# Impact of the pathogen *Pyrenophora semeniperda* on *Bromus tectorum* seedbank dynamics in North American cold deserts

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## Summary

*Bromus tectorum* is a dominant winter annual weed in cold deserts of western North America. We followed patterns of seed carry-over and abundance of the pathogen *Pyrenophora semeniperda* over 5 years at *B. tectorum*-dominated shadscale (*Atriplex confertifolia*) and sagebrush (*Artemisia tridentata*) sites in southern Idaho. We hypothesised that more seeds could potentially carry over at the drier shadscale site because of minimal autumn precipitation, but that *P. semeniperda*, a pathogen that primarily kills dormant seeds, would have more impact at the drier site, where a higher density of dormant seeds would likely be present in the early spring seedbank. Successful first-year seed carry-over was higher in years with below-average autumn precipitation. It was lower at the shadscale site than at the sagebrush site (9% vs.16%). The number of seeds killed during incubation by *P. semeniperda* averaged three times higher at the drier site and the number of field-killed seeds averaged almost five times higher. This suggests that pathogen-related mortality caused the greater decrease in seed carry-over at the drier site. Mortality risk increased dramatically with seed age. This climate–pathogen interaction apparently limits *B. tectorum* seedbank carry-over in cold deserts to 3 years or less. *Pyrenophora semeniperda* shows potential as a biocontrol agent for *B. tectorum* in these habitats.

**Keywords:** biological control, cheatgrass, dormancy, downy brome, *Drechslera campanulata*, drooping brome, germination, sagebrush, shadscale.

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## Introduction

*Bromus tectorum* L. (cheatgrass, downy brome, drooping brome) was introduced into western North America from Eurasia in the late 1800s, probably as a contaminant in grain seed (Mack, 1981). It rapidly expanded its range, reaching its current geographic distribution within 30 years. It now occupies over 40 million ha in the Intermountain West area of the USA alone (Rosentreter, 1994). It is the dominant species on at least 200 000 $km^2$, making it the most common vascular plant species in the region. The establishment and spread of *B. tectorum* in the interior West has been called perhaps the most significant plant invasion in the modern history of North America (D'Antonio & Vitousek, 1992).

The ecology of many of the areas inhabited by *B. tectorum* has been permanently altered (Mack *et al.*, 2000; Harrod, 2001). The disturbance created by severe overgrazing permitted *B. tectorum* to become dominant in the understory of many cold desert shrubland communities. Once established, this species creates the disturbance it needs to thrive by forming a continuous fine fuel layer that dries early in the fire season. Fire return times in sagebrush steppe ecosystems have been shortened from 60–110 years or more to intervals as short as 3–5 years (Whisenant, 1990). Most of the *B. tectorum* seedbank is destroyed in these fires, but

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enough seeds survive for the species to establish dominance in the years immediately following a burn (Young & Evans, 1978). The result is wildfire on ever-increasing areas, replacement of native vegetation with *B. tectorum* monocultures and accelerated loss of cold desert shrubland habitat.

*Bromus tectorum* is a facultative winter annual. Its seeds possess varying degrees of dormancy at dispersal in early summer, but this primary dormancy, which functions to prevent precocious germination in response to summer rain, is lost through dry after-ripening at warm temperatures (Meyer *et al*., 1997; Bauer *et al*., 1998). When the first frontal storms of autumn arrive, the seeds are poised to germinate quickly. In years and habitats with adequate autumn precipitation, most or all of the seeds germinate. Only those seeds suspended in the litter or in other quick-drying microsites may fail to germinate. In years and habitats with little or no autumn precipitation, most of the seeds remain ungerminated until temperatures warm in the spring (Mack & Pyke, 1983). The non-dormant fraction usually germinates in spring, while the remaining seeds, which remain in a state of secondary dormancy induced by winter conditions, represent the fraction that has the potential to carry over as a persistent seedbank (Evans & Young, 1975; Young & Evans, 1975).

