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Secondary Dormancy and Summer Conditions Influence Outcomes in the Pyrenophora semeniperda - Bromus tectorum Pathosystem

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ABSTRACT

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Variable mortality of *Pyrenophora semeniperda*–infected *Bromus tectorum* seeds has been referred to as a "race for survival." Dormant seeds are highly susceptible to *P. semeniperda* infection. While much is known about primary dormancy little is known about secondary dormancy in *B. tectorum* seeds. Dormancy status is not the only variable determining outcomes within the *Bromus* - *Pyrenophora* pathosystem. Varying temperature and intermittent hydration may strongly influence germination outcomes of *B. tectorum* in the presence of *P. semeniperda*. While it has long been assumed that *B. tectorum* seeds are infected by *P. semeniperda* in the fall it was recently suggested that seeds may be infected in the summer; however, there is little evidence to support this.

To further characterize the *Pyrenophora semeniperda* - *Bromus tectorum* pathosystem two studies were conducted to address the following: (1) characterization of secondary dormancy in *B. tectorum* seeds and (2) summer interactions between host and pathogen after summer inoculation. Studies were conducted using dormant and/or non-dormant *B. tectorum* (along with *B. rubens* in one study) seeds and two strains of *P. semeniperda*. Study one used laboratory and field experiments to characterize secondary dormancy in *B. tectorum* seeds in terms of temperature (0.5-20^{0}C), and water potential (-2.0-0 MPa). Data was used in repeated probit regression analysis to determine hydrothermal parameters ($\Psi_b(50)$, $\sigma_{\Psi b}$, θ_{HT}) for secondary dormancy induction and loss. In the second study seeds were inoculated with one of two strains of *P. semeniperda* then exposed to intermittent hydration or dry storage at warm temperatures (30-60°C). After treatment seeds were rehydrated and outcomes observed.

Optimum conditions for secondary dormancy induction were incubation at -1.0 MPa at 5°C. Seeds were likely to enter secondary dormancy through the cold winter months indicated by an increase or more positive $\Psi_b(50)$, while a decrease or more negative $\Psi_b(50)$ is associated with dormancy loss which is generally observed in the hot, dry summer months. When seeds were inoculated in the summer they only escaped death when summer conditions were ideal for after-ripening which allowed them to germinate rapidly under favorable autumn conditions. However, the pathogen caused high seed mortality no matter the treatment when disease progression advanced enough to inhibit seed germination.

Thus this research shows that in areas with frequent summer rain storms, it would be highly advantageous to apply *P. semeniperda* as a biocontrol on seeds at maturity.

Keywords: *Bromus tectorum*, disease development, secondary dormancy, embryo, germination, hydration, mortality, pathosystem, *Pyrenophora semeniperda*, water potential, carryover

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The role of low temperature and water potential in secondary dormancy of *Bromus tectorum* seeds as explained by hydrothermal modeling

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ABSTRACT

Bromus tectorum L. is a facultative winter annual that has invaded millions of hectares in western North America. This paper extends previous efforts to describe dormancy cycles of *B. tectorum* seeds by accounting for the induction and loss of secondary dormancy (i.e., the process whereby non-dormant seeds return to a dormant state). Laboratory and field experiments were conducted to characterize the induction of secondary dormancy, as well as create hydrothermal time models to quantify secondary dormancy induction and loss. We used two hydrothermal models; the first allowed mean base water potential ($\Psi_b(50)$) to vary while holding hydrothermal time (θ_{HT}) and standard deviation ($\sigma_{\Psi b}$) constant, and the second allowed all model parameters to vary through time. In the laboratory, seeds were exposed to various temperatures and water potentials. In the field, seeds were placed in the seed bank in late autumn and retrieved monthly to determine dormancy status. In the laboratory it was determined that optimum conditions for inducing secondary dormancy were incubation at -1.0 MPa at 5°C where a maximum of 78% of seeds became dormant. The field study confirmed the laboratory results: ungerminated seeds became increasingly dormant through the winter at low temperatures and water potentials. Hydrothermal parameters were determined from lab and field experiments. Parameters were used to create predictive germination time courses which were compared to observed germination. Results from this study illustrate that hydrothermal modeling effectively characterizes secondary

dormancy induction and loss, and mean base water potential is an effective index of seed germination progression.

KEYWORDS

invasive weed, seed dormancy cycle, seed bank, secondary after-ripening

INTRODUCTION

Seed dormancy affects both germination rate and percentage (Allen and Meyer 2002), and reduces accuracy in predicting germination timing (Batlla *et al*., 2007; Bochenek *et al*., 2007; Batlla and Benech-Arnold, 2010). Defined as the condition of a seed that inhibits germination under otherwise sufficient environmental conditions, dormancy can be classified as primary (i.e., present in seeds at maturity) or secondary (i.e., induced in previously non-dormant seeds or re-induced in seeds that have lost primary dormancy) (Benech-Arnold *et al*., 2000). Dormancy is not an "all or nothing" seed characteristic, but varies over a continuum between some maximum and minimum level (Batlla *et al*., 2004). Dormancy levels can vary among seeds within a population (Bradford, 2002; Batlla *et al*., 2004), as well as seasonally throughout the year (Finch-Savage and Leubner-Metzger, 2006).

Like many other winter annuals, the grass *B. tectorum* L. produces seeds with high primary dormancy upon maturity in early summer (Benech-Arnold, 2000; Bair *et al.,* 2006), which is lost through dry-after-ripening in as little as four weeks (Christensen *et al.*,1996). Seeds can then enter secondary dormancy during winter if conditions are not favorable for germination in autumn. Because *B. tectorum* seeds lose secondary dormancy through the same dry summer

conditions that result in loss of primary dormancy (i.e., after-ripening), this paper will refer to the loss of secondary dormancy as secondary after-ripening.

B. tectorum has invaded millions of hectares in the western United States (Meyer *et al*., 2006). This weed finds success through its ability to dominate an area after disturbance (Knapp, 1996), and further hinders native re-establishment through rapid germination, an ability to produce root growth even at cold winter temperatures (Aguirre and Johnson, 1991; Pyke and Novak, 1994), and a capacity to remain viable in the seed bank across years via dormancy (Meyer *et al.,* 2006).

Volumes have been written to capture fundamental characteristics of seed dormancy (Baskin and Baskin, 1996; Allen *et al*., 2006). Population based threshold models are increasingly popular as they allow simple mathematical equations to describe dormancy status, including primary dormancy release (Steadman and Pritchard, 2004; Alvarado and Bradford, 2005; Bair *et al*., 2006; Wang *et al*., 2009; Ichihara *et al*., 2009).

Hydrothermal time is a population-based threshold model that describes time-normalized progress towards germination as a function of incubation temperature and water potential. The use of hydrothermal time to describe seed germination was introduced by Gummerson (1986) and expanded by Bradford (1990, 1995). Key concepts of this modeling technique include a base or threshold temperature and water potential below which seeds do not germinate. Thus the time to germination for a specific fraction of a seed population can be calculated using the following equation:

$$\theta_{HT=}(\Psi-\Psi_b(g))(T-T_b)t_g \quad (1)$$

where θ_{HT} is the hydrothermal time requirement for the germination (hydrothermal time constant), Ψ and T are the actual water potential and temperature of the environment, Ψ_b is the base water potential for germination fraction g, T_b is the base temperature for hydrothermal time to accumulate, and t is the actual time to germination for fraction g of the given population.

To extend this model to account for an entire population Gummerson assumed that the distribution of base water potentials, the variable factor in a population, is approximately normal with mean base water potential ($\Psi_b(50)$) and standard deviation ($\sigma_{\Psi b}$). This assumption allowed him to use probit transformation, which linearizes the cumulative normal distribution associated with germination time courses, to characterize germination for the entire seed population by the equation:

$$\text{Probit}\ (g/g_m) = [(\Psi - \Psi_b(50) - \theta_{HT})/((T-T_b)t_g]/\sigma_{\Psi b} \qquad (2)$$

where (g/g_m) is the fraction of viable seeds in the given population, and $\sigma_{\Psi b}$ is the standard deviation of the $\Psi_b(50)$)for fraction g of the population.

The mean base water potential $\Psi_b(50)$ has been shown to be a valuable index for characterizing seed populations with both ecological (Allen *et al,*. 2006; Köchy and Tielbörger, 2007) and physiological (Bradford 1986; Groot and Karssen, 1992) relevance. More recently, changes in $\Psi_b(50)$ have been used to describe primary dormancy release through dry after-ripening (Christensen *et al*., 1996; Bair *et al*., 2006). Here we aim to extend these models to account for the induction and release of secondary dormancy in *B. tectorum* seeds under field conditions. As a secondary objective, we report on laboratory experiments designed to test the hypothesis that

the key factors responsible for inducing secondary dormancy included the combination of low temperatures and low water potentials.

MATERIALS AND METHODS

This study was carried out in three phases. In the first phase we tested our hypothesis that a combination of low temperatures and water potentials will induce secondary dormancy. In the second phase we conducted laboratory experiments to establish hydrothermal parameters for two seed populations. In the third phase we carried out a field retrieval experiment to determine *in situ* dormancy changes and obtain data to validate the model for secondary dormancy induction and release.

General Approach to Experimental Design and Statistical Analysis

Experimental data were analyzed as fully randomized designs using the analysis of variance (ANOVA) procedure of SAS 9.2, 2007 (SAS Inc., Cary, North Carolina, USA). Data were arcsine transformed for analysis to account for heterogeneity of variance; however, original means are reported. All experiments in this study included factorial combinations for each treatment as subsequently described.

Laboratory Experiments

All seeds used in laboratory experiments were cleaned by hand after collection and allowed to after-ripen under ambient laboratory conditions, rendering seeds non-dormant at the onset of each experiment.

Secondary dormancy induction at 5°C in various potentials

Bromus tectorum seeds were collected in June 2011 from the Brigham Young University research farm (Spanish Fork, Utah, USA). To test our hypothesis that a combination of low temperatures and low water potentials would induce secondary dormancy, seeds were incubated (5°C, continual darkness) for 28 days at one of five water potentials (0, -0.5, -1.0, -1.5, or -2.0 MPa) obtained using solutions of polyethylene glycol 8000 (Michel and Kaufmann1972, 1983). Solution water potentials were verified with a Water Activity Meter (Dewpoint PotentiaMeter WP4, Decagon Devices, Pullman, Washington, USA). For each treatment, four replications of 25 seeds each were placed in covered 100 mm Petri dishes on the surface of two blue germination blotters (Anchor Paper, St. Paul Minnesota, USA) saturated with the appropriate solution. In order to minimize any changes in water potential due to drying, petri dishes were stacked in sealed plastic sleeves on top of a water-saturated paper towel before being placed in an incubator. Germination (radicle emergence ≥ 1mm) was recorded weekly. After 7,14 or 28 d, seeds in negative water potentials were transferred to water and incubated (20°C, alternating 12 h fluorescent light / 12 h dark) for an additional 28 d. Seeds were scored for germination on days 1, 2, 4, 7, 11, 14, 21, and 28. Remaining seeds were considered viable and dormant on day 28 if firm when pressed which we have found to be comparable to a cut test (Ooi et al., 2004) for *B. tectorum* seeds.

Secondary dormancy induction of multiple genotypes at low temperatures and various water potentials

To test whether lower temperatures would improve secondary dormancy induction, four genotypes were subjected to a factorial combination of low temperatures and water potentials. Seeds were collected from four individual breeding lines from greenhouse grown plants in 2010. Specific lines were chosen to compare dormancy induction of two salt desert genotypes (Hot Springs Mountains, Nevada; Dugway Proving Grounds, Utah) with two cold desert generalist genotypes (Desert Experimental Range; Cricket Mountains, Utah). All seed lots had an initial viability >95% at the onset of the experiment.

