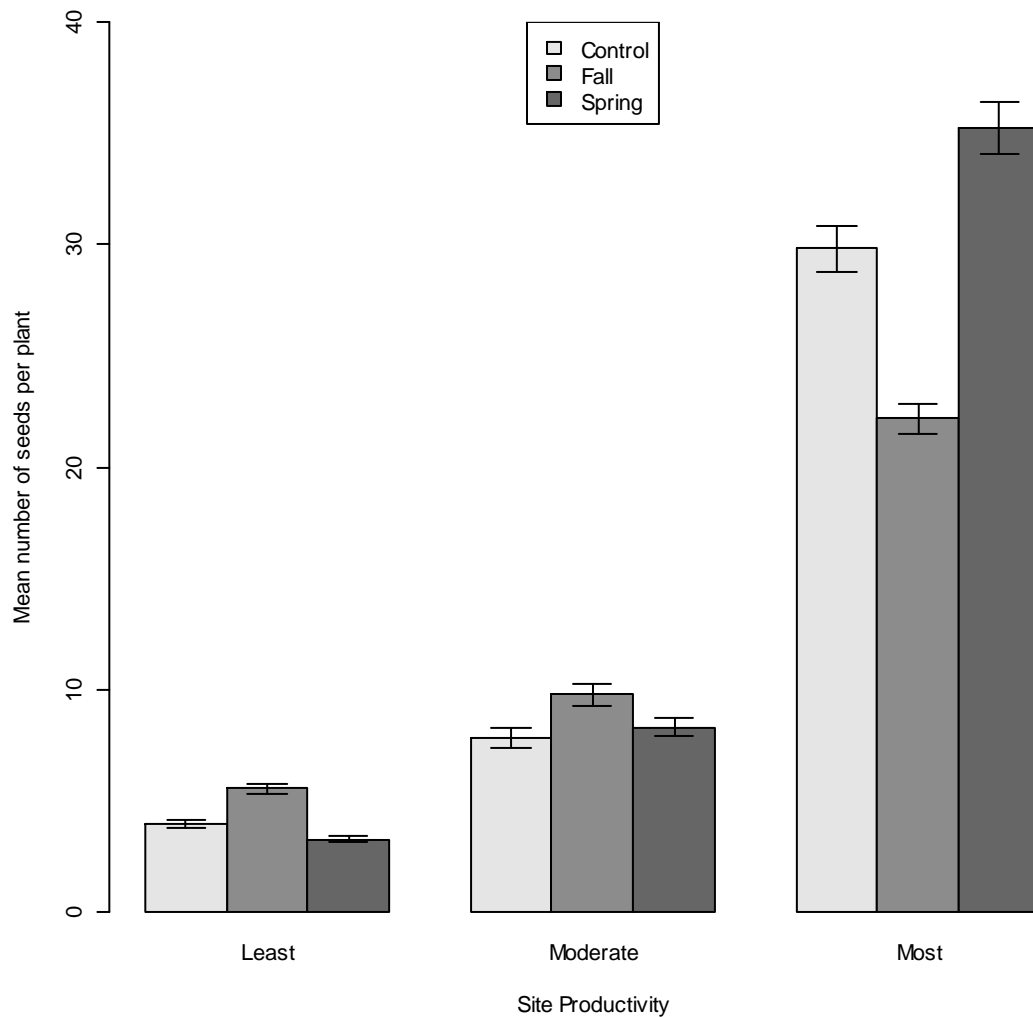


Figure 1.2: Effect of *S. fimicola* (CID323) on *B. tectorum* seeds per plant (bars indicate means and whiskers indicate SEM) at different sites (ranked from least to most productive) and time of inoculation (Fall=More Stressful; Spring=Less Stressful)

1.4 Discussion and Conclusions

The results indicate that infection by *S. fimicola* can have both negative and positive results on *B. tectorum* fitness depending on the interaction of habitat type and timing of inoculation. The strain used in our experiments (CID 323) fits the description of a Class 2 endophyte by Rodriguez *et al.* (2009), or those likely to be involved in habitat-adapted symbioses with hosts, in all but expected effects. The fungus colonizes root and shoot tissues,

Table 1.2. Mean results at Site X Treatment combinations. Results significantly different from control at 0.05 level by Tukey's HSD are marked with a *.

Site	Treatment	Mean Weight (g)	Difference from Control	Mean Number of Seeds	Difference from Control
<i>Most Stressful</i>	Control	0.0241	_____	3.9936	_____
	Fall	0.0359	+0.0118g * +48.96% p<0.0007	5.5528	+1.5592 * +39.04% p<0.0001
	Spring	0.0248	+0.0007g +02.90% p<0.9879	3.2716	-0.7220 * -18.08% p<0.0062
<i>Moderately Stressful</i>	Control	0.0643	_____	7.8127	_____
	Fall	0.0814	+0.0171g +26.59% p<0.2055	9.7701	+1.9574 * +25.05% p<0.0010
	Spring	0.0692	+0.0049g +07.62% p<0.9911	8.3050	+0.4923 +06.30% p<0.9992
<i>Least Stressful</i>	Control	0.2244	_____	29.8182	_____
	Fall	0.1380	-0.0864g * -38.50% p<0.0001	22.1875	-7.6307 * -25.59% p<0.0002
	Spring	0.2649	+0.0405g * +18.05% p<0.0216	35.2279	+5.4097 * +18.14% p<0.0320

produces extensive, systemic infections (indicated by isolation from surface sterilized shoot tissue several days after root inoculation), and is horizontally transmitted through either spores ejected onto the leaf surface or direct contact between mycelium and roots. The expectation, derived from the habitat-adapted symbiosis hypothesis, that infection by this type of endophyte should have the greatest positive effect on growth and fecundity of the host in the most stressful conditions and fewer or less-pronounced positive effects as host conditions become less stressful, however, is not supported in a clear manner by the results of infection of *B. tectorum* by *S. fimicola*. If *S. fimicola* was acting as a habitat-adapted symbiont of *B. tectorum*, then the treatments would be expected to have unambiguously positive effects in the most stressful conditions and less positive effects in the least stressful conditions. However, the results show that the significant effects of infection on host fitness in the most stressful site were mixed depending on the timing of inoculation; positive effects on biomass and seed number after fall inoculation, and negative on number of seeds after spring treatment. In the moderately stressful site *S. fimicola* had a positive effect on seed number in the fall treatment, but no significant effects in spring. At the least stressful site *S.*

fimicola strongly reduced biomass and seed number of *B. tectorum* after fall inoculation, but significantly increased both biomass and seed production after spring inoculation. The results in this case do not represent a clear, unambiguous case of an endophyte having greater positive effects in the most stressful environment (low productivity sites after fall emergence) and reduced positive effects as additive stress is lessened along a productivity gradient. What the results do indicate is a need for a general hypothesis of context dependency for the effects of endophytes (and other fungal symbionts) on their hosts. The habitat-adapted symbiosis hypothesis is very specific in its formulation: type II endophytes confer certain types of stress resistance to hosts under extreme biotic conditions. Our observations of the effects of *S. fimicola* don't meet those strict criteria, but they are certainly suggestive that the *context of symbiosis* matters enormously in determining outcome for the host.

The effects of *S. fimicola* on the growth and fecundity of *B. tectorum*, then, appear to be more in line with studies showing that endophytic and other fungi (mycorrhizal, in particular) can have a range of effects on host fitness depending on environmental factors. Clavicipitaceous endophytes are usually described as being defense mutualists, but have been shown to have other positive effects on host fitness, including increasing host biomass and competitive ability, under certain environmental conditions (Marks, Clay, & Cheplick 1991). *Neotyphodium coenophialum* has been implicated in helping an exotic grass (*Lolium arundinaceum*) establish in existing plant communities in a novel range (Rudgers, Mattingly, & Koslow 2005). On the other hand, endophytic fungi from the Epichloae do not always have similar effects on host fitness, and can decrease host fitness in alternate hosts (such as the native *Festuca arizonica*) or under different herbivory levels, at different levels of site factors such as soil moisture, and in conditions characterized by interactions of herbivory and water stress (Saikkonen et al. 1999; Koh & Hik 2007; Miranda, Omacini, & Chaneton 2011). Environmental conditions and complex biotic interactions can result in the effects of other members of the Epichloae ranging from mutualistic to parasitic (Saikkonen, Wali, Helander, & Faeth 2004; Schardl, Leuchtman, & Spiering 2004; Saikkonen, Gundel, & Helander 2013). It has been shown that *Epichloë festucae* can have either positive or negative effects on *Festuca rubra* in Sweden: in dry, nutrient rich conditions hosts infected by the endophyte had increased productivity, while in wetter or poorer soils infected plants fared poorly compared to uninfected plants (Saona et al. 2010). Schulz and Boyle (2005) document that

some non-clavicipitaceous endophytes may cause disease symptoms when hosts are placed in conditions of extreme physical or environmental stress, although the stated assumption is that the endophytic fungi responsible do not produce positive effects on hosts in normal conditions. We have also seen other fungi isolated as endophytes, but more commonly described as dung fungi, have antagonistic effects on host fitness similar to those described here from *S. fimicola* at certain site and timing combinations (such as the least stressful site and fall inoculation). Redman, Dunigan, & Rodriguez (2001) suggest that in some cases host plant physiology can determine the outcome of symbiosis (ranging from pathogenic to mutualist) of infection by endophytic *Colletotrichum* species. This is consistent with findings from other studies of fungal-plant symbiosis, including those of mycorrhizal associations which again span from mutualistic to antagonistic in outcome depending on various biotic and abiotic factors (Johnson, Graham, & Smith 1997). Additionally, investigations into plant-fungal-pathogen interactions, such as mycoparasitism of powdery mildew disease organisms (Erysiphaceae), have shown that symbiotic outcomes for plants and pathogens can be dependent on abiotic and biotic factors, ranging from humidity of the growth conditions to the genetics of the organisms involved (Kiss 2003).

In the results reported here, the most stressful site and time period did indeed have the most positive effects on host growth and fecundity, but the least stressful site and timing also showed positive effects on the host plant from treatment with the fungus. The results of treatments at the intermediate-stress conditions represented on the gradient (spring inoculation at most stressful site, both inoculations at the moderately stressful site, and the fall inoculation at the least stressful site) are not entirely consistent with the habitat-adapted symbiosis hypothesis. Another question arises from the difference in effect between spring and fall treatments in both the least and most stressful sites: is the difference in result likely to be caused by different temperature effects on the fungus itself? We find this unlikely, since if true we'd expect to see a similar pattern from fall to spring inoculation across each of the sites along the productivity gradient. It is also possible that the ambiguous effects may be a result of most of the experimental sites being at the most stressful side of an environmental productivity gradient for the host plant. The location in Montana is certainly in the Northern regions where *B. tectorum* is reported as being widely invasive and highly productive. Although highly plastic in response to environmental conditions, the 'normal range' of seed

production is between 25 and 5,000 seeds per plant, with average plants in open conditions producing 300-400 seeds per plant (Zouhar 2003). This suggests that all of the test sites are on the lower end of a regional productivity gradient for *B. tectorum*, and in fact, it isn't uncommon in greenhouse and field conditions to see plants with thousands of seeds produced on more than 10 culms per plant. If the change in host response to infection at different inoculation times seen in the more productive sites on our gradient (the least stressful site) is typical of *B. tectorum* response at the middle and higher ends of the productivity gradient (i.e., so that fall inoculation with *S. fimicola* would have a predictable negative impact on biomass and seed production), then there could be some implications of this that are relevant to the management and control of *B. tectorum* infestations.

Research into biocontrol options for *B. tectorum* in the US have focused largely on microbial pathogens in the invaded range (i.e., not classical biocontrol which would identify pathogens from the invasive plant's native range in Eurasia) that may reduce productivity or seed banks. At this point, there have been no very successful biocontrol methods developed, but there are a few microbial agents being actively researched. *Pyrenophora semeniperda*, sometimes called "The Black Fingers of Death," is a N. American native soil fungus that has been identified as a seed-bank pathogen of *B. tectorum* in the invaded range with less significant negative effects on perennial, native grass competitors (Beckstead et al. 2014). *Ustilago bullata*, the head smut pathogen, has been shown to be endemic in most *B. tectorum* populations in N. America, but efforts to use it as an effective biocontrol has been hampered by confounding environmental conditions (Meyer, Nelson, & Clement 2001; Meyer et al. 2008). Chestnut Bunt Pathogen, *Tilletia fusca*, is potentially useful to control *B. tectorum*, but is again most effective under very specific circumstances; in this case, infection and control is best in mesic conditions with constant snow cover over the winter, which is not the type of environment in which *B. tectorum* is most in need of control (Meyer et al. 2008). A native rhizobacterium, *Pseudomonas fluorescens* (D7), has been shown to be effective in certain environmental conditions (fall application just before first snow, similar to the fall treatment with *S. fimicola* described in this paper), and has the added advantage of controlling *Aegilops cylindrica*, an invasive plant which is commonly co-occurring with *B. tectorum* in the American West (Stubbs & Kennedy 2012; Stubbs, Kennedy, & Skipper 2014). Since many of the native microbes evaluated are most effective in specific environmental conditions, it

may be that *S. fimicola* could be combined with another agent to increase control of *B. tectorum* in some areas of its range. In particular, the conditions under which *P. fluorescens* D7 best controls the invasive grass are similar to those of the fall treatment at our least stressful site; this suggests that a combination treatment of D7 with *S. fimicola* in fall treatments at high productivity sites would be worth exploring.