Little has been published on seedbank longevity for *B. tectorum*. Hulbert (1955), working in eastern Washington, reported that seeds in the *in situ* seedbank germinated to 100% in response to autumn rain the first year. Chepil (1946) reported similar results for early-sown seeds in Saskatchewan, while seeds sown late in the autumn averaged 20% carry-over to the second year. Less than 1% of the total number of seedlings emerged the third year. Young *et al*. (1969) reported that density of carry-over seeds in the seedbank in May on sagebrush sites in Nevada in an average year was as high as 5000 seeds per $m^2$, while in a year with a long, wet spring there was very little seed carry-over. Hull and Hansen (1974) measured first-year *in situ* seedbank carry-over of 1.7–17% at sagebrush sites in northern Utah and southern Idaho, while Wicks (1997) measured carry-over as high as 30% in Nebraska. These studies indicate that *B. tectorum* populations frequently carry seeds over to the second year following production, but there is little evidence for seedbank persistence beyond 3 years.

One factor that may limit the lifespan of *B. tectorum* seeds is attack by the seed pathogen *Pyrenophora semeniperda*. This species is commonly observed on grass seeds as stromata of the asexual (anamorph) state of the fungus, *Drechslera campanulata* (Kreitlow & Bleak, 1964; Young *et al*., 1969). This generalist pathogen can attack seeds of a wide range of grass genera and also occurs as a leaf spot pathogen on seedling grasses (Medd *et al*., 2003). Biotypes that have been studied in Australia infect developing ovules when the plant is in flower (Medd & Campbell, 2005). Florally infected seeds first show signs of the disease when they are hydrated post-dispersal in the seedbank or in a Petri dish. The pathogen sporulates in the seedbank, and conidia, or possibly ascospore products of the sexual (teleomorph) state, which also grows on seeds, carry out the life cycle by infecting a new generation of developing ovules. Medd and Campbell (2005) were not able to cause seed death by inoculating mature seeds directly. But with biotypes of the pathogen that occur on *B. tectorum* in western North America, conidial inoculation of uninfected mature seeds led readily to infection, the production of fungal stromata and frequently to seed death (Beckstead *et al*., 2006). Most seeds killed by the pathogen in these inoculation trials were dormant; non-dormant seeds germinated quickly and were generally able to escape the disease.

Medd and Campbell (2005) studied the epidemiology of *P. semeniperda* on a range of weedy grass species as well as cereal crops in Australia, with the aim of developing this pathogen as a bioherbicide. The possibility for use of such a bioherbicide against *B. tectorum* is intriguing. In order to pursue this possibility, we first need a better understanding of *B. tectorum* seedbank dynamics and of the impact of *P. semeniperda* on *B. tectorum* seeds under natural conditions in the field. Interestingly, this pathogen is not known to occur in the Old World range of *B. tectorum*, so that it represents a natural enemy first encountered in its invaded range (Yanow *et al*., 2004). Presumably, North American populations of *P. semeniperda* on *B. tectorum* originated on seeds of native grass species.

We designed our research to test four hypotheses. Because all *B. tectorum* seeds are highly germinable in autumn, we expected that seedbank carry-over from autumn to spring would be negatively correlated with autumn precipitation, which directly affects the probability of autumn germination. We therefore predicted that potential first-year seed carry-over would be higher at the drier shadscale site than at the semiarid sagebrush site, as well as being higher in years of below-average autumn precipitation. Because *P. semeniperda* is postulated to attack dormant seeds in the spring seedbank, which remain hydrated but ungerminated for relatively long periods, we would expect its impact to be greater under conditions of higher potential seed carry-over, namely at the drier shadscale site. We also hypothesised that the risk of pathogen attack would be cumulative through time, so that pathogen impact would increase with seed age.