Seeds from the four genotypes were incubated in each of five water potentials (0, -0.5, -1.0, -1.5, and -2.0) at one of three low temperatures (0.5, 2, and 5°C). For each treatment, four replications of 25 seeds were placed in covered 100 mm Petri dishes and incubated for 28 d. After 28 d seeds in negative water potentials were transferred to water and incubated (20°C, alternating 12 h fluorescent light/ 12 h dark) for an additional 28 d. Germination was recorded as previously described.

Field Study

Seeds from two populations of *B. tectorum* were collected in June 2011: one from the Brigham Young University research farm (Spanish Fork, Utah, USA) and the other from Tooele County, (Whiterocks, Utah, USA). To test whether results from laboratory studies could be repeated in the field, on November 8, 2012 seeds from each population were divided and placed into 40 nylon mesh bags (300 seeds/bag). Bags were placed on the soil surface (after the top 2 cm of

soil were removed in order to reduce *in situ* seed mortality caused by *Pyrenophora semeniperda)* under 2 cm of autoclaved *B. tectorum* litter at the Whiterocks study site (40° 19.680'N 112° 46.680'W elevation 1446 m, average annual precipitation of 19.9 cm). Bags and litter were held in place by nylon mesh secured with metal nails. A digital data logger (Em50, Decagon Devices, Pullman, Washington) was installed at the site immediately adjacent to the field plot, which recorded hourly temperature and soil water potential during the duration of the experiment using a soil water content sensor (5TM Soil Moisture and Temperature Sensor, Decagon Devices, Pullman, WA).

Subsamples (two random bags from each population) were retrieved and returned to the laboratory at monthly intervals beginning December 10, 2012 and ending August 8, 2013. Retrieved seeds were randomly assigned to one of three laboratory incubation water potentials (0, -0.5, or -1.5 MPa) and one of two incubation temperatures (15 or 25°C) in order to determine if these variables would contribute to the expression of secondary dormancy induction. A subset of seeds (0-22%), ones which had germinated in the field or been killed by *P. semeniperda* were removed from the retrieval set. For each treatment, four replications (an equal number of the remaining seeds, generally at least 20) of each seed population were placed in covered Petri dishes on the surface of two blue germination blotters (Anchor Paper, St. Paul Minnesota, USA) saturated with the appropriate solution. After 28 d, seeds at low water potentials were transferred to water and incubated at the same incubation temperatures of 15 or 25°C for an additional 28 d. The following seed outcomes were recorded on days 1, 2, 4, 7, 11, 14, 21, and 28: germination (radicle emergence ≥ 1mm), and seed dormancy (ungerminated but viable as determined by firmness of seeds).

Hydrothermal Model Development

Hydrothermal time equation determination

The parameters $\theta_{HT,}$ $\sigma_{\Psi b}$ and $\Psi_b(50)$ for fully after-ripened seeds of each seed population were determined as described in detail by Christensen *et al*. (1996). A probit model was created for each seed collection by collapsing germination curves obtained from incubation at two constant temperatures (15 and 25°C) and a range of water potentials (0, -0.5, -1.0, -1.5, -2.0 MPa) into a single regression. This resulted in an estimated θ_{HT}, $\sigma_{\Psi b}$ and $\Psi_b(50)$ value for each population.

Modeling technique 1

We used two different approaches to characterize acquisition and loss of secondary dormancy in the field using hydrothermal concepts. The first model assumed that θ_{HT} and $\sigma_{\Psi b}$ remained constant across field retrievals, while $\Psi_b(50)$ was allowed to fluctuate as an indication of dormancy status. These assumptions were previously validated for primary dormancy loss in *B. tectorum* (Christensen *et al*., 1996; Bair *et al*., 2006). Hydrothermal parameters were initially calculated at the onset of the experiment for the Spanish Fork and Whiterocks populations. When germination was > 50% for viable field-retrieved seeds imbibed in water, time to 50% relative germination was determined from germination time courses. In order to calculate $\Psi_b(50)$ the following relationship was used:

$$\Psi_b(g) = -\theta_{HT}/ (T(t_g)) \quad (3)$$

Values for t_{50}, were determined using linear interpolation between the two points surrounding the 50% fraction. When final germination did not reach 50% of viable seeds, but was between 5 and 50%, the time to reach 75% relative germination was used as described in detail by Bauer *et al*. (1998) with the following equation to determine the $\Psi_b(g)$ of the relative 75% fraction

$$\Psi_b(g) = \Psi_b(50) + (\text{probit}(g))(\sigma_{\Psi b}) \quad (4)$$

By substituting the calculated value for $\Psi_b(g)$, $\sigma_{\Psi b}$, which both come from the slope of the regression line, and the probit value for the corresponding 75% fraction of the given seed population into the equation we were able to estimate $\Psi_b(50)$.

Modeling technique 2

As an alternative to allowing only $\Psi_b(50)$ to change we also used the same data set but recalculated all parameters ($\theta_{HT,}$ $\sigma_{\Psi b}$ and $\Psi_b(50)$) following each seed retrieval for laboratory-incubated seeds. We included this technique because an inherent limitation in using hydrothermal concepts to model secondary dormancy induction involves the simultaneous processes of hydrothermal time accumulation and secondary dormancy induction. Thus in our second modeling technique we assumed θ_{HT} changed over time as seeds progressed towards germination, while $\Psi_b(50)$ changed as secondary dormancy was induced in seeds. We acknowledge that an inherent limitation with this approach is that hydrothermal time accumulation and secondary dormancy induction occur simultaneously and cannot be distinguished one from the other.

Germination curve predictions

To evaluate the success of each model, we compared predictions of germination time courses for each of the hydrothermal models with actual observed germination for laboratory-germinated seeds following retrieval from the field.

In order to create prediction curves using each of the modeling techniques mentioned above, probit values for various incubation times were calculated using equation (2). For modeling technique 1, where only $\Psi_b(50)$ changed across retrievals, new $\Psi_b(50)$ values for each seed retrieval were used in equation (2) along with the original values of θ_{HT} and $\sigma_{\Psi b}$. Using technique 2, where all hydrothermal parameters changed, new values of $\theta_{HT,}$ $\sigma_{\Psi b}$ and $\Psi_b(50)$ were estimated and used in equation (2). Probit values were transformed into the corresponding germination fractions. Predicted t_g values were compared with actual germination time course curves for each treatment combination.

RESULTS

General Approach to Experimental Design and Statistical Analysis

All findings reported in the results were statistically significant at the $P<0.01$ level unless otherwise noted.

Laboratory experiments

Secondary dormancy induction at 5°C in various water potentials

Seeds incubated at 20°C did not become dormant. They either germinated under initial incubation conditions or following transfer to water (Figure 1A). Seeds incubated at 5°C temperatures and water potentials for 7 or 14 d before transfer to water showed similar results; seeds failed to become dormant (Figure 1B, C); however, seeds incubated at 5°C at low water potentials for 28 d had a decrease in total germination of viable seeds, which indicated that many of the seeds had been induced into secondary dormancy (Figure 1D). Dormancy levels varied significantly by Ψ where maximum induction of dormancy occurred at -1.0 MPa. No secondary

dormancy induction was observed in any low water potentials in other treatments indicating the significance of the treatment time x Ψ interaction. Seeds incubated in water at 0 MPa and 5°C failed to enter secondary dormancy, as indicated by complete germination (Figure 1D). These data suggest an optimum water potential of -1.0 MPa for inducing secondary dormancy at 5°C, at least for the Spanish Fork collection.

Secondary dormancy induction for multiple genotypes at low temperatures and various water potentials

Incubation at 5°C was the best for inducing secondary dormancy of multiple genotypes (Figure 2). All seed genotypes incubated at 0.5°C had high germination after transfer to water (Figure 2; A, B, C, D). Seeds incubated at 2°C showed a slight decrease in germination, but all populations still germinated to above 80% (Figure 2 E, F, G, H). As in the first experiment, significant secondary dormancy induction was only observed at 5°C, thus indicating that lower temperatures do not lead to increased dormancy under the experimental conditions used (Figure 2 I, J, K, and L).

Incubation at -1.0 MPa was the most successful at inducing secondary dormancy in the Hot Springs Mountains genotype, where only 40% of the seeds germinated. However, -1.5 MPa was slightly more effective for the Dugway Proving Grounds genotype. Seeds from the Desert Experimental Range had a small decrease in germination at -1.5 MPa while Cricket Mountain seeds were not induced into secondary dormancy under any conditions included.

Field Experiment

Soil water potentials remained close to -1.0 MPa much of the time during the first six weeks in the field (Figure 3), and diurnal temperature fluctuations encompasses 5°C for much of the same period. Thus, while highly variable, the field soil conditions still approximated those that produced secondary dormancy induction in the laboratory. The soil surface froze in mid-December and by early January we observed snow cover to a depth of approximately 30 cm. These conditions kept the soil frozen at an unsaturated soil moisture condition until mid-March, when the snow melted and saturated the soil for approximately 10 days (not shown). Soil gradually dried to <-0.5 MPa by mid-May, and remained dry and warm (20-60 degrees) throughout the summer months.

Seeds were 100% germinable before being placed in the field on Nov 8, 2013 (indicated by control treatments on Figure 4 A-F), but germinability varied significantly depending upon retrieval date. Seeds became increasingly dormant through the winter and early spring months, with a loss of dormancy during summer. By March > 95% of seeds that had not germinated in the field or been killed by the fungus *Pyrenophora semeniperda* (<5%) were dormant, and a majority of the seeds remained dormant until June. At this point retrieved seeds increased in total germination percentage until August, when 100% of seeds imbibed in water germinated.

Water potential had a significant effect on seeds retrieved from the field and incubated in the laboratory. Upon return to the laboratory following one month in the field, expression of dormancy induction was more complete when seeds were incubated at -0.5 or -1.5 MPa (Figure 4 C, D, E, and F) than when incubated directly at 0 MPa. This was observed with both seed

populations and with post-retrieval incubation at 15°C or 25°C (only data for 25°C are shown, since results at 15°C did not differ statistically, P=.31). Following two months in the field, however, virtually all viable, ungerminated seeds were dormant regardless of post-retrieval incubation conditions. Seeds remained dormant over the period of January to June, after which seeds became increasingly germinable in water. By August, a majority of the seeds had lost dormancy and germinated. The small subset of seeds which did not germinate were those incubated at low water potentials in the laboratory, suggesting that secondarily after-ripened seeds remain sensitive to low water potentials. Results from the field study confirm laboratory findings: acquisition of secondary dormancy is associated with a combination of low temperatures and limited water availability.

Hydrothermal Model Development

Hydrothermal time equation determination

Hydrothermal parameters were calculated for both seed populations (Whiterocks and Spanish Fork). At the onset of the field experiment, θ_{HT} values were similar for both seed populations (Table 1). However, $\sigma_{\Psi b}$ for Spanish Fork was approximately half the value of the Whiterocks collection, which indicates higher uniformity of seed germination. Since $\Psi_b(50)$ was much lower for Whiterocks than Spanish Fork (-1.61 vs. -1.35 respectively) Whiterocks seeds progressed more rapidly towards germination at lower water potentials than did seeds of the Spanish Fork collection.

