Secondary Dormancy of Seeds in Relation to the *Bromus tectorum–Pyrenophora semeniperda* Pathosystem

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Abstract

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Bromus tectorum is a highly invasive annual grass. The fungal pathogen *Pyrenophora semeniperda* can kill a large fraction of *B. tectorum* seeds. Outcomes in this pathosystem are often determined by the speed of seed germination. In this paper we extend previous efforts to describe the pathosystem by characterising secondary dormancy acquisition of *B. tectorum*. In the laboratory approximately 80% of seeds incubated at –1.0 MPa became dormant. In the field, seeds were placed in the seed bank in late autumn, retrieved monthly and dormancy status determined. The field study confirmed the laboratory results; ungerminated seeds became increasingly dormant. Our data suggest that secondary dormancy is much more likely to occur at xeric sites.

Keywords: biological control; invasive annual grass

Bromus tectorum (cheatgrass) has invaded millions of hectares in western North America. Seed production on heavily invaded sites can approach 50 000 seeds/m^2 (Smith *et al.* 2008). The ascomycete fungus *Pyrenophora semeniperda* infects *B. tectorum* seeds, killing them through competition for endosperm reserves (Beckstead *et al.* 2007) and production of toxins that prevent cell division (Evidente *et al.* 2002). Our laboratory is investigating the *B. tectorum–Pyrenophora semeniperda* pathosystem, with the goal of developing *P. semeniperda* for use as a bio-herbicide against *B. tectorum* seeds. Following infection, the competitive outcome in this pathosystem is associated with host seed germination rate; rapidly germinating seeds escape death while slowly germinating or dormant seeds are killed (Beckstead *et al*. 2007). Characterising the *B. tectorum* seed dormancy cycle is therefore critical in understanding this pathosystem as well as optimising biological control efforts.

As a facultative winter annual grass, *B. tectorum* seeds are at least conditionally dormant at maturity. Populations lose primary dormancy through dry after-ripening, and are then able to

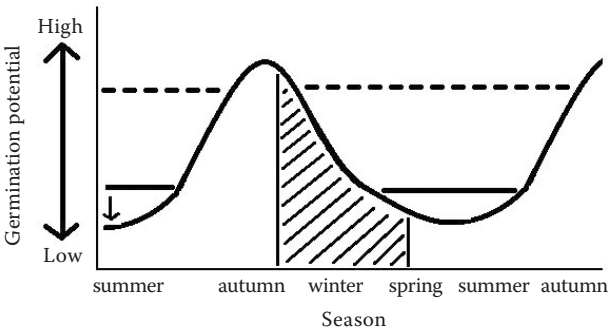

Figure 1. The annual dormancy cycle of *Bromus tectorum* seeds and vulnerability to *Pyrenophora semeniperda*. Seed populations mature in early summer (vertical arrow) and are characterised by high primary dormancy. The figure illustrates germination potential of ungerminated seeds during the first 15 months following maturation and dispersal. The hatched area beneath the curve indicates when seeds become secondarily dormant. Horizontal lines represent periods of maximum vulnerability to infection by *P. semeniperda* for a nonvirulent strain of fungus (solid lines) or a virulent strain (dashed lines). For a given strain of *P. semeniperda*, rapidly germinating, non-dormant seeds are more likely to escape death following infection because the growing embryo more quickly utilises endosperm reserves

germinate in the autumn with adequate precipitation (Figure 1). When precipitation is inadequate, seeds may become secondarily dormant and carry over across years as components of the soil seed bank. Secondarily dormant seeds are particularly vulnerable to attack by *P. semeniperda*.

Our efforts to predict current-year germination of *B. tectorum* seeds under both laboratory (Christensen *et al.* 1996; Bauer *et al*. 1998) and field (Meyer & allen 2009) conditions have been successful in characterising primary dormancy loss and germination during the summer and autumn. Here we report data sets that extend our understanding to account for the acquisition and loss of secondary dormancy.

MATERIAL AND METHODS

We conducted laboratory and field studies using two populations of *B. tectorum* seeds collected from separate sites (Whiterocks and Spanish Fork Farm, Utah) in June 2011. Seeds were cleaned and after-ripened under laboratory conditions; seeds were therefore in a non-dormant condition when these studies were conducted.

Laboratory study. Seeds collected from the Spanish Fork Farm site were randomly assigned to one of five water potentials (0, –0.5, –1.0, –1.5, or –2.0 MPa) and incubated at 5°C for four weeks. For each treatment, four replications of 25 seeds each were placed in covered Petri dishes on the surface of two blue germination blotters (Anchor Paper, St. Paul, USA) saturated with the appropriate solution. Germination was recorded on the first day of each week. After the allotted time, seeds in negative water potentials were switched to water and 20°C incubation. Seeds were scored for germination on days 1, 2, 4, 7, 11, 14, 21, and 28. Viability of ungerminated seeds was determined on day 28.