 

## Materials and methods

We quantified *B. tectorum* autumn seedbanks over a 5-year period, determined how much of the autumn seedbank was carried over as viable ungerminated seeds until late the following spring and examined the impact of *P. semeniperda* on survival of seeds in the seedbank. The study was carried out in *B. tectorum* monocultures at two sites on the Snake River Plains of southern Idaho, USA, North Standifer, a semiarid site formerly dominated by Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) and South Grass, a somewhat more arid site formerly dominated by shadscale (*Atriplex confertifolia*) (Table 1). A supplemental 2-year study was carried out in a *B. tectorum* monoculture on a shadscale site at Whiterocks in Skull Valley, Utah (Table 1).

### *Southern Idaho study*

Seedbank sampling at the southern Idaho sites was initiated in the autumn of 2000. Autumn sampling took place in early September each year, after dispersal of current-year seeds was complete, but prior to any germination-triggering precipitation. The intent was to measure the current-year autumn seedbank plus any older seeds that had been carried over from previous years. Late spring sampling took place in early May, after all germination was complete, but before dispersal of current-year seed and was intended to measure only carry-over seeds at least 1 year old. Sampling took place each autumn and spring until spring 2005. The exception was autumn 2001, when early autumn precipitation triggered emergence before samples could be obtained.

At each of the two study sites (Table 1), the sample area was defined as a rectangular plot 20 m wide and 50 m long (0.1 ha). On each sampling date and location, 20 randomly located samples were collected within the area. The samples were taken with a steel soil moisture can 6 cm in diameter and 4 cm deep. The can was inverted and pressed into the soil until the bottom was flush with the surface. The surrounding area was then cleared with a mason's trowel down to mineral soil to prevent seed contamination, and the trowel was used to lift the can with its soil core intact, so that the soil core could be transferred to a labelled paper sack. The samples were usually dry at the time of collection; moist samples were allowed to air-dry before storage. Collections from May dates were stored over the summer under laboratory conditions, so that any dormant seeds would lose their dormancy through dry after-ripening (Bauer *et al*., 1998), while September collections, which contained only non-dormant seeds, were processed within a few weeks of collection. Both spring and autumn samples were processed in early winter each year. Preliminary data indicated that the pathogen was not impacted by storage at laboratory temperatures.

To extract the seeds, the soil and accompanying litter and seeds were placed on a coarse screen. Soils at both study sites are comprised of fine loess that passes readily through a screen with little or no pressure needed to break up aggregates. The litter and seed material remaining on the screen were processed by hand to identify and remove apparently viable seeds. These seeds were then placed on moistened germination blotters in plastic Petri dishes and incubated at 20°C for 2 weeks. All viable seeds germinated within 3 days; these were counted daily and removed. The remaining seeds were scored at the end of the 2-week period as non-viable or as killed by *P. semeniperda*. By the end of 2 weeks, the black stromata of the fungus were evident on killed seeds. Seeds killed by the pathogen were still turgid, unlike seeds that were non-viable, which were generally limp and empty. We tested for the possibility of cross-contamination by inoculating uninfected seeds and measuring the time from inoculation to production of conidia under laboratory conditions. This process took

**Table 1** Location and habitat information for two study sites in south western Idaho and one study site in west-central Utah

| | North Standifer | South Grass | Whiterocks |
|---|---|---|---|
| Location | 43.28765°N 116.103°W | 43.15765°N 116.23815°W | 40.32820°N 112.77816°W |
| County and state | Ada, Idaho | Ada, Idaho | Tooele, Utah |
| Elevation (m) | 987 | 933 | 1450 |
| Habitat type | Wyoming big sagebrush | Shadscale salt desert | Shadscale salt desert |
| Disturbance regime | Burn, monoculture | Burn, monoculture | Burn, monoculture |
| Average August to November precipitation (mm)* | 44 | 34 | 62 |
| Average December to January precipitation (mm) | 50 | 37 | 28 |
| Average February to March precipitation (mm) | 94 | 74 | 81 |
| Average total August to May precipitation (mm) | 188 | 145 | 171 |

*Precipitation mean values for North Standifer and South Grass are based on 15-year data from nearby National Guard-maintained rain gauges; precipitation mean values for Whiterocks are based on the Dugway NOAA reporting station.

at least 3 weeks, making it extremely unlikely that any of the killed seeds were infected during incubation. In fact, the seeds germinated so quickly that only those already infected in the field were likely to be killed.

