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A Race for Survival: Can *Bromus tectorum* Seeds Escape *Pyrenophora semeniperda*-caused Mortality by Germinating Quickly?

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• *Background and Aims* Pathogen–seed interactions may involve a race for seed resources, so that seeds that germinate more quickly, mobilizing reserves, will be more likely to escape seed death than slow-germinating seeds. This race-for-survival hypothesis was tested for the North American seed pathogen *Pyrenophora semeniperda* on seeds of the annual grass *Bromus tectorum*, an invasive plant in North America. In this species, the seed germination rate varies as a function of dormancy status; dormant seeds germinate slowly if at all, whereas non-dormant seeds germinate quickly.
• *Methods* Three experimental approaches were utilized: (*a*) artificial inoculations of mature seeds that varied in primary dormancy status and wounding treatment; (*b*) naturally inoculated undispersed seeds that varied in primary dormancy status; and (*c*) naturally inoculated seeds from the carry-over seed bank that varied in degree of secondary dormancy, habitat of origin and seed age.
• *Key Results* In all three approaches, seeds that germinated slowly were usually killed by the pathogen, whereas seeds that germinated quickly frequently escaped. *Pyrenophora semeniperda* reduced *B. tectorum* seed banks. Populations in drier habitats sustained 50 times more seed mortality than a population in a mesic habitat. Older carry-over seeds experienced 30 % more mortality than younger seeds.
• *Conclusions* Given the dramatic levels of seed death and the ability of this pathogen to reduce seed carry-over, it is intriguing to consider whether *P. semeniperda* could be used to control *B. tectorum* through direct reduction of its seed bank.

Key words: Biocontrol, biotic resistance, cheatgrass, *Drechslera campanulata*, invasive species, mycoherbicide, pathogen, seed bank, seed-borne.

INTRODUCTION

For many fungal seed pathogens, the disease life cycle is largely unknown. This is especially true for non-crop seeds, where the soil seed bank makes up an important stage in species life history. *Pyrenophora semeniperda* is one seed pathogen for which there exists some knowledge on ecology and disease life cycle, although primarily on crop seeds. This study explores the role *P. semeniperda* plays in the disease ecology of a non-crop species that forms seed banks.

Pyrenophora semeniperda has been considered a weak pathogen that infects seeds and causes seedling leaf spotting on cool season cereal crops and several of their co-occurring grass weeds; dicot species can occasionally be infected (Medd, 1992). The fungus has a wide distribution that includes North and South America, South Africa, Australia and New Zealand. At present there is no evidence that *P. semeniperda* occurs in Europe or Asia (Yonow *et al.*, 2004). Most records of its distribution are obtained from infected seeds from field surveys or grain testing (Medd, 1992). The fungus, most generally seen as the anamorph *Drechslera campanulata*, has distinctive black stromata (up to 1 mm diameter and several centimetres long) that protrude from infected seeds. The stromata bear conidiophores with elongate multiseptate conidia. The conidiophores are hairy in appearance and readily visible.

Medd and colleagues (2003) proposed a disease life cycle for *P. semeniperda* that includes leaf infection of seedlings, resulting in ring spots, and also infection of developing seeds (also called floret or floral infection), which results in temporarily reduced seedling growth or sometimes in seed death. Studies have also shown that post-dispersal infection of mature seeds (as would occur in seed banks) is possible, but does not result in seed death; instead infection results in a temporary reduction in seedling growth (Wallace, 1959; Barreto and Fortugno, 1994; Campbell and Medd, 2003). In contrast, Kreitlow and Bleak (1964) demonstrated high mortality from this pathogen for seeds of native North American grasses sown in the field, resulting from post-dispersal infection, and Meyer *et al.* (2007) have produced evidence that this pathogen is a major cause of mortality in seed banks of *Bromus tectorum*. If *P. semeniperda* can infect seeds while they are in the seed bank (post-dispersal infection) and cause seed death, then it could be a strong pathogen on non-crop seeds that form seed banks in wildland systems. This dimension of the pathogen life cycle would involve an additional window of infection that could be crucial to the seed bank dynamics of the host species.

An important part of any disease life cycle is the way in which the pathogen interacts with its host. In a pathogen–seed interaction, the disease development process may simply be a race for survival. It is predicted that seeds that germinate more quickly, mobilizing the seed resources,

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will be more likely to escape seed death than slow-germinating seeds. It is hypothesized that the germinating seed competes with the pathogen for seed resources and that the outcome of this race determines whether the seed will survive.

