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A proposed mechanism for high pathogen-caused mortality in the seed bank of an invasive annual grass

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ABSTRACT

Pyrenophora semeniperda can infect nondormant *Bromus tectorum* seeds under optimal germination conditions, but most escape mortality. This reduces pathogen fitness relative to infection of dormant seeds, which are almost always killed. However, field experiments showed that a large proportion of seeds killed following inoculum addition were not accounted for as dormant seeds, but instead were likely nondormant seeds that would have germinated without inoculum addition. We hypothesized that widely fluctuating water availability to seeds would favor pathogenesis by delaying germination and allowing disease progression at water potentials below those that permit radicle emergence. To test this, nondormant host seeds were inoculated, imbibed for 8 or 24 h, subjected to controlled dehydration for 1–21 d, rehydrated, and scored for mortality. With dehydration at −4 MPa, mortality increased with dehydration duration after short or long imbibition. At −40 MPa, mortality increased with dehydration duration only after long imbibition. At −150 MPa, there was no effect of dehydration duration on generally low mortality. These results illustrate that fluctuating moisture can cause high nondormant seed mortality, explaining how this pathogen kills nondormant seeds.

1. Introduction

The importance of soil-borne fungal plant pathogens in structuring natural plant communities has been increasingly recognized in recent years, largely through studies on plant-soil feedback (Bever et al., 2010; Ke et al., 2015). In these studies, the positive or negative legacy effects of soil fungi on plants subsequently grown in those soils are measured, usually in greenhouse or growth chamber experiments (Bever, 1994; Klironomos, 2002). Another common technique for specifically measuring the effects of soil-borne fungi is the use of fungicide treatments as controls to quantify, through elimination, the impact of fungal organisms (e.g., Blaney and Kotanen, 2001; Orrock and Damschen, 2005). These kinds of studies focus on the effect of soil-borne pathogens on plants, rather than explicitly addressing the ecology of the fungi themselves. The great majority of studies that address soil-borne pathogens in natural systems use some variant of this 'black box' approach. There are few mechanistic studies of natural pathosystems, and most of these involve foliar or floral pathogens that are more amenable to experimental approaches (e.g., Alexander et al., 1996; Gilbert and Webb, 2007). Even when causal soil-borne organisms are identified, there is generally little or no exploration of life cycles or mechanisms of pathogenesis (Klironomos, 2002). The need for more mechanistic studies of pathogens and other organisms causing soil legacy effects in natural systems has recently been recognized (Van der Putten et al., 2016). Mechanistic or even descriptive studies of natural pathosystems involving seeds as hosts are even less common, despite the recognition that plant-pathogen interactions at the seed stage can have major impacts on population dynamics of both host and pathogen species and also on processes mediating community structure (Chambers and MacMahon, 1994; Gilbert, 2002).

One of the few seed pathogens that has been studied in detail is *Pyrenophora semeniperda*, an ascomycete that primarily attacks grass seeds in the soil seed bank, particularly seeds of weedy annual brome grasses that produce large seed crops (see Meyer et al., 2016 for review). Unlike most soil pathogens, which must be cultured for identification, this fungus produces macroscopic stromatal structures that are easily recognized on killed seeds *in situ*, a

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morphological feature that has facilitated ecological investigations. These investigations have primarily addressed the ecology of *P. semeniperda* on the seeds of *Bromus tectorum* (cheatgrass, downy brome), a highly invasive winter annual grass that occurs in monocultures over tens of millions of hectares in semi-arid western North America (Chambers et al., 2014).

Pyrenophora semeniperda achieves its greatest success through its impacts on *B. tectorum* seed banks, with densities of killed seeds sometimes in excess of 15,000 seeds m^{-2} (Meyer et al., 2007). Laboratory studies on pathogenesis suggested that its primary prey would be dormant seeds (Beckstead et al., 2007). While conidia located on the seed surface germinate beginning 6 h following the onset of imbition (Finch-Boekweg et al., 2016), the fungus requires 14 d from conidial inoculation to appearance of stromata on seeds under optimal temperature and moisture conditions (Finch et al., 2013). Nondormant *B. tectorum* seeds germinate in 1–3 d under these conditions (Allen et al., 1994; Meyer et al., 2016). Thus, almost all nondormant seeds escape pathogen-caused mortality, although the fungus can infect germinating seeds and sometimes sporulate on seeds that produce viable seedlings. In contrast, dormant *B. tectorum* seeds germinate very slowly if at all, and the pathogen can cause near-complete mortality at low inoculum loads.

The process of pathogenesis has been termed a 'race for survival'. This race is usually won by the pathogen on dormant seeds but almost always won by host seeds that are nondormant (Beckstead et al., 2007; Finch et al., 2013). The pathogen is in direct competition with the host seedling developing from a nondormant seed for endosperm resources that are the primary source of nutrition (Finch-Boekweg et al., 2016). This means that pathogen fitness as measured by reproductive output (conidial production) is generally greatly decreased after infection of a germinating seed relative to its fitness on a dormant seed.

In semi-arid habitats where both pathogen and host are abundant, *B. tectorum* seeds are dormant at dispersal in early summer. Following dispersal, wide fluctuations of temperature and moisture characterize the soil seed zone (Meyer et al., 2007; Meyer and Allen, 2009). Regardless of whether inoculation occurs during summer, fall or winter, the interaction between pathogen and host occurs in the context of a soil environment that can be wet and dry repeatedly (Finch et al., 2013).

Bromus tectorum seeds gradually lose dormancy through dry after-ripening over the summer, and are poised to germinate very rapidly with the first germination-triggering autumn rains (Meyer and Allen, 2009). Seeds that do not germinate in autumn usually enter secondary dormancy under late fall/winter conditions (Hawkins et al., 2017). This phenological pattern suggests that most successful pathogen attack in natural seed banks would involve either seeds still in primary dormancy in the summer (Hawkins, 2014) or carryover seeds in a state of secondary dormancy in late fall through early spring (Finch et al., 2013). However, preliminary evidence from seed bank studies suggested that there is considerable *P. semeniperda*-caused mortality in early autumn, presumably on nondormant seeds (Hawkins, 2014).

