Title: Cheatgrass Stand Failure in the Great Basin: Fungal Pathogens, Carbon Dynamics, and Fungistasis

Type: Science Project 1A (1)

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Project Objectives:

The main project objective is to advance the knowledge of the causes and consequences of cheatgrass die-offs to the point that both the occurrence of die-offs and probable post-die-off successional trajectories can be predicted. A second objective is to learn whether die-off scenarios can be deliberately manipulated to meet management goals.

Management Objectives:

This project supports the development of scientific information, tools, and technical products to inform and augment conservation decisions and actions by natural resource managers. It targets individuals involved in strategic decision making with regard to the management of sagebrush rangelands infested with the invasive annual *Bromus tectorum* (cheatgrass), with the goal of increasing ecosystem resilience. Specifically, it addresses problems and opportunities presented by the cheatgrass die-off phenomenon, which may impact large areas in some years. By developing predictive tools, this proposal will provide managers with the ability to manage die-offs in a larger temporal and spatial context instead of as unpredictable and isolated catastrophic events.

Project Description:

A cheatgrass die-off occurs when this annual grass exhibits complete stand failure, resulting in areas devoid of vegetation that are especially conspicuous in spring, when the gray litter from the previous year contrasts strongly with the green color of adjacent current-year stands (Fig. 1; Baughman and Meyer 2013, Meyer et al. 2014a). Such stand failure is also a common problem in agriculture, especially with annual cereal grains, and has been demonstrated to be caused by a variety of different soilborne fungal pathogens (Bockus et al. 2010). The cheatgrass die-off phenomenon has been familiar to range scientists for decades (e.g., Piemeisel 1951). However, the first concerted scientific effort to understand die-offs mechanistically was not initiated until 2011, under the aegis of the USDI BLM Integrated Die-off Project. A series of large-scale die-offs in north-central Nevada in the preceding years stimulated management interest and resulted in the initiation of this multifaceted research effort. The proposed project represents a continuation of research we have completed as part of this BLM initiative.

We have identified many key pieces of the die-off puzzle in three years of research effort. We now know that die-offs are usually, but not always, a transient phenomenon, i.e., cheatgrass usually re-establishes from the *in situ* seed bank the year following a die-off. We have demonstrated experimentally that both cheatgrass and native grasses can readily establish into previous-year die-offs (Fig. 2; Meyer et

al 2012, 2013; Nicholson 2014, Baughman 2014). We also know that, when die-offs fail to recover, the soil quickly becomes bare of litter. Older die-offs are often invaded by dicot weeds (e.g., tumblemustard, bur buttercup, annual kochia) with seeds that are better adapted than those of cheatgrass for retention and establishment on bare soil (see hypothesized post-die-off successional trajectory scheme in Fig. 3).

Another piece of the puzzle is that some cheatgrass monoculture areas experience recurrent die-offs separated by one or more years of successful cheatgrass establishment, suggesting that die-off causal agents can persist through time in healthy cheatgrass stands until environmental conditions promoting stand failure once again occur. Other areas apparently rarely if ever experience die-off events. A third piece of the puzzle is that die-offs are not the all-or-nothing events that they appear to be. Die-off causal agents can interact with weather patterns such as sporadic autumn precipitation to reduce cheatgrass stand density and seed production to varying degrees. They may impact establishment to some level every year, especially in die-off prone areas.

Much of our research the last three years has focused on identifying the soilborne fungal pathogens that may be responsible for cheatgrass stand failure (Meyer et al. in press). We have identified four principal ascomycete pathogens that are implicated in the die-off phenomenon. These appear to work in tandem to cause the cycles of die-off and recovery that we observe in die-off prone areas. The first pathogen, a currently undescribed species of *Fusarium* (fusarium seed rot), primarily kills germinating seeds and may be the primary pathogen that prevents stand establishment (Figure 4A; Meyer et al. 2014b, Franke et al. in press). The second pathogen, *Epicoccum nigrum* (yellow patch), appears to kill both germinating seeds and pre-reproductive plants (Fig. 5B). The third pathogen, an undescribed species in the family Rutstroemiaceae, attacks established cheatgrass plants at the root-crown interface and causes sterility and premature drying and lodging of plants (bleach blonde syndrome; Fig. 5A). It can cause major decreases in seed production and may also create a litter environment conducive to fusarium seed rot and yellow patch disease (see below). The fourth pathogen, *Pyrenophora semeniperda* (‘black fingers of death’), kills primarily dormant seeds and cannot cause die-offs directly, but it may reduce the residual seed bank to such low levels that post-die-off recovery does not occur (Fig. 4B; Baughman and Meyer 2013). A fifth pathogen, the basidiomycete *Ustilago bullata*, causal agent of head smut disease, is also common but apparently cannot cause stand failure in the xeric environments where die-offs commonly occur (Fig. 4C; Meyer et al. 2010).