To test the race-for-survival hypothesis, *B. tectorum* (cheatgrass, downy brome), which is a host for *P. semeniperda* (Shoemaker, 1966) and has seeds that lose dormancy during dry storage (Allen *et al.*, 1995), was focused on in this study. *Bromus tectorum* is a winter annual grass of European origin that invaded the western USA in the late 1800s (Mack, 1981). Recently harvested *B. tectorum* seeds are dormant and germinate slowly if at all, whereas seeds that have experienced a period of warm, dry conditions are not dormant and germinate quickly (Bauer *et al.*, 1998). This strategy prevents precocious summer germination, but allows for timely germination in response to autumn rains. Seeds that do not germinate in the autumn may enter secondary dormancy in response to winter conditions or may remain non-dormant and germinate in spring (Young *et al.*, 1969; Mack and Pyke, 1983). Secondarily dormant seeds have the potential to remain in the seed bank as carry-over seeds, i.e. seeds that remain in the seed bank for an additional period of time along with current-year seeds (Evans and Young, 1975). The distinctive stromata of *P. semeniperda* have been found on *B. tectorum* seeds from a wide range of populations in desert, shrub-steppe and mountain habitats throughout the Intermountain West (S. E. Meyer, US Forest Service, Shrub Sciences Laboratory, Utah, USA, unpubl. res.).

Three types of studies were used to test the race-for-survival hypothesis. In the first study, artificial inoculations were performed using mature seeds, to simulate processes that might take place after dispersal in the seed bank (post-dispersal infection). A test of Koch's postulates was completed, establishing this pathogen as the cause of seed death, and the effects of wounding and seed dormancy status on disease levels and seed mortality were also measured. In the second study, mortality levels on populations of naturally inoculated undispersed seeds were compared when incubated before and after dormancy loss. In the third study, disease levels were quantified on populations of naturally inoculated seeds from the carry-over seed bank at three contrasting sites, and the effect of dormancy status on disease levels and mortality was determined.

Seed bank studies have indicated that *B. tectorum* populations in drier environments have larger potential seed bank carry-over than those in more mesic environments (Young *et al.*, 1969; Meyer *et al.*, 2006). This is because complete autumn germination is more likely to occur in mesic environments. If *P. semeniperda* diminishes the seed bank by killing secondarily dormant carry-over seeds in the spring, then seed banks in drier habitats, where seed bank carry-over is greater, should contain larger numbers of seeds killed by *P. semeniperda* than seed banks in mesic environments. A prediction extending this hypothesis is that sites containing older seeds in the seed bank will have a greater proportion of seeds killed by *P. semeniperda* than sites with younger seed banks. Field

seed bank carry-over data from three contrasting sites were used to test these predictions.

MATERIALS AND METHODS

Laboratory inoculation experiments on post-dispersal infection

Bromus tectorum seeds (technically florets) and *P. semeniperda* (Brittlebank and Adam) Shoemaker isolates were obtained from a monospecific stand of *B. tectorum* located in Spokane, Washington, USA (47°61'N, 117°31'W; elevation 873 m). Mature *B. tectorum* seeds were bulk-collected in July 2003. Dead *B. tectorum* seeds with *P. semeniperda* stromata were hand-extracted from soil samples collected in June 2003 using a soil core (10 cm diameter and 2 cm depth to mineral soil). Both *B. tectorum* seeds and *P. semeniperda* stromata on seeds were stored at room temperature for approx. 1 year prior to the first experiment.

To prepare the inoculum, *P. semeniperda* stromata were cut from dead *B. tectorum* seeds, surface-sterilized (1 min in 70 % ethanol, 1 min in 0·0525 % sodium hypochlorite), and placed onto V8® agar. The mycelium produced conidia within 5–7 d. To remove the conidia from the agar, 5 mL of sterile water were added and a rubber scraper was used to rub the mycelium gently. This conidia–mycelium–water mixture was decanted into a large test tube through a double layer of cheesecloth to obtain a solution primarily of conidia. Conidial concentration was quantified using a haemocytometer, and sterile water was added or decanted until a concentration of 5000 conidia mL^{-1} was achieved. A mock inoculum (control) was prepared following the same procedures, except that the V8® agar was not inoculated with stromata.

In June 2004, the ability of *P. semeniperda* to infect mature, non-dormant *B. tectorum* seeds (post-dispersal infection) that were either intact or wounded by scarification (seeds were lightly rubbed with 600 ultra fine sandpaper to simulate natural wounding) was tested. Twenty-five *B. tectorum* seeds surface-sterilized as described above were placed in each of ten replicated plastic dishes (59·2 mL) for each of the two inoculation treatments (i.e. mock-inoculum or *P. semeniperda* inoculum) and each of the two scarification treatments (i.e. intact seeds or scarified), for a total of 40 plastic dishes and 1000 seeds. To each plastic dish containing seeds, 5 mL of pathogen inoculum or mock-inoculum was added and seeds were allowed to imbibe the solution on a shaker for 14 h. Following the imbibing period, seeds were placed in Petri dishes (100 × 15 mm) on two germination blotters (Anchor Paper, St Paul, MN, USA) saturated with water. Petri dishes were randomly stacked in plastic bags closed with rubber bands to retard water loss and incubated for 30 d at 15/25 °C under 12 h diurnal photoperiods. Dishes were rewetted as needed. Inoculation and wounding effects on germination were determined by counting the number of germinated seeds (radicle ≥1 mm) weekly up to day 30. The number of seeds killed by *P. semeniperda* was determined at the end of the incubation period by counting the number of

ungerminated seeds that exhibited stromatal development. To confirm the cause of seed death (completing Koch's postulates), sub-samples of surface-sterilized dead and germinated seeds from the above treatments were plated on V8® agar to isolate the fungus. The appearance of fungal stromata on dead seeds also confirmed the cause of death. The experiment was repeated in July 2004 following the same protocol, except that five instead of ten replicates were included and more detailed seed germination data were collected over time. All ungerminated seeds lacking fungal stromata were checked for viability with a cut test at the end of the incubation period; dormant seeds were defined as viable seeds that did not germinate within 30 d.