One hypothesis to explain how *P. semeniperda* could kill nondormant seeds relates to field conditions under which inadequate rainfall prevents seeds from completing germination. We hypothesized that the fungus could remain active at water potentials below those that permit seed germination, so that pathogenesis would be favored under conditions of limited water availability. We modeled the ability of the fungus to germinate and grow at suboptimal water potentials based on laboratory experimental data using a hydrothermal time approach (Bauer et al., 1998; Barth et al., 2015). We demonstrated that *P. semeniperda* could remain active at water potentials far below the threshold for *B. tectorum* seed germination, with conidial germination at potentials as low as −6 MPa and some mycelial growth even at −11 MPa as achieved with polyethylene glycol. Our hypothesis that this would affect successful attack on nondormant seeds was tentatively supported in inoculation experiments at water potentials just below the limit for seed germination (i.e., −1.5 to −2.0 MPa; Finch et al., 2013). However, seeds in the field seed bank spend very little time at such high suboptimal water potentials (Meyer and Allen, 2009), making this a somewhat unrealistic test.

Here we report results from inoculum addition experiments that estimate pathogen-caused nondormant seed mortality in natural seed banks, to demonstrate that significant nondormant seed mortality occurs across multiple sites with different ecological settings. We then test the hypothesis that the pathogen would be favored over nondormant seeds under intermittent hydration conditions approximating those likely to be encountered in autumn field seed banks. We hypothesized that longer post-inoculation imbibition periods prior to dehydration, higher dehydration water potentials, and longer dehydration periods would all result in greatly increased pathogen success evident as killed nondormant seeds with stromata in post-treatment rehydration.

2. Materials and methods

2.1. Field inoculum addition studies

Inoculum addition studies were carried out at five field sites in the western United States from 2008 to 2010 (Table 1). The primary purpose was to determine the feasibility of eliminating the dormant carryover seed bank of *B. tectorum* by augmenting natural *P. semeniperda* inoculum loads through addition of laboratory-produced bulk inoculum. Inoculum addition treatments varied among experiments but always included a control treatment with no inoculum addition and a high inoculum treatment with 45 g of bulk inoculum per 0.1 m^2 plot. Only the results from these two inoculum treatments are analyzed and presented here. Experiments at two sites (Davis Mountain and Santaquin Canyon, 2008) included only inoculum addition (strain WRK0; Finch et al., 2013, Barth et al., 2015). At a third site (Whiterocks, 2009) we carried out a large factorial experiment with burn and herbicide treatments in addition to inoculum addition treatments (Table 1). Because the herbicide treatments produced no significant effects on the pathogen, inoculation treatments were pooled across herbicide treatment in the analysis to increase statistical power. Similarly, we pooled results across four pathogen strains included in experiments at two additional sites (Lytle Ranch and Haven Flats, 2010), as differences among strains were not the emphasis of the current analysis. Burn treatments were also excluded from analysis.

At each site, the inoculum addition experiment had a randomized block design with 10 blocks. Each block contained one experimental unit of each treatment combination. An experimental unit consisted of a 0.1 m^2 plot surrounded by a buffer area to prevent cross-contamination. Each of these experimental units received an addition of 100 nondormant *B. tectorum* caryopses (hereafter seeds) prior to treatment application in an effort to ensure the presence of seeds on every experimental plot. This addition (1000 seeds m^2) made a largely negligible contribution to the seed bank, as seed densities in autumn normally range from 10,000 to 40,000 seeds m^{-2} (Meyer et al., 2007; Smith et al., 2008).

Bulk inoculum was prepared by growing the fungal strain in potato dextrose broth (PDB) culture (Meyer et al., 2010), then inoculating an inert carrier (calcined montmorillonite clay) supplemented with fresh PDB. The bulk inoculum was incubated at room temperature (approximately 25 °C) under UV light to induce sporulation, then dried, sieved, and weighed as described in Meyer et al. (2014a). Each autumn prior to the first germination-triggering

Table 1
Details for five field inoculation experiments used to estimate the effect of added *Pyrenophora semeniperda* bulk inoculum (45 g 0.1 m^{-2}) on *Bromus tectorum* late spring carryover seed bank size and composition. All experiments had 10 block replicates and included inoculated plots and uninoculated controls.

	Davis Mountain UT 2008	Santaquin Canyon UT 2008	Whiterocks UT 2009[a]	Lytle Ranch UT 2010[b]	Haven Flats WA 2010[b]
Latitude	40.121036°	39.940874°	40.328000°	37.143317°	46.311490°
Longitude	−112.663919°	−111.769201°	−112.778000°	−114.019170°	−119.494195°
Elevation	1579 m	1705 m	1445 m	876 m	200 m
Vegetation Type	sagebrush steppe	mountain brush	cold desert shrubland	warm desert shrubland	palouse prairie
Inoculum Strain	WRK0	WRK0	WRK0	Four strains[b]	Four strains[b]
Total Plot Number	20 (10 control)	20 (10 control)	80[a] (40 control)	50 (10 control)	50 (10 control)

[a] At Whiterocks in 2009, four herbicide treatments were factorially combined with inoculum treatments; these herbicide treatments had no significant effect, so inoculation treatments were pooled across herbicide treatments to increase sample size.
[b] At Lytle Ranch and Haven Flats in 2010, strains WRK0, TMC23, TMC16, and DOG3 were included; the inoculum addition treatment is pooled across strains in the analysis to increase sample size.

rainstorms (Meyer and Allen, 2009), bulk inoculum (or carrier without inoculum for the control treatment) was applied to each plot, and the experiment was left to experience natural weather conditions through autumn, winter, and spring.