Field study. Seeds from each population were placed in each of 40 mesh bags (300 seeds/bag) on the soil surface under 2 cm of autoclaved *B. tectorum* litter at our xeric Whiterocks study site (40°19.680'N, 112°46.680'W elevation 1446 m, average annual precipitation of 19.9 cm) on November 8, 2012. At monthly intervals thereafter, subsamples (two bags from each population) were retrieved and returned to the laboratory. Seeds were scored for field germination, and remaining seeds were randomly assigned to one of two water potentials (0 or –1.5 MPa) and to one of two incubation temperatures (15 or 25°C). For each treatment, four replications of 25 seeds each were placed in covered Petri dishes on the surface of two blue germination blotters (Anchor Paper, St. Paul, USA) saturated with the appropriate solution. After 28 days, seeds at –1.5 MPa were transferred to water, and all dishes were incubated for an additional 28 days with germination scored as previously described.

RESULTS AND DISCUSSION

At 5°C *B. tectorum* seeds incubated in water (0 MPa) germinated almost entirely within the first week (Figure 2). Germination rates and percentages for seeds at negative water potentials were much lower than those in water, and were lowest for seeds in –1.0 MPa. This water potential is likely near the optimum for secondary dormancy induction in *B. tectorum*. Germination at –1.0 MPa was only 20% by the end of the experiment, indicating that 80% of the seeds became secondarily dormant after four weeks at this water potential. Secondary dormancy appears to be induced by a combination of low temperature and limited water availability.

In the field, above-zero temperatures coupled with intermittent moisture availability allowed approximately 5% of seeds to germinate during the first month following installation (Figure 3).

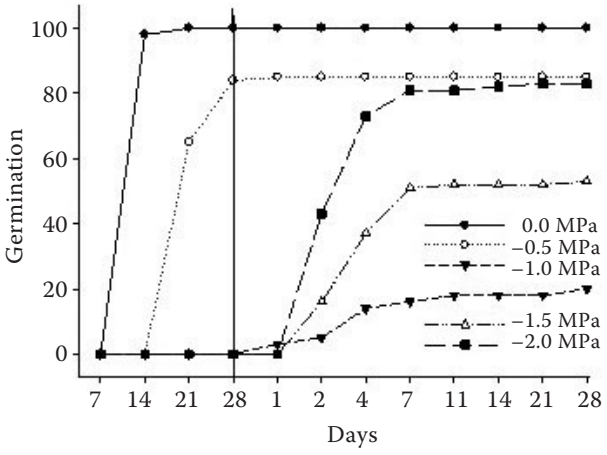

Figure 2. Laboratory germination percentages for initially nondormant seeds of *Bromus tectorum* seeds. Seeds were incubated at 5°C for 28 days at water potentials of 0, –0.5, –1.0, –1.5, or –2.0 MPa. The solid vertical line indicates the point at which the seeds were transferred from negative water potentials to water and then incubated at 20°C. Ungerminated seeds became secondarily dormant