Modeling technique 1

Our first modeling technique required that only $\Psi_b(50)$ (of water-germinated seeds) be re-calculated after each seed retrieval to account for seeds entering and exiting secondary dormancy. Because germination of at least 5% was required to calculate hydrothermal time parameters, we were unable to calculate $\Psi_b(50)$ during winter and spring months. This is indicated by missing data from March to May (February – June for 25C incubation; Figure 5; Table 1). $\Psi_b(50)$ values for both seed populations were initially low, but increased dramatically as seeds were induced into dormancy through the winter. Values again but decreased as seeds lost dormancy through secondary after-ripening as summer progressed (Figure 5). Whiterocks seeds had an initially lower $\Psi_b(50)$ value than Spanish Fork at -1.62 MPa, but eventually rose higher than Spanish Fork seeds to above 0.5 MPa (Table 1). Seed germination is prohibited when Ψ_b rises above 0. Thus, a majority of both seed populations entered dormancy after one month in the field (December) and lost dormancy by July as $\Psi_b(50)$ values decreased to below 0. $\Psi_b(50)$ values in July and August were not as low as they were in the previous November, which helps to explain why seeds remained sensitive to incubation at low water potentials.

Modeling technique 2

This modeling technique required that all hydrothermal parameters be re-calculated after each seed retrieval. Whiterocks and Spanish Fork seeds displayed similar trends as in modeling technique 1; specifically, $\Psi_b(50)$ increased through the winter months and decreased through the summer months, while changes in hydrothermal time were in the opposite direction (Table 2). Due to high seed dormancy, parameters could not be calculated for seeds retrieved from February through June. By retrieval 9 (August) the θ_{HT} had increased significantly while $\Psi_b(50)$

had decreased to well below 0, indicating that seeds were losing dormancy and able to germinate under a larger range of conditions (i.e. lower water potentials). All regressions run were statistically significant with the exception of the regression for Spanish Fork seeds in the January retrieval (R=.23), which is likely due to an extremely small sampling size, n = 8.

Germination curve predictions

Comparison of predicted versus observed values showed that both modeling techniques allowed for good prediction in most cases (Figure 6). Modeling technique 2 was more accurate in December for three out of four germination curves (Figure 6 A, I, C, D). This result was due to the conflict of the simultaneous process of hydrothermal time accumulation and secondary dormancy induction. Modeling technique 2 better predicts germination in this situation as it accounts for hydrothermal time accumulation, while modeling technique 1 assumes that hydrothermal times stays constant. However, modeling technique 1, which was the simplest approach, was at least as accurate at predicting germination at all other times.

DISCUSSION

Under constant laboratory conditions secondary dormancy induction was most successful at -1.0 MPa and a temperature of 5°C. In the field temperatures and water potentials fluctuated around these same conditions, as temperatures became colder and water potentials more negative during the first six weeks. These conditions still rendered a large fraction of the seeds dormant after one month and all previously ungerminated seeds dormant after two months. Field results confirm laboratory results: namely, that secondary dormancy is induced via low temperatures and low

water potentials under fluctuating conditions as well as constant conditions. Thus we can detect seed dormancy changes in the lab or field even when conditions aren't exactly the same.

B. tectorum populations vary in their susceptibility to enter secondary dormancy. Results from multiple studies (reviewed by Allen *et al.* 2013) suggest that seed populations from drier sites are more likely to enter secondary dormancy than those from mesic sites, which is supported by the very limited set of genotypes included in the present study. Two *B. tectorum* seed populations which showed a high degree of secondary dormancy induction were the desert populations (Hot Springs and Dugway) while the shrubland (i.e., more mesic; Cricket Mountain) population displayed lack of dormancy.

The ability of seeds to become secondarily dormant affects seedbank carryover across years. The predisposition of seeds to go secondarily dormant differs among genotypes as Weber *et al*. (2010) reported for oilseed rape. However, it may also be possible that some *B. tectorum* seed populations cannot be induced into secondary dormancy, which may explain why seeds from some sites (e.g., mesic sites) have little carryover. In this study as temperatures rose in the field and soils neared saturation in early spring seeds did not germinate, because they were dormant. Secondary dormancy does not prepare seeds for germination in the spring but rather for successful germination the following autumn. Only seeds which do not enter dormancy in the winter are capable of spring germination, as we have observed in the field following dry winters (S. Meyer, unpublished). Hence some *B. tectorum* populations have much greater carryover than others (Smith *et al*., 2008).

The successful use of $\Psi_b(50)$ to indicate secondary dormancy status (modeling technique 1 herein) adds further support to the value of using this parameter as an index of germination status in several contexts (Christensen *et al*., 1996; Bauer *et al*., 1998; Bair *et al.,* 2006; Meyer and Allen, 2009; Alvarado and Bradford, 2005; Meyer *et al*., 2000). The second and more complex modeling technique involved re-calculation of all hydrothermal parameters to describe seed dormancy status, which has rarely been used (Bazin *et al*., 2010) but may produce more accurate predictions in some cases. Though using $\Psi_b(50)$ as the index has been established as a reputable standard, secondary dormancy has some inherent complexities that are hard to account for; as seeds accumulate hydrothermal time they will in some situations (winter conditions) be induced to secondary dormancy. These two events can occur simultaneously making it impossible decipher between the two different processes. The second modeling approach does a better job accounting for each of these processes. Results suggest that "fast" seeds germinate while "slow" seeds go dormant. As hydrothermal time accumulates in the field, the apparent θ_{HT} is reduced for seeds which have not gone dormant. On the other hand $\Psi_b(50)$ increases which accounts for the seeds which have been induced into secondary dormancy.

During November and December field conditions became more non-optimal for germination so that only the "fast" seeds accumulated sufficient hydrothermal time to germinate, while the slowest seeds apparently failed to escape the dormancy re-inducing mechanisms. These mechanisms likely have a molecular explanation. Jones (1997) and Holdsworth (1999) determined that in wild oat seeds reinduction of dormancy by exposure to non-optimal germination conditions is associated with synthesis of A*f*VP1 transcripts. The so called "fast" seeds don't react to the secondary dormancy cues and thus do not respond to these transcripts.

Modeling technique 2 was the best predictor for germination curves following one month in the field and modeling technique 1 was best for all subsequent months. After one month many of the seeds had been induced into secondary dormancy, but the "fastest" seeds had accumulated enough hydrothermal time to germinate. Following one month in the field, the first modeling technique was a better predictor of seed germination because hydrothermal was no longer accumulating in the cold. Over the winter and into the summer months it is likely the hydrothermal was lost or "forgotten", as occurs when imbibed seeds that have nearly completed germination are subjected to rapid drying (Debaene-Gill *et al.* 1994).

To our knowledge, this is the first time hydrothermal modeling has been used to explain secondary dormancy induction and loss in seeds of any kind. Previous hydrothermal modeling efforts have helped to explain primary dormancy loss in a variety of species (Christensen et al., 1996; Bauer, 1998; Bair *et al.,* 2006; Meyer and Allen, 2009; Alvarado and Bradford, 2005; Meyer *et al*., 2000; Gianinetti and Cohn, 2007). Here we found that changes in $\Psi_b(50)$ are useful for predicting secondary dormancy induction and loss as has previously been reported for primary dormancy. The conceptual simplicity of the hydrothermal time approach to predict seed germination modeling makes it a logical tool for examination of secondary seed dormancy acquisition and loss in other species.

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TABLES

Table 1

Changes in mean base water potential ($\Psi_b(50)$) for field-retrieved *Bromus tectorum* seeds during secondary dormancy induction and release using modeling technique 1, where $\Psi_b(50)$) was allowed to change while holding other parameters (θ_{HT} and $\sigma_{\Psi b}$) were held constant. Seeds were incubated at either 15 or 25°C. Missing data and missing months (March-May) indicate insufficient germination occurred to estimate $\Psi_b(50)$.

	$\Psi_b(50)$ (Initial)		$\Psi_b(50)$ (December)		$\Psi_b(50)$ (January)		$\Psi_b(50)$ (February)		$\Psi_b(50)$ (June)		$\Psi_b(50)$ (July)		$\Psi_b(50)$ (August)	
Population	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C
	MPa													
Whiterocks	-1.62	-1.62	-0.95	-0.09	0.60	0.72	-	-	0.17	-	-0.98	-0.10	-0.77	-0.45
Spanish Fork Farm	-1.35	-1.35	-0.87	-0.11	-0.18	0.15	0.21	-	-	-	-0.27	0.09	-0.50	-0.40

Table 2

Changes in hydrothermal time parameters for two *Bromus tectorum* seed collections when all model parameters were allowed to change during secondary dormancy induction and release (Modeling technique 2). Missing data and missing months (March-May) indicate insufficient germination occurred to estimate $\Psi_b(50)$. Units of measurement are MPa-degree-days for θ_{HT} and MPa for $\sigma_{\Psi b}$ and $\Psi_b(50)$.

	Initial				December				January				August			
	θ_{HT}	$\sigma_{\Psi b}$	$\Psi_b(50)$	R^2	θ_{HT}	$\sigma_{\Psi b}$	$\Psi_b(50)$	R^2	θ_{HT}	$\sigma_{\Psi b}$	$\Psi_b(50)$	R^2	θ_{HT}	$\sigma_{\Psi b}$	$\Psi_b(50)$	R^2
Whiterocks	66	.59	-1.61	.75	11	.54	-.16	.87	-	-	-	-	46	.29	-.51	.79
Spanish Fork Farm	56	.26	-1.35	.79	12	.30	-.02	.90	6	.29	-.04	.23	66	.39	-.74	.78

FIGURES

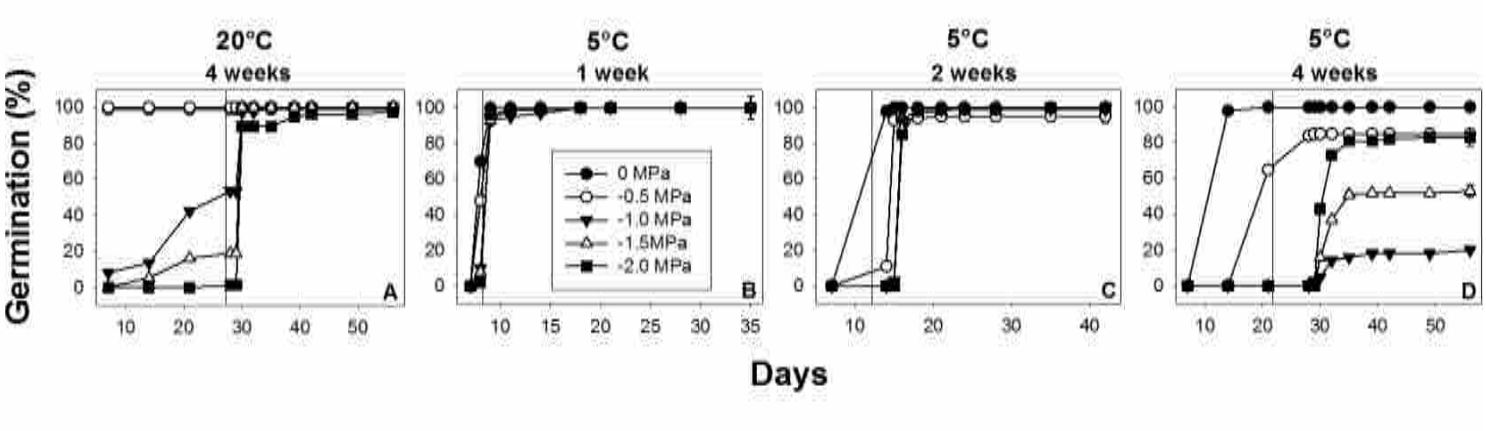

Figure 1

Secondary dormancy induction of seeds subject to various temperatures and water potentials as indicated by reduced germination. *Bromus tectorum* seeds collected from the Brigham Young University research farm (Spanish Fork, Utah, USA) were incubated at 20° C in water for 28 days (A) or at 5° C for either 7 (B), 14 (C) or 28 days (D) at various water potentials (0, -0.5-1.0, -1.5, -2.0 MPa). After 7(B), 14(C), or 28 days (A, D) seeds at all negative water potentials were transferred to water and incubated for an additional 28 days at 20° C (transfer represented by the vertical line on each graph).