To evaluate the status of the late spring seed bank, a single sample was taken from the center of each experimental unit prior to any dispersal of current-year seeds. Samples were obtained with a steel can (6 cm diameter × 4 cm high). After a two-month period in warm storage to simulate the summer conditions that remove secondary dormancy (Hawkins et al., 2017), samples were screened to remove mineral soil and hand-sorted to extract pathogen-killed and apparently viable seeds (see Meyer et al., 2007 for details). Killed *B. tectorum* seeds (with visible fungal stromata) were counted and apparently viable seeds (firm but ungerminated with no visible stromata) were incubated for 2 wks at 10/20 °C alternating temperature (12-h photoperiod corresponding to the higher incubation temperature) and scored twice a week for germination or pathogen-caused mortality. Because newly-infected seeds require >2 wk to exhibit stromatal development under this temperature regime, seeds that exhibited stromata within 2 wk were considered to have been killed in the field. At the end of the incubation period all remaining seeds were cut to determine viability. Nearly all remaining viable seeds germinated within 3 d.

For each experiment, the treatments to be included in the subset for analysis as described above (Table 1) were analyzed using mixed model Poisson regression for count data (SAS PROC GLIMMIX) with inoculum treatment (no inoculum control vs. high inoculum addition) as the fixed main effect and block as the random effect. Results are presented as density per dm^2, which can be converted to density per m^2 by multiplying by 100. Response variables included killed seed density, viable seed density, and total seed density.

2.2. Laboratory intermittent hydration experiments

Seeds of *B. tectorum* were collected from a wild population at the Brigham Young University Research Farm (Spanish Fork, Utah, USA) in June 2011. Filled seeds were separated by hand and stored under ambient laboratory conditions (approximately 25 °C and 32% relative humidity) to allow seeds to after-ripen. The *P. semeniperda* inoculum strain was WRK0. Conidial inoculum was produced as previously described by Meyer et al. (2010). Seeds were inoculated with a 1:100 spore:talc mixture (w/w) by placing seeds and an excess of inoculum in a test tube vial and rapidly shaking for 30 s. Inoculated seeds were then exposed to initial hydration (i.e., imbibition) followed by controlled dehydration, then rehydration. Seed water content was determined by weighing as described by Allen et al. (1993).

Nondormant (fully after-ripened) seeds were imbibed in Petri dishes on the surface of two blue germination blotters (Anchor Paper, St. Paul, Minnesota, USA) at constant 20 °C (12 h fluorescent light/12 h dark cycle), which is near optimum for both pathogen (Campbell et al., 1996) and host (Meyer and Allen, 2009). Each treatment included four replicates of 25 well-spaced seeds. Seeds were hydrated either continuously or for two periods based on earlier studies (Allen et al., 1993): for 8 h, when the rapid phase of imbibition was nearing completion and conidia exhibited visible germ tubes or for 24 h, nearing the time when radicle emergence was first observed and appresoria had penetrated the caryopsis wall (Finch-Boekweg et al., 2016). Dehydration water potentials included −4, −10, −40, and −150 MPa, achieved above saturated salt solutions (K_2SO_4, KNO_3, NaCl and $MgCl_2$, respectively) in sealed containers. The physiological significance of these water potentials is described in Bair et al. (2006) and references cited therein. Dehydrating seeds were placed on plastic weighing trays and floated above salts at a carefully controlled temperature of 20 °C in sealed jars for durations of either 1, 7, 14, or 21 d (Allen et al., 1992, 1993). No seeds germinated during any of the dehydration treatments.

Following dehydration, seeds were returned to water-saturated blotters and rehydrated, again at 20 °C. Seeds were scored as germinated (radicle protruded at least 1 mm) or killed (macroscopic *P. semeniperda* stromata visible with no radicle present) after 2, 4, 7, 11, 14, 21 and 28 d. At 28 d, viability of remaining ungerminated seeds was determined using a cut test.

Both prolonged storage at relatively high water content and rapid dehydration have the potential to damage seeds and potentially make them more susceptible to pathogen attack. In order to eliminate seed damage as a significant variable in explaining the results and evaluate any vigor loss that might have occurred during the dehydration treatments, germination time courses for seeds not killed by the pathogen were examined. Mortality and viability were evaluated as percentage of total seeds killed by the pathogen (mortality) or remaining germinable (viable seeds) and was compared with initial final germination percentages for the seed lot. Vigor was evaluated by examining the germination rate of non-killed seeds; loss of vigor is typically accompanied by a reduction in germination rate (Bewley et al., 2013). Germination rate was evaluated as the percentage of total germinable seeds with radicles emerged on days 2 and 4 of the rehydration period.

Seed mortality data from the intermittent hydration experiment were analyzed as a completely randomized design using analysis of covariance (ANCOVA; SAS PROC GLIMMIX). Pathogen-killed seed proportion was analyzed as the binomial response variable. The independent variables were dehydration duration (continuous variable) and imbibition period and dehydration water potential

(class variables). Water potential was not treated as a continuous variable in the analysis because we had no expectation that response to this variable would be linear. The resulting means were converted to percentages for graphical display following analysis.

3. Results

3.1. Field inoculum addition studies

Pyrenophora semeniperda bulk inoculum addition prior to the first germination-triggering rainfall in autumn resulted in a significant increase in pathogen-killed seed density the following spring (Table 2, Fig. 1). This was observed at all five sites, with two- to five-fold increases. A concurrent significant decrease in viable carryover seed density was also observed at all five sites. Viable seed density was reduced with inoculum addition from an average of 35 seeds dm^{-2} to an average of 12 seeds dm^{-2}. This provided evidence that pathogen inoculum addition could effectively decrease the density of viable carryover seeds by increasing mortality of dormant seeds, indicating that seed mortality was inoculum-limited at natural inoculum loads.

