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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