It appears that the strain of *S. fimicola* we worked with in this study has some of the characteristics of a habitat-adapted symbiont in the broadest sense, but there isn't strong evidence from the experiments reported here to assume that it plays an important role in adaptation of *B. tectorum* to stressful environmental conditions, generally. It is also possible that the effects of endophyte infection are confounded by site characteristics not accounted for in our crude estimation of expected productivity; microbial interactions and competition seem most likely to interfere with the effects on biomass and fecundity, and these are worth explicitly exploring in future work. It is clear, however, that the context of symbiosis between fungus and plant host is important in determining the effects on the plant host's growth and fecundity. Although we didn't test any effects on the productivity or persistence of the fungal symbiont, it is likely that context of the symbiosis has effects there as well. It is clear that a broader and more inclusive hypothesis to explain the context dependency of symbiotic outcomes is necessary. In order to assess the specific positive and negative effects of infection on the host, it will be necessary to conduct experiments on longer, better defined and controlled environmental gradients. If the sites we used are generally characteristic of the more stressful conditions under which *B. tectorum* is found in the region, what would the effects of infection by *S. fimicola* be in more productive sites? It will also be important to design greenhouse or field experiments that manipulate biotic and abiotic environmental variables in order to determine what factors determine the effect of infection by *S. fimicola* on *B. tectorum*. Since we have documented both positive and negative effects of this endophyte on host growth and fecundity in greenhouse and field conditions, but haven't isolated factors which specifically determine the sign of effect, further studies are warranted. We have been able to add evidence that *S. fimicola*, which is usually described as an obligate coprophilous species, can clearly infect some hosts as an endophyte and significantly affect host fitness. If additional studies of this particular system were to determine the conditions under which the strong negative influences on growth and fecundity occur, *S. fimicola* could

likely join the short list of native microbes with potential for controlling *B. tectorum* in the Interior Pacific Northwest.

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Chapter 2: Apparent competition in grasses may be driven by endophytes with cryptic effects on emergence and early growth

Abstract

Apparent competition favors disease-tolerant hosts; when plants tolerate pathogens that affect competitors, apparent competition can be a mechanism of invasion. Grasses in the Palouse Prairie region of the US are commonly affected by *Fusarium*-related diseases. *Bromus tectorum* and *Ventenata dubia* have different histories of invasion in the Pacific Northwest and susceptibilities to *Fusarium* diseases. Twenty strains of *Fusarium* isolated from *V. dubia* and *B. tectorum* plants in Idaho were used to test the hypothesis that *V. dubia* tolerates infection by *Fusarium* better than *B. tectorum* and native grass *Pseudoroegneria spicata*. *Fusarium* isolates had strongly negative effects on emergence and seedling biomass of *B. tectorum* and *P. spicata*. In contrast, *Fusarium* had significant positive effects on emergence of *V. dubia*, and strongly positive effects on seedling biomass. The results provide limited support for the hypothesis that *V. dubia* benefits from apparent competition with competitors as mediated through interaction with *Fusarium* species.

Keywords: *Fusarium*, *Ventenata dubia*, *Bromus tectorum*, Apparent competition, Endophytic fungi, Plant invasion

2.1 Introduction

Competition between organisms is generally considered of great importance in determining plant community structure and the outcomes of processes from succession to invasion by exotic organisms. As Paul Keddy suggests in *Competition, 2nd edition* (2001), “in order to maintain access to resources, organisms sometimes must interfere with their neighbors... [and this is] the state of affairs of both the simplest bacterium and the most self-assured twentieth-century human” (pg. 1). Competition in plant communities is often described in terms of direct competition between neighbors for resources (water, sunlight, or soil nutrients) or direct negative effects of exuded chemicals (termed “allelopathy”) exerted on members of the same or different species (Choler *et al.* 2001; Fowler 1986; Holmgren *et al.* 1997; Inderjit *et al.* 2011). Competition has been suggested as a key mechanism in maintaining species richness in plant communities, and “intransitive competition” in

particular has been demonstrated to maintain diversity in a community (Soliveres *et al.* 2015). Indirect competition between species or individuals happens when negative effects on neighbors are mediated through another species or the environment. One particular type of indirect competition is apparent competition, in which competing species don't exert direct effects on each other, but rather experience interaction effects which may favor one species as a result of interaction with other organisms. Apparent competition was originally described in terms of common enemies influencing population dynamics of competitors (for example a predator or herbivore), but is also applicable to situations where other types of symbiosis (e.g., mutualism) are involved (Aschehoug *et al.* 2012; Connell 1990; Holt & Lawton 1994).

During the summer of 2012, 28 strains of fungi from the genus *Fusarium* were isolated from *Bromus tectorum* and *Ventenata dubia* plants growing in an invaded native Palouse Prairie fragment on Paradise Ridge, Idaho. These two annual grass invaders of the inland Pacific Northwest are considered direct threats to the endangered Palouse Prairie ecosystem due to their ability to alter fire-cycles and outcompete native perennial bunchgrasses in certain circumstances (Nyamai *et al.* 2011; Wallace *et al.* 2015). After growing the fungi in culture for two weeks, we observed that *B. tectorum*-associated *Fusarium* isolates were morphologically distinct from the *V. dubia*-associated *Fusarium* isolates. An observation was also made while collecting the plants that *Fusarium*-infected plants were of differing heights from non-infected plants (infected *B. tectorum* plants were significantly shorter than non-infected plants, while infected and non-infected *V. dubia* plants had no significant difference in height). The seemingly distinct *Fusarium* endophyte communities of an older and a newer invader, and the possible negative effects on *B. tectorum* growth, suggests that differential effects of infection by *Fusarium* might favor *V. dubia*, the newer invader. This led us to conduct a series of experiments in order to explore the effects on emergence and biomass of the *Fusarium* isolates on *V. dubia*, *B. tectorum*, and *P. spicata* seedlings.

Ten isolates from each host, designated as *Fusarium ex Ventenata* (FEV) and *Fusarium ex Bromus* (FEB), were grown in axenic culture and used to challenge each grass species. Since fungi from the genus *Fusarium* are often described as pathogens of both agricultural and wild grasses (and far less often described as non-disease causing

endophytes), the possibility that *V. dubia* has acquired tolerance of, immunity from, or a different type of symbiosis with these fungi is of particular interest in the face of an expanding *V. dubia* invasion. The experiments in this study were designed to assess whether *V. dubia* responds differently to infection by *Fusarium* isolates than competitors *B. tectorum* and *P. spicata*. Two common measures of seedling health that directly affect competition, seedling emergence and seedling aboveground biomass, were used as responses. Treatments included bulk inoculations (combined spore suspensions from all 10 *Fusarium* isolates of FEV and 10 *Fusarium* isolates of FEB) and individual isolates (each of 10 *Fusarium* isolates from each host). Since one of the most common disease-causing *Fusarium* species (*F. culmorum*) was absent from the isolates cultured from *B. tectorum* and *V. dubia* in the field, we also conducted a small serial-infection experiment to determine whether certain isolates from the invasive grasses would protect *V. dubia* from infection or effects of *F. culmorum*. We hypothesized that if *V. dubia* is benefiting from apparent competition, then symbioses between *Fusarium* isolates from *B. tectorum* and *V. dubia* should favor *V. dubia* growth and emergence while providing either smaller positive or negative effects on competitors *B. tectorum* and *P. spicata*. Additionally, we hypothesized that if *V. dubia* has a mutualistic interaction with *Fusarium* isolates, that the host may be derive a certain amount of protection from disease caused by *F. culmorum*.

2.2 Materials and Methods

2.2.1 Isolation of *Fusarium* from *V. dubia* and *B. tectorum* and inoculum production

B. tectorum and *V. dubia* plants were collected in June and July of 2012 from a remnant Palouse Prairie fragment on Paradise Ridge (46.677559, -116.976893; 1128m). Root and culm (between 1st and 2nd node above crown) segments of ~5cm were surface sterilized using 50% ethanol solution for 5 minutes and then washing in sterile DI water for 1 minute (Baynes *et al.* 2012). Sterilized segments were plated on Potato Dextrose Agar and incubated at 20°C on a lab bench at the University of Idaho's College of Natural Resources. Fungi emerging from the plant segments were sub-cultured and re-plated in order to produce pure cultures. After two weeks growth at 20°C, fungal cultures were separated by genus and morphotyped, based on colony appearance, growth rate, and spore characteristics. As is often the case, many endophytic fungi were isolated from both exotic grass species: a total of 118

morphotypes out of 223 subcultures, with 32% of successfully isolated strains being from genus *Fusarium* (Arnold 2007; Leighton 2012). After initial assays were conducted (see results below) and confirmed that *Fusarium* was of interest in regards to *V. dubia*, efforts were made to sample populations around Moscow, ID in order to determine whether *Fusarium spp.* were commonly found as endophytes of stems in the area. Using the methods described above, the roots and culm sections from 100 plants at each of 12 different populations were sampled, all within a radius of 100 miles from Paradise Ridge.

Interest in the isolated fungi was focused on the *Fusarium* genus based on two factors: there was a perceived correlation between plant height in the field at time of collection and presence or absence of *Fusarium*; and there were marked differences in morphology of the *Fusarium* isolated from *V. dubia* (FEV) and *B. tectorum* (FEB) (Leighton 2012). FEV grew close to the surface of the agar and produced red or purple exudates, while FEB produced abundant aerial mycelia and were often cream- or pale yellow-colored with no exudates produced in agar. Species within genus *Fusarium* are often pathogenic to wild and cultivated grasses. The *Fusarium* collection was narrowed to 10 isolates from *V. dubia* and 10 isolates from *B. tectorum*, based on morphological similarities. Bulked inoculum was created from FEV and FEB after being grown at ~20°C on potato dextrose agar on lab benches for 30 days. Spore solutions were created by wetting plates with sterile DI water, gently agitating mycelia on surface of the agar with a glass rod, and filtering through cheesecloth to remove large pieces of mycelia or agar. Inoculum volume for each spore suspension (FEV and FEB) was brought to 1000mL with sterile DI water; final concentration of each inoculum was approximately 1.1 million spores and hyphal fragments / mL, as determined by hemocytometer.

Inoculum for each individual *Fusarium* isolates was produced in a similar manner, after having been grown for 30 days. Final volume of each of the 20 inocula produced (10 FEV, 10 FEB) were brought to 100mL by addition of sterile DI water; final concentration of each inoculum was approximately 1.1 million spores and hyphal fragments / mL, as determined by hemocytometer. All inocula (both bulked and individual isolate) were produced from cultures that had been sub-cultured only twice in order to reduce chances of loss of virulence through serial sub-culturing: all cultures used to produce inoculum were

sub-cultured directly from plates derived from initial sub-cultures from endophytes emerging from plant segments in agar.

2.2.2 Seed collection of *V. dubia*, *B. tectorum*, and *P. spicata*

A collection of *V. dubia* was made at Virgil Phillips Farm Park, Latah County, Idaho in July 2012 (46.814048, -117.014165; 875m). *V. dubia* plants were grown in the greenhouse in 2012 and 2013 from this initial stock and experiments reported here were performed with these greenhouse-grown *V. dubia* seeds. *B. tectorum* seeds were collected at Parker Experimental Farm in Moscow, ID in July, 2011 (46.725397, -116.954912; 796m). *P. spicata* seeds were collected at Asotin, WA (46.331859, -117.041966; 975m) in 2008 and provided to the authors by the USDA Forest Service Rocky Mountain Research Station (Boise, ID).

2.2.3 Bulk inoculum emergence study

Single *V. dubia*, *B. tectorum*, and *P. spicata* seeds were planted in 200 cell 1.75" depth plug-flats filled with wetted potting mix (Sunshine #1 soilless), and were treated with bulked *Fusarium* spore solutions (FEV and FEB). 300µl of spore suspension were directly applied to the surface of each seed and then covered with wetted potting mix to a depth of ~2mm. Each bulked spore suspension treatment was applied to 200 seeds of each grass species (n=200 per treatment, with FEV and FEB treatments for each grass species and one control treatment per species). The control for each grass species included the application of 300µl of sterile DI water to each seed before covering with wetted potting mix to a depth of ~2mm. Plants were grown in greenhouse conditions (20°-24°C, 16:8 day:night cycle) until seed emergence stopped. Emergence of seedlings was tallied each day, and emergence was determined to have stopped when no seedlings emerged for three consecutive days (thus, time of total experiment was different for each species and ranged from 14 days for *B. tectorum* to almost 1 month for *V. dubia*).