To deal with the issue of possible cross-contamination among seeds within a Petri dish in these experiments, some preliminary trials were performed to determine the period of time from inoculation to conidial production under the present experimental conditions. Inoculated seeds do not exhibit stromatal development prior to 2 weeks of incubation at 20–25 °C, but most seeds develop stromata by 4 weeks. Conidia, which are needed for infection (Medd *et al.*, 2003), have not been seen on stromata until after 4 weeks of incubation and usually only after water availability decreases within the dish. A minimum of 6 weeks of incubation would therefore be required for cross-contamination to confound the results (i.e. 4 weeks for conidial production plus 2 weeks for new conidial infections to show disease signs).

The data from this experiment were analysed using analysis of variance (ANOVA) for a completely randomized design, with trial, inoculation and wounding treatments as fixed main effects (PROC GLM; SAS, 2000). The two trials were not significantly different (d.f. 1,40, $F = 2.23$, $P = 0.14$) and data were combined for this study. To improve homogeneity of variance, proportional data were arcsine square-root transformed.

In July 2005, the effect of dormancy status on susceptibility to attack by *P. semeniperda* was assessed using dormant (recently harvested) seeds and non-dormant (fully after-ripened) *B. tectorum* seeds, which have contrasting germination time courses and germination percentages (Fig. 1). Seed samples for the dormant treatment were collected in July 2005 from the same location mentioned above, and seeds were inoculated within 1 week of collection and incubated and scored as described above. The only exception to the above protocol was that ten replicates of 25 seeds were used. Data from the non-wounded treatment in trial 2 were used to represent the non-dormant treatment in the analysis of the effects of dormancy status. The data were analysed as a two-way ANOVA for a completely randomized design, with dormancy status and inoculation treatment as the fixed main effects (PROC GLM; SAS, 2000).

Disease levels on pre-dispersal naturally inoculated seeds

In the summer of 1992, *B. tectorum* seeds were harvested (bulk collections from a minimum of 30 individuals) from four populations that represent a range of natural habitats (Table 1) as part of a large laboratory study on
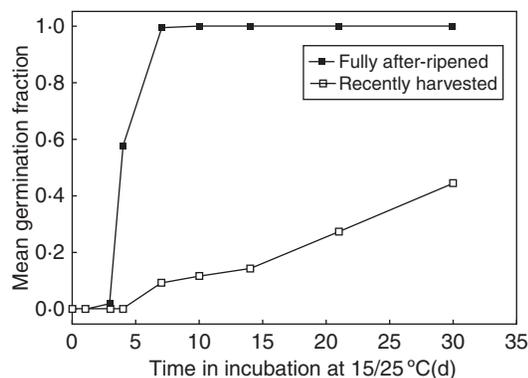

Fig. 1. Changes in germination patterns during incubation at 15/25 °C for *B. tectorum* seeds incubated within 2 weeks of harvest (recently harvested) and after 10 months of storage at 20 °C (fully after-ripened); sample sizes are 250 and 125 seeds, respectively. Seed were not inoculated with the fungal pathogen.

B. tectorum seed germination regulation (Beckstead *et al.*, 1996; Meyer *et al.*, 1997; see these papers for experimental details). In addition to collecting germination data in these studies, *P. semeniperda*-caused mortality was also quantified. For the present analysis, data from four seed populations incubated for 28 d when recently harvested (within 2 weeks of collection) and after 16 weeks of storage at 20 °C were used. Incubation was carried out at four temperature regimes: 5/15, 10/20, 15/25 and 20/30 °C (12/ 12 h). For each treatment, a seed population was represented by four replications of 25 seeds. Germination was scored 2, 4, 7, 11, 21 and 28 d after initiation. On day 28, the remaining ungerminated seeds were examined for stromata of *P. semeniperda* and were scored as dormant, killed by the pathogen or initially non-viable (due to causes other than this disease). Ungerminated seeds that developed stromata were presumed to have been killed by the pathogen.