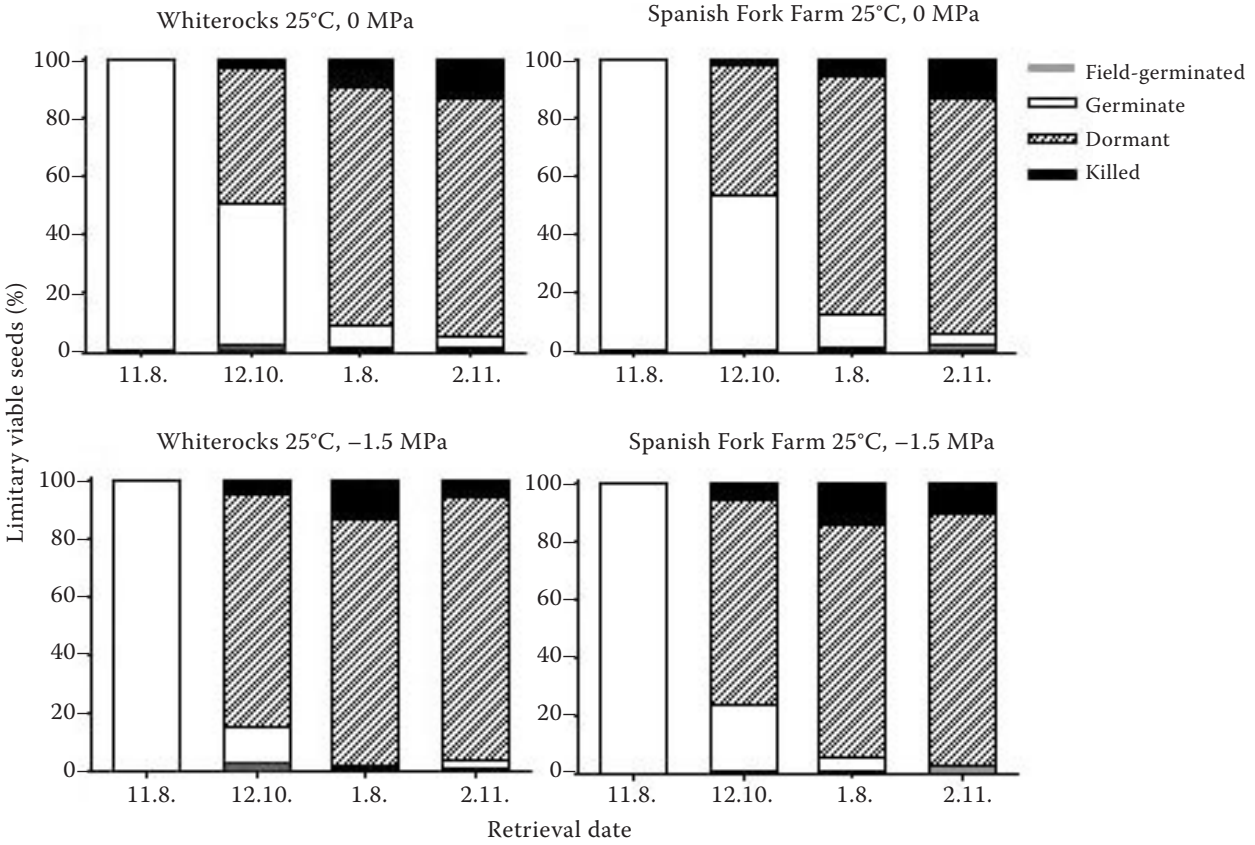

Figure 3. Secondary dormancy acquisition in the field for two populations of *Bromus tectorum* seeds. Seeds were initially non-dormant at 0 MPa, as illustrated by the left column (control – no exposure to field conditions) in each graph. Seeds were placed on the soil surface under 2 cm of litter on November 8, retrieved on the dates indicated, incubated in the laboratory at 25°C and water potentials of 0 or –1.5 MPa, then transferred to 0 MPa. Viability loss (killed seeds) was almost entirely due to attack by the fungus *Pyrenophora semeniperda*

Seeds in the field became increasingly dormant over time. Upon return to the laboratory following one month in the field, dormancy induction was more complete when seeds were incubated at –1.5 MPa prior to transfer to 0 MPa than when incubated directly at 0 MPa. This was observed with both seed populations and with post-retrieval incubation at 15°C as well (not shown). Following two months in the field, however, virtually all viable seeds were dormant regardless of the laboratory treatment used to assess dormancy. Results from the field study confirm findings in the laboratory experiment; namely, that acquisition of secondary dormancy is associated with a combination of low temperatures and limited water availability.

The timing and sufficiency of autumn precipitation determines whether a seed germinates, remains germinable, or enters secondary dormancy. Seeds that become secondarily dormant are highly susceptible to death from *P. semeniperda* (Finch *et al.* 2013b). This may help explain the high levels of killed seeds found in soil seed banks at xeric sites (Allen & Meyer 2013). Until seeds are released from secondary dormancy during late spring and summer (Allen *et al.* 2010), seeds remain highly vulnerable to infection and death by the fungus (Figure 1).

B. tectorum has invaded a wide range of habitats in the Western United States. In our attempts to characterise the *B. tectorum–P. semeniperda* pathosystem we have sampled many invaded sites (Allen & Meyer 2013). At almost all sampling locations, we found the presence of at least some seeds killed by *P. semeniperda*. However, the highest levels of killed seeds are associated with xeric sites. At these sites, the probability of intermediate hydration during summer and autumn, as well as secondary dormancy induction during late autumn and winter, are greatest (Finch *et al.* 2013b). These are all conditions that favour the fungus in the competition for seed endosperm storage reserves.

Therefore, use of *P. semeniperda* as a bio-herbicide may be most successful in xeric locations where incomplete autumn germination is likely, and a fraction of the seed population carries over by becoming secondarily dormant.

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