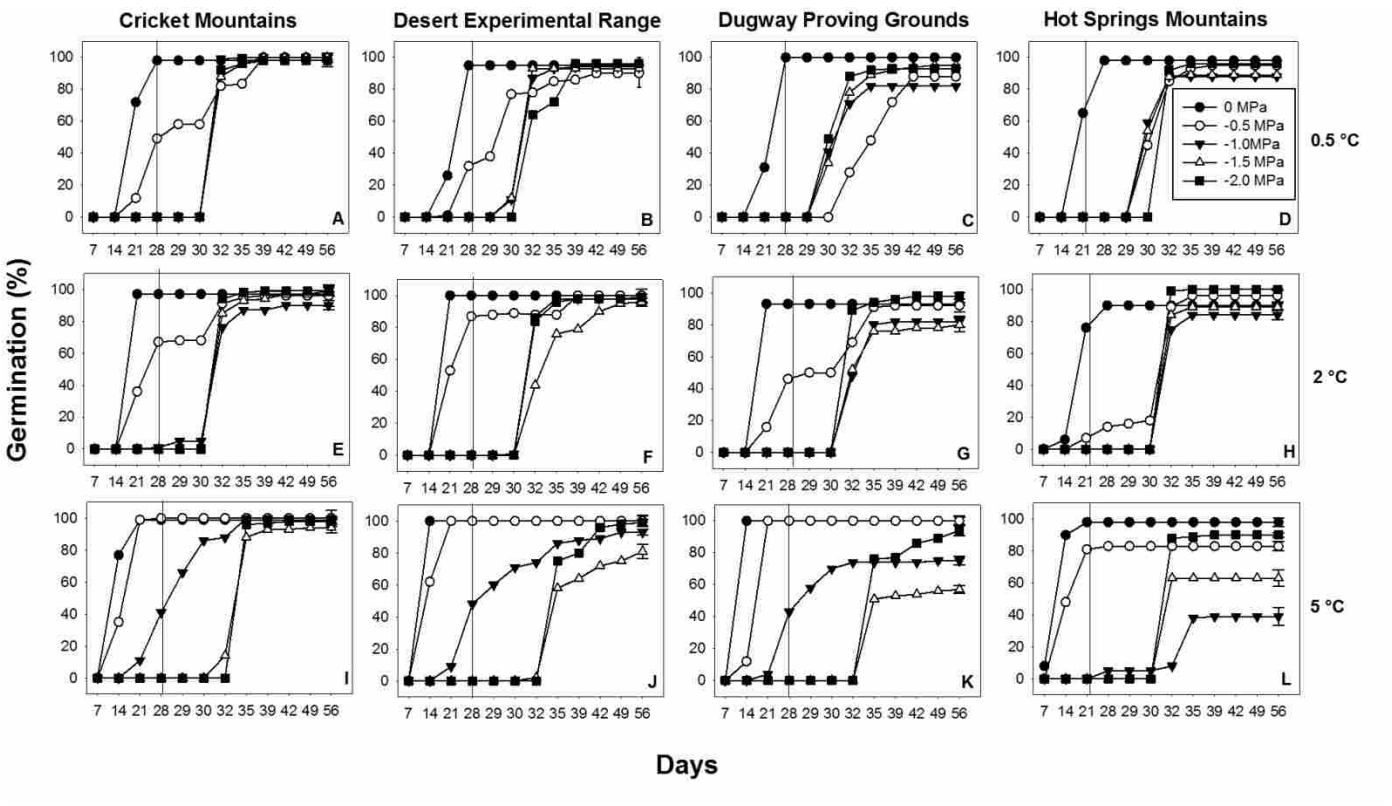

Figure 2

Secondary dormancy induction as indicated by reduced germination in four genotypes of *Bromus tectorum* seeds subject to near-freezing temperatures and various water potentials. Seeds originally collected from four sites: Cricket Mountains, Utah (A,E,I); Desert Experimental Range, Utah (B, F, J); Dugway Proving Grounds, Utah (C, G, K); and Hot Springs Mountains, Nevada (D, H, L) were incubated at various water potentials (0, -0.5, -1.0 , -1.5 -2.0 MPa) and low temperatures: 0.5°C (A-D), 2°C (E-H), 5°C (I-L), for 28 days. Seeds were then transferred from low temperatures and water potentials to free water and incubated at 20°C for another 28 days (represented by the vertical line on each graph). Following transfer, seeds were scored for germination (radicle > 1mm).

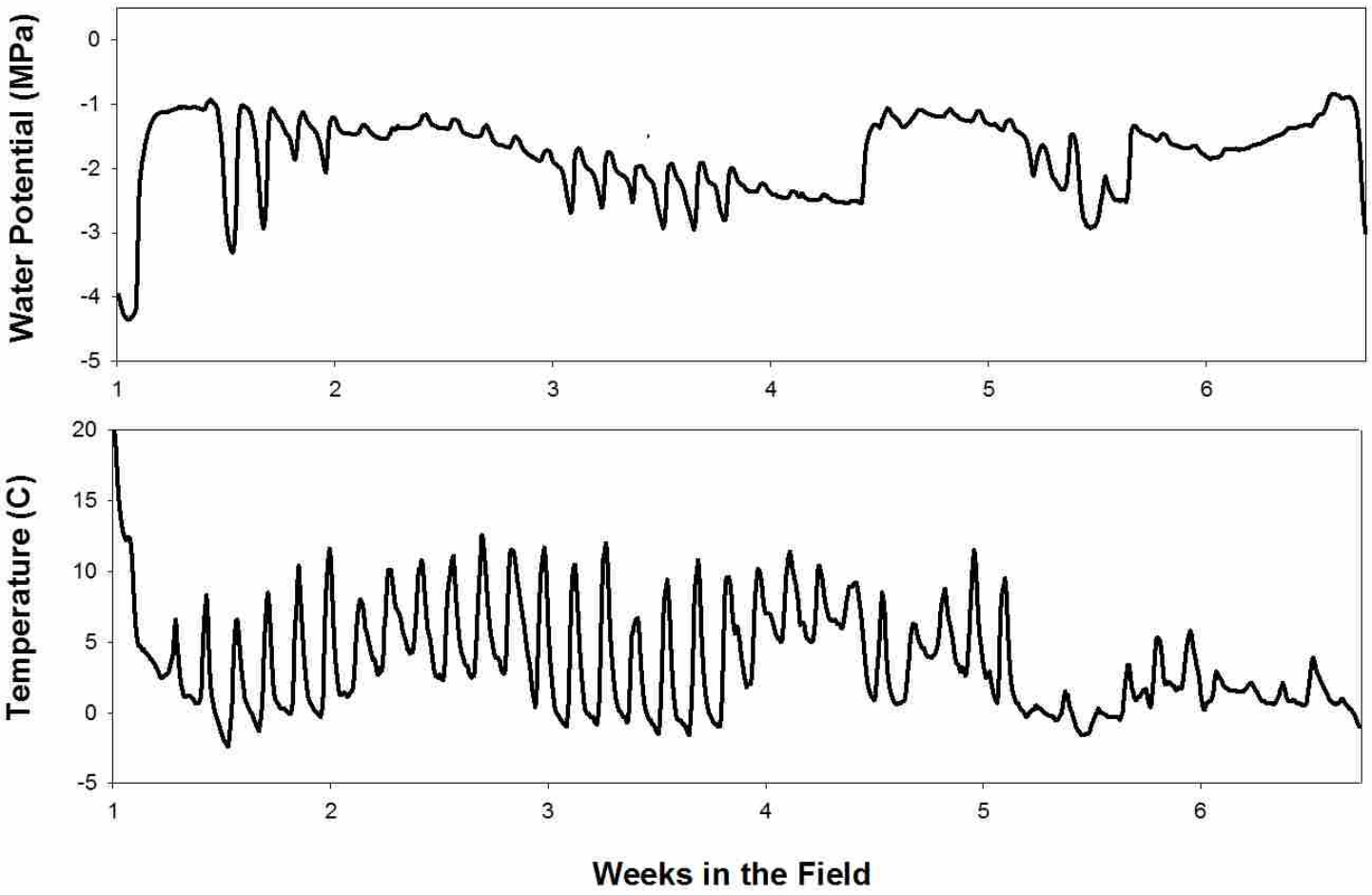

Figure 3

Field temperatures (top graph) and water potentials (bottom graph) at the Whiterocks study site measured at a depth of 1 cm for the 6-week period from November 8, 2012 to December 19, 2012. This is the period during which secondary dormancy induction was induced.