More interesting from the perspective of the present study, however, was the fact that inoculum addition not only caused mortality of dormant seeds that would otherwise have carried over, but also increased the total density of seeds in the late spring seed bank, that is, the sum of killed and viable seeds (Fig. 1). This effect was also significant at all five sites (Table 2). Inoculum addition increased the density of killed seeds far more than could be accounted for by the decrease in density of viable carryover seeds. The simplest explanation for this result is that, with added inoculum, the pathogen could kill seeds that were able to escape through germination at natural inoculum loads. These seeds are shown as 'escaped seeds' in the control treatment (Fig. 1). This assumes that initial autumn seed densities were similar for control and inoculated treatments. The ability of the pathogen to cause mortality of large numbers of seeds that apparently escaped through germination at lower inoculum loads strongly implies that it was able to kill nondormant seeds. The density of seeds shown as killed in the inoculum added treatment but escaped in the control treatment varied from 13 seeds dm^{-2} at Lytle Ranch, where densities in the spring seed bank were low, to 82 seeds dm^{-2} at the Santaquin Canyon site. The average density of putatively nondormant killed seeds was 49 seeds dm^{-2}.

3.2. Laboratory intermittent hydration experiments

Water uptake of *B. tectorum* seeds during initial hydration followed a characteristic triphasic pattern that included a rapid imbibition phase (0–8 h) followed by a period of slower water uptake (8–32 h) after which radicle emergence and an increased rate of water uptake in developing seedlings was observed (Fig. 2). Dehydration initiated after 8 or 24 h resulted in rapid seed water loss. At each dehydration water potential, seeds reached near-equilibrium water contents after 6 or 14 h, respectively. Late-dehydrated seeds equilibrated at slightly higher water contents than did early-dehydrated seeds. Dehydration at −150 MPa returned seeds to near air-dry weights within 24 h.

Inoculated nondormant seeds germinated to near 100% within 3 d of continuous hydration at 20 °C and experienced very low pathogen-caused mortality (<2%), confirming earlier findings that typical strains of the pathogen do not cause significant nondormant seed mortality under optimum conditions for seed germination (Beckstead et al., 2007; Finch et al., 2013).

In the test of dehydration treatment effects on seed viability and vigor, an average of 1% of total seeds remained ungerminated at the end of the post-dehydration incubation period, comparable to the percentage of nonviable seeds present at the beginning of the experiment. This indicates that seeds not killed by the pathogen

Table 2
F-tests for the inoculum addition main effect from mixed model Poisson regression analysis for count data using SAS Proc GLIMMIX for *Pyrenophora semeniperda* experiments at five sites across three years (2008–2010). See Fig. 1 for effects.

Site	Year	Killed Seed Density			Viable Seed Density			Total Seed Density		
		d. f.	F	P	d. f.	F	P	d. f.	F	P
Davis Mtn.	2008	1,9	198.0	<0.0001	1,9	86.2	<0.0001	1,9	46.6	<0.0001
Santaquin Cyn.	2008	1,9	489.6	<0.0001	1,9	141.2	<0.0001	1,9	255.7	<0.0001
Whiterocks	2009	4184	85.1	<0.0001	4184	14.6	<0.0001	4184	40.6	<0.0001
Haven Flats	2010	1,39	667.3	<0.0001	1,38	538.1	<0.0001	1,38	258.8	<0.0001
Lytle Ranch	2010	1,39	157.4	<0.0001	1,39	169.9	<0.0001	1,39	46.7	<0.0001

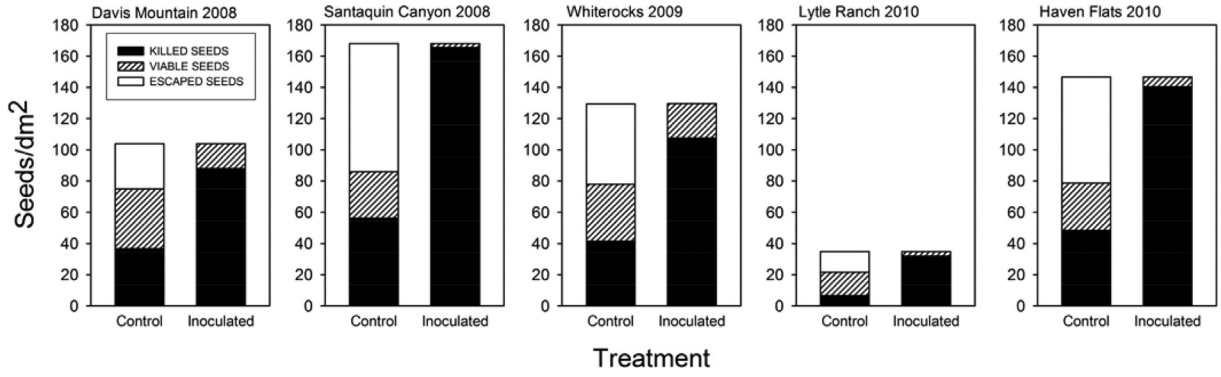

Fig. 1. Effect of *Pyrenophora semeniperda* inoculum addition in early autumn on the size and composition of the *Bromus tectorum* carryover seed bank in late spring the following year at five experimental sites. Escaped seeds were not measured directly, but were instead calculated based on the assumption that seedbank density was similar for inoculated and non-inoculated controls at the beginning of autumn. See Table 2 for statistical analysis.