The number of viable and pathogen-killed seeds in each sample was expressed on a per $m^2$ basis using the surface area of the sample can (28.3 $cm^2$) as a conversion factor. We also expressed number of seeds killed by the pathogen as a fraction of total viable plus killed seeds. Samples with no seeds were treated as missing values in the analysis of this variable. Starting in spring 2003, we quantified field-killed seeds with protruding stromata of the pathogen in each sample. This quantification was carried out only on spring seedbank samples. The number of field-killed seeds was also expressed on a per $m^2$ basis.

The seedbank data were analysed using analysis of variance for a completely randomised design, using the SAS General Linear Model (GLM) procedure. We chose to exclude the spring 2002 data, because we had no corresponding autumn 2001 data for comparison; these data are included on the graphs but not in the analysis. We used the GLM procedure, because the design was not balanced; there were six missing samples across four sampling dates because of the errors in the field. Model fixed effects included site, year and season (autumn or spring). Year is defined as the autumn of 1 year combined with spring of the next year and designated as the second year, e.g. 2001 includes autumn 2000 and spring 2001. In order to improve homogeneity of variance, the two density (count) variables were log transformed and the dependent variable killed seed proportion was arcsine square root transformed prior to analysis (Quinn & Keough, 2002). Mean values of the untransformed data are presented.

### *Utah study*

A supplementary seedbank study at Whiterocks in Skull Valley, Utah, was initiated in summer 1999 (Table 1). We took advantage of a natural grasshopper herbivory event that totally prevented seed set in 1999 over an area of several square kilometres. We obtained seedbank samples from a location near the centre of the denuded area in August. These samples included only carry-over seeds of age 1 year or older. For comparison, we also sampled an area about 5 km distant where seed set had proceeded normally and that included current-year seeds in addition to older seeds. Finally, we returned to the original sample location in the denuded area the following spring and took another set of seedbank samples prior to any seed fall of the current-year crop (on plants that had established from carry-over seeds the previous autumn). These samples contained only seeds of age 2 years or older. The sampling and processing protocol was similar to that described for the Idaho sites, except that the samples were taken with a larger diameter (10 cm) sampling device, and 50 samples rather than 20 samples were taken from the denuded area in 1999. This data set was analysed using the GLM procedure for a completely randomised design, with dependent variable transformations as described.

## Results

### *Southern Idaho study*

The most obvious result of the Idaho seedbank study was the large and highly significant difference in density of viable seeds present in autumn vs. spring seedbanks (Fig. 1A and Table 2). This result was not surprising, as most *B. tectorum* seeds are expected to germinate in the first year following production and to be absent from the seedbank by late spring. Mean viable seed density in the autumn was 20 400 seeds per $m^2$, while mean density in spring was only 2900 seeds per $m^2$.

Seed density varied significantly across years and the highly significant year by season interaction indicated that both autumn seedbank size and seedbank carry-over varied as a function of year (Fig. 1A; Table 2). There was a significant positive correlation between seed density in the autumn and seed density in the spring, using mean values for each site and year ($r = 0.812$, d.f. $= 6$, $P = 0.0147$), but this relationship only explained 66% of the variation in spring seedbank size. The remaining variation is due to differences in seed carry-over between sites and years. There were substantial differences in seed carry-over among years, as supported by the significant year by season interaction for seed density (Table 2). The highest carry-over (mean 20.8%) occurred in 2003, which had autumn precipitation well below average and spring precipitation near the average, while the lowest carry-over (6.8%) occurred in the year 2005, which experienced an autumn with nearly double the average precipitation and a spring near the average (Fig. 2). These results support the hypothesis that seedbank carry-over tends to be greater in years with below-average autumn precipitation.