ANOVA was conducted for a completely randomized design, with dormancy status and population as fixed main

TABLE 1. *Location and habitat information for Utah* B. tectorum *seed collections used in the study of* P. semeniperda *disease incidence on undispersed seeds*

Location	County	Habitat	Elevation (m)	Mean annual precipitation (mm)*
Fairview Top	Sanpete	Mountain meadow	2790	702
Provo	Utah	Mountain brush	1410	405
Snow's Canyon	Washington	Blackbrush shrubland	1150	263
Green River	Grand	Salt desert shrubland	1270	161

Locations are ranked by total mean annual precipitation.
*Precipitation means based on nearest NOAA or Snotel reporting station: Provo, Green River and St George UT NOAA stations and Buck Flat UT Snotel Station.

effects (PROC GLM; SAS, 2000). Incubation temperature was not included as a main effect, but data from all four incubation temperatures were included. The response variables were germinable fraction, dormant fraction and fraction killed by the pathogen; these were transformed as described above to improve homogeneity of variance prior to analysis.

Disease incidence on naturally inoculated seeds from the carry-over seed bank

Seed bank samples were obtained within *B. tectorum* monocultures for three Utah populations from contrasting environments in spring 2003 (Table 2). To measure disease incidence on carry-over seeds at least 1 year old, all seed bank samples were obtained in late spring (May), after germination was complete but prior to dispersal of current-year seed. For each population, 20 randomly located samples were collected. Samples were taken with a steel can (6 cm in diameter and 4 cm deep) by inverting the can and pressing it into the soil. A mason's trowel was used to lift the can with its soil core intact for transfer to a paper sack. Samples were usually dry at collection; moist samples were allowed to air-dry before processing. The samples were screened to remove loose soil, then processed by hand within 2 weeks of collection to identify, remove and quantify apparently viable (intact) seeds and also any field-killed seeds with protruding stromata.

To test the effect of variable germination rate determined by dormancy status for secondarily dormant seeds on disease incidence and seed mortality, apparently viable (intact) seeds extracted from the samples were subjected to two storage treatments. Half the seeds from each sample were stored at 20 °C for 4 months to allow loss of secondary dormancy, while the remaining seeds were stored at 2 °C to maintain secondary dormancy. Following the storage treatments, seeds were placed on moistened germination blotters (Anchor Paper, St Paul, MN, USA) in plastic Petri dishes (15 × 100 mm) and incubated at 20 °C for 2 weeks. Germinated seeds (radicle >1 mm) were counted and removed after 3, 7 and 14 d of incubation. On day 14, the remaining ungerminated seeds were scored as dormant, killed by the pathogen (i.e. with stromata visible) or initially non-viable (due to causes other than this disease). There were no viable dormant (ungerminated) seeds. Those that developed stromata within the 14 d incubation period were assumed to have been infected prior to sample collection. The total number of seeds in each storage treatment was seven, 125 and 74 for the Spanish

Fork Farm, Whiterocks upland and Whiterocks valley populations, respectively. The effect of secondary dormancy status and population on the fraction of seeds killed during incubation was analysed using two-way ANOVA for a completely randomized design, with dormancy status and population as the fixed main effects (PROC GLM; SAS, 2000). The response variable was transformed for analysis as described above.

The seed bank carry-over data for field samples from the three populations were also used to test whether seed bank carry-over and density of field-killed seeds varied among populations from contrasting habitats (Table 2). To examine habitat effect, the mesic Spanish Fork Farm population was compared with the xeric Whiterocks populations. For this analysis, seed variables were expressed on a per m^2 basis using the surface area of the sample can (28·3 cm^2) as a conversion factor. The response variables were density of field-killed seeds with stromata, density of viable seeds and density of seeds killed during incubation; variables were square-root transformed prior to analysis to improve homogeneity of variance. One-way ANOVA was used for a completely randomized design, with population as the fixed main effect (PROC GLM; SAS, 2000). Differences between populations were determined using a multiple comparison test (Ryan–Einot–Gabriel–Welsch *F* test).

In this experiment, it was possible to test for the effect of seed age (Table 2) on disease incidence and seed mortality. To investigate seed age, advantage was taken of a natural experimental difference between the two Whiterocks sites. Although the sites are within 10 km of each other, the elevational difference produces environments with slightly different weather. In 2002, the Whiterocks valley site experienced a localized drought that resulted in no seed production, whereas the Whiterocks upland site escaped the drought, and seed production in 2002 was similar to that in previous years. These contrasting seed production scenarios in 2002 resulted in differences in the age of seed bank carry-over seeds in spring 2003, with all seeds at the valley site at least 2 years old and the upland site containing mostly 1-year carry-over seeds.