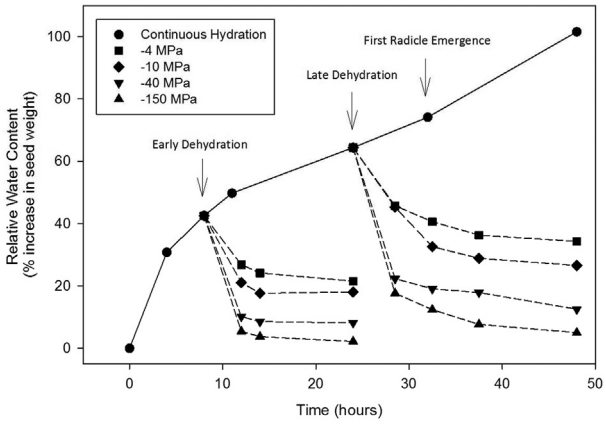

Fig. 2. Change in water content of nondormant *Bromus tectorum* seeds hydrated continuously (solid line) or dehydrated at indicated water potentials following hydration for 8 or 24 h (dashed lines) at 20 °C. Early dehydration was timed to begin approximately when the rapid phase of imbibition was completed, while late dehydration was timed to occur shortly before the first radicle emerged.

remained germinable in all dehydration treatments. Most remaining viable seeds (86%) germinated within 2 d after transfer to water, and 97% germinated within four days. These are germination rates typical of healthy nondormant *B. tectorum* seeds at 20 °C (Allen et al., 1994), suggesting that negligible loss of vigor occurred during dehydration. There were no evident patterns associated with imbibition period, dehydration duration, or dehydration water potential.

The effect of dehydration duration on pathogen-caused seed mortality as measured during subsequent rehydration was a function of both pre-dehydration imbibition period (8 or 24 h) and dehydration water potential, resulting in a significant three-way interaction among these three variables in ANCOVA (Table 3). Patterns of response to imbibition period and dehydration duration contrasted strongly among dehydration water potentials (Fig. 3). At −4 MPa, pathogen-caused mortality increased linearly with dehydration duration regardless of imbibition period (8 versus 24 h) until it reached a maximum of >90% (Fig. 3A). With dehydration at −10 MPa, there was an apparent interaction between dehydration duration and imbibition period. High mortality (i.e., approaching that achieved at −4 MPa) was observed only with the longer imbibition period (Fig. 3B). With short imbibition followed by dehydration at −40 MPa, there was no infection, no disease progression through time, and consequently no seed mortality, whereas after the long imbibition period mortality increased linearly with dehydration duration and reached levels comparable to those reached at −4 MPa (Fig. 3C). With dehydration at −150 MPa, there was no effect of dehydration duration on subsequent seed mortality (Table 3, Fig. 3D). There was a significant effect of imbibition period, with little or no mortality following short imbibition but mortality averaging 35% across all dehydration durations following long imbibition.

Another measure of disease progression during dehydration is the interval between the initiation of rehydration and the appearance of fungal stromata on killed seeds. This is most clearly demonstrated after the long 21- d dehydration period (Fig. 4). For dormant seeds in continuous hydration, 14 d at 20 °C were required for stromata to appear (Finch et al., 2013). Seeds dehydrated at −4 MPa exhibited rapid stromatal formation (4 d) with either short or long imbibition followed by 21- d dehydration (Fig. 4A). Seeds dehydrated at −10 MPa exhibited stromatal formation within 7 d in the long imbibition treatment and after 11 d in the short imbibition treatment (Fig. 4B). Seeds dehydrated at −40 and −150 MPa after long imbibition (Fig. 4 C, D) also exhibited stromata at 11 d, whereas after short imbibition seeds were not infected and did not exhibit any stromatal development.

4. Discussion

The field inoculum addition studies reported here showed that a sizeable fraction of pathogen-killed seeds present in the spring seed bank likely suffered mortality as nondormant seeds. This phenomenon was consistently observed at sites across a range of environments and in multiple years (Tables 1 and 2; Fig. 1). These putatively nondormant killed seeds represented from 18 to 78% of total killed seeds present in the spring seed bank and averaged 59% of total killed seeds, showing that the pathogen could greatly increase its fitness by killing nondormant seeds in autumn across a wide range of environments.

Our indirect measure of the effect of the pathogen on mortality of nondormant *B. tectorum* seeds is likely an underestimate, as it does not account for nondormant seeds killed in control treatments. Seeds killed by the pathogen at natural field inoculum loads could have been killed either when nondormant or when dormant. Our methodology did not permit this distinction, but some fraction of these seeds were probably also killed when nondormant. Thus the nondormant seed mortality observed with inoculum addition is probably not unique to situations with high inoculum loads. The inoculum addition treatment made it possible to detect the mortality of nondormant seeds, rather than necessarily representing a required condition for pathogen-caused nondormant seed mortality.

The laboratory experiment manipulating patterns of intermittent hydration provided ample evidence to support the hypothesis that the pathogen has an advantage over the host seed at low water potentials. We believe this is likely the most important mechanism to explain pathogen-caused mortality of nondormant *B. tectorum* seeds in the autumn seed bank. Many experimental scenarios resulted in high mortality, unlike the continuous hydration treatment with inoculated nondormant seeds, in which mortality was

Table 3
Analysis of covariance (ANCOVA) for the intermittent hydration experiment using SAS Proc GLIMMIX for a binomial response variable, with dehydration duration (1–21 d) as the continuous variable, imbibition time (8 or 24 h) and dehydration water potential (−4, −10, −40 or −150 MPa) as the class variables, and pathogen-killed seeds as a function of total seeds as the response variable (see Fig. 3 for graphical display of results).

Source	DF	F-Value	P
Dehydration duration	1, 112	156.90	<.0001
Imbibition time	1, 112	24.64	<.0001
Dehydration water potential	3, 112	1.84	0.1436
Dehydration duration x imbibition time	1, 112	11.32	0.0010
Dehydration water potential x imbibition time	3, 112	14.55	<.0001
Dehydration duration x dehydration water potential	3, 112	32.32	<.0001
Dehydration duration x dehydration water potential x imbibition time	3, 112	4.18	0.0076