2.2.4 Biomass studies

In the seedling, 14-day biomass experiment, plants grown in the bulked inoculum emergence study in greenhouse conditions (20°-24°C, 16:8 day:night cycle) were collected 14 days after emergence had ceased by clipping at the soil surface. Seedlings were dried in paper pouches at 70°C in a forced-air dryer for 48 hours and then weighed. Number of

replicates per treatment and species combination were necessarily unbalanced, since emergence rates were different between species and treatments within species.

2.2.5 Individual isolates emergence study

Ten *Fusarium* isolates from *V. dubia* (FEV) and ten *Fusarium* isolates from *B. tectorum* (FEB) were grown on potato dextrose agar for 30 days. Inoculum was prepared as described above. Single *V. dubia*, *B. tectorum*, and *P. spicata* seeds were planted in 200 cell 1.75" depth plug-flats filled with wetted potting mix (Sunshine #1 soilless), and were treated with individual *Fusarium* spore solutions (10 each of FEV and FEB). 300µl of spore suspension were directly applied to the surface of each seed and then covered with wetted potting mix to a depth of ~2mm. Each bulked spore suspension treatment was applied to 200 seeds of each grass species (n=200 per treatment, with FEV and FEB treatments for each grass species and one control treatment per species). The control for each grass species included the application of 300µl of sterile DI water to each seed before covering with wetted potting mix to a depth of ~2mm. Plants were grown in greenhouse conditions (20°-24°C, 16:8 day:night cycle) until seed emergence stopped. Emergence of seedlings was tallied each day, and emergence was determined to have stopped when no seedlings emerged for three consecutive days (thus, time of total experiment was different for each species and ranged from 14 days for *B. tectorum* to almost 1 month for *V. dubia*).

2.2.6 Bulked FEV and FEB effects on above ground biomass 90 days after emergence

Three *V. dubia*, *B. tectorum*, and *P. spicata* seeds were planted in each of 50 4x4" pots for each of 3 different treatments (Control, FEB, FEV) in wetted potting mix (Sunshine #1 soilless). 300µl of spore suspension or sterile DI water was applied to the surface of each seed and then covered with wetted potting mix to a depth of ~2mm. After seedling emergence, pots were thinned to one seedling per pot, so that there were a total of 300 plants (n=50 per treatment, with control, FEV, and FEB treatments for each grass species). Seedlings were grown in greenhouse conditions (20°-24°C, 16:8 day:night cycle) for 90 days after emergence, and were then collected by clipping at soil surface. Seedlings were dried in paper pouches at 70°C in a forced-air dryer for 48 hours and then weighed.

2.2.7 Competition intensity in the presence of *F. culmorum*, and serial inoculation with selected FEV isolates and *F. culmorum* inoculum on above ground biomass 90 days after emergence.

Two isolates from *V. dubia* which had positive effects on *V. dubia* emergence (FEV2b and FEV8b) and negative or neutral effects on competitors emergence were chosen for a serial inoculation experiment with *F. culmorum* (see Results and **Table 2.1**, below). The *F. culmorum* strain used (WSU ID: 70110097) was obtained from Dr. Paulitz of the USDA Agricultural Research Service (Pullman, WA) and Washington State University; the strain is known to be highly virulent to hard red winter wheat. Before conducting the serial infection experiment described below, a greenhouse experiment was conducted to determine whether *F. culmorum* could directly alter the competition between *Triticum aestivum* and *V. dubia*. Four different treatments, with 10 replicates each, were established in 1 gallon pots filled with Sunshine Mix no. 1: three seeds of *V. dubia* were planted in the center, surrounded by 24 seeds of *T. aestivum* in groups equidistant from the central group and each other; three seeds of *T. aestivum* were planted in the center, surrounded by 24 seeds of *V. dubia* in groups equidistant from the central group and each other; three seeds of *V. dubia* were planted in the center, surrounded by 24 seeds of *T. aestivum* in groups equidistant from the central group and each other, and each seed was inoculated with 300 μ l of *F. culmorum* inoculum, prepared as described above; and three seeds of *T. aestivum* were planted in the center, surrounded by 24 seeds of *V. dubia* in groups equidistant from the central group and each other, and each seed was inoculated with 300 μ l of *F. culmorum* inoculum, prepared as described above. Each pot was thinned after seeds emerged so that the central seedling (*V. dubia* or *T. aestivum*) in each treatment was surrounded by eight equidistant competitor seedlings, and plants were grown for 90 days with the intention of collecting, drying, and measuring biomass as described below in the serial-infection experiment.

In the serial infection experiment, three *V. dubia* seeds were planted in each of 10 4x4" pots for each of 6 treatments (Control, FEV2b, FEV8b, Control + *F. culmorum*, FEV2b + *F. culmorum*, and FEV8b + *F. culmorum*) in wetted potting soil (Sunshine #1 soilless). 300 μ l of spore suspension (for all four FEV2b and FEV8b treatments) or sterile DI water (for both positive and negative controls) was applied to the surface of each seed and then covered with wetted potting mix to a depth of ~2mm. Seedlings were grown in greenhouse conditions

(20°-24°C, 16:8 day:night cycle) for 90 days after emergence, and were then collected by clipping at soil surface. Seedlings were dried in paper pouches at 70°C in a forced-air dryer for 48 hours and then weighed.

2.2.8 DNA extraction, amplification, and sequencing

In order to estimate taxonomic identity of each isolate, the ITS region of nuclear ribosomal DNA were sequenced (White *et al.* 1990). Qiagen DNeasy 96 Plant Kits (Qiagen, Valencia, CA, USA) were used with standard protocols to extract DNA from fresh cultures (grown on PDA). ITS1F and LR3 primers were used in PCR reactions, where the volume was 20 µl and included 2 µl of genomic DNA. Cycle sequencing reaction volume was 10 µl, including 1 µl of the PCR product. Forward and reverse sequencing was conducted using an ABI PRISM 3730 Genetic Analyzer, and Geneious® 6.1.7 was used to trim regions with more than a 5% chance of an error per base. For each isolate, forward and reverse reads were paired to generate a consensus sequence. Finally, consensus sequences were used as the basis for BLAST queries to determine closest positively identified taxa (Altschul *et al.* 1990). All sequences have been deposited to NCBI GenBank (Accessions KT347149-KT347168). Sequences of FEV and FEB isolates were aligned using Clustal Omega for multiple sequence alignment on the EMBL-EBI website (Sievers *et al.* 2010; Li *et al.* 2015). A rough phylogenetic tree was constructed using software on servers at *phylogeny.fr*, along with a sequence of *Fusarium culmorum* (GenBank accession DQ459870.1 from Starkey *et al.* 2007). Multiple sequence alignments were computed using MUSCLE within Geneious version 6.1.7, curation with Gblocks, Maximum Likelihood estimation of the tree with PhyML (min of SH-like and Chi2) with 100 bootstraps, and tree visualization was performed with TreeDyn (Dereeper *et al.* 2008; Kearse *et al.* 2012; Edgar *et al.* 2004; Castresana 2000; Guindon *et al.* 2010; Anisimova & Gascuel 2006; and Chevenet *et al.* 2006).

2.2.9 Statistical Analyses

All data was analyzed using R 3.0.1 (R Core Team 2013). In the emergence experiments, treatment and control proportions (how many seedlings emerged of 200 seeds per treatment) were analyzed using chi-square proportion tests, with Yates correction where appropriate (population means within 0.05 of 1.0 or 0.0). Above ground biomass from the bulked inoculum experiments was analyzed using 1-way ANOVA and Tukey's HSD. Differences from control were considered statistically significant at the $p < 0.05$ level.

2.3 Results

The survey of *V. dubia* populations around Paradise Ridge indicates that *Fusarium spp.* associated with the grass is not unusual, nor the norm: *Fusarium spp.* were isolated from 25% of the twelve sampled populations in the region.

2.3.1 Bulk *Fusarium inoculum* effect on *P. spicata*, *V. dubia*, and *B. tectorum* emergence

A bulk of 10 *Fusarium* isolates from *V. dubia* a bulk of 10 *Fusarium* isolates from *B. tectorum* were tested for effects on emergence against *P. spicata*, *B. tectorum*, and *V. dubia*. Both FEV and FEB had significant negative effects (-40%, $p < 0.0001$ and -18%, $p < 0.0001$, respectively) on *P. spicata* emergence. See **Tables 2.1** and **2.2** (below). Neither FEV nor FEB in bulked inoculum had a significant negative effect (-2%, $p = 0.4381$ and -3%, $p = 0.0880$, respectively) on *B. tectorum* emergence. FEV had a significant negative effect (-11%, $p = 0.0002$) on *V. dubia* emergence, while FEB had no significant effect (+5%, $p = 0.0904$) on *V. dubia* emergence.

2.3.2 Individual *Fusarium* isolates effect on *P. spicata*, *V. dubia*, and *B. Tectorum* emergence

10 *Fusarium* isolated from *V. dubia* were tested for effects on emergence against *P. spicata*. Five isolates, FEV51A, FEV8B, FEV38A, FEV2B, and FEV34B, had significant negative effects (-30%, $p < 0.0001$; -23%, $p < 0.0001$; -21%, $p < 0.0001$; -17%, $p < 0.0001$; and -11%, $p = 0.0009$, respectively) on *P. spicata* emergence, decreasing emergence proportions compared to population proportion (established by control). See **Table 2.1**. 10 *Fusarium* isolated from *B. tectorum* were tested for effects on emergence against *P. spicata*. None of the isolates from *B. tectorum* had a significant negative effect on *P. spicata* emergence. See **Table 2.1**.

Table 2.1 Effects of *Fusarium ex Ventenata* (FEV) on emergence of three grasses. Each cell shows emergence rate as difference from control; statistically significant results ($p < 0.05$ by χ^2 proportion tests) are marked with an *; positive, significant results are shown in **BOLD**.

<i>Fusarium ex Ventenata</i>	Bulk FEV	FEV 51a	FEV 8b	FEV 38a	FEV 2b	FEV 34b	FEV 19a	FEV 30a	FEV 22a	FEV 12a	FEV 31a
<i>Pseudoroegneria spicata</i>	-.40 *	-.30 *	-.23 *	-.21 *	-.17 *	-.11 *	-.06	-.05	-.04	-.04	+.01
<i>B. tectorum</i>	-.02	-.02	0.0	-.03	-.02	+.02	-.04 *	-.05 *	-.04 *	0.0	-.01
<i>V. dubia</i>	-.11 *	-.05	+.08 *	+.02	+.11 *	-.07	-.04	+.02	+.01	+.04	+.03

10 *Fusarium* isolated from *V. dubia* were tested for effects on emergence against *V. dubia* and *B. tectorum*. Two isolates, FEV8B and FEV2B, had significant positive effects (+8%, $p=0.0234$ and +11%, $p=0.0029$, respectively) on *V. dubia* emergence, increasing emergence proportions compared to population proportion (established by control). See **Table 2.1**. Three isolates, FEV19A, FEV22A, and FEV30A, had significant negative effects (-4%, $p=0.0266$; -5%, $p=0.0266$; and -4%, $p=0.0037$, respectively) on *B. tectorum* emergence, decreasing emergence proportions compared to population proportion (established by control). See **Table 2.1**.

10 *Fusarium* isolated from *B. tectorum* were tested for effects on emergence against *V. dubia* and *B. tectorum*. Three isolates, FEB5A, FEB14G, and FEB107B had significant negative effects (-12%, $p=0.0006$; -11%, $p=0.0016$; and -10%, $p=0.0041$, respectively) on *V. dubia* emergence, decreasing emergence proportions compared to population proportion (established by control). See **Table 2.2**. Eight isolates, FEB5a, FEB10C, FEB43B, FEB57A, FEB14C, FEB107B, FEB18A, and FEB5C, had significant negative effects (-11%, $p=0.0028$; -15%, $p<0.0001$; -12%, $p=0.0006$; -8%, $p=0.0230$; -13%, $p=0.0004$; -7%, $p=0.0466$; -12%, $p=0.0011$; and -9%, $p=0.0157$, respectively) on *B. tectorum* emergence, decreasing emergence proportions compared to population proportion (established by control). See **Table 2.2**.

Table 2.2 Effects of *Fusarium ex Bromus* (FEB) on emergence of three grasses. Each cell shows emergence rate as difference from control; statistically significant results ($p<0.05$ by χ^2 proportion tests) are marked with an *.