RESULTS

Laboratory inoculation experiments on post-dispersal infection

Laboratory inoculation experiments showed that mature *B. tectorum* seeds can be infected by *P. semeniperda* in

TABLE 2. *Location and habitat information for* B. tectorum *seed bank samples used in carry-over seed study*

Location	County/state	Habitat	Elevation (m)	Mean annual precipitation (mm)*	Age of seed carry-over
Spanish Fork Farm	Utah/UT	Sagebrush steppe-mountain brush	1420	486	≥1 year
Whiterocks Upland[†]	Tooele/UT	Sagebrush steppe-pinyon juniper	1610	199	≥1 year
Whiterocks Valley[†]	Tooele/UT	Salt desert shrubland	1455	199	≥2 years

*Precipitation means based on nearest NOAA reporting station: Spanish Fork Powerhouse UT for Spanish Fork Farm and Dugway UT for both Whiterocks sites.
[†]The two Whiterocks sites are 8·6 km apart; the nearest NOAA reporting station is the same for both sites.

the imbibed state, often resulting in seed death and subsequent development of pathogen stromata (Fig. 2). Very few seeds in the control treatments were killed by the pathogen, while seed mortality in the fungal inoculum treatments averaged 57 % (inoculum main effect, d.f. 1,40, $F = 360 \cdot 97$, $P = 0 \cdot 0001$). Seed wounding resulted in a 38 % increase in seeds killed by *P. semeniperda* relative to intact seeds (wounding main effect, d.f. 1,40, $F = 13 \cdot 66$, $P = 0 \cdot 0007$). Most of the seeds not killed by the pathogen germinated, although a somewhat lower percentage of wounded seeds than intact seeds germinated in the control treatment, showing some direct damage from scarification. Control seeds not inoculated with the pathogen germinated to an average of 89 %, whereas seeds inoculated with the pathogen only germinated to 43 % (Fig. 2; inoculum main effect, d.f. 1,40, $F = 118 \cdot 01$, $P \leq 0 \cdot 0001$).

Isolation of *P. semeniperda* from seeds during laboratory experiments showed that inoculation treatments resulted in infection. All germinated seeds sampled that were inoculated with the pathogen contained the pathogen within their tissues (20/20 seeds), whereas none of the germinated seeds from the control inoculation treatment contained the fungus. For ungerminated seeds sampled on day 14 of incubation, all but two of the sampled inoculated seeds contained the fungus within their tissues (22/24). Seeds in the control treatment were not completely free from the presence of *P. semeniperda* (3/27), indicating that a few seeds were florally infected with the fungus prior to the experiment. To complete Koch's postulates, *P. semeniperda* was successfully isolated from all seeds killed by the pathogen as determined by the distinctive stromata on day 30 (36/36), indicating that *P. semeniperda* was the pathogen responsible for seed death. Together, these findings indicate that *P. semeniperda* successfully infected all inoculated seeds but did not kill all infected seeds.

Inoculation results for recently harvested and fully after-ripened seeds showed that seed death varied dramatically as a function of primary dormancy status. Pathogen-caused seed mortality was nearly twice as high for recently harvested (dormant) seeds, with 83 % of seeds killed by the pathogen during incubation ($n = 250$ seeds), in comparison with fully after-ripened (non-dormant) seeds, with 44 % of seeds killed by the pathogen during incubation ($n = 275$ seeds) (dormancy status main effect, d.f. 1,39, $F = 20 \cdot 38$, $P = 0 \cdot 0001$). This mortality difference is clearly linked to the major difference in germination rate between the two dormancy states (Fig. 1). Pathogen-caused mortality in the control treatment was zero for both recently harvested (dormant) seeds and fully after-ripened (non-dormant) seeds, and was significantly different from the fungal inoculum treatment (d.f. 1,39, $F = 352 \cdot 79$, $P = 0 \cdot 0001$).