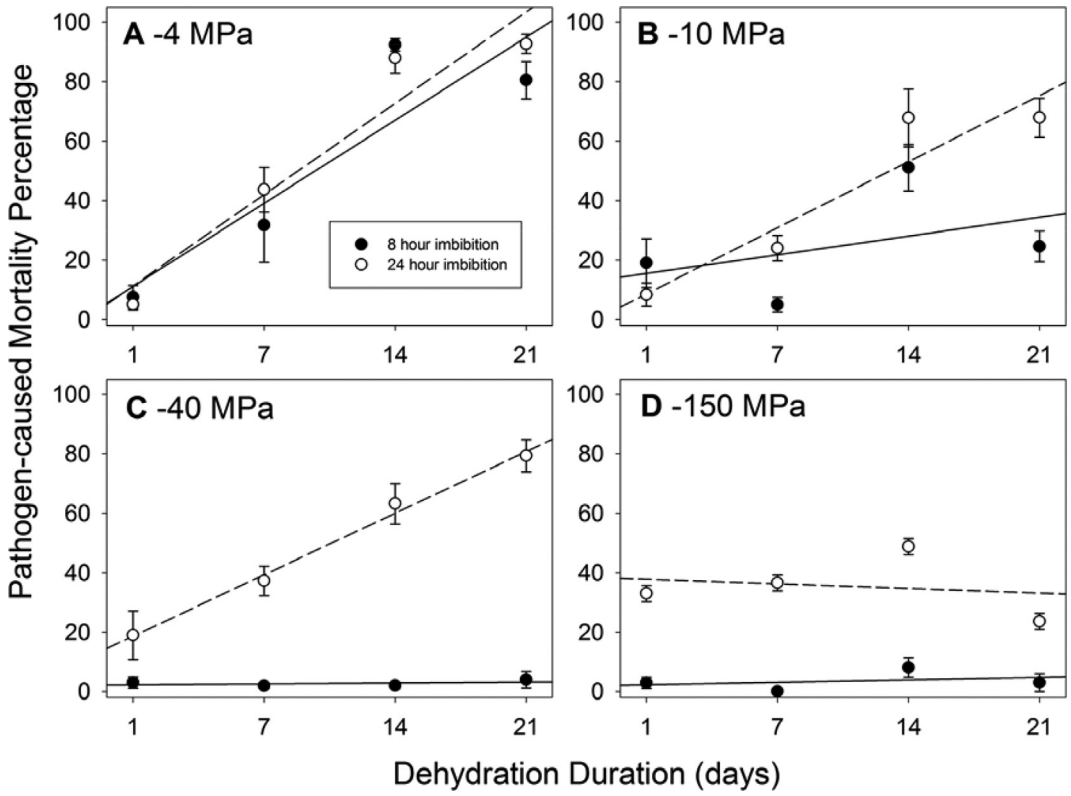

Fig. 3. *Pyrenophora semeniperda*-caused mortality of *Bromus tectorum* seeds at 20 °C after imbibition for one of two periods (8 or 24 h), followed by dehydration at one of four water potentials for periods of 1–21 d, and subsequent rehydration for 28 d: (A) −4 MPa, (B) −10 MPa, (C) −40 MPa, (D) −150 MPa. Lines are fitted regression lines for each imbibition period from analysis of covariance (ANCOVA) at each dehydration water potential. Error bars = standard error of the mean. See Table 3 for statistical analysis.

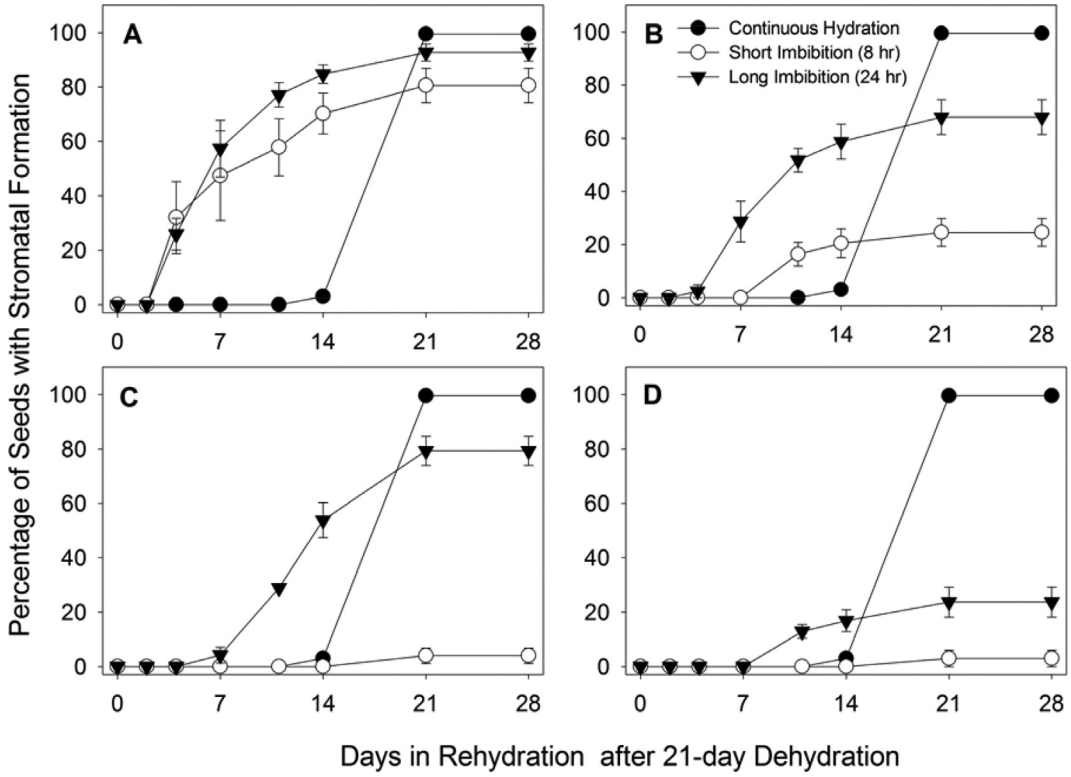

Fig. 4. Time courses of visible stromatal formation on *Pyrenophora semeniperda*-killed *Bromus tectorum* seeds during rehydration after a short (8 h) or long (24 h) imbibition period followed by a dehydration treatment of 21 d at: (A) −4 MPa, (B) −10 MPa, (C) −40 MPa, and (D) −150 MPa. The time course for stromatal formation under conditions of continuous hydration on dormant seeds (Finch et al., 2013) is shown for comparison. Error bars represent standard error of the mean.