The site by season interaction for the seed density variable was also highly significant (Fig. 1A; Table 2). This suggests that seed carry-over differed between sites. Although there is no direct test of this difference because of design limitations, the trend was lower for mean successful seed carry-over at the arid shadscale site (9%) than at the sagebrush site (16%). The significant three-way interaction for seed density suggests that the degree to which the sites differed in seed carry-over varied by year. This interaction is evident in Fig. 1A; for 2001 and

 

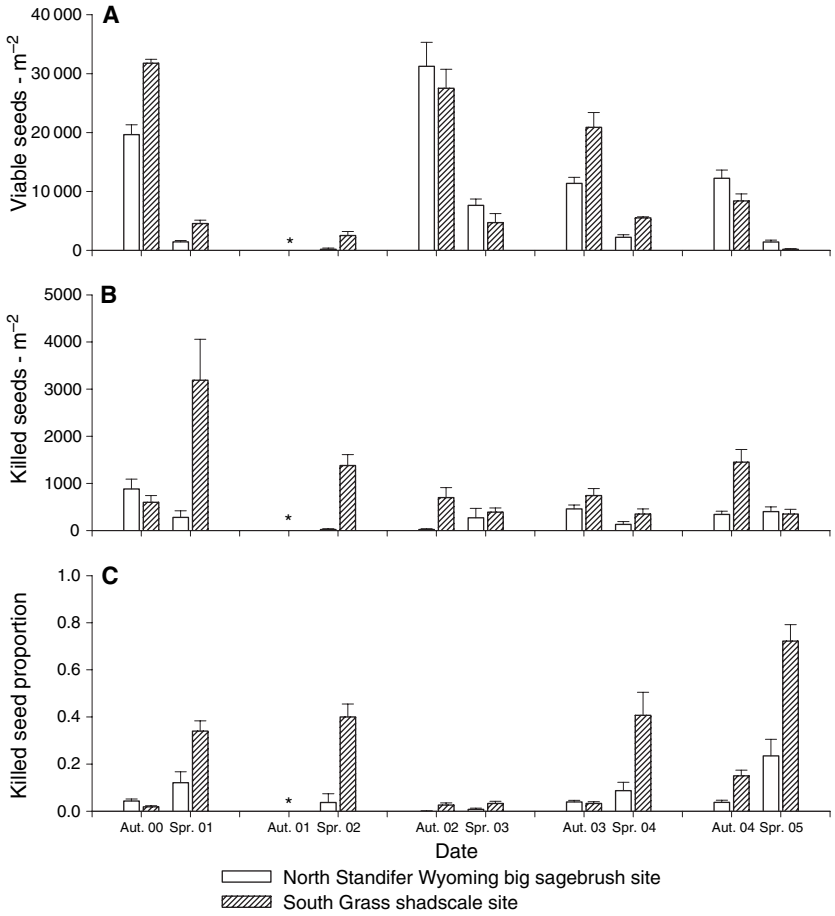


**Fig. 1** (A) Viable *Bromus tectorum* seed density in autumn before germination and in late spring before seed dispersal over the period September 2000 to May 2005 at the North Standifer sagebrush and the South Grass shadscale sites on the Snake River Plains in southern Idaho, (B) density of seeds killed by *Pyrenophora semeniperda* during incubation and (C) proportion of apparently viable seeds that were killed by *P. semeniperda* in incubation. (*indicates missing data; error bars represent standard error of the mean of untransformed data).