Disease levels on pre-dispersal naturally inoculated seeds

Pathogen-caused mortality varied as a function of primary dormancy status as well as among populations for *B. tectorum* seeds that were naturally inoculated prior to dispersal (Fig. 3). All four populations contained sizeable dormant fractions when recently harvested, but were completely non-dormant after 16 weeks of storage at 20 °C. All populations were characterized by the slow, asynchronous germination when recently harvested and the rapid, synchronous germination when fully non-dormant that is shown in Fig. 1. The four populations showed sizeable differences in the fraction of recently harvested seeds killed by the pathogen (population main effect; d.f. = 3,120; $F = 45 \cdot 15$; $P = 0 \cdot 0001$), although disease levels were generally low (<20 %). Seeds of populations from more xeric habitats (Green River and Snow's Canyon) showed higher levels of disease when dormant than those from more mesic habitats
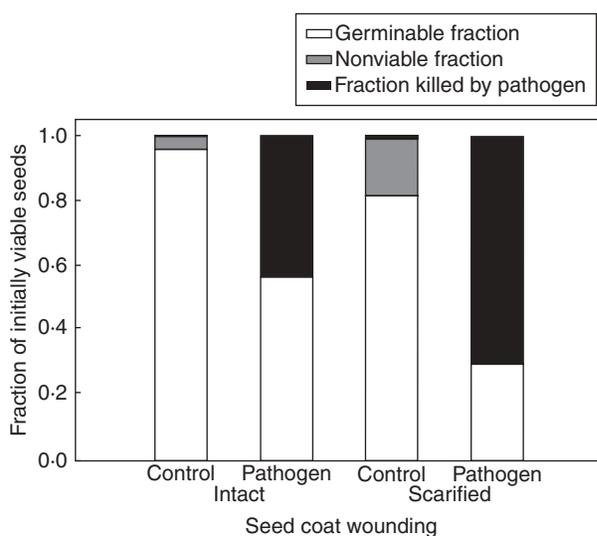
FIG. 2. Effect of inoculation (control vs. pathogen) and wounding (intact vs. scarified) treatments on seed fractions that are germinable, non-viable and killed by *P. semeniperda* ($n = 275$ seeds). Data are means of two trials.
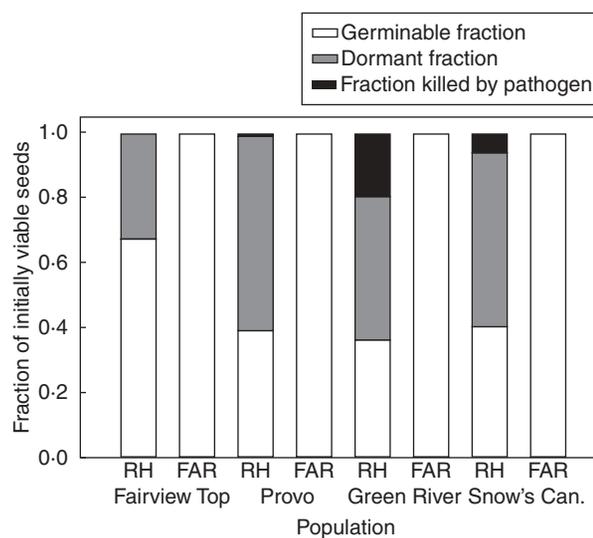
FIG. 3. Fraction of initially viable seeds collected from *B. tectorum* plants at the time of seed dispersal that are germinable, dormant or killed by *P. semeniperda* for four populations subjected to incubation when recently harvested (RH; incubated within 2 weeks of harvest) and fully after-ripened (FAR; stored for 16 weeks at 20 °C). Data represent the means of germination tests at four incubation temperatures ($n = 400$ seeds).

(Hobble Creek and Fairview Top). Once seeds were fully non-dormant, however, no killed seeds with pathogen stromata were seen in any collection (dormancy status main effect; d.f. = 1,120; $F = 144.42$, $P = 0.0001$). These samples were drawn from the same bulk collections and presumably had equivalent pathogen loads, but only dormant seeds were killed by the pathogen. Non-dormant seeds were able to escape pathogen-caused mortality. Again, this difference was clearly linked to a difference in seed germination rate.

Disease incidence on naturally inoculated seeds from the carry-over seed bank

In experiments with carry-over seeds from soil seed banks, the fraction of apparently viable seeds killed by *P. semeniperda* during incubation varied both according to dormancy status and among populations (Fig. 4A). Carry-over seeds stored at 2 °C to maintain dormancy showed higher levels of post-incubation mortality than those stored at 20 °C to allow dormancy loss (44·8 % mortality for more dormant seeds vs. 32·6 % for less dormant
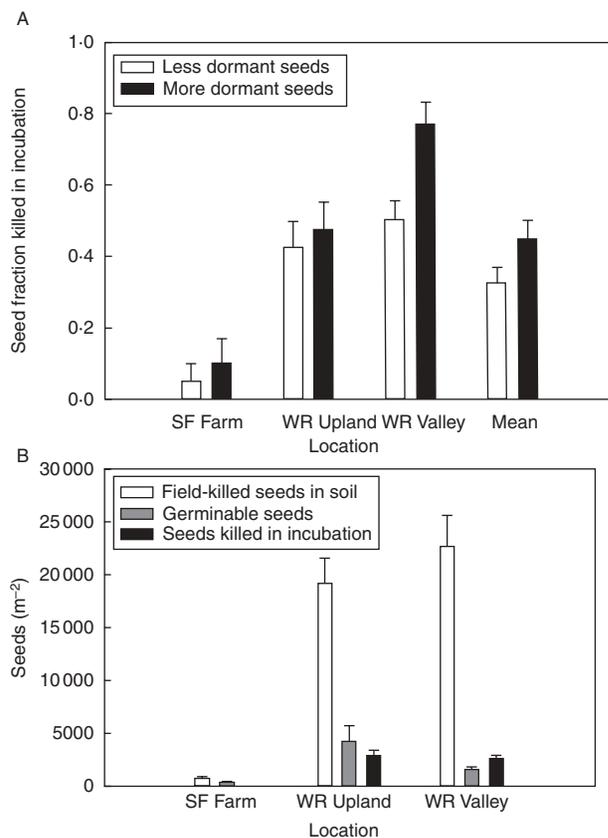
FIG. 4. (A) Fraction of initially viable seeds obtained from late spring *B. tectorum* seed banks at three contrasting sites and stored at 20 °C (less dormant) or at 2 °C (more dormant) that were killed by *P. semeniperda* during incubation (mean ± 1 s.e.; for sample sizes, see Materials and Methods). (B) Density of field-killed seeds in soil, viable seeds and seeds killed by *P. semeniperda* in incubation for *B. tectorum* seed bank samples from three contrasting sites (mean ± 1 s.e.; *n* = 20 sampling units per site).