<i>Fusarium ex Bromus</i>	Bulk FEB	FEB 5a	FEB 14g	FEB 10c	FEB 43b	FEB 57a	FEB 6a	FEB 14c	FEB 107b	FEB 18a	FEB 5c
<i>Pseudoroegneria spicata</i>	-.18 *	-.06	-.06	-.05	-.03	-.03	-.03	-.02	-.01	+.01	+.02
<i>B. tectorum</i>	-.03	-.11 *	-.06	-.15 *	-.12 *	-.08 *	-.06	-.13 *	-.07 *	-.12 *	-.09 *
<i>V. dubia</i>	+.05	-.12 *	-.11 *	+.03	+.04	-.06	-.01	+.02	-.10 *	+.03	-.06

It is important to note that with the FEV individual isolates tested against *B. tectorum* that the control and treatment emergence rates were much lower than in previous experiments (a control emergence proportion of ~0.45, as opposed to the FEB vs *B. tectorum* control proportion of ~0.95). This difference is a possible effect of having done the FEV vs *B. tectorum* experiment later in the year (August 2013) than the FEB vs *B. tectorum* (June

2013) and the resulting difference in temperature in the partially climate controlled greenhouse (average high temperatures and humidity in August can be considerably higher than those in May or June in the greenhouse); they could also be attributable to a decline in the viability of the seed associated with time. However, the results of the χ^2 proportion tests depend on the difference between control and treatment within an experiment rather than a difference in controls between experiments, so this should not have a significant effect on the validity of the results.

2.3.3 Bulked *Fusarium* inoculum effect on *P. spicata*, *V. dubia*, and *B. tectorum* above ground seedling biomass

A bulk of 10 *Fusarium* isolates from *V. dubia* and a bulk of 10 *Fusarium* isolates from *B. tectorum* were tested for effects on post-emergence above ground biomass against *P. spicata*, *B. tectorum*, and *V. dubia* seedlings. Results of 1-way ANOVA indicate that treatment with bulked inoculum was statistically significant ($p < 0.0001$) for *P. spicata*, and Tukey's HSD indicates that both FEV and FEB had significant negative effects (-36% with $p < 0.0001$ and -30% with $p < 0.0001$, respectively) on *P. spicata* above ground biomass compared to control. See **Table 2.3** and **Figure 2.1**, below.

Table 2.3 Effects of *Fusarium* on seedling biomass (14 days after emergence) of three grasses. Each treatment cell shows difference from control biomass; treatment for each host and treatment combination significant by one-way AOV ($p < 0.05$); statistically significant differences ($p < 0.05$ by Tukey's HSD) are marked with an *; positive, significant results are shown in **BOLD**.

	Control	Bulk FEB	Bulk FEV
<i>Pseudoroegneria spicata</i>	0.0138g	-0.0042g *	-0.0050g *
<i>B. tectorum</i>	0.0199g	-0.0053g *	-0.0022g *
<i>V. dubia</i>	0.0025g	+0.0039g *	+0.0019g *

Results of 1-way ANOVA indicate that treatment with bulked inoculum was statistically significant ($p < 0.0001$) for *B. tectorum*, and Tukey's HSD indicates that both FEV and FEB had significant negative effects (-27% with $p < 0.0001$ and -11% with $p = 0.0047$, respectively) on *B. tectorum* above ground seedling biomass compared to control. See **Table 2.3** and **Figure 2.1**. Results of 1-way ANOVA indicate that treatment with bulked

inoculum was statistically significant ($p < 0.0001$) for *V. dubia*, and Tukey's HSD indicates that both FEV and FEB had significant positive effects (+76% with $p < 0.0001$ and +156% with $p < 0.0001$, respectively) on *V. dubia* above ground seedling biomass compared to control. See **Table 2.3** and **Figure 2.1**.

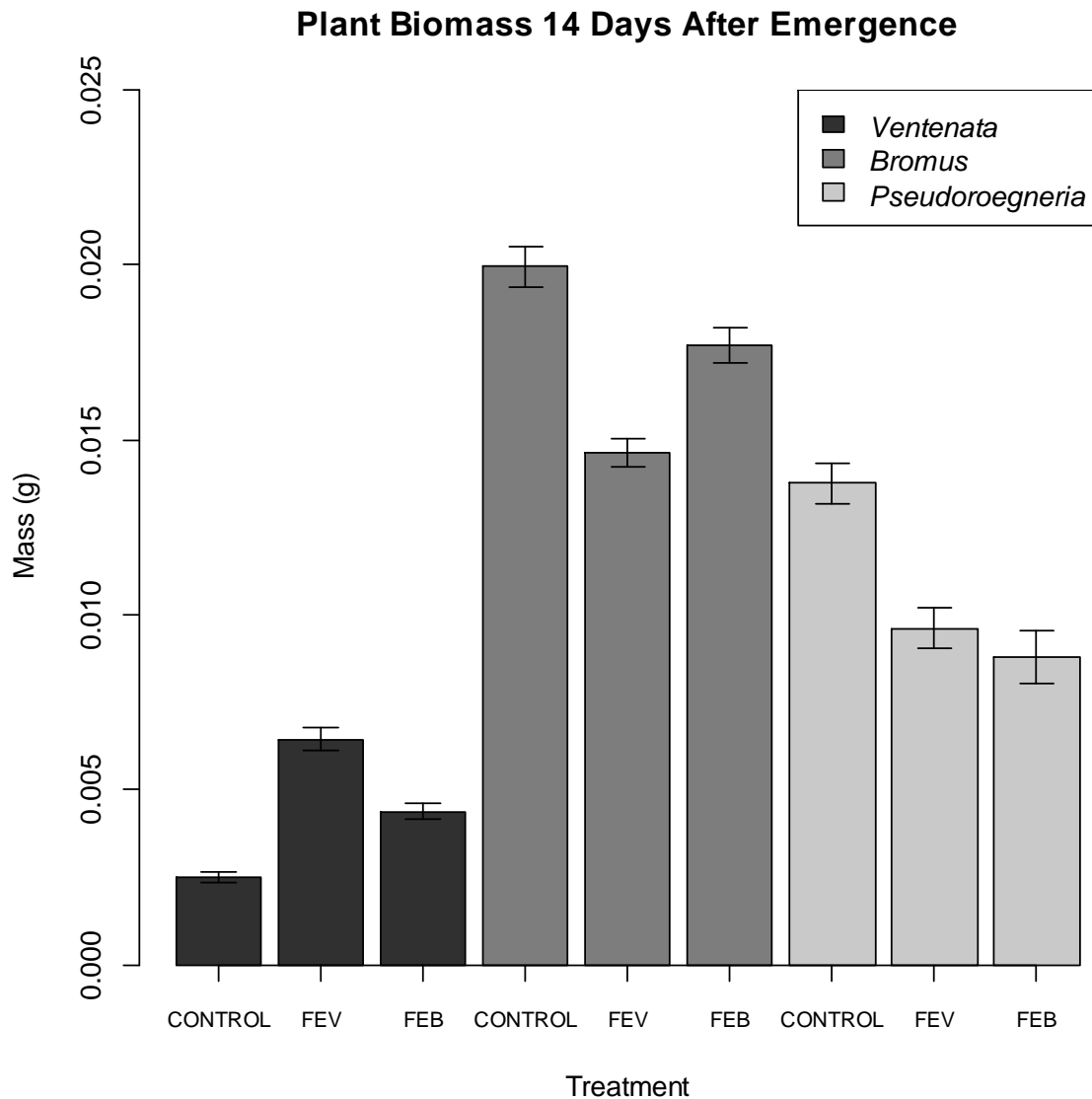


Figure 2.1 Mean aboveground biomass of seedlings 14 days after emergence. Each grass (*V. dubia*, *B. tectorum*, and *P. spicata*) had a control (sterile DI water), *Fusarium ex Ventenata* (FEV), and *Fusarium ex Bromus* (FEB) treatment applied to seeds during planting. Plants were collected 14 days after emergence had ceased for each treatment, dried, and weighed.

2.3.4 Bulked *Fusarium* inoculum effect on *P. spicata*, *V. dubia*, and *B. tectorum* above ground biomass, 90 days after emergence

A bulk of 10 *Fusarium* isolates from *V. dubia* and a bulk of 10 *Fusarium* isolates from *B. tectorum* were tested for effects on above ground biomass after 90 days of growth in the greenhouse on *P. spicata*, *B. tectorum*, and *V. dubia*. Results of 1-way ANOVA indicate that treatment with bulked inoculum was statistically significant ($p=0.0185$) for *P. spicata*. Tukey's HSD indicates that neither FEV nor FEB had significant effects on *P. spicata* above ground biomass compared to control, but that the FEV and FEB treatments were significantly different from each other (difference of FEV-FEB was -0.1151g , $p<0.0224$). See **Table 2.4** and **Figure 2.2**, below. Results of 1-way ANOVA indicate that treatment with bulked inoculum was not statistically significant ($p=0.359$) for *B. tectorum*, and Tukey's HSD indicates that neither FEV nor FEB had significant effects on *B. tectorum* above ground biomass compared to control. See **Table 2.4** and **Figure 2.2**. Results of 1-way ANOVA indicate that treatment with bulked inoculum was not statistically significant ($p=0.519$) for *V. dubia*, and Tukey's HSD indicates that neither FEV nor FEB had significant effects on *V. dubia* above ground biomass compared to control. See **Table 2.4** and **Figure 2.2**.

Table 2.4 Effects of *Fusarium* on aboveground biomass of three grasses grown for 90 days after emergence. Each treatment cell shows difference from control biomass; treatment for *P. spicata* was significant by one-way AOV ($p=0.019$); no significant differences between treatment and control means within species were found using Tukey's HSD.

	Control	Bulk FEB	Bulk FEV
<i>Pseudoroegneria spicata</i>	0.3743g	+0.0950g	-0.0201g
<i>B. tectorum</i>	0.9981g	+0.0292g	+0.0703g
<i>V. dubia</i>	0.5156g	-0.0458g	-0.0416g

Plant Biomass 90 Days After Emergence

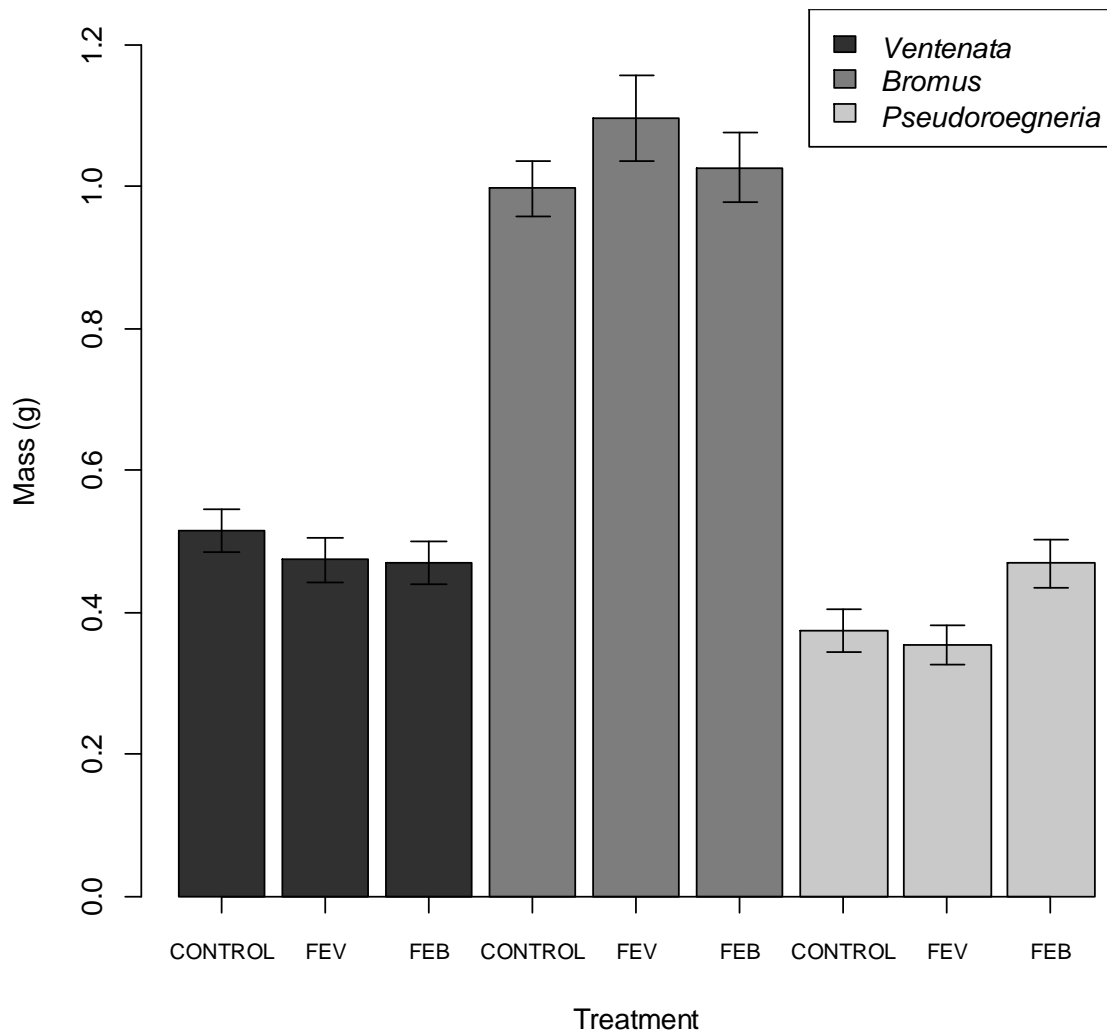


Figure 2.2 Mean aboveground biomass of seedlings 90 days after emergence. Each grass (*V. dubia*, *B. tectorum*, and *P. spicata*) had a control (sterile DI water), *Fusarium ex Ventenata* (FEV), and *Fusarium ex Bromus* (FEB) treatment applied to seeds during planting. Plants were collected 90 days after emergence had ceased for each treatment, dried, and weighed.