To complete the picture of die-off causality, we need an overarching hypothesis that can explain how these pathogens can interact to cause a die-off, yet allow stand establishment in the year following epidemic disease. We propose to test the hypothesis that, under ‘normal’ conditions, fungistasis inhibits disease development (Lockwood 1977, Nelson 2004, Garbeva et al. 2011), i.e., the soil microbial community suppresses disease development caused by fungal pathogens through competition for nutrients or through the release of suppressive microbial metabolites, even when these pathogens are present. In order for a die-off to occur, some factor must release the pathogen(s) from fungistasis, allowing disease to become epidemic. The year following a die-off, fungistasis is usually re-imposed, again suppressing disease development and allowing stand establishment from the carryover seed bank. One line of evidence that fungistasis is imposed after a die-off is that we have not detected changes in emergence in response to *Fusarium* inoculum addition (or fungicide addition) in field small plot studies, in spite of the fact that this same inoculum can cause seed mortality in autoclaved soil in growth chamber experiments.

A very important factor in the release from fungistasis is available ‘labile carbon’, i.e., carbohydrate carbon, that is the main energy substrate for both soil microbes and germinating pathogen propagules (Garbeva et al 2011, Bonanomi et al. 2013). This carbon must come ultimately from

cheatgrass, which is the only abundant photosynthetic organism in the system, in the form of either litter or leakage from cheatgrass seeds or roots. We have data to support the hypothesis that the litter created by a bleach blonde epidemic, which likely contains the carbohydrates and other nutrients that would otherwise have been transported to seeds, could be the source of this 'labile carbon'. We propose that such an epidemic will facilitate the activity of pathogens (fusarium seed rot, yellow patch) that attack germinating seeds (Fig. 5). The propagules of the bleach blonde pathogen (persistent resting structures called sclerotia) are themselves hypothesized to be released from fungistasis through a specific cue provided by host roots after the establishment of dense cheatgrass stands following favorable autumn precipitation.

We will approach the fungistasis hypothesis by addressing a series of six questions:

1) Do pathogen propagules experience fungistasis in field soils, and if so, how are they released from fungistasis? We will use techniques referenced in Garbeva et al. (2011) to test the germinability of pathogen propagules in physical contact with unaltered field soils, sterilized field soils, and field soils with added carbohydrates. The prediction is that pathogen propagule germination will be suppressed in unaltered soils but will be permitted in sterilized soils (removal of the microbial community) or in unaltered soil with carbohydrate addition (removal of nutrient limitation). We will also test for germinability on low-carbohydrate and high-carbohydrate sterile laboratory media, to test whether fungistasis is imposed by a soil microbial mechanism other than direct nutrient competition (e. g., production of suppressive metabolites).

2) Does fungistasis in field soils suppress disease development and resulting seed mortality, and how can this suppression be overcome? We will use a modification of the method of Slykhuis (1947) that utilizes seedbed microcosms collected from the field (Beckstead et al. 2010). Nondormant cheatgrass seeds will be planted into microcosms in a randomized block experiment with ten blocks and the following factorial design: unaltered vs. sterilized soil, with or without carbohydrate addition, and with no added inoculum, added fusarium seed rot inoculum, or added yellow patch inoculum. Seedling emergence success and disease-caused seed mortality will be measured after seven days. The prediction is that the pathogens will not be able to cause disease in unaltered soil, but that either carbohydrate addition or sterilizing to remove the microbial community will alleviate fungistasis and result in high seed/seedling mortality.

3) Can release from fungistasis be explained by litter dynamics? Cheatgrass disease cycles have a major impact on litter dynamics. Conversely, the level of carbohydrate-C available to organisms in the seed zone will depend on cheatgrass litter quality as well as quantity. We will quantify litter in the field under intact stands, after die-off, and in bleach blonde and yellow patch areas. We will then analyze samples of litter, and of seed zone soil immediately beneath the litter, for organic matter and carbon and nitrogen composition (Blair et al. 1995, Robertson et al. 1999). We will also include soils from persistent die-offs that no longer have litter. Our hypothesis is that the litter created by both the bleach blonde pathogen and the yellow patch pathogen will be higher in carbohydrate-C and nitrogen than the litter from healthy cheatgrass plants, and that this difference will also be evident in the seed zone soil beneath the litter in fall, when the litter begins to break down after precipitation events.