seeds; dormancy status main effect; d.f. 1,117, $F = 4.02$, $P = 0.0472$). Viable seeds in samples stored at 20 °C germinated to >90 % within 3 d, while those stored at 2 °C took from 3 to 7 d longer, supporting the idea that the higher pathogen-caused mortality in the high-dormancy treatment was related to slower seed germination rate. All initially viable seeds not killed by the pathogen germinated within 14 d. The proportion of seeds that were killed during incubation also varied among populations (population main effect; d.f. 2,117, $F = 43.82$, $P = 0.0001$). Mortality was lowest for the mesic Spanish Fork Farm population and highest for the xeric Whiterocks valley population, where seeds in the seed bank were oldest.

The density of viable seeds in the spring seed bank also varied significantly among populations (d.f. 2,57, $F = 16.23$, $P = 0.0001$; Fig. 4B). As expected, the seed bank at the mesic Spanish Fork Farm site had a much lower density of viable carry-over seeds than either Whiterocks site. The Whiterocks valley site, where no seeds had been produced the previous year, also had significantly lower viable seed densities than the upland site. The density of seeds killed by *P. semeniperda* (both previously field killed and killed in incubation) also varied among sites (previously field-killed seeds, d.f. 2,57, $F = 67.09$, $P = 0.0001$; seeds killed during incubation, d.f. 2,57, $F = 48.09$, $P = 0.0001$). The mesic Spanish Fork Farm site had significantly fewer field-killed seeds and seeds killed in incubation in comparison with the xeric Whiterocks sites.

Seed age affected the proportion of seeds killed by the pathogen (Fig. 4B). The Whiterocks valley seed bank, which contained only seeds ≥2 years old, showed a significantly higher fraction of seeds killed by the pathogen in comparison with the Whiterocks upland population, where the seed bank was dominated by 1-year-old carry-over seeds.

DISCUSSION

These results build upon and extend the known disease life cycle of *P. semeniperda* to show conclusively that this pathogen can infect mature seeds that have been dispersed from the maternal plant, such as seeds residing in the seed bank, and that it can cause death of these post-dispersal-infected seeds. Most previous studies on *P. semeniperda* have focused on seed death as a consequence of pre-dispersal infection (i.e. floret infection; Medd *et al.*, 2003). It has been established here that this pathogen can also cause significant seed mortality when mature seeds are infected post-dispersal.

In support of previous studies, it is shown that pathogen-caused mortality can occur for *B. tectorum* seeds infected prior to dispersal. Some floret infection was detected within the laboratory inoculation control treatments, in which seeds were surface-sterilized; however, it was rare (1 %). With the second experimental approach, which measured disease levels on seeds naturally inoculated prior to dispersal, pathogen-caused seed mortality was detected. It is possible that these seeds were infected during seed maturation on the maternal plant; however, conidia undoubtedly can disperse to mature seeds while they are still on the plant. The seeds in this second experimental approach were

not surface-sterilized prior to testing, so it was not possible to determine definitively whether disease development was due to pre-dispersal floret infection or simply post-harvest infection of mature seeds that carried conidia superficially at the time of collection.

Some fungal pathogens are dependent on existing wounds to penetrate and cause disease (Levin, 1976; Wu and Bradford, 2003). It was found that seed coat wounding was not necessary for *P. semeniperda* infection and the resulting seed death, although pathogen-caused mortality did increase with seed coat wounding.

The present findings on post-dispersal infection of mature seeds support previous work that seed bank (or soil-borne) infection can occur in addition to floret infection, although the findings contradict most previous inoculation studies, which found that disease-free seeds inoculated with *P. semeniperda* post-harvest were little affected by the fungus (Wallace, 1959; Barreto and Fortugno, 1994; Campbell and Medd, 2003). In contrast, it was found that germination was substantially reduced for inoculated mature *B. tectorum* seeds and the percentage of pathogen-caused seed mortality was quite high. The differences between the present results and those of previous studies may be explained by the use of different host species (primarily, wheat vs. *B. tectorum*), or perhaps the pathogen biotypes utilized varied in virulence. A more likely explanation is that seed collections used in these studies varied in germination rate. As in previous studies, it was found that all seeds inoculated with *P. semeniperda* eventually developed the distinctive black stromata, whether or not they germinated or produced normal seedlings.

The prediction that rapidly germinating seeds will be more likely to escape seed death than slowly germinating seeds was well supported by the present data. This prediction was supported by all three experimental approaches, which included both laboratory and field-based studies. In addition, the pattern held for variable germination rates as a function of dormancy status for both primary and secondary dormancy. The simplest hypothesis to explain these findings is that a germinating seed competes with the infecting fungal pathogen for seed resources (Medd and Campbell, 2005). Rapidly germinating seeds, mobilizing the seed resources, will be more likely to escape seed death than slowly germinating seeds. The data do not indicate which seed resources are limiting, but it is likely that the competition is for the stored resources in the endosperm.