2.3.5 Competition in the presence of *F. culmorum* and effects of serial inoculation of FEV2b, FEV8b, and *F. culmorum* on *V. dubia* 90-day biomass

The preliminary competition in the presence of *F. culmorum* experiment was terminated early due to widespread development of disease in both *T. aestivum* and *V. dubia*. In the greenhouse competition assays, *F. culmorum* was shown to be highly virulent to both

T. aestivum and *V. dubia* (> 50% of all *V. dubia* seedlings inoculated with *F. culmorum* developed ‘crown rot’ disease and died within 60 days, the surviving plants were severely curtailed in growth and generally had more than ¾ leaves killed). The widespread external symptoms of crown rot made collecting specimens and drying them impractical and unnecessary, as the seedlings didn’t survive long enough to make measurement of competition intensity meaningful. It is fair to qualitatively assess the effect of this particular pathogenic strain of *F. culmorum* on *V. dubia* as highly virulent and particularly deadly.

Seeds of *V. dubia* were inoculated with DI water, FEV2b, or FEV8b on planting, and then challenged with inoculum of *F. culmorum* after seedling emergence. Plants were grown for 90 days, collected, dried and weighed in order to assess whether either of these two *Fusarium* isolates would protect *V. dubia* seedlings from disease caused by *F. culmorum*. Previous work (described above) indicates that *V. dubia* is highly susceptible to disease caused by *F. culmorum*, and it has been demonstrated in other host plants that prior infection by a mutualist endophyte (*Piriformospora indica*, in this case) can protect wheat plants from crown rot disease caused by *F. culmorum* and *F. graminearum* (Rabiey *et al.* 2015). Results of 1-way ANOVA indicate that treatment was significant ($p < 0.001$), but it is very clear that prior infection by FEV2b or FEV8b did not provide protection from the effects of infection by *F. culmorum*. Tukey’s HSD reveals that the only significantly positive effect between treatment groups was a +79% increase ($p < 0.001$) in biomass between FEV8b and Con+, the positive control plants treated with *F. culmorum*. Significantly different negative effects include: Con and Con+ (-40%, $p = 0.004$); Con and FEV2b+ (-54%, $p < 0.001$); Con and FEV8b+ (-55%, $p < 0.001$); FEV2b and FEV2b+ (-49%, $p = 0.002$); and FEV8b and FEV8b+ (-58%, $p < 0.001$). See **Figure 2.3** and **Tables 2.5a** and **2.5b** for mean weights and differences.

Table 2.5a Mean aboveground biomass of *V. dubia* after serial infection, with: no treatment (“Con”); inoculation by *F. culmorum* (“Con+”); inoculation by *Fusarium* isolates FEV2b and FEV8b; and inoculation by FEV2b and FEV8b followed by inoculation with *Fusarium culmorum* 10 days after seedling emergence (“FEV2b+” and “FEV8b+”).

	Con	Con +	FEV2b	FEV8b	FEV2b +	FEV8b +
Mean Weight	0.622g	0.371g	0.547g	0.665g	0.281g	0.277g

Table 2.5b FEV and *F. culmorum* Serial infection treatment pairs compared: with differences in weight in grams shown in the second column; treatments significantly different from control at $p < 0.05$ using Tukey's HSD marked with an *; and p values are listed in the third column.

Treatments Compared	Difference in weight (g)	Tukey HSD p adj =
con+-con	-0.251*	0.004
FEV2b-con	-0.075	0.858
FEV2b+-con	-0.341*	0.000
FEV8b-con	0.043	0.985
FEV8b+-con	-0.345*	0.000
FEV2b-con+	0.176	0.078
FEV2b+-con+	-0.090	0.739
FEV8b-con+	0.294*	0.000
FEV8b+-con+	-0.094	0.678
FEV2b+-FEV2b	-0.266*	0.002
FEV8b+-FEV8b	-0.388*	0.000

2.3.6 Identification of *Fusarium* isolates

DNA from 19 of the FEV and FEB isolates were successfully sequenced on the ITS region with primers 1F and LR3 (extraction from FEB 43B was unsuccessful, and there were two reads from FEV 31A, labeled in the sequences and resulting trees as “FEV 31A1” and “FEV 31A2”). Edited sequences were approximately 1,100bp in length, and were compared to submitted sequences on the NCBI database using the BLAST tool. The accession numbers for the *Fusarium* sequences used in these experiments, and the closest BLAST match in the database for each sequence, are shown in **Table 2.6**. An unrooted phylogenetic tree with bootstrap values of the sequences used in the experiments plus a *F. culmorum* sequence from a published study is shown in **Figure 2.4**. Although this preliminary phylogenetic tree shouldn't be used to draw inferences about evolutionary relationships between the isolates used in our experiments, it is useful to note here that the FEB and FEV sequences do not neatly segregate into separate branches. Sequences which match closely with known *F. accuminatum* strains, in particular, were isolated from both *B. tectorum* (FEB14g) and *V. dubia* (FEV51a, FEV8b, FEV12a, and FEV31a).

Ventenata and *Fusarium culmorum*

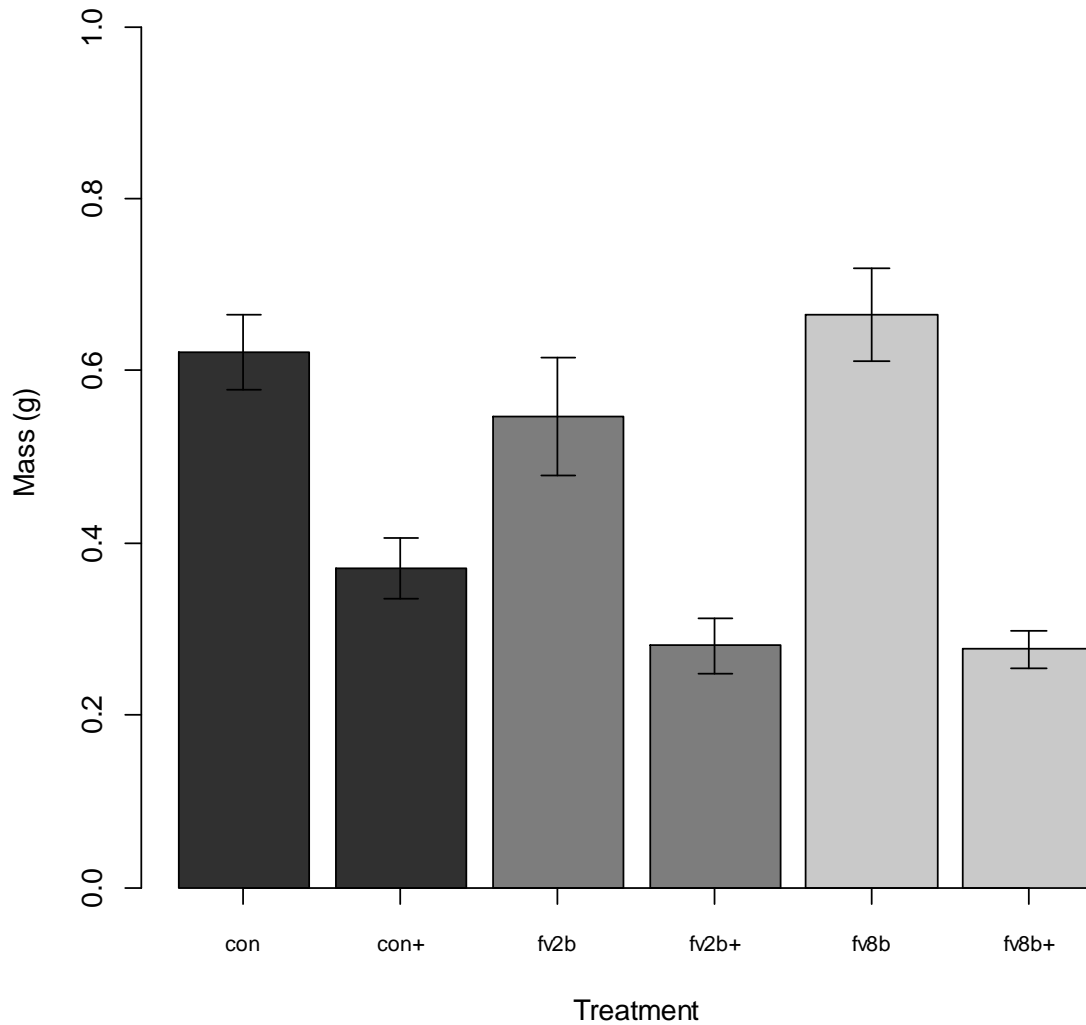


Figure 2.3 Mean aboveground biomass of serially infected *V. dubia* plants 90 days after emergence. Controls (“con” and “con+”) were treated with sterile water and *Fusarium culmorum* inoculum, respectively. Treatment groups were inoculated with FEV2b (“fv2b”), FEV2b and *Fusarium culmorum* (“fv2b+”), FEV8b (“fv8b”), or FEV8b and *Fusarium culmorum* (“fv8b+”). Plants were collected 90 days after emergence had ceased for each treatment and then dried and weighed.

Table 2.6 GenBank Accession numbers for the referenced *Fusarium* spp. used in experiments in this study. Aligned sequences were matched using the BLAST algorithm on the GenBank website; numbers given are the percentage identity to the listed sequence(s) in the database.

Culture ID	GenBank Accession	Closest Match and Sequence Identity from BLAST
FEB6A	<u>KT347149</u>	100/99 <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> AY188919.1
FEB57A	<u>KT347150</u>	100/98 <i>Fusarium flocciferum</i> strain NRRL 45999 GQ505465.1
FEB14C	<u>KT347151</u>	99/99 <i>Fusarium flocciferum</i> strain NRRL 45999
FEB5C	<u>KT347152</u>	100/99 <i>Fusarium</i> sp. WF157 HQ130713.1 ex <i>Warburgia</i> Uganda
FEB5A	<u>KT347153</u>	100/99 <i>Fusarium</i> sp. WF157 HQ130713.1 ex <i>Warburgia</i> Uganda
FEB14G	<u>KT347154</u>	100/99 <i>Fusarium acuminatum</i> NRRL 54212 HM068320.1 in <i>Sclerotinia</i>
FEB18A	<u>KT347155</u>	100/99 <i>Fusarium</i> sp. WF157 HQ130713.1 ex <i>Warburgia</i> Uganda
FEB107B	<u>KT347156</u>	100/99 <i>Fusarium</i> sp. WF157 HQ130713.1 ex <i>Warburgia</i> Uganda
FEB10C	<u>KT347157</u>	99/99 <i>Fusarium oxysporum</i> f. sp. <i>rapae</i> Japan AB586994.1
FEV30A	<u>KT347158</u>	100/100 <i>Fusarium</i> sp. WF150 HQ130706.1 ex <i>Warburgia</i> Uganda
FEV51A	<u>KT347159</u>	100/99 Uncultured <i>Gibberella</i> clone AC_2w_D08 Austria Tyrol forest leaf litter JF449525.1; 100/99 w <i>Fusarium acuminatum</i> NRRL 54212 HM068320.1 in <i>Sclerotinia</i>
FEV8B	<u>KT347160</u>	100/99 <i>Fusarium acuminatum</i> strain NRRL 54212 HM068320.1 in <i>Sclerotinia</i>
FEV34B	<u>KT347161</u>	100/99 <i>Fusarium flocciferum</i> strain NRRL 45999 GQ505465.1
FEV38A	<u>KT347162</u>	99/99 Uncultured <i>Gibberella</i> clone AC_2w_D08 Austria Tyrol forest leaf litter JF449525.1
FEV19A	<u>KT347163</u>	99/99 Uncultured <i>Gibberella</i> clone AC_2w_D08 Austria Tyrol forest leaf litter JF449525.1
FEV12A	<u>KT347164</u>	99/100 <i>Fusarium acuminatum</i> strain NRRL 54212 HM068320.1 in <i>Sclerotinia</i>
FEV31A1	<u>KT347165</u>	100/99 <i>Fusarium acuminatum</i> strain NRRL 54212 HM068320.1 in <i>Sclerotinia</i>
FEV31A2	<u>KT347166</u>	100/99 <i>Fusarium acuminatum</i> strain NRRL 54212 HM068320.1 in <i>Sclerotinia</i>
FEV22A	<u>KT347167</u>	100/99 <i>Fusarium</i> sp. NRRL 34036 GQ505451.1
FEV2B	<u>KT347168</u>	100/99 <i>Fusarium</i> sp. NRRL 34036 GQ505451.1