**Table 2** Analysis of variance for the Idaho *Bromus tectorum* seedbank study using the GLM procedure

| Model effects/dependent variables | Model d.f. | *F*-value | *P*-value |
|---|---|---|---|
| Log viable seed density (error d.f. = 298) | | | |
| Site | 1 | 21.48 | <0.001 |
| Year | 3 | 27.52 | <0.001 |
| Season | 1 | 248.82 | <0.001 |
| Site × year | 3 | 10.53 | <0.001 |
| Site × season | 1 | 26.05 | <0.001 |
| Year × season | 3 | 10.70 | <0.001 |
| Site × year × season | 3 | 6.48 | <0.001 |
| Log killed seed density (error d.f. = 298) | | | |
| Site | 1 | 15.12 | <0.001 |
| Year | 3 | 7.58 | <0.001 |
| Season | 1 | 4.07 | 0.045 |
| Site × year | 3 | 1.68 | n.s. |
| Site × season | 1 | 0.06 | n.s. |
| Year × season | 3 | 5.48 | 0.001 |
| Site × year × season | 3 | 8.81 | <0.001 |
| Transformed killed seed proportion (error d.f. = 276) | | | |
| Site | 1 | 76.34 | <0.001 |
| Year | 3 | 30.36 | <0.001 |
| Season | 1 | 114.48 | <0.001 |
| Site × year | 3 | 6.22 | <0.001 |
| Site × season | 1 | 44.23 | <0.001 |
| Year × season | 3 | 6.86 | <0.001 |
| Site × year × season | 3 | 3.69 | 0.013 |

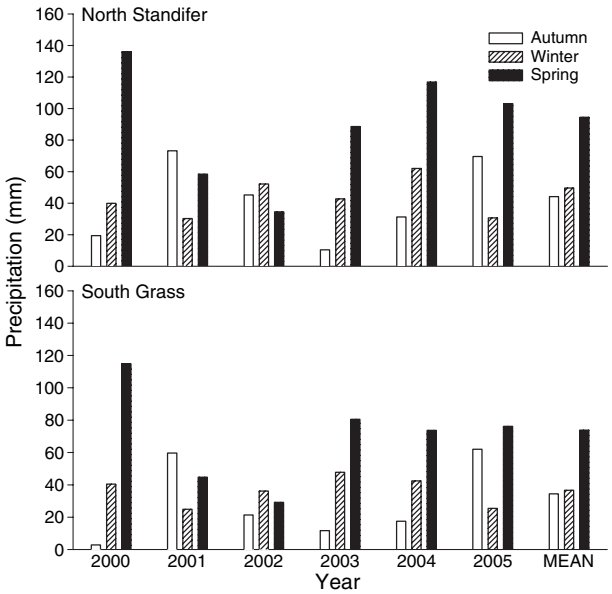


**Fig. 2** Mean seasonal precipitation totals and yearly seasonal totals for rain gauges near the North Standifer sagebrush study site and the South Grass shadscale study site for each year (August through May) over the 6-year period of the study. Autumn precipitation includes August through November, winter precipitation includes December and January and spring precipitation includes February through May.

2003, both sites show similar diminution in seedbank size from autumn to spring, while in 2004 and 2005, the shadscale site showed much greater diminution than the sagebrush site.

The number of seeds killed by *P. semeniperda* during incubation was significantly higher at the shadscale site (mean 930 vs. 330 seeds per $m^2$ at the sagebrush site; Fig. 1B; Table 2). The difference between seasons was only marginally significant, with mean values of 620 seeds per $m^2$ in the autumn and 650 seeds per $m^2$ in the spring. The season higher order interactions were all significant, but are difficult to interpret (Table 2; Fig. 1B). In some years, the difference between sites was greater in the autumn and in other years this difference was greater in the spring. Seeds that showed signs of the disease in spring samples in a given year and those that showed disease signs the following autumn are in all likelihood from the same subset of seeds, namely carry-over seeds that became infected in the spring seedbank prior to sample collection, but which dried before disease signs appeared in the field. This would explain why the average absolute number of seeds that developed the disease in incubation was essentially the same in spring and autumn samples. Current-year seeds in the autumn seedbank would be unlikely to contract the disease because of their quick germination, and previously uninfected carry-over seeds that have been allowed to after-ripen also germinate quickly. Given our procedure of allowing all seeds to after-ripen prior to performing germination tests, most seeds in both spring and autumn samples that developed disease signs were probably infected in the spring prior to sampling.