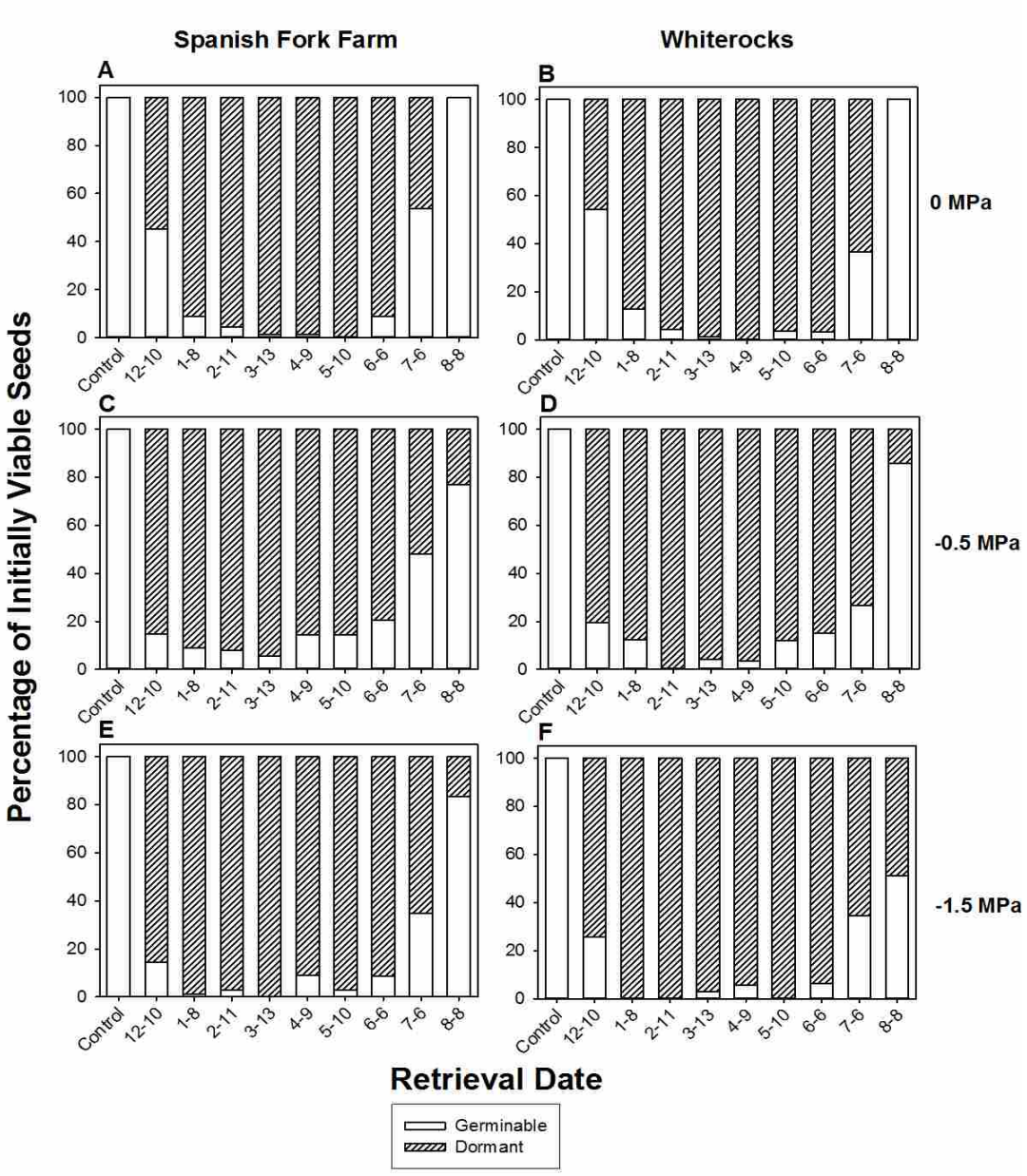

Figure 4

Status of ungerminated Spanish Fork (A, C, E) and Whiterocks (B, D, F) *Bromus tectorum* seeds installed in November and retrieved on dates indicated. Retrieved subsamples were returned to the laboratory and incubated at 25°C in water potentials of 0 (A, B), -0.5(C, D), or -1.5 (E, F) MPa. After 28 d seeds at low water potentials were transferred to new Petri dishes and incubated in water for an additional 28 days. Seed status was determined as germinable (germinated following retrieval and laboratory incubation) or dormant (viable but ungerminated).

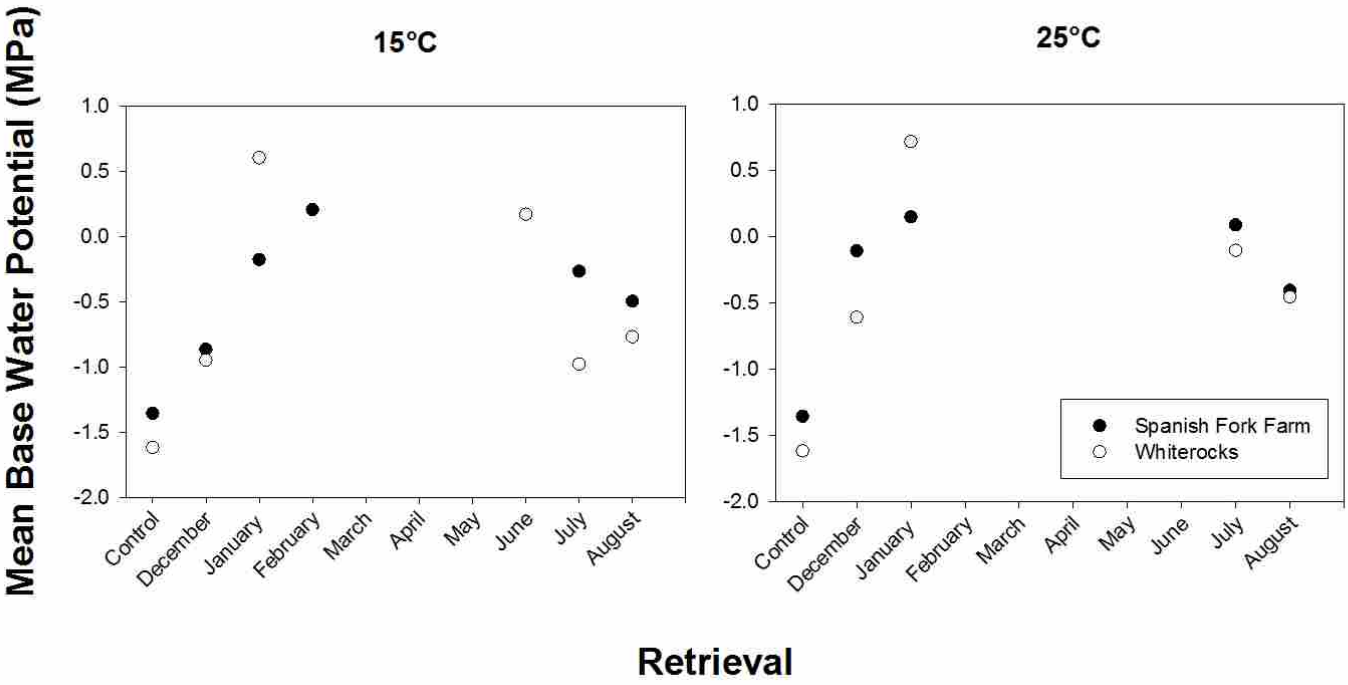

Figure 5

Seed dormancy status as indicated by changes in mean base water potential ($\Psi_b(50)$) of *Bromus tectorum* seeds for the Whiterocks (open circles) and Spanish Fork (filled circles) collections. Retrieved seeds were incubated at 15°C (left) or 25°C (right). Missing data points from February to June indicate insufficient germination to estimate $\Psi_b(50)$.

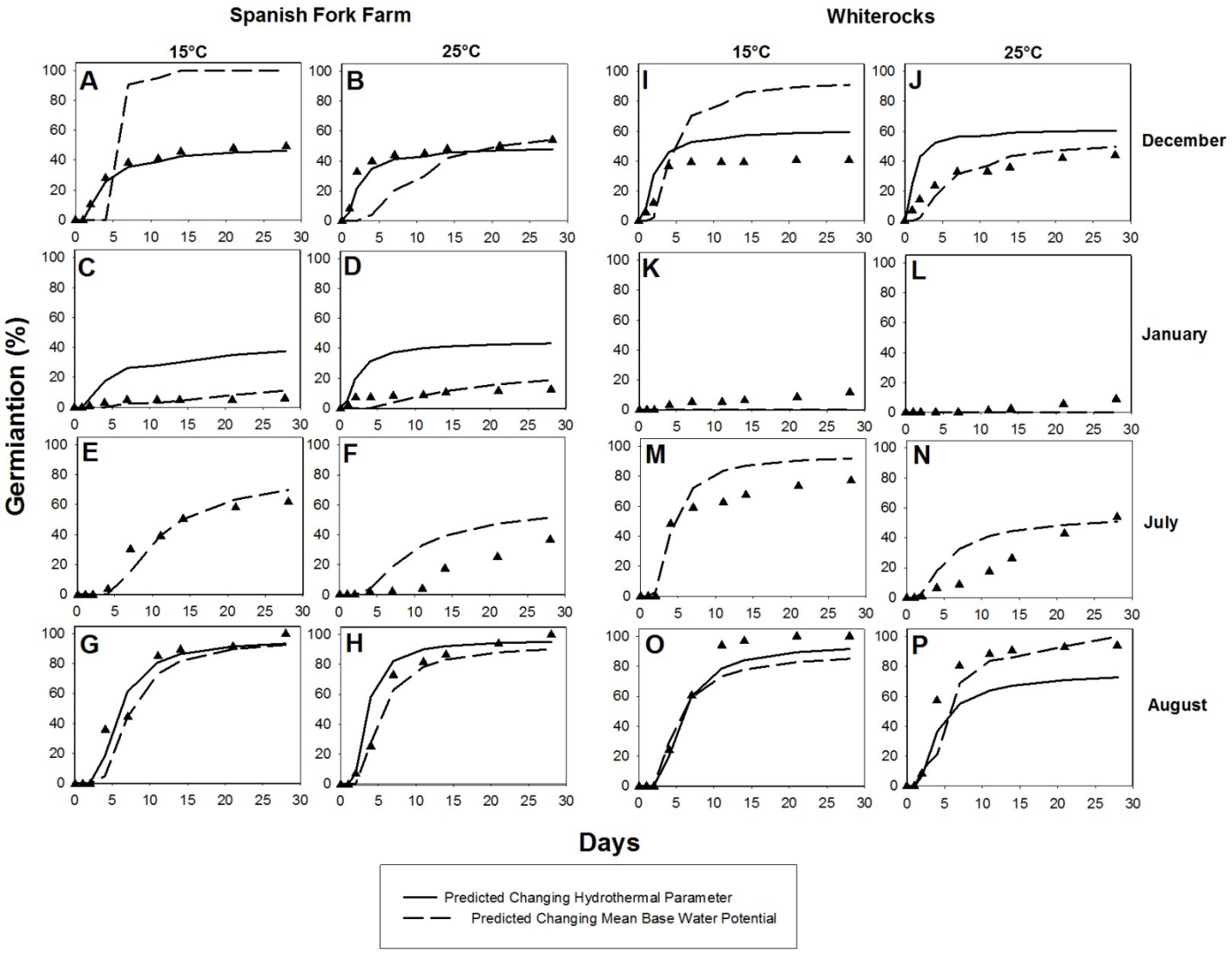

Figure 6

Predicted (lines) and observed (symbols) time-course curves for germination of Spanish Fork and Whiterocks *Bromus tectorum* seeds. Curves were obtained by fitting each of the two hydrothermal time models to the observed laboratory data. Dashed lines indicate the model which allowed only mean base water potential ($\Psi_b(50)$) to change and solid lines represent the model which allowed all hydrothermal parameters (θ_{HT}, $\Psi_b(50)$, $\sigma_{\Psi b}$) to change. Each row represents seeds from a different seed retrieval as follows: row 1, December (A, B, I, J), row 2, January (C, D, K, L), row 3, July (E, F, M, N), row 4, August (G, H, O, P). Missing data points from January to July indicate insignificant germination occurred to estimate hydrothermal parameters (θ_{HT}, $\Psi_b(50)$, $\sigma_{\Psi b}$) .

Summer interactions between host and pathogen in the *Pyrenophora semeniperda* - *Bromus* pathosystem

Hawkins, K.K., Allen, P.S., Meyer, S.E.

ABSTRACT

Bromus tectorum and *Bromus rubens* are highly invasive winter annual grasses in the western United States and *Pyrenophora semeniperda* an ascomycete fungus which can kill infected *Bromus* seeds. This paper aims to test whether seeds that are infected during early summer and then exposed to intermittent hydration will experience sufficient disease progression to kill seeds, or whether subsequent after-ripening will result in rapid germination allowing infected seeds to escape death. Laboratory experiments were conducted to determine how periodic hydration followed by drying at various storage temperatures (30-60°C) affects mortality of initially infected seeds. Experiments at two contrasting field sites were conducted to allow infected seeds to experience natural temperature and moisture fluctuations. Both laboratory and field data confirmed that *P. semeniperda* kills initially infected seeds that experience intermittent hydration – episodes of wetting followed by drying. An initial infection period of 24 hours resulted in subsequent seed mortality > 60% across all treatments; seeds with an initial infection period of 72 hours experienced higher mortality (>80% and often near 100%). Dry storage of infected seeds led to varying levels of mortality from *P. semeniperda* depending on storage duration and temperature, which confirms results from previous studies showing that *P. semeniperda* is favored under dormancy conditions that do not allow for rapid completion of germination. This suggests that biocontrol efforts using *P. semeniperda* may be effective when applied during the summer, at least in the habitats that experience precipitation during this season.