essentially nil as shown in previous work (Beckstead et al., 2007; Finch et al., 2013). All variables that were manipulated in the intermittent hydration experiment (imbibition duration, dehydration duration, and dehydration water potential) had significant effects on the outcome and also interacted strongly with each other. We previously used hydrothermal modeling to predict dormancy loss and germination of *B. tectorum* seeds in the field (Meyer and Allen, 2009). While pathogen-host interactions were not included in that study, extensive monitoring of field temperature and moisture conditions demonstrated that wide diurnal as well as post-rainfall fluctuation in soil moisture indeed characterizes the soil environment where this pathosystem operates in semi-arid habitats. More recent field studies demonstrated that nearly complete mortality occurred when inoculated *B. tectorum* seeds were subjected to wide fluctuations in soil moisture during summer months (Hawkins, 2014).

Disease incidence generally increased with duration of both imbibition and dehydration, at water potentials as low as −40 MPa. The ability of some fungi to grow inside seeds and even to infect and to sporulate at water potentials as low as −50 MPa is well-documented (Halloin, 1986; Magan and Lacey, 1988). However, most of these are 'storage fungi' best studied as organisms that cause diseases of stored crop seeds such as grains and legumes. They usually infect dry seeds post-harvest, in contrast to 'field fungi', which often infect developing crop seeds and are not normally active at water potentials <-15 MPa.

At the lowest water potential (−150 MPa), seeds were essentially air-dry; there was no disease progress during dehydration and thus no storage duration effect. Seeds that experienced short imbibition prior to this dehydration treatment did not become infected, but once the seeds were successfully infected during long imbibition, the pathogen was apparently poised inside the seed to take advantage of any germination delay to complete pathogenesis. It was able to cause 35% mortality during the rehydration period, showing that it could survive inside the seed during extended dehydration at this very low water potential, then resume the process of pathogenesis upon rehydration.

In earlier studies on water partitioning within seeds during dehydration following imbibition, Allen et al. (2000) showed that water in barley seeds moves preferentially to the embryo during dehydration. It is possible that pathogenesis that targets the embryo is favored during wetting and drying cycles as a result of water movement to the embryo during drying. During continuous hydration the endosperm appears to be catabolized first and the embryo later (Finch-Boekweg et al., 2016).

In summary, we have shown that *P. semeniperda* can successfully attack and kill nondormant *B. tectorum* seeds in the fall, in contrast to our original hypothesis that the pathogen could successfully cause mortality only on dormant seeds. We have further shown that under conditions of fluctuating water availability that follow one or more precipitation events inadequate to trigger full seed germination, the pathogen is able to infect seeds and to continue the process of pathogenesis at water potentials far below those that permit seed germination.

Fungal diseases of plants are often assumed to be more important in mesic environments, and this is likely true for many foliar and damping off diseases whose causal agents depend on high humidity or free water for infection (e.g., Augspurger and Kelly, 1984; Magarey et al., 2005). Fungal seed pathogens that can operate at low water potentials are much less subject to these constraints, however. Instead, low water potential may limit seed escape through germination and facilitate success for many seed pathogens that operate in semi-arid environments. For example, a seed pathogen on *B. tectorum* belonging to the *Fusarium tricinctum* species group caused higher mortality at water potentials that suppress seed germination than in free water (Meyer et al., 2014b), and current evidence suggests that large-scale *B. tectorum* stand failure in which this pathogen is implicated is more likely to occur in dry years (Weisberg et al., 2017). Whether this mechanism for pathogen success is a general feature of seed pathosystems in semi-arid environments will require examination of a much wider range of pathogens affecting seeds in these environments.