4) How does the soil microbial activity that putatively causes fungistasis vary with soil/litter conditions in field soils and in the laboratory? These experiments will quantify soil microbial activity using soil respiration (Robertson et al. 1999). First, we will measure microbial activity in the seed zone as a function of the soil/litter conditions described under Question 3. Second, we will perform laboratory pulse experiments with carbohydrate addition to field soils from intact stands, previous-year die-offs, and persistent die-offs, to determine the timeline of microbial response to carbon addition (peak followed by decline in respiration) in each soil condition. The objective of these experiments is to determine how closely soil microbial activity tracks labile carbon availability in time in these soils, both on the short time scale of cheatgrass emergence and on longer time scales involving the loss of litter from previous years.

5) How does pathogen abundance (inoculum load) interact with soil carbohydrate level and litter characteristics to mediate cheatgrass emergence, survival, stand density and biomass production in the field? We will utilize a field small plot study to determine whether carbohydrate addition can release the resident pathogen population and cause emergence failure, and whether inoculum addition will interact synergistically with carbohydrate addition to cause higher levels of fusarium seed rot or yellow patch disease in previous-year die-off areas and intact stands. We will also test the effect of litter removal on disease incidence. We already have some evidence that the severity of a die-off is reduced with litter removal. We will include a watering treatment to manipulate emergence timing and examine its effect on disease incidence and stand establishment. The design will include broadcast-seeded plots as well as plots precision-seeded with cheatgrass (Meyer et al. 2014b; see Table 1a for experimental design). The response variables will be emergence and survival on the precision-seeded plots, and tiller density and total biomass on the broadcast-seeded plots. One objective will be to separate the negative effect of carbohydrate addition on cheatgrass biomass production (presumably operating through nitrogen limitation; Perry et al. 2010) from its effect on stand density, which is more likely an outcome of pathogen activity. Another objective is to determine whether it is possible to cause a die-off just by 'waking up' the resident pathogen population, without adding inoculum. The ability to create die-offs on demand could be a useful restoration tool, especially if causal pathogens return to a quiescent state due to the reimposition of fungistasis following an epidemic, as suggested by our small plot studies (Fig. 2).

6) **Can die-off recovery failure be explained by seed bank and/or litter dynamics?** While most die-offs recover from the carryover seed bank, sometimes die-offs fail to recover (Fig. 3). As we know that we can seed successfully after a die-off, the most likely explanation for die-off persistence across multiple years is inability to establish a stand because of a lack of carryover seed bank. The black fingers of death pathogen is also favored by high litter (Beckstead et al. 2012), and may reduce the carryover seed bank to very low levels during a die-off. Without subsequent input from a current-year stand, litter cover is quickly lost, leaving soils that may remain bare for many years, in spite of possible seed influx from adjacent areas. We hypothesize that added litter will provide a roughened surface to increase seed retention, and will also tend to ameliorate the harsh physical environment of bare soil. Conversely, litter could also have negative effects, possibly associated with increased activity of pathogens that attack seeds. To test this hypothesis, we will perform a small-plot study on a persistent die-off (bare soil), manipulating seed availability (no added seeds, broadcast seeds, and precision-planted seeds) and litter cover (using additions of autoclaved litter), and measuring seedling emergence/survival in precision-seeded plots and stand density/biomass production in unseeded and broadcast seeded plots (see Table 1b for experimental design). The hypothesis is that thin litter will increase retention of both

naturally dispersed and broadcast seeds and will also increase emergence and survival of planted seeds, but that thick litter will inhibit emergence and survival (Meyer et al. 2014).

All primary studies will be carried out at the Whiterocks study site in Skull Valley, Utah, where we have a 22-year history of cheatgrass research. This site is in a die-off prone area; several cycles of die-off and recovery have been observed there over the years. Based on pathogen surveys in three states (Utah, Nevada, Washington; Meyer et al. 2012), we expect that results obtained in Skull Valley will be relevant to managers wherever cheatgrass die-offs occur. We may also make use of die-offs as they occur in other areas, for example, in northern Nevada this year (2014), as a source of material for the laboratory and growth chamber experiments.

Project Products:

At the end of this two-year research effort, we will have a clear idea of how cheatgrass die-off and recovery processes are regulated on a local scale. Furthermore, by integrating our findings with results from other components of the Cheatgrass Integrated Die-off Project, including the restoration plantings and geospatial modeling carried out by our colleagues at the University of Nevada, Reno, we will be able to scale up our understanding to the landscape level. This will increase our ability to predict die-offs, to manage them as they occur, and potentially to create die-offs with the explicit goal of restoration.