KEY WORDS

Bromus tectorum, Bromus rubens, seed bank, carryover

INTRODUCTION

*Bromus tectorum (*Allen and Meyer, 2014) and *Bromus rubens* (Hunter 1991) are highly invasive winter annual grasses. While both of these species have invaded large areas in the western U.S., *B. tectorum* is found across a wide variety of habitats (Meyer *et al*., 2006) while *B. rubens* is most commonly found in warm deserts (Brooks and Berry, 2006). As prolific seed producers, *B. tectorum* can yield up to 50,000 seeds m^{-2} (Meyer *et al*., 2006) while *B. rubens* has been reported to produce a maximum of 950 seeds per plant (Huxman *et al*., 2002) and (separately) up to 6000 plants m^{-2} (Salo 2004). In the absence of natural enemies these weeds can flourish and dominate in part due to greatly increasing the frequency of wildfires (Esque and Schwalbe, 2002; Blank *et al*., 2006).

The ascomycete fungus *Pyrenophora semeniperda* has been investigated as a possible biological control agent for killing *B. tectorum* seeds (Meyer *et al*., 2006; Meyer *et al*., 2008). Under natural field conditions (Allen and Meyer, 2014a) or controlled laboratory and field experiments, the *P. semeniperda,* also known as the Black Fingers of Death Fungus (BFOD), killed up to 100% of *B. tectorum* seeds (Meyer *et al*., 2010; Finch *et al*., 2013a). However, under certain conditions infected seeds can escape death (Beckstead *et al*., 2007). Variable mortality is explained by Beckstead *et al.* (2007) as a "race for survival," where outcome between host and pathogen is dependent on the ability to mobilize endosperm reserves. Seeds which are able to germinate quickly utilize the endosperm and escape death, while slow-germinating seeds are

more likely to be killed. Slow germination is typically associated with primary (Allen and Meyer, 2002) or secondary seed dormancy (Hawkins *et al*., 2013), or incubation under conditions that do not allow rapid germination such as unfavorable temperatures, or hydration - dehydration cycles (Finch et al., 2013a). *Bromus* seeds lose primary dormancy through dry after-ripening (Christensen *et al*., 1996; P. Allen, unpublished). *B. tectorum* seeds have been reported to enter secondary dormancy through the winter if conditions are not favorable for germination in autumn (Hawkins *et al*., 2013). It is not known whether *B. rubens* seeds become secondarily dormant.

Outcomes in this pathosystem are also influenced by the occurrence and severity of disease in a particular habitat. Higher levels of the fungus are associated with dry sites, where inadequate autumn precipitation results in a greater percentage of seeds becoming secondarily dormant (Meyer *et al*., 2006; Allen *et al*., 2014). Many fungi including *P. semeniperda* can grow and thrive at water potentials (Marin *et al*., 1996; Lahlaili *et al*., 2005; Ji *et al*., 2007) lower than those that allow *B. tectorum* seeds to germinate (C. Barth, 2014 unpublished; Finch *et al*., 2013a).

Three main interaction periods have been characterized for the *P. semeniperda – B. tectorum* pathosystem (Figure 6; Finch *et al.,* 2013a): 1) summer, 2) autumn, and 3) winter/spring. While the interaction between *P. semeniperda* and *B. rubens* has received less attention to date, preliminary data (P. Allen, unpublished) suggest that the three interaction periods are conceptually similar. In early summer seeds are primarily dormant as they reach maturity and then lose dormancy through the summer. In autumn infected seeds escape death due to rapid

germination with sufficient rainfall or may be killed if radicle emergence is delayed by repeated exposure to water potentials too low to permit germination. If the soil remains dry in the autumn, seeds may enter secondary dormancy during winter and, because they are unable to germinate, can be killed during winter or spring. Generally it has been assumed that seeds are infected in the fall and killed in the seed bank the following spring (Beckstead *et al.,* 2010). More recently Finch et al. (2013a) hypothesized that seeds may be infected during summer and die if intermittent hydration (i.e., imbibition periods followed by drying) leads to sufficient disease progression, though the data to support this are lacking. In order to test this hypothesis we conducted both laboratory and field experiments using *B. tectorum* and *B. rubens* seeds. Our approach in the present study was to determine how periodic hydration followed by drying at various storage temperatures (30- 60°C) affects seed mortality in the laboratory. We also conducted experiments at two contrasting field sites where infected seeds experienced natural and minimally supplemented soil moisture. We hypothesized that summer infection would favor the pathogen because seeds would be unable to germinate while the fungus could progress to some degree at low water potentials that restrict seed activity (Finch *et al*., 2013a). While *B. tectorum* has been the focus of previous research, we included *B. rubens* seeds in the field experiments to learn whether outcomes would be similar for both species. Differences in response could help to explain the unexpected observation that *in situ* levels of seeds killed by *P. semeniperda* are lower in Mohave Desert sites than in the Great Basin (S. Meyer, unpublished).

MATERIALS AND METHODS

For laboratory and field experiments seeds of two *Bromus* species were collected from three contrasting Utah locations. *B. tectorum* seeds were collected from a mesic sagebrush steppe habitat in central Utah County (Spanish Fork), a cold desert habitat in west-central Tooele

County, (Whiterocks), and a Mohave Desert population in southern Washington County (St. George). *B. rubens* seeds were also collected from the same Mohave location. All seeds were collected at maturity during late May or early June in 2013. Seeds were cleaned by hand and used immediately or stored in a freezer (-10°C) to prevent dormancy loss.

P. semeniperda inoculum was originally produced from stromata obtained from killed seeds at Whiterocks Road (Whiterocks, Utah, USA) and Tenmile Creek (Snowville, Utah, USA) in 2010. Isolated strains from these populations (WRR 10-14 and TMC 10-16 respectively) were developed by agar culture and spore productions as described by Meyer *et al.* (2010). Inoculation consisted of placing 25 seeds into a test tube vial containing 3 mg. of 1:100 *P. semeniperda*: talc mixture, then shaking for 30 s.

General Approach to Experimental Design and Statistical Analysis

Experimental data were analyzed as fully randomized designs using the analysis of variance (ANOVA) procedure of SAS 9.2, 2007 (SAS Inc., Cary, North Carolina, USA). Data were arcsine transformed for analysis to account for heterogeneity of variance. However, original means are reported. Means separations were performed as appropriate using Least Squares Means test. All experiments in this study included factorial combinations for each treatment as subsequently described.

Laboratory experiments

Determining effects of storage temperatures

Seeds from all three lots were inoculated with one of the two strains of *P. semeniperda* that were produced as previously described. In addition a non-inoculated control treatment was included. Seeds were then initially imbibed for 24 h (25°C, dark) before being air-dried at 25°C and approximately 25% relative humidity for a period of 48 h. For each treatment, four replications of 25 seeds each were placed in covered 100 mm Petri dishes on the surface of two blue germination blotters (Anchor Paper, St. Paul Minnesota, USA) saturated with water. Inoculated (or imbibed in the case of controls) and dried seeds were placed back into 100 mm Petri dishes and stacked in plastic containers. Seeds were then stored at one of 4 temperatures (30, 40, 50, and 60°C) for a period of either 4 or 8 w. After storage seeds were re-imbibed in water and incubated at 20°C (alternating 12 h fluorescent light/ 12 h dark) for 4 w and periodically scored as germinated (radicle > 1mm), killed by *P. semeniperda* (visible macroscopic stromata > 1 mm) or killed by heat (no radicle or stromata).

Determining effects of intermittent hydration

The two strains (WRR 10-14 and TMC 10-16) of *P. semeniperda* were produced and used to inoculate *Bromus* seeds as previously described. In addition a non-inoculated control treatment was included. Seeds were initially imbibed for a period of 24 or 72 h. For each infection treatment, four replications of 25 seeds each were placed in covered 100 mm Petri dishes on the surface of two blue germination blotters saturated with water. After imbibition seeds were air-dried under ambient conditions (approximately 25°C and 30% humidity) for a period of 48 h before being placed back into dry 100 mm Petri dishes and stored at 25°C (alternating 12 h

fluorescent light/ 12 h dark). The experiment was carried out over 16 w. After the initial infection period half of the seeds were re-imbibed three times for the same 24 or 72 h period at three week intervals (i.e., weeks 3,6, and 9). After each imbibition period seeds were again air-dried for a period of 48 h before being placed in dry Petri dishes to be stored at 25°C. At week 12 all seeds were again imbibed and incubated for another 4 w at 25°C (alternating 12 h fluorescent light/ 12 h dark) and scored for germination (radicle > 1mm), death (visible macroscopic stromata > 1 mm), or germination + infection (both radicle + stromata observed).

Field experiment

Seeds of *B. tectorum* (Whiterocks, Spanish Fork, and St. George) and *B. rubens* (St. George) were inoculated with one of two strains (WRR 10-14 and TMC 10-16) of *P. semeniperda* as previously described, while no inoculum was used on the control seeds. Previous research (Beckstead *et al*., 2014) indicates that *P. semeniperda* of the same strain can infect seeds of a wide range of species. Seeds were then placed inside mesh bags before being placed in covered 100 mm Petri dishes on the surface of two saturated blue germination blotters. For each treatment, four replications of 100 seeds were imbibed in water for a period of 24 or 72 h. After the initial infection period seeds were installed in habitats near their respective collection site: northern populations (Spanish Fork and Whiterocks) at the university research plots located in Provo, UT, USA, on May 22, 2013 and southern populations (St. George) at the Lytle Ranch study site (Washington County, UT, USA) on May 27, 2013. Mesh bags containing inoculated seeds were placed on the soil surface under 2 cm of autoclaved *B. tectorum* litter. Bags and litter were held in place by netting with metal nails. Seeds experienced natural precipitation with 25 mm of water manually added to the plots on August 13 at the Provo site and August 18th at Lytle

Ranch. This water was added to ensure disease progression, since minimal natural precipitation had occurred up to this point in the summer. Seeds were retrieved from Lytle Ranch on September 14 and Provo on September 18, as significant autumn precipitation was occurring at both sites. Retrieved seeds were brought to the laboratory where they were removed from the mesh bags and placed on two blue germination blotters saturated with water in 100 mm Petri dishes. Dishes were incubated at 25°C (alternating 12 h fluorescent light/ 12 hour dark) and scored for germination and infection as previously described.

RESULTS

General Approach to Experimental Design and Statistical Analysis

All findings reported in the results were significant at the $P<0.01$ level unless otherwise noted.

Laboratory experiments

Determining effects of storage temperatures

Uninoculated seeds (i.e. controls, not shown) from all populations were 100% germinable after 4 w of storage at 30-60°C. Infection followed by 4 w of storage led to a majority of seeds being killed by *P. semeniperda* upon rehydration for all treatment variables (Figure 1). Temperature, storage duration, and seed population had significant effects on the number of seeds killed by *P. semeniperda*. For infected seeds stored from 30-50°C, the percentage of seeds killed was > 70% except Spanish Fork seeds stored at 30°C and inoculated with the WRR 10-14 pathogen strain (Figure 1A-C). Following storage at 40 and 50°C >90% were killed (Figure 1B, C). Seeds stored at 60°C resulted in significantly fewer seeds killed by *P. semeniperda* for all populations (Figure 1: D). While St. George seeds largely escaped death (Figure 1 D) (<15% of seeds were killed at this temperature) there was a slight trend for lower mortality with Whiterocks and Spanish Fork

as well. Apparently, storage at 60°C likely began to impair fitness of the fungus, while also allowing a fraction of seeds to complete after-ripening and subsequently germinate before *P. semeniperda* could complete the disease cycle.