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References

Alexander, H.M., Thrall, P.H., Antonovics, J., Jarosz, A.M., Oudemans, P.V., 1996. Population dynamics and genetics of plant disease: a case study of anther-smut disease. Ecology 77 (4), 990–996.
Allen, P.S., White, D.B., Markhart, A.H., 1993. Germination of perennial ryegrass and annual bluegrass seeds subjected to hydration-dehydration cycles. Crop Sci. 33, 1020–1025.
Allen, P.S., Debaene, S.B., Meyer, S.E., 1994. Regulation of germination timing in facultatively fall-emerging grasses. In: Monsen, S.B., Kitchen, S.G. (Eds.), Proc. Symposium on Ecology, Management, and Restoration of Intermountain Annual Rangelands, Boise, Idaho, 17-21 May 1992, pp. 215–219.
Allen, P.S., White, D.B., Russer, K., Olsen, D., 1992. A system for controlling water potential in seed germination research. Hortscience 27, 264–366.
Allen, P.S., Thone, E.T., Gardener, J.S., White, D.B., 2000. Is the barley endosperm a water reservoir for the embryo when germinating seeds are dried? Int. J. Plant Sci. 161 (2), 195–201, 2000.
Augspurger, C.K., Kelly, C.K., 1984. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. Oecologia 61, 211–217.
Bair, N.B., Meyer, S.E., Allen, P.S., 2006. A hydrothermal after-ripening time model for seed dormancy loss in *Bromus tectorum* L. Seed Sci. Res. 16, 17–28.
Barth, C.W., Meyer, S.E., Beckstead, J., Allen, P.S., 2015. Modeling temperature and water potential effects on conidial germination and mycelial growth for a fungal seed pathogen using hydrothermal time. Fungal Biol. 119, 720–730.
Bauer, M.C., Meyer, S.E., Allen, P.S., 1998. A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. J. Exp. Bot. 49, 1235–1244.
Beckstead, J., Meyer, S.E., Molder, C.J., Smith, C., 2007. A race for survival: can *Bromus tectorum* seeds escape *Pyrenophora semeniperda*-caused mortality by germinating quickly? Ann. Bot. 99, 907–914.
Bever, J.D., 1994. Feedback between plants and their soil communities in an old field community. Ecology 75, 1965–1977.
Bever, J.D., Dickie, I.A., Facelli, E., Facelli, J.M., Klironomos, J., Moora, M., Rillig, M.C, Stock, W.D., Tibbett, M., Zobel, M., 2010. Rooting theories of plant community ecology in microbial interactions. Trends Ecol. Evol. 25, 468–478.
Bewley, J.D., Bradford, K., Hilhorst, H., Nonogaki, H., 2013. Seeds: Physiology of Development, Germination and Dormancy, third ed. Springer. 392 pp.
Blaney, C.S., Kotanen, P.M., 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. J. Appl. Ecol. 38 (5), 1104–1113.
Campbell, M.A., Medd, R.W., Brown, J.F., 1996. Growth and sporulation of Pyrenophora semeniperda in vitro: effects of culture media, temperature and pH. Mycol. Res. 100 (3), 311–317.
Chambers, J.C., Bradley, B.A., Brown, C.S., D'Antonio, M.J., Grace, J.B., Hardegree, S.P., Miller, R.F., Pyke, D.A., 2014. Resilience to stress and disturbance, and resistance to *Bromus tectorum* L. invasion in cold desert shrublands of western North America. Ecosystems 17, 360–375.
Chambers, J.C., MacMahon, J.A., 1994. A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. Annu. Rev. Ecol. Systemat. 25, 263–292.
Finch, H., Allen, P.S., Meyer, S.E., 2013. Environmental factors influencing *Pyrenophora semeniperda*-caused seed mortality in Bromus tectorum. Seed Sci. Res. 23, 57–66.
Finch-Boekweg, Heather, Gardner, J.S., Allen, P.S., Geary, B., 2016. Postdispersal infection and disease development of Pyrenophora semeniperda in Bromus tectorum seeds. Phytopathology 106 (3), 236–243.
Gilbert, G.S., 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annu. Rev. Phytopathol. 40, 13–43.
Gilbert, G.S., Webb, C.O., 2007. Phylogenetic signal in plant pathogen–host range. Proc. Natl. Acad. Sci. Unit. States Am. 104 (12), 4979–4983.
Halloin, J.M., 1986. Microorganisms and seed deterioration. In: McDonald.Jr, M.B., Nelson, C.J. (Eds.), Physiology of Seed Deterioration, Special Publication #11.

Crop Science Society of America, Inc, Wisconsin, Madison, pp. 89–99.
Hawkins, K.K., 2014. Secondary dormancy and summer conditions influence outcomes in the *Pyrenophora semeniperda - Bromus tectorum patho* system. Thesis. Brigham Young University, USA.
Hawkins, K.K., Allen, P.S., Meyer, S.E., 2017. Secondary dormancy induction and release in *Bromus tectorum* seeds: the role of temperature, water potential, and hydrothermal time. Seed Sci. Res. 27, 12–25.
Ke, P.J., Miki, T., Ding, T.S., 2015. The soil microbial community predicts the importance of plant traits in plant–soil feedback. New Phytol. 206 (1), 329–341.
Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417, 67–70.
Magan, N., Lacey, J., 1988. Ecological determinants of mould growth in stored grain. Int. J. Food Microbiol. 7 (3), 245–256.
Magarey, R.D., Sutton, T.B., Thayer, C.L., 2005. A simple generic infection model for foliar fungal plant pathogens. Phytopathology 95 (1), 92–100.
Meyer, S.E., Merrill, K.T., Allen, P.S., Beckstead, J., Norte, A.S., 2014a. Indirect effects of an invasive annual grass on seed fates of two native perennial grass species. Oecologia 174, 1401–1413.
Meyer, S.E., Allen, P.S., 2009. Predicting seed dormancy loss and germination timing for *Bromus tectorum* in a semi-arid environment using hydrothermal time models. Seed Sci. Res. 19, 225–239.
Meyer, S.E., Beckstead, J., Franke, J., 2016. Community ecology of fungal pathogens on *Bromus tectorum*. Chapter 7. In: Germino, Matthew J., Chambers, Jeanne C., Brown, Cynthia S. (Eds.), Exotic Brome-grasses in Arid and Semiarid Ecosystems of the Western US: Causes, Consequences, and Management Implications. Springer: Series on Environmental Management, pp. 193–221, 2016.
Meyer, S.E., Franke, J.L., Baughman, O.W., Beckstead, J., Geary, B., 2014b. Does *Fusarium*-caused seed mortality contribute to *Bromus tectorum* stand failure in the Great Basin? Weed Res. 47, 511–519.
Meyer, S.E., Quinney, D., Nelson, D.L., Weaver, J., 2007. Impact of the pathogen *Pyrenophora semeniperda* on *Bromus tectorum* seed bank dynamics in North American cold deserts. Weed Res. 47, 54–62.
Meyer, S.E., Stewart, T.S., Clement, S., 2010. The quick and the deadly: growth vs. virulence in a seed bank pathogen. New Phytol. 187, 209–216.
Orrock, J.L., Damschen, E.I., 2005. Fungi-mediated mortality of seeds of two old-field plant species1. J. Torrey Bot. Soc. 132 (4), 613–617.
Smith, D.C., Meyer, S.E., Anderson, V.J., 2008. Factors affecting Bromus tectorum seed bank carryover in western Utah. Rangel. Ecol. Manag. 61 (4), 430–436.
Van der Putten, W.H., Bradford, M.A., Pernilla Brinkman, E., Voorde, T.F., Veen, G.F., 2016. Where, when and how plant–soil feedback matters in a changing world. Funct. Ecol. 30 (7), 1109–1121.
Weisberg, P.J., Dilts, T.E., Baughman, O.W., Meyer, S.E., Leger, E.A., Van Gunst, K.J., Cleeves, L., 2017. Development of remote sensing indicators for mapping episodic die- off of an invasive annual grass (*Bromus tectorum*) from the Landsat archive. Ecol. Indicat. 79, 173–181.