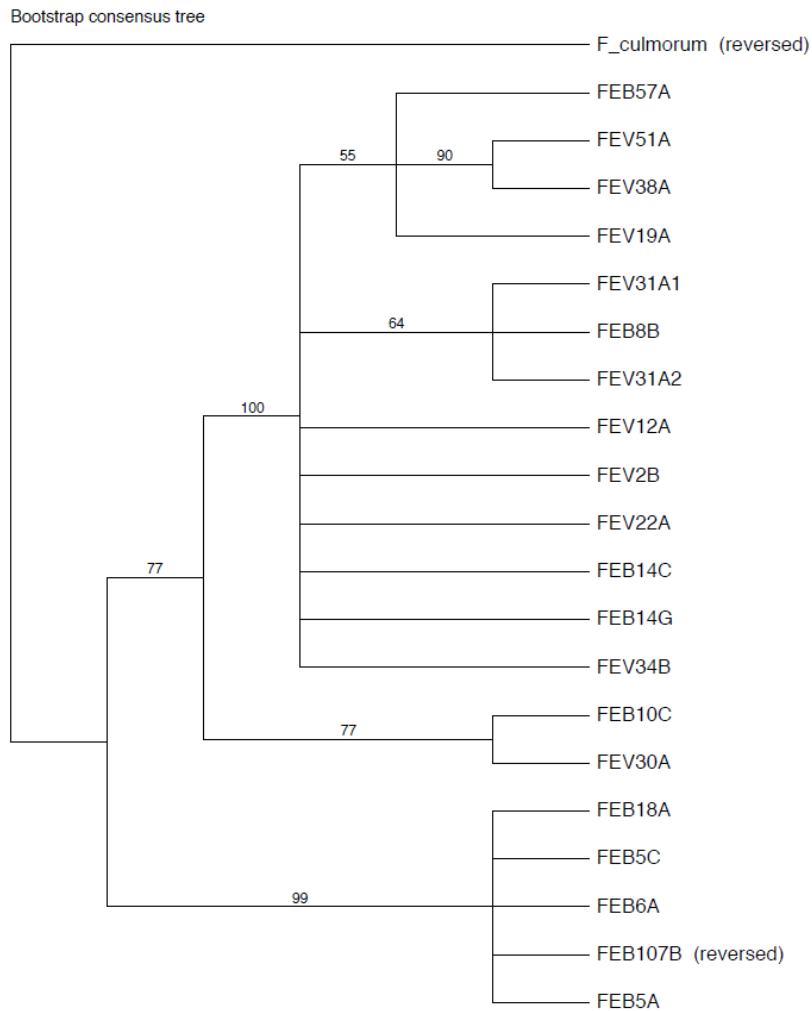


Figure 2.4 A maximum likelihood based phylogenetic tree (branch lengths are proportional to number of substitutions per site) of FEV and FEB ITS sequences (~1100 bp), along with a *F. culmorum* sequence from Starkey *et al.* (2007). Branch support values are from 100 bootstrap repetitions.

2.4 Discussion

Our results provide evidence that *V. dubia* may be benefitting from association with endophytic *Fusarium* strains through apparent competition with competitors *B. tectorum* and *P. spicata*. While none of the *Fusarium* strains isolated from *B. tectorum* or *V. dubia* caused external symptoms of disease in *V. dubia*, both *B. tectorum* and *P. spicata* showed signs of the development of crown rot under certain greenhouse conditions. Additionally, the only positive, statistically significant effects of *Fusarium* inoculation of seeds were on *V. dubia*. Two individual isolates (FEV8b and FEV2b) from *V. dubia* increased emergence of *V. dubia*

seedlings, and the bulked inoculum of isolates from both *V. dubia* (FEV) and *B. tectorum* (FEB) increased seedling biomass 14 days after emergence. All other significant results were negative (in either reducing biomass or decreasing proportion of emerged seeds). This suggests that *V. dubia* has a different symbiotic relationship with *Fusarium* (under some conditions) than two competitors. At the same time, however, *V. dubia* is clearly both highly susceptible to disease caused by *F. culmorum* (as > 50% of all infected plants died within 90 days of emergence during the competition experiment) and not protected by prior infection from the two individual FEV isolates with the most positive effects on *V. dubia*. Taken together, the bulked inoculum, biomass, and individual isolate experiments described above indicate that fungal endophytes from the *Fusarium* genus associated with exotic grasses (*V. dubia* and *B. tectorum*) can have different effects on those grasses and a native competitor, *P. spicata*. The cryptic, positive effects of *Fusarium* infection on *V. dubia* may also suggest that endophytes can have a larger role in driving apparent competition than has previously been demonstrated; this, combined with the ubiquity of endophytes in natural systems, may mean that the general effects of apparent competition have been underestimated in previous studies.

It is clear from the bulked and individual inoculum experiments that the native grass *P. spicata* is more negatively affected by *Fusarium* isolates than either of the exotic grasses *B. tectorum* or *V. dubia*. *P. spicata* biomass was significantly reduced by both FEV and FEB inoculation, and emergence was also reduced by both treatments in the bulked inoculum experiment. Although not analyzed quantitatively, there was also an observed post-emergence mortality amongst *P. spicata* seedlings treated with FEV individual isolates consistent with fusarium crown rot (or foot rot) disease (Baldwin 1990 and Cook 1980). *B. tectorum* seedlings were most affected by the FEV bulked inoculum in terms of biomass reduction, with no significant effect from the bulked inoculum of either FEV or FEB on emergence. Individual isolates from both FEV and FEB significantly reduced *B. tectorum* emergence to a greater extent than in *V. dubia* emergence. Amongst treated *B. tectorum* seedlings that were later placed in a vernalization chamber (at 4°C, 10:14 day-night cycle, for 60 days) there was a noticeable exterior colonization of the stem immediately above the root crown, followed by death of seedlings (again, consistent with fusarium crown rot disease). This was consistent in seedlings treated with either FEV or FEB isolates. *V. dubia* seedlings

exhibited no post-emergence mortality or obvious signs of disease during vernalization, although they did exhibit similar symptoms and (even greater incidence of) seedling death when challenged with *F. culmorum*.

One possible outcome of apparent competition involving symbioses between plant and microbial communities is facilitation of invasion by exotic plants, whether through direct positive effects of mutualism on an exotic plant or direct negative effects on native competitors (Bever et al. 2012; Van der Putten & Peters 1997). The Palouse Prairie remnants in the interior Pacific Northwest have perennial grass-native shrub communities that are actively threatened by exotic winter-annual grasses *B. tectorum* and *V. dubia* (Nyamai et al. 2011). *V. dubia* is a relatively new invader of the intermountain Western United States, and is of particular concern in eastern Washington, eastern Oregon, and western Idaho (Noone et al. 2013; Wallace et al. 2015). It is commonly found in association with *B. tectorum*, along roadways and agricultural field edges, and invading perennial bunchgrass and prairie habitat. In the last decade, anecdotal reports have indicated that *V. dubia* can successfully invade *B. tectorum* monocultures in north-central Idaho and eastern Washington (Finch 2011 and Fitzsimmons 2012). One of the persistent questions regarding *V. dubia* is: how is it able to invade *B. tectorum* monocultures? Additionally, how does it successfully compete with native perennial grasses (such as *Pseudoroegneria spicata*) which are often more productive individually and produce many more seeds per plant?

V. dubia is not a prolific seed producer, and it is not known to be capable of vegetative reproduction (Scheinost, Stannard, & Prather 2008). Microbes have been shown to affect competition between annual and perennial grasses in other N. American contexts; California's central valley grasslands have experienced a major change in community composition from native perennial grasses to dominance by exotic annual grasses. It has been demonstrated that healthy perennial grasses are highly resistant to invasion by annual grasses in that system, and that a symbiosis between exotic grasses (including *Avena fatua*) and barley and cereal yellow dwarf viruses, vectored by exotic aphids, has disproportionate effects on the native perennial grasses (Malmstrom et al. 2005). The exotic annual grasses are unable to successfully compete for resources with native perennials in the absence of the virus and aphid, but when widespread infection by the virus occurs in both native and exotic grasses the exotic grasses are favored (Borer et al 2007). This supports an argument for

examination of microbial mechanisms for how *V. dubia* is able to successfully invade both *B. tectorum* monocultures and native bunchgrass-shrub communities; it is possible that microbial soil-feedback or the effects of fungal endophytes are facilitating the invasion of *V. dubia*.

There are precedents in the literature for fungi increasing the growth of an invasive and reducing growth of competitors, or for altering growth and fecundity characteristics in such a way that a species benefits from apparent competition. One study has found that non-native invasive species *Vincetoxicum rossicum* has increased biomass when challenged with soil-borne fungi (including *Cadophora orchidicola*, *Ilyonectria radiculicola*, and *Macrophomina phaseolina*) that are pathogenic to native competitors in Ontario, Canada (Day et al. 2015). Endophytes from genus *Alternaria* have been shown to directly increase the competitive ability of *Centaurea stoebe* against native grasses in its invaded range of N. America (Aschehoug et al. 2012). Replacement experiments utilizing sterilized and unsterilized coastal soils have shown that pathogenic soil-fungi associated with a plant species can have differential effects on competing species; *Ammophila arenaria* and *Festuca rubra*, often found in competition in coastal dunes in the Netherlands, were differently affected by a host of soil-borne pathogenic fungi including *Alternaria alternate*, *Cylindrocarpon dydium*, and *Fusarium equisetum* (Van der Putten and Peters 1997). In regards to *Fusarium* species, Mangla et al. (2008) found that invasive plant species *Chromolaena odorata* accumulated high concentrations of *Fusarium* spores in the soil around its roots in invaded areas of India; the same *Fusarium* strains accumulating in *C. odorata* rhizosphere proved pathogenic to native competitors, including *Amaranthus spinosus* and *Bambusa arundinacea*).

In general then, the results of our experiments are supportive of the hypothesis that a differential relationship between *V. dubia* and *Fusarium* isolates could be contributing to the plant's successful competition with native grass *P. spicata*. Some strains of *Fusarium* have been investigated to determine whether they are causal agents of *B. tectorum* dieoffs in the N. Great Basin and have been found to be highly virulent seed pathogens, and it seems plausible that the newer invader (*V. dubia*) may benefit from *Fusarium*-mediated competition with the older invader, which it has been seen to displace in the Palouse Prairie region of the Pacific Northwest (Meyer et al. 2013). It is noteworthy that while *B. tectorum* is noted in the USDA's plant-fungus host database as hosting seven taxa from *Fusarium* (including both *F.*

accuminatum and *F. oxysporum* which secondary root rot in the invasive grass, and are similar in ITS sequences to eight of the 20 *Fusarium* isolates used in our experiments), *V. dubia* has zero records with *Fusarium* in the same database. Isolate FEV8b may be of particular interest, in this sense: it significantly increased emergence of *V. dubia* (+8%) but had no effect on *B. tectorum* emergence; and it can cause crown-rot like disease leading to fatality in *B. tectorum* seedlings vernalized at 4°C for 40 days. Although FEV8b did not protect *V. dubia* from the effects of *F. culmorum* in the serial-infection experiment, it did have a slightly positive (but not statistically significant) effect on biomass in that same experiment. From our small survey of *V. dubia* populations in the vicinity of Paradise Ridge, it is clear that the association between *Fusarium spp.* and *V. dubia* isn't limited to that site. There is a clear argument to be made for sampling *V. dubia* across the invaded range to determine how common the association is and whether it is contributing to the invasion. In conclusion, it seems that *V. dubia* may indeed be experiencing the benefits of apparent competition in its interactions with fellow invasive *B. tectorum* and native grass *P. spicata*; specifically, the only positive effects of infection by any *Fusarium* isolate used in our study was on *V. dubia*, and it overall experienced fewer and smaller negative effects of infection than its competitors. Further experiments to determine the extent of positive interaction of *V. dubia* with other *Fusarium* isolates (from plant hosts and soil samples) in the invaded range are warranted, as are comprehensive surveys to determine the extent to which *V. dubia* individuals (and competitors) are infected by *Fusarium* as an endophyte in the invaded range.