Uninoculated seeds stored for 8 w began to lose viability at 60°C. Germination reached only 72% and t50 was approximately 5 d. Infection followed by 8 w of storage produced highly variable results upon rehydration. Storage at the higher temperatures (40-60°C) led to decreased vigor as indicated by reduced germination rates (Table 1) as well as a fraction of seeds being killed by heat following continuous exposure to high temperatures (Figure 1E-H). Following storage at 30°C germination ranged from 55-95%, suggesting that many seeds were able to fully after-ripen before rehydration which allowed them to germinate quickly and escape the fungus (Figure 1 E). Remaining seeds stored at 30°C were killed by *P. semeniperda*. Following 8 w of storage at 40°C a fraction of seeds (7-39%) were killed due of the heat (i.e., seeds failed to germinate but no fungal stromata emerged, Figure 1 F). Seed outcomes following 50°C storage varied by population. The Whiterocks seed population had up to 43% killed by heat, while heat kill for the other seed populations was negligible. Germination and death due to infection varied among populations with 50°C storage. Following storage at 60°C much higher germination (35-76%) and death due to heat (4-50%) was observed. Mortality due to *P. semeniperda* was relatively low (Figure 1 H). This suggests that seeds were after-ripening but also losing vigor and viability due to continual exposure to heat or the interaction between high temperature storage and *P. semeniperda* infection. The t50 values for seeds incubated at 60°C for 8 w were two to four times as long as seeds incubated at 30°C (Table 1). We acknowledge that prolonged storage

at constant high temperatures is an artificial condition that seeds do not experience under field conditions.

Determining effects of intermittent hydration

All single interactions (seed population, imbibition time, number of hydration-dehydration episodes, pathogen strain) had a highly significant effect on seed mortality due to *P. semeniperda*. Seeds not inoculated with *P. semeniperda* (i.e. control treatments) had high germination following one or three hydration-dehydration episodes at the end of the experiment (Figure 2 A, D), with a small background level of seeds killed by *P. semeniperda.* In contrast, all seeds subjected to the infection treatments had high mortality rates due to *P. semeniperda*. Initial imbibition time was highly significant for both germinated seeds and *P. semeniperda*-killed seeds. Seeds initially imbibed for 72 h showed much higher mortality than seeds imbibed for 24 h. However, fungal stromata often appeared following radicle emergence with the 24 h infection treatment. An initial infection period of 72 h apparently resulted in better disease progression, as more seeds were killed by infection. Population variation regarding percentage of seeds killed by *P. semeniperda* was also highly significant. *B. rubens* seeds generally had lower seed mortality than the other populations, ranging from 50-60% for seeds imbibed for 24 h whereas mortality for all other populations ranged from 60-100% mortality. Though there were some highly significant two-way interactions (strain x imbibition time and population x imbibition time) these differences were generally small and did not appear to have significant biological importance.

Field experiment

Both field sites received little rain from June to August (Figure 3), which is why we chose to add an artificial precipitation event (dates indicated by vertical arrows). Rain was substantial (> 7 precipitation events at both sites) during September which was likely to cause germination of seeds and/ or spores, so we retrieved seeds at this time in order to determine outcomes in the laboratory. While dry summer conditions were conducive for seed after-ripening to occur as indicated by both field germination and high post-retrieval germination in the laboratory (as seen in the controls), seeds that received infection treatments were nearly all either killed by the fungus or had stromata appear following radicle emergence (Figure 4). Seeds initially imbibed for only 24 h had greater survival (germination percentages ranged from 5-40%; Figure 4: B, C), while seeds imbibed from 72 h had survival of 0-25%. Though strain differences were statistically significant the data failed to show any clear patterns from which we could draw relevant biological conclusions.

DISCUSSION

Infection of *B. tectorum* seeds by *P. semeniperda* followed by dry storage interspersed with hydration - dehydration episodes, resulted in high seed mortality under both laboratory and field conditions. Our hypothesis that summer infection would favor the pathogen was strongly supported by the results; seeds were unable to germinate under conditions that allowed for significant disease progression. This finding also supports the conceptual model of Finch *et al*. (2013a), who proposed that summer-infected seeds may not after-ripen sufficiently to germinate and escape disease progression.

Finch *et al*., (2013b) reported that *P. semeniperda* spores germinate within 6 - 8 h under conditions similar to the infection treatments used in this study. Following germination, conidia produce germ tubes or hyphae that branch, giving rise to several penetration sites, and generally penetrate caryopses within 24 h. Significantly advanced infection occurs after 72 h. As the disease progresses appressoria form on the ends of the hyphae and also penetrate the seeds through openings such as stomata and broken trichomes. Following 8 d of disease development under continuous hydration the endosperm was largely consumed by *P. semeniperda*. Results in the present study suggest that disease progression can occur during extended periods of dryness punctuated by intermittent hydration. In this study we found the pathogen is favored when infection of dormant seeds is followed by summer or summer-like conditions. Loss of dormancy through after-ripening (control treatments in this study) results in seeds capable of rapid germination in autumn (Finch *et al*., 2013a). *Bromus* seeds can survive extended dry conditions where temperatures reach 60°C at the soil surface (Meyer and Allen, 2009). However, constant extreme temperatures are detrimental to both seeds and the fungus. Both can survive short periods of intense heat. In a study on the effects of fire on survival of *B. tectorum* seeds and *P. semeniperda* propagules, the mean thermal death point (TDP_{50}) following a 5-minute exposure was 164°C for pathogen propagules and 148°C for host seeds (Beckstead *et al*., 2010), Our data suggest that the fungus has a lower tolerance for prolonged storage at moderately high temperatures (50-60°C) than do *Bromus* seeds, at least under the conditions of this study.

Several studies conclude that *P. semeniperda* can kill *B. tectorum* seeds *in situ* (Meyer *et al*., 2006; Meyer *et al*., 2008a; Meyer *et al*., 2008b; Beckstead *et al*., 2010; Finch *et al*., 2013a; Allen *et al*., 2014a). Efforts to characterize the *Pyrenophora – Bromus* pathosystem have previously

focused on autumn soil conditions as key variables, and seed mortality has been positively correlated with low autumn precipitation (Meyer *et al.,* 2006; Meyer *et al*., 2008b; Allen *et al*., 2014a).Similarly, research studies to date involving the use of *P. semeniperda* as a bio-herbicide have focused on autumn applications (Meyer *et al*., 2008). However, the present study shows that summer biocontrol application may be preferable in locations where summer thunderstorms are likely to occur before seeds after-ripen.

ACKNOWLEDGEMENTS

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TABLE

Table 1

Time to 50% relative germination (t(50)) for *Bromus tectorum* seeds stored at 30 or 60°C for 8 weeks following an initial infection period of 24 hours followed by drying for 48 hours. After storage seeds were again imbibed in water and germination (radicle emergence) was scored for 28 days

	WR TMC	**WR WRR**	**SF TMC** t(50) value	**SF WRR** Days	**SG- TMC**	**SG- WRR**
30°C	5.15	5.09	4.44	4.70	4.84	4.94
60°C	23.14	22.24	13.09	10.07	11.16	11.85

FIGURES

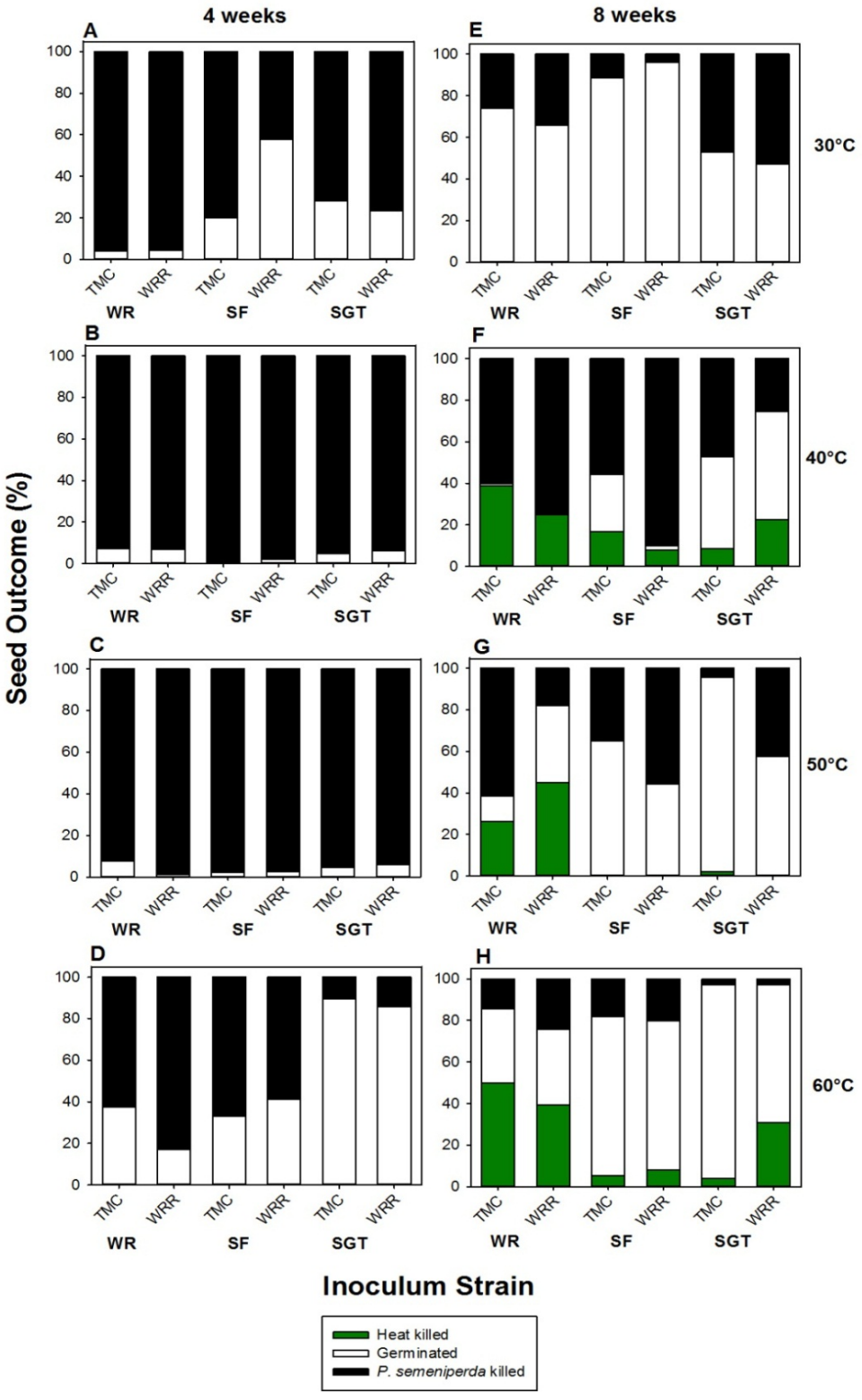
4 weeks
8 weeks
A
E
B
F
C
G
D
H
30°C
40°C
50°C
60°C
Seed Outcome (%)
0
20
40
60
80
100
TMC
WRR
WR
SF
SGT
Inoculum Strain
Heat killed
Germinated
P. semeniperda killed

Figure 1

Percentage of germinated and killed *Bromus tectorum* seeds following infection by *Pyrenophora semeniperda* and dry storage. Seeds were collected from Spanish Fork, (SFF), Whiterocks (WR), and St. George (SGT) in early summer of 2013, inoculated with one of two strains of *Pyrenophora semeniperda* (WRR 10-14Road or TMC 10-16) as indicated on the x-axis, imbibed in water for 24 hours at 25°C to allow infection to occur, and dried for 48 hours at 25°C. Seeds were then stored at 30(A, B), 40(C, D), 50(E, F), or 60°C (G, H) for a period of 4 (A, C, E, G) or 8 (B, D, F, H) weeks. Following storage, seeds were imbibed in water and incubated (20°C) for four weeks, then scored for germination (radicle > 1mm), killed by *P. semeniperda* (stromata > 1mm), or heat-killed (no radicle or stromata).