The exact mechanism that drives the race for survival remains to be discovered. *Pyrenophora semeniperda* is known to release phytotoxic metabolites that kill plant tissues (Evidente *et al.*, 2002; Capio *et al.*, 2004). A phytotoxic metabolite found in *P. semeniperda*, cytochalasin B, has been shown to inhibit *Pisum sativum* seed germination by interfering with the mobilization and utilization of seed reserves (Tamagnini *et al.*, 1983). It has been proposed that the exudates from germinating seeds trigger the pathogen–seed interaction and fungal production of phytotoxic metabolites (Campbell and Medd, 2003). Rapidly germinating seeds may release fewer exudates than slowly germinating seeds and therefore insufficient toxins would be released

to kill the seed prior to germination. The relative importance of simple resource competition vs. more complex metabolite-mediated pathogen–seed interactions in determining the outcome of the race for survival is a topic for further research.

The strong support for the race-for-survival hypothesis indicates that germination rate is a critical factor determining whether seed death will occur following infection by this pathogen. Given that most of the previous research on *P. semeniperda* has been conducted on crop seeds, primarily wheat (for a review, see Medd *et al.*, 2003), which generally lack seed dormancy mechanisms and germinate quickly, it is not surprising that this race-for-survival hypothesis has not been previously explored or supported. This pathogen is widespread in non-crop plant communities within the western USA and has the ability to negatively impact seed banks of the exotic *B. tectorum* as well as native perennial grasses (Kreitlow and Bleak, 1964; Meyer *et al.*, 2007). The existence of variable seed dormancy and contrasting germination rates for various wildland grass species indicates that the race-for-survival hypothesis has important implications for the host–pathogen dynamics of different host species as well as for the community ecology of the plant associations of arid and semi-arid ecosystems, where the impacts of *P. semeniperda* are likely to be greatest.

The prediction that *B. tectorum* seed banks in drier habitats would show higher seed carry-over and would contain more seeds killed by *P. semeniperda* in comparison with mesic habitats where there is little seed bank carry-over was supported. It was found that *P. semeniperda* played an important role in reducing *B. tectorum* seed banks by killing secondarily dormant carry-over seeds. The wet autumn weather at the Spanish Fork Farm probably allowed the non-dormant seeds to germinate quickly, preventing seed carry-over and *P. semeniperda*-caused mortality. In contrast, the Whiterocks sites received low levels of autumn precipitation and therefore many seeds were prevented from germinating. These carry-over seeds became secondarily dormant and were then subject to pathogen-caused mortality.

Data from other studies have also indicated that the risk of *P. semeniperda*-caused seed death is higher at drier sites. In a 5-year *B. tectorum* seed bank study, Meyer *et al.* (2007) found that *P. semeniperda* reduced the seed carry-over at a salt desert shrubland site to a greater degree than at a more mesic Wyoming big sagebrush site; three times as many carry-over seeds were killed in incubation and five times as many field-killed seeds with stromata were obtained from seed bank samples from the drier site than the mesic site. Similarly, Kreitlow and Bleak (1964) found that *P. semeniperda*-caused seed death for several grass species in artificial seed bank studies was high at the most xeric sagebrush site, low at the more mesic mountain brush site, and non-existent at the most mesic montane aspen–conifer site. Interestingly, all of the perennial grasses tested by Kreitlow and Bleak (1964) have slower mean seed germination rates than non-dormant *B. tectorum* seeds (SE Meyer, US Forest Service, Shrub Sciences Laboratory, Utah, USA, unpubl. res.).

It was found that the risk of *P. semeniperda*-caused seed death to carry-over seeds, was greater for older than for younger seeds. Meyer *et al.* (2007) also found that seed death caused by *P. semeniperda* increased with seed age. This seed age effect could be due to greater leakage of solutes from older seeds (Harman *et al.*, 1978) which could signal spore germination of the pathogen (Nelson, 1990), or it could be due to increased probability of pathogen contact for older seeds due to the cumulative risk over time.

In conclusion, it was found that *B. tectorum* seeds can escape *P. semeniperda*-caused seed death by germinating quickly. This escape is available for seeds infected post-dispersal (as would occur in a seed bank) as well as for seeds infected prior to dispersal (as would occur for floret infection), and also potentially for carry-over seeds within the seed bank. The findings also indicate that even though *B. tectorum* can generally escape this pathogen by germinating quickly, many seeds fall prey to the fungus, as evidenced by the high numbers of field-killed seeds, especially in drier environments where secondary dormancy is common. Medd and Campbell (2005) explored the use of *P. semeniperda* as a biocontrol agent for annual grass weeds within winter cereal crops, primarily through the means of pre-dispersal floret infection. Given the sometimes dramatic levels of seed death following inoculation of mature seeds found in the present study and the ability of this pathogen to reduce seed carry-over, it is intriguing to consider whether *P. semeniperda* could be used as a mycoherbicide to help control *B. tectorum* through the direct reduction of its seed bank.

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