In addition to a series of peer-reviewed scientific publications and presentations at professional meetings, the take-homes from our project will be made available in the form of user-friendly reports that will be posted online and advertised widely to the relevant client groups. We plan to engage resource managers interested in cheatgrass die-off and its possible implications for restoration through our website at cheatgrassbiocontrol.org (currently being updated), and through webinars, as well as through workshops, field tours, and regional professional meetings as opportunities present themselves, with a minimum commitment shown in the timeline attached in the budget section.

Communication and Engagement:

The die-off research program which includes the proposed project was developed over the last several years through close cooperation with Mike Pellant and Don Major of the Idaho State Office of the BLM and was initiated in direct response to the needs of land managers in areas affected by cheatgrass die-off. We will remain in close communication with land managers interested in this problem (see timeline and deliverables), as well as with research colleagues involved with other aspects of the BLM Integrated Cheatgrass Die-off Project, which is still ongoing, and who are engaged in geospatial modeling aspects of the research as well as on-the-ground restoration projects that are scheduled to be implemented this coming year.

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Disclaimer Regarding Data Sharing: As this work will be carried out in part by US government employees on official time, there are no issues with data sharing. All information generated will ultimately be in the public domain.

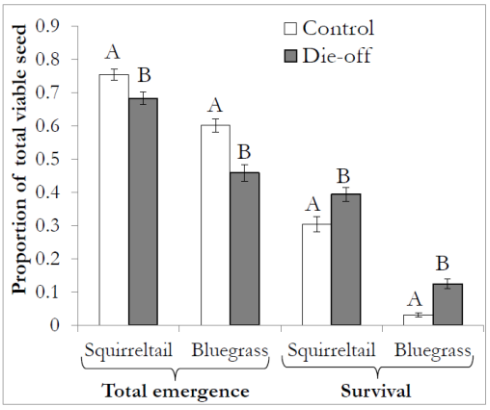
Tables and Figures

Table1. Experimental designs for the field small plot experiments outlined under Questions 5 and 6.

Question 5			
Experimental Design	Split Plot		
Experimental Unit	0.10 m^2 plot		
Replication	10 blocks		
Total Experimental Units	720		
		Treatment Levels	
Main Plot Factor			
Die-off Condition	Previous-year die-off	Intact stand	
Subplot Factors			
Labile Carbon	No labile carbon added	Labile carbon added	
Water	No added water	Water added	
Inoculum	No inoculum	*Fusarium* inoculum	*Epicoccum* inoculum
Litter Manipulation	No litter manipulation	Litter removal	Litter addition
Question 6			
Experimental Design	Randomized Block		
Experimental Unit	0.10 m^2 plot		
Replication	10 blocks		
Total Experimental Units	90		
		Treatment Levels	
Seeding Treatment	No added seeds	Broadcast seeds	Precision-planted seeds
Litter Treatment	No added litter	Thin litter (1 cm)	Thick litter (3 cm)

Figure 1. A die-off in Eden Valley near Winnemucca, Nevada, in 2009, showing the apparently sharp boundary between the die-off and the adjacent healthy cheatgrass stand (Photo by Owen Baughman).

Figure 2. Seedling emergence and survival for two native grasses and for cheatgrass after precision-planting into a die-off and into an adjacent intact cheatgrass stand (control) at the Dun Glen study site near Winnemucca, Nevada in 2012-2013 (Baughman 2014, Nicholson 2014), showing only slightly reduced emergence but increased survival on the die-off for natives, and both increased emergence and increased survival on the die-off for cheatgrass.



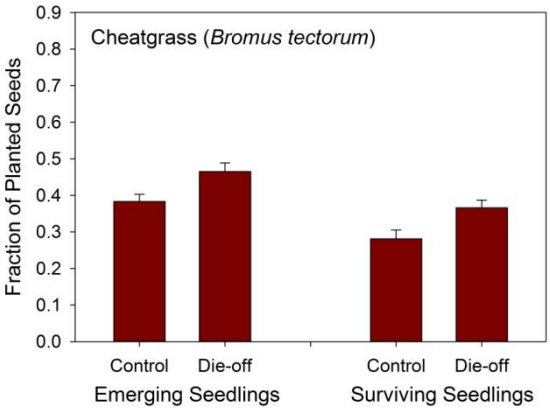


Figure 3. Proposed alternative successional trajectories following a cheatgrass die-off:

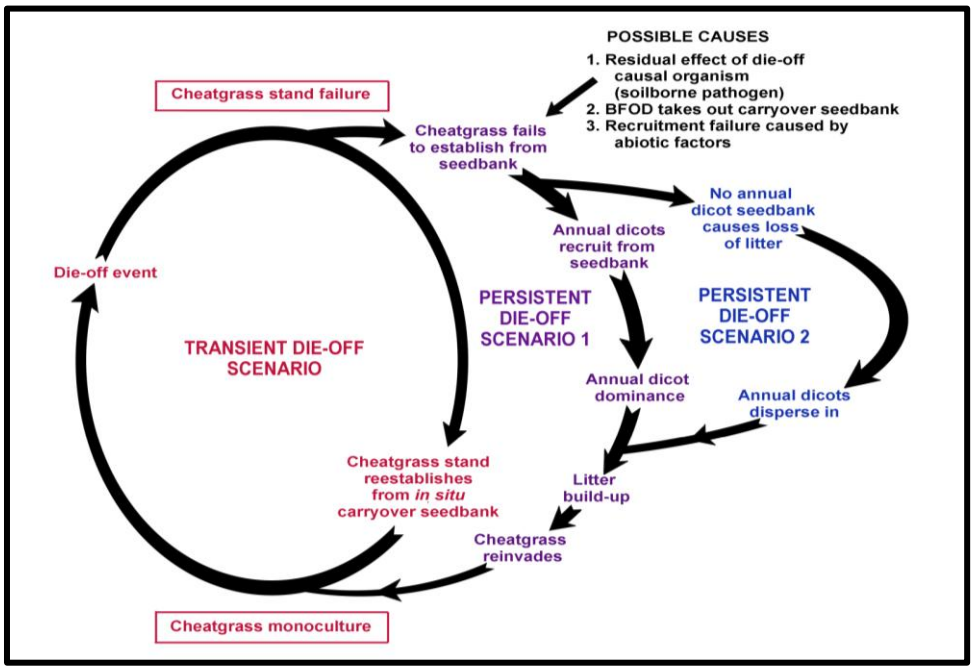


Figure 4. Disease signs on cheatgrass caused by: (A) the fusarium seed rot pathogen (in the laboratory), (B) the black fingers of death pathogen (seed from field seed bank), and (C) the head smut pathogen (field-collected smutted tiller).

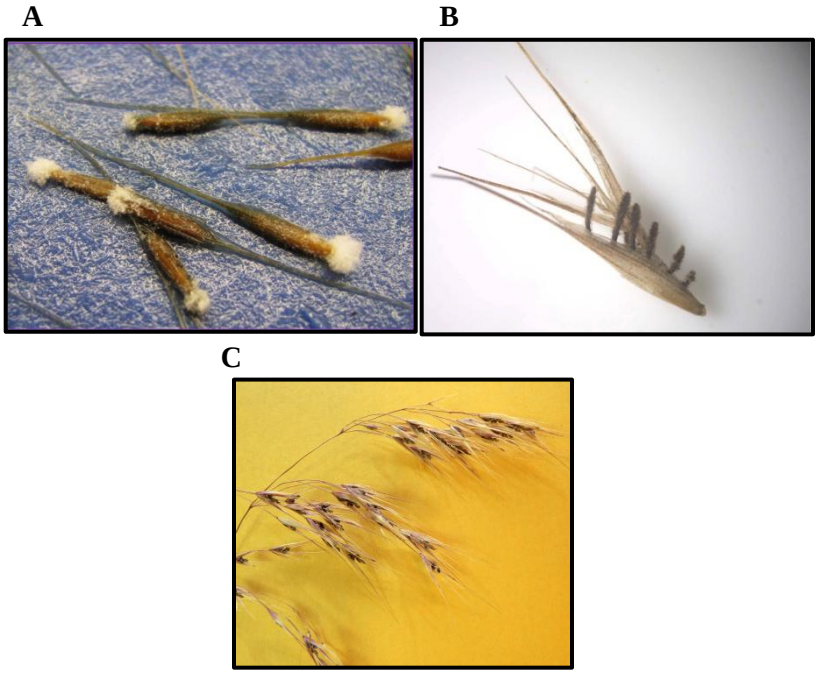


Figure 5. Disease signs on cheatgrass in the field caused by: (A) the bleach blonde pathogen (an undescribed taxon in the Rutstroemiaceae) and (B) the yellow patch pathogen (*Epicoccum nigrum*).

A **B**

Figure 6. Fertile, smutted, and bleach blonde tiller densities in the long-term small plot study at the Whiterocks Study Site (Skull Valley, Utah) over a three-year period: very high densities of bleach blonde tillers in 2012 were followed by a near-die-off (very low tiller densities overall) in 2013 and by stand recovery but with a high proportion of bleach blonde tillers in 2014 (n = 150 plots). The 2013 die-off was complete over much of the site; the small plot area had a very sparse stand.