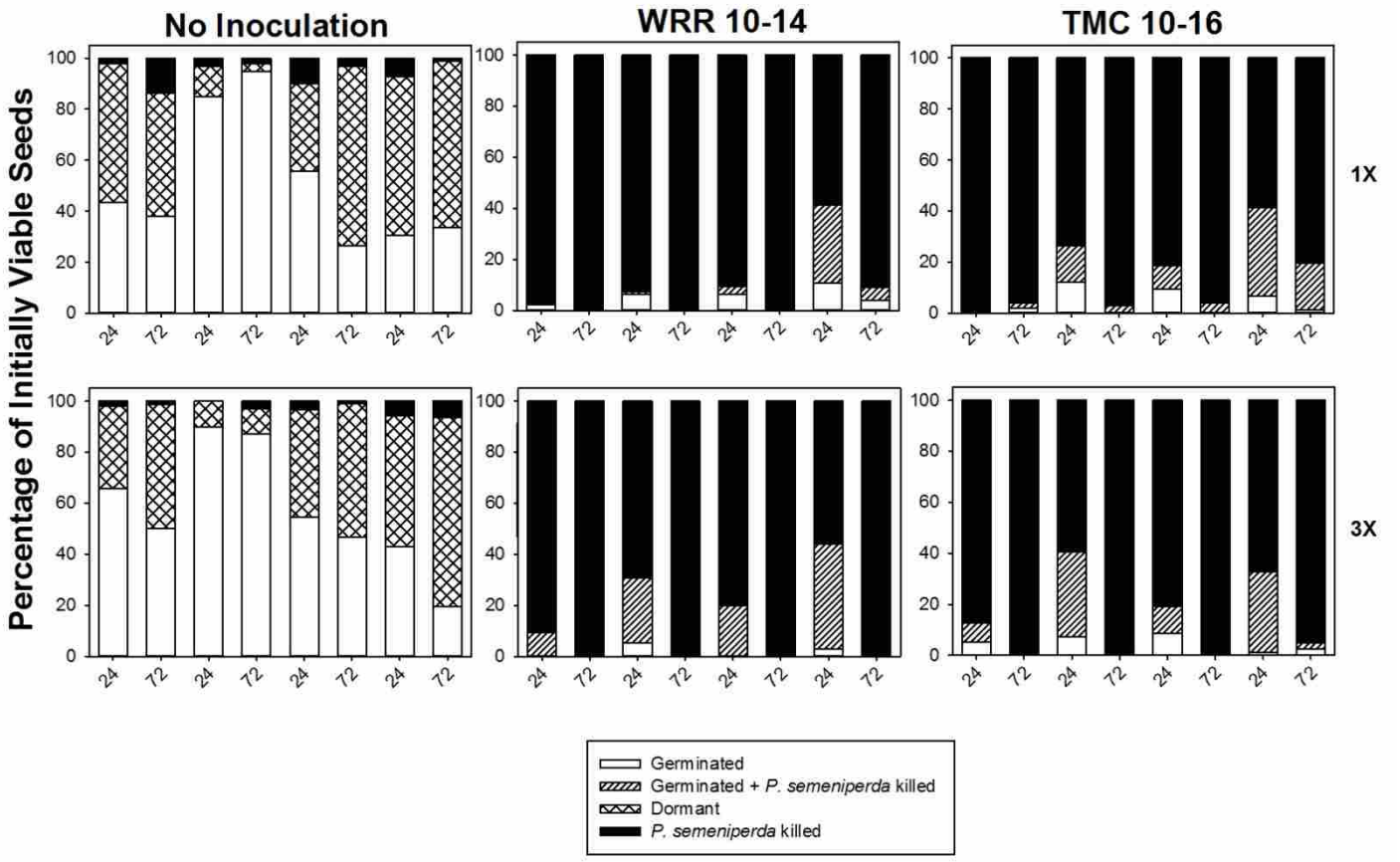

Figure 2

Bromus tectorum and *Bromus rubens* seed outcomes following infection by *P. semeniperda* and 0 or 3 additional periods of hydration (24 or 72 h) followed by dehydration. *B. tectorum* seeds were collected from Whiterocks (WR), Spanish Fork (SF), and St. George (SGT), while *B. rubens* seeds were collected from St. George (SGR). Primarily dormant seeds were inoculated with one of two strains of *Pyrenophora semeniperda* (WRR 10-14 or TMC 10-16). The experiment was carried out over the course of 12 weeks. At the start (week 0) all seeds were imbibed for a period of 24 or 72 hours in free water and then dried for a 24 hour period at 25°C. Seeds were then stored at 25°C. Half of the seeds were imbibed only initially (A-C), the other half of the seeds were went through three hydration-dehydration episodes (once every three week at weeks 3,6, and 9) following the same 24 or 72 hours (D-F) of imbibition as initially experienced. At week 12 all seeds in the experiment were imbibed and incubated for another 4 weeks and subsequently scored for germination (radicle > 1mm), *P. semeniperda* killed (fungal stromata > 1mm with no radicle), dormant (no radicle but still viable), or germination + stromata.

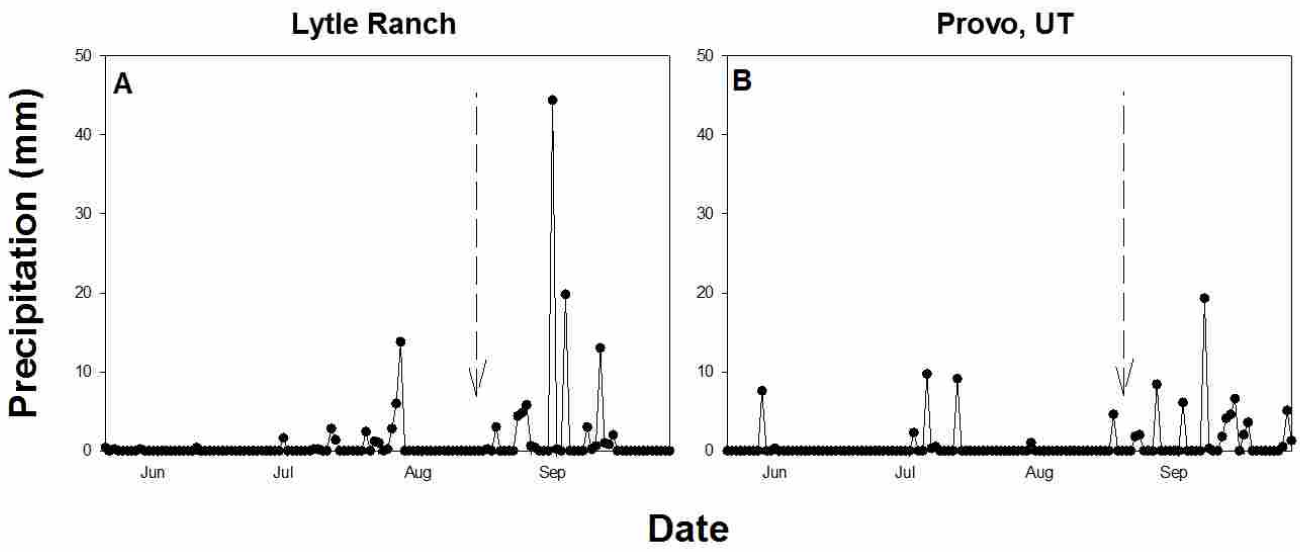

Figure 3

Summer precipitation at the two field sites. A single artificial precipitation event of 25 mm was added to plots on dates indicated by vertical dashed arrows.

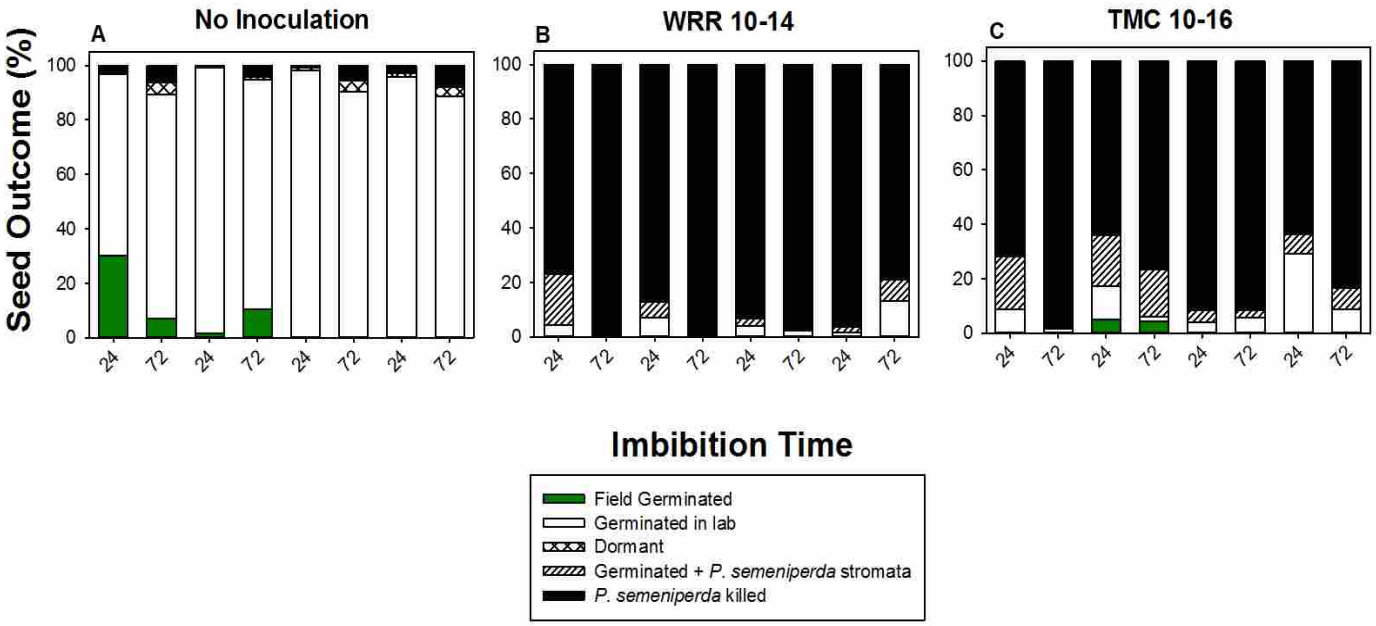

Figure 4

Outcomes for infected seeds at the two field sites. Recently-harvested *Bromus tectorum* seeds collected from Whiterocks, Spanish Fork, and St. George and *Bromus rubens* seeds collected from St. George were inoculated with strains of *Pyrenophora semeniperda* (WRR 10-14 or TMC 10-16), or had no inoculation and were then imbibed for a period of 24 or 72 hours in free water. Seeds were then placed back in the field near their respective locations; Whiterock and Spanish Fork seed were placed in university research plots at Provo, Utah, USA and St. George seeds (*B. tectorum and B. reubens*) were placed at the Lytle Ranch Preserve plots near St. George, Utah, USA. Seeds were thus exposed to ambient conditions throughout the summer. Seeds received 25 mm of supplemental irrigation indicated by the vertical arrow on Fig. 3. With the onset of autumn rains, seeds were retrieved from the field and taken to the laboratory. Field-germinated seeds were counted and removed. Remaining seeds were incubated for 28 days at 25°C in water and regularly scored for germination (radicle > 1mm), death (fungal stromata > 1mm with no radicle), dormancy (no radicle but still viable), or both germination + stromata.