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Chapter 3: The potential use of Community-Based Observing Networks (CBONs) in monitoring and managing plant invasions in arid environments of the American West

Abstract

The management of invasive plant species in the Intermountain West has high annual costs and relatively modest positive effects on the overall problem. This is partly due to mismatches in scale between existing policy and effective management actions, a lack of knowledge of the linked social-ecological processes of invasion, and the lack of coordinated response at the community and regional level. Community-Based Observing Networks are a tool developed to provide high resolution, reliable data on linked social and ecological phenomena in difficult to access or expensive to monitor landscapes. Such a tool is well-suited to addressing the problems with plant invasion, and can potentially provide a network on which to build an early warning system to increase food security in the American West.

3.1 Introduction

Biological invasions by exotic plants are estimated to cost the US economy tens of billions of dollars in damages and control efforts per year, and have been identified as primary drivers of environmental change both in the US and globally (Pimentel *et al.* 2005; Vitousek *et al.* 1996). These invasions have transformed ecosystems at landscape and regional levels, as seen in the domination of millions of hectares of forest by kudzu (*Pueraria montana*) in the southeastern US and in the fundamental change of fire cycles in the arid steppes of the western US by repeated invasions of annual grasses such as cheatgrass (*Bromus tectorum*) (Forseth & Innis 2004; D'Antonio & Vitousek 1992). Recognition of these pervasive impacts and challenges has resulted in massive investment into preventing and managing exotic plant invasions in recent decades. Early scientific studies on the ecology and control of plant pests led to the development of invasion biology as a distinct field by the 1980s, and funding, research, and readership has expanded dramatically over subsequent years. Despite these efforts and the wealth of ecological knowledge that has been accumulated over the years, invasion science has so far struggled to develop an integrated ecological approach to the study of exotic plant populations (Heger *et al.* 2013). As such, we still lack a strong fundamental understanding of what makes a particular plant invasive and what makes particular ecosystems vulnerable to invasion (Moles *et al.* 2012).

Politically, the signing of Executive Order 13112 in 1999 resulted in invasive species becoming an important national regulatory goal (Invasive Species 1999). In its wake, the prevention, eradication, and control of plant pests has experienced increased funding at multiple levels, and has encouraged burgeoning development of management structures across federal agencies, state departments, academic institutions, and county governments. However, many of these bodies fail to regularly integrate current ecological knowledge and to apply it in the context of policy and management (Simberloff *et al.* 2013). As a result, the success stories of prevention and eradication efforts remain rare, and the economic costs of invasive plant infestations continue to rise year after year (Pimentel *et al.* 2005). In short, there remains little systematic evidence that national economic, political, and social efforts as they relate to invasive plants have resulted in desired management outcomes at the local, regional, or national levels.

We suggest that Community-Based Observing Networks (CBONs), distributed networks of community members who act as monitors of linked social and ecological drivers, are ideal tools to inform policy decisions, monitor the presence and spread of species of interest, and improve management outcomes at local and regional scales. We next describe in greater detail the difficulties in dealing with plant invasions from policy and management perspectives, focusing in particular on complications arising from managing resources in the complex social, political, and ecological landscapes of the American West. We will discuss CBONs and the potential benefits of their application to the problems associated with plant invasion. We will end with by making recommendations on how to implement a CBON in the Intermountain West and how to leverage the data from such a network for increasing food security.

3.2 The problems with successful management of invasive species

There are three primary difficulties with implementing successful invasive plant management programs in the Intermountain West. First is a mismatch in scale between the laws and policies affecting management of invasive plants (which is often at the federal, continental, or statewide scale) and the scale at which actual plant invasions happen and management actions are implemented (which is usually at the field, local, and county levels). Effective invasive plant policy and management also faces issues arising from complex

jurisdictional, ecological, and ownership patterns across invaded landscapes; in short, two contiguous parcels invaded by the same species may be governed by different laws and have different incentives for control depending on land-use type (i.e., if one is farmed and one is an amenity). Finally, much of the research on invasive plant biology has focused primarily on biophysical, ecological, and habitat characteristics, while there are good logical and empirical reasons to think that plant invasions are actually a linked social and ecological phenomenon.

A significant part of the difficulty in managing plant invasions is due to a mismatch in scale between governmental policies and effective management actions. Policies at the national level for controlling invasive species are limited to black lists (prohibitions on certain species being imported), which focus only on plants already described as damaging to the environment and economy and which are often established and widespread to a point that precludes effective control (Simberloff et al 2013; Rejmánek and Pitcairn 2002). These black lists, which prohibit the importation of problem plants and require the quarantine of others, are administered by the USDA under the authority of the Plant Protection and Quarantine (PPQ) division of APHIS. The PPQ issues Federal Orders when APHIS determines that regulatory action is necessary to control the import of a specific plant, and this authority derives from the Plant Protection Act of June 20, 2000, as amended, 412(a), 7 U.S.C. § 7712(a). State-level designations of exotic plants as either “noxious” or “weedy” have large implications for subsequent regulatory action and management capacity despite being heavily affected by public perception and political interests (Nunez and Simberloff 2005). In some states, such as Idaho, invasive species of concern are designated as noxious weeds at the state level, and all land owners and managers in the state are required to eradicate, contain, or control populations. Washington State, on the other hand, has county, regional, and state lists of noxious weeds in an effort to better match the scale of policy to the scales at which actual infestations occur.

This disparity in approaches can lead to difficulties in implementing management approaches at the proper scale and across legal boundaries (which often don't reflect ecological boundaries). State and county level designations are inconsistent within ecotypes or habitat types that cross boundaries. For example, there are several plant species which are listed as Class C Noxious Weeds in Washington, including absinth wormwood (*Artemisia absinthium*), common teasel (*Dipsacus fullonum*), and bull thistle (*Cirsium vulgare*), which

are common on the Palouse, an ecoregion spanning the border between Washington and Idaho. Under Washington State noxious weed laws, a landowner in Whitman Co. who knows of the existence of such weeds but fails to control them is subject to a fine of \$250.00 per day, rising to \$1,000 per day after four offenses within five years, while a landowner of a parcel one hundred meters to the east in Latah Co. will not receive so much as a notice from the county weed superintendent (17.10 RCW. WSR 99-24-029, § 16-750-020; WSR 97-06-108, § 16-750-020; & WSR 93-01-004, § 16-750-020).

This problem of weeds having little regard for jurisdictional boundaries is compounded by interaction with land tenure systems and boundaries. Adequate control is often possible at the field and population level for many invasive plants by using Integrated Pest Management (IPM) techniques, but stories of successful regional eradication efforts remain scarce (but see Anderson *et al.* 2003). This is often because management actions differ between owners and managers of parcels; responses to plant invasion are constrained by access to resources, unequal enforcement of noxious weed laws in different localities, and differing incentives to control plants. This characterization is particularly relevant in the complex social and ecological landscapes of the American West. Much of the area between the coast range and the continental divide is characterized by steep environmental gradients and is heavily utilized for natural resource extraction and agricultural production. The resulting complexity of different land uses and land tenure increases uncertainty about the effectiveness of control efforts and the dynamics of invasions, further complicating management decision-making (Liu *et al.* 2011). These landscapes are also increasingly being converted into mixed ownership communities as a result of land subdivision for development and amenity migration (Yung *et al.* 2015). One result of such subdivision is an increase in both the number of plots and the number of managers (producers, amenity owners, and institutional managers); this leads to a fragmented, complex landscape composed of different land uses and parcel sizes. In such “management mosaics,” as described by Epanchin-Niell *et al.* (2010), the proliferation of ever smaller parcels has significant effects on the control of invasive plants: it decreases the total economic impact of invasion on individual owners, producers, or managers, thus reducing a key incentive for controlling weeds; and it increases the control costs per unit of land for managers, due to reinvasion from neighboring properties and insufficient bases to achieve economies of scale. This means that successful, long term

control of a species is dependent not just on the decisions and actions of the owner of a particular parcel, but also on the owners of the neighboring parcels: thus, successful invasive species management is increasingly understood as a collective action issue (Herbsdorfer *et al.* 2006). Furthermore, additional barriers to collective action necessary for successful control at the regional level derive from the increasing prevalence of absentee landowners and the greater diversity of views regarding land and nature resulting from ever larger numbers of individuals making management decisions (Klepeis *et al.* 2009).

In the policy and management sense, plant invasions are difficult to deal with as a result of the complex systems of laws, regulations, and ownership structures. They are also difficult to describe in a purely biological sense, although much effort has been expended on biological research into invasive plants and invadable ecosystems in the last few decades (Simberloff *et al.* 2013). Part of the difficulty in characterizing invasive species biologically and ecologically is that they are not biological phenomenon, in the strictest sense. Rather, plant invasions are more accurately described in terms of systems characterized by high complexity and high uncertainty, and they tend to exhibit non-linear dynamics resulting from social and ecological feedbacks across multiple temporal and spatial scales (Levin *et al.* 2013). In other words, human action is equally important in both introducing and establishing populations of specific invasive species, whether such actions be intentional or accidental. An example of this can be seen with a relatively new plant invader of the Intermountain West, wiregrass (*Ventenata dubia*): while much work has been done to determine how it establishes in plant communities, how it invades different habitats, and what habitats are most susceptible to invasion, it is becoming clear that *V. dubia* is primarily dispersed and established through hay and grass seed sales within the invaded range (Wallace, Pavek, & Prather 2015). The biology of the grass and the biophysical nature of invaded habitats are important to understand, but so are the anthropogenic drivers of invasion (in this case, resistance by producer associations to listing *V. dubia* as a noxious weed at state and county levels, which means that it continues to be dispersed and established in an ever wider range on the Palouse and surrounding areas). It is our belief that to be successful, invasive plant science and management needs to be reinvented as a transdisciplinary endeavor that conceives of invasion as a socioecological process. These types of social-ecological systems (SES) approaches promote integrative science that can help guide management and research

priorities, but also can help to develop human networks to maximize social and ecological resilience in the face of environmental change (Folke *et al.* 2005). In addition, SES science has the potential to expand our understanding of change and consequence in the context of ecological thresholds and complex feedbacks while providing a template for place-based, adaptive decision-making (Schluter *et al.* 2012; Caplet *et al.* 2012).

3.3 Community based observing networks and integration with existing institutions for invasive plant management

A Community based observing network (CBON) is an SES inspired methodology and system developed for the integrated monitoring of social and biophysical variables associated with environmental changes caused by natural and anthropogenic processes. CBONs are composed of trusted, highly trained observers distributed across a landscape of interest and connected by networks enabling communication in any direction, and they are useful in improving our understanding of and resilience to environmental change (Alessa *et al.* 2015). They are particularly useful in high resolution monitoring taking advantage of local and traditional ecological knowledge. Established CBONs, such as the Bering Sea Sub-Network in the Arctic, have been used to improve knowledge of resource extraction and climate change driven environmental changes to sea mammal and fish systems that directly affect subsistence and commercial activities (Fidel *et al.* 2014). CBONs are fundamentally centered and structured on the interests, observations, and input of communities impacted by environmental change. A key and distinguishing feature of CBONs is that they locate and engage the cooperation of community residents with established expertise in the environmental issue of interest (Johnson *et al.* 2015). If there are objections to enlisting community members, whether they be farmers, ranchers, extension agents, school-children or teachers, it might be wise to recall the words of Steinbeck in *Cannery Row*: when Doc wanted to understand the “grass and birds and trees” in their entirety (and escape “love trouble”), he left the University of Chicago and “walked among farmers and mountain people, among swamp people and fishermen” (Steinbeck 1945). There are few better observers of the land than those who live on it, and Steinbeck knew this well. The character of Doc was based on his best friend, Ed Ricketts, and the two of them (with very little formal biological education between them and no advanced degrees) accomplished considerable

feats of what we would now call systems ecology. In particular, they researched and published *The Log from the Sea of Cortez* together, which had a remarkable level of detail on food-web dynamics and system-wide fluxes for the early 1940s (Levy 2007). Although the profession of farmer or writer or college dropout octopi-collector doesn't immediately suggest itself as the ideal occupation for a field scientist, and enlisting (or accepting) the help of such folks is out of fashion in academic circles, we'd be much poorer in our knowledge of many ecological phenomena without the contributions of such interested community members.

The use of CBONs could be instrumental in reinvigorating collaborations between community members and scientists. They are an optimal tool for use when collecting desired data would be prohibitively expensive due to the costs of sending scientific field crews to collect data at high resolutions in difficult or inaccessible terrain. Additionally, they require cooperation from local community members across the spatial extent of the network through the duration of a project, and this leads to continuous and in depth engagement of scientists and managers with the landowners and communities most affected by environmental change (Johnson *et al.* 2015). These traits of CBONs are what make them an ideal tool for monitoring and managing invasive plants. The establishment of community based monitoring approaches have to this point been limited to developing countries or regions experiencing rapid environmental or social change. In the United States, CBONs have almost exclusively been established in the Arctic due to the difficulties of monitoring environmental and social factors in that landscape. These and similar communities often have a strong need for real time environmental data and lack the funds to support data collection by scientific professionals, and such methods have proved very useful in increasing the effectiveness of natural resource management interventions (Danielsen *et al.* 2007). Over the long term, however, these programs sometimes struggle with sustained funding or collapse from a lack of integration into existing management structures (Danielsen *et al.* 2005).

One of the great difficulties in establishing a distributed network such as a CBON is in identifying community-based personnel with the expertise and willingness to provide high quality data over extended periods of time. This makes integration and cooperation with existing institutional weed management programs of utmost importance to the successful implementation of a CBON focused on invasive plants. With the recognition that invasive

plants represent a problem beyond the control of individual landowners or agencies, cooperative management efforts in western regions have expanded significantly in recent years. Cooperative weed management areas (CWMAs) are institutions defined as a collective partnership between government agencies, tribes, landowners, and stakeholders working to manage noxious weeds in a defined geographic or ecological area (Gunderson-Izurieta *et al.* 2008). There are now well over 100 recognized CWMAs in the Western United States covering the vast majority of public and private land, many of which have been formed in the last 15 years (Fuller 2011). CWMAs have become such key elements in western weed management efforts that membership in a CWMA is often seen as a prerequisite for acquiring state or federal funding for weed control efforts. Their importance in securing funding isn't to be taken in such a way as to devalue the level of local and institutional knowledge encapsulated in most CWMAs: the concept grew out of a desire to maximize success by engaging in collaborative action across a region while tapping into the diverse expertise available from both diverse individuals and institutions (Gunderson-Izurieta *et al.* 2008). Despite their key role, CWMAs exhibit large variation in funding, sustainability, and organizational structures throughout the regions and there is little documented understanding of the ecological impact and improvement in management outcomes from the development of CWMAs. But what CWMAs undoubtedly *have* done, and done well, since their creation is develop and document networks of interested landowners, managers, and community members at the scales where a CBON could be the most useful in monitoring invasive plants and collecting data to inform management decisions.

3.4 How the establishment of a CBON can address the difficulties of managing invasive plants in complex landscapes

As outlined above, effective control of invasive plants will require: (1) high resolution monitoring of invasive species distributions, land use history, control efforts, and management capacity at spatial and temporal scales relevant to both policy and management efforts; (2) knowledge of the linked social, ecological, and biophysical drivers of invasion and community response to invasion; and (3) coordinated action across diverse landscapes, legal jurisdictions, and management institutions; collective, coordinated action across diverse landscapes, legal jurisdictions, and management institutions. The establishment of a

Community-Based Observing Network (CBON) in the Intermountain Pacific Northwest will allow communities, policy makers, researchers, and managers to simultaneously address these needs. CBONs have the potential to provide significant scientific and management benefits in dealing with the challenges presented by plant invaders in complex landscapes.

A. The establishment of a CBON will allow for detailed spatial and temporal monitoring of linked social and environmental factors (land-use type, site characteristics, weed abundance, specific control efforts, management capacity, etc.). Landowners, community members, and land managers are often well positioned to quickly recognize environmental changes and assess the risk of exotic plant species, and this information will enable researchers and state-level managers to respond to new infestations in a timely fashion (i.e., before an exotic plant is established and so widely spread that it is impossible to control). This information, and the networks that support it, will encourage the rapid response of weed science professionals and allow local knowledge and experience to inform regulatory designations of plants as “invasive,” “noxious,” or “weedy.” This will allow a CBON focused on invasive species to develop into the backbone of a functional early warning system (often called an early detection and rapid response (EDRR) system in the context of invasive species), which has been a primary goal of federal and state agencies and policies for more than a decade (DiTomaso 2000 and Pyšek & Richardson 2010).

B. CBONs will allow for a consistent methodological approach to data collection that will provide high resolution data that would be prohibitively expensive for scientific professionals to obtain through systematic surveys. This approach to data collection which can focus on simultaneously collecting ecological and social data at identical scales can be used to identify areas vulnerable to or resistant to infestation at scales from local to the landscape and regional. Such information will provide natural resource managers the tools to visualize plant dynamics at the landscape level and prioritize control efforts accordingly, and it will give policy makers the information necessary to coordinate noxious weed laws across jurisdictional boundaries. Ultimately, this data can be integrated across local levels to regional and landscape scales in order to increase scientific understanding of the joint social and ecological drivers of and responses to plant invasions, and better direct state and federal management priorities (Cummings, Cummings, & Redman 2006).

C. CBONs will allow and encourage the production of community-informed, co-produced sets of management and socio-economic priorities. Such templates will enable landowners, food producers, communities, managers, and researchers to collaboratively monitor and respond to invasion, while providing adaptive management structures to cope with the long-term consequences of plant invasion. Such a network allows for the simultaneous dissemination of co-produced knowledge in both directions: from researchers, policy makers, and managers to communities and landowners; *as well* as from landowners and producers to scientists and natural resource professionals. This will create incentives for landowners and community members to participate in CBONs, as it will empower stakeholders to be engaged in the process of managing invasive plants from the identification of invaders stage to the implementation of policies and IPM strategies. These efforts will help to maintain a focus on cooperative partnerships, consistent participation, and outreach efforts that are the hallmark of effective CWMA and successful weed management actions (Fuller 2011; Gunderson-Izurieta *et al.* 2008; Herbsdorfer *et al.* 2007).

3.5 Conclusion and recommendations

Although there has been an increase in federal and state level attention to the phenomenon of plant invasion in the last couple of decades, the increased effort and resources devoted to the problem doesn't seem to have accomplished as much as was initially hoped for. This is due to several factors, including a mismatch between the scales of noxious weed policy and successful management actions, a lack of knowledge on how social and ecological interactions drive invasion and responses to invasion, and a lack of coordinated action across complex landscapes characterized by multiple legal jurisdictions, management goals, and ownership structures. The creation of a Community-Based Observing Network (CBON) devoted to monitoring and responding to plant invasion in the Intermountain PNW will enable new avenues of research into the science of biological invasion and social-ecological systems and provide a methodology to improve management outcomes and guide the creation of more effective noxious weed policies at local and state levels. Such a CBON will also empower members of communities most affected by plant invasion to participate fully in the response, from designating exotic species of concern, to monitoring and mapping infestations, to coordinating management actions within communities and across

jurisdictional and ownership boundaries. This will invert the classic, federal and state level paradigm of top-down response to environmental change, where government researchers identify environmental issues and require landowners, land managers, and communities to respond or face penalties. A CBON relies on the expertise that already exists or can be created within a community to respond to environmental change and direct government research and policy to support collective response.

In order to establish a CBON focused on long-term monitoring of environmental and social data associated with plant invasion, we can recommend a general action plan, informed by our experience and study of the literature:

1. Identify a particular landscape of interest, which should be diverse enough to reflect the complexity of social-ecological processes for the region and be compact enough to establish a meaningful network of people who can regularly (yearly or bi-yearly) meet in person. For the purposes of this example, we'll consider the 'tri-state' area of the Palouse, Snake River, and Blue Mountains ecoregions, where the borders of Washington, Oregon, and Idaho. The area covered by the existing Tri-State Demo Cooperative Weed Management Area (CWMA) is ideal for this example.
2. Identify and reach out to stakeholder groups in the communities of the landscape of interest (again, the existing CWMA), land-manager groups or individuals (from county, state, or federal agencies, and NGOs), and scientists interested in working in the landscape. In the particular area we have designated for this example, the stakeholder groups would likely include individual landowners, producer groups (cattlemen's association, growers, etc.), the forestry industry, and hunting/amenity groups (Rocky Mountain Elk Foundation, etc.). The agencies would include local and county weed boards and extension offices, state resource agencies, federal land-managers (for example, from the US Forest Service Starkey Experimental Forest and the Army Corps of Engineers, who control land surrounding the dams on the Snake and Columbia Rivers). Scientists would likely be solicited for participation from the land-grant universities (University of Idaho, Washington State University, and Oregon State University), as well as the federal agencies (US Forest Service Pacific Northwest Research Station, etc.), and NGOs (The Nature Conservancy Zumwalt Prairie Preserve).

3. Hold a set of facilitated stakeholder meetings (with members from step 2), and identify an issue or set of issues which are of interest and utility to both scientists, land managers, and community members in the landscape designated in step 1. An example might be the invasion of sagebrush steppe and Palouse ecosystems by invasive annual grasses, such as cheatgrass (*Bromus tectorum*), medusahead wildrye (*Taeniatherum caput-medusae*), and wiregrass (*V. dubia*). It is critical that, in establishing an actual CBON, this step is conducted through holding stakeholder meetings with scientists, managers, and community members in attendance. CBONs are best considered as equal partnerships between scientists and communities, and if one side dictates the research agenda, or any other term, it is likely to fail when one party realizes its needs are not being met.

4. Establish a leadership team comprised of equal numbers of community members, land-managers, and scientists working on the project. It is important in any kind of collaborative research project to have clear lines of communication, level-headed decision making, and fiduciary responsibility shared by a group of trusted individuals. It is important that the leadership team be representative, so that the CBON does not fail in a case where one class of participants is favored over another.

5. Establish an agreed upon set of data standards, metadata standards, data sharing agreements, and privacy assurances between all members of the CBON. This is of critical importance for both ensuring high-quality data and building trust between the community, land-managers, and scientists. Since CBON data is generated by community members, it is at least equally 'owned' by those communities, and as such that ownership must be respected. Community members and researchers should work together to publish good science while respecting privacy and acknowledging that some community data is not suitable for public distribution.

6. Create protocols for what types of data need to be collected to address the issues identified in step 3. Integrated social-ecological data is complex and sometimes difficult to produce in interoperable sets, and these issues should be addressed at the outset. If, for example, it is decided that data on environmental variables (precipitation, ground cover, species diversity, phenology, etc.) should be analyzed alongside and with social data (land-use change, land-use history, cultural practices,

changes to land-tenure, amenity based uses, transportation patterns and corridors, etc.), they must be collected in such a scale and manner that they can be usefully analyzed together. In this step it will also be necessary to decide on how data will be archived, and how data is to be collected and uploaded to the archive. Are there mobile applications for collecting site information, and if so, who is responsible for developing such an (or adapting an existing) application? Develop a spatially and temporally explicit data collection plan.

7. Establish a team to hold training sessions for community observers. Graduate students might be ideal for this task, in which trainers are responsible for traveling to communities and training participants who will be making observations in protocols, data standards, and submission procedures. Topics in this example might include how to accurately identify the difference between *V. dubia* and *B. tectorum*, how to estimate plant cover in a plot, how to take soil samples, or how to collect, store, and submit vegetation samples to a collaborating university lab. Social science topics might include how to document land-use changes both historical and contemporaneous, or how to report amenity use in fair, private, and non-intrusive manner.

8. Develop a plan for distribution of products, data, and visualizations to policy makers, community members, and the scientific community. In this sort of cooperative research venture, it is important to consider a range of products. Extension professionals are likely available in the area covered by the CWMA, and they are trained in how to distribute ecological and economic information to communities and producers. Scientists should communicate with their community and manager partners about publications and presentations. Tools should be developed collaboratively so that the information collected is first and foremost made available to the communities which collected (and own) it. Easily accessible online archives, visualizations, and other such tools should be discussed and planned.

All of these steps are merely suggestions for how one might go about establishing a CBON, and, they are driven by some overarching considerations and commitments. CBONs *should be* collaborative not just in name but in practice, and ideally should act as real partnerships between community members affected by environmental change, land-managers responsible

for resource use and/or protection, and scientific researchers. The products of CBONs *must be* useful to all partners, whether it be in terms of access to new data or types of data for community members, recommendations to inform policy makers or models of invasion processes for land managers, or publications and outreach products which will count towards career advancement for research scientists. The issues involved must be of real and sustained interest to the community that will be providing the majority of observations, and products must be owned and distributed by all partners equally. These are not necessarily easy ideals to achieve, but we think that they will ensure the long-term success and utility of CBONs deployed in new areas and concerned with novel social-ecological systems and issues.

We recommend, then, the establishment of a CBON for monitoring and intervening in plant invasion in the Intermountain Pacific Northwest, and we further recommend that the network structure be informed by the Cooperative Weed Management Areas (CWMA) that are already established in the area. By cooperating with CWMA, the new CBON will have already gone some way to identifying key members of communities that have the expertise and willingness to act as sensors in such a distributed array. By enabling the monitoring of linked social and ecological drivers of invasion at scales relevant to both ecology *and* management response, CBONs will move us away from centralized, top-down, resource intensive models for dealing with plant invasion and towards sustainable, community-based, integrated control of plant pests (Epanchin-Niell & Wilen 2015). If established and maintained for the long term, a CBON additionally has potential to act as critical data collection and repository mechanism for the creation of integrated early warning systems that will ultimately enhance the food security of the American west.

3.6 References

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