A more easily interpretable disease variable is the fraction of total apparently viable seeds (viable + killed seeds), which were killed during incubation (Fig. 1C; Table 2). Spring values were almost always higher than autumn values for this variable (mean 0.226 vs. 0.044), and the season main effect is highly significant (Table 2). The proportion of killed seeds was higher at the arid shadscale site than at the sagebrush site (mean 0.186 vs. 0.071). The mean proportions were higher at the shadscale site in both spring (0.358 vs. 0.116) and autumn (0.057 vs. 0.031), but the difference was much more pronounced in spring, resulting in a significant two-way interaction. The exact nature of these relationships varied by year (e.g. the differences were not particularly apparent in 2003), making the three-way interaction significant as well.

In analysis of variance for density of field-killed seeds with stromata for spring samples from 2003 to 2005, site and year main effects were significant, while the site by year interaction was not (Fig. 3). On average, there were almost five times as many field-killed seeds with stromata in samples from the shadscale site (mean 14 600 vs. 3200 seeds per $m^2$ for the sagebrush site; site main effect: d.f. = 1, 108; $F = 14.54$; $P = 0.0002$). The mean density of field-killed seeds with stromata was much higher in spring 2003 and 2004 than in spring 2005 (year main effect: d.f. = 2, 108; $F = 4.68$; $P = 0.0113$), but the difference between sites was apparent even in 2005.

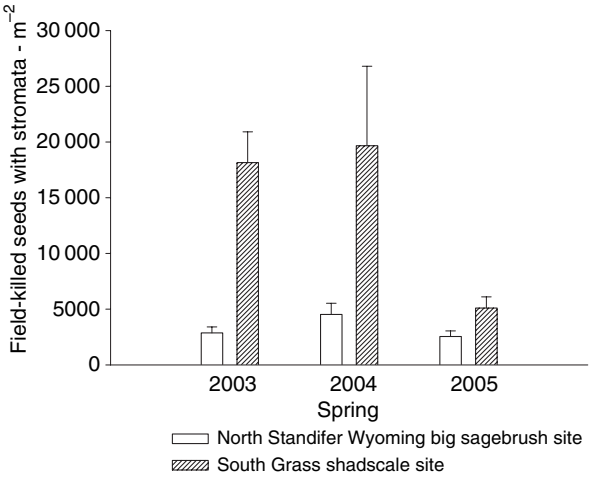


**Fig. 3** Density of field-killed *Bromus tectorum* seeds with fungal stromata in seedbank samples in each of three springs at the North Standifer sagebrush and South Grass shadscale sites on the Snake River Plains in southern Idaho. (Error bars represent standard error of the mean).

### *Utah study*

In our study of seedbank size and pathogen impact for seedbanks containing seeds of different known ages conducted at Whiterocks in Skull Valley, Utah, the seed age main effect was highly significant for viable seed density (d.f. = 2, 84; $F = 157.37$; $P < 0.0001$) and for the proportion of seeds killed by *P. semeniperda* during incubation (d.f. = 2, 84; $F = 13.18$; $P < 0.0001$). Differences in absolute densities of seeds killed in incubation were not significant.

As expected, the highest viable seed density (13 800 seeds per $m^2$) was found where the current-year seedbank had been sampled along with older seeds, while intermediate seed density (3100 seeds per $m^2$) was encountered where the seedbank was at least 1 year old (Fig. 4). Low seed density (230 seeds per $m^2$) was encountered where the seedbank was at least 2 years old. We have no estimate of first-year carry-over from autumn 1998 to spring 1999 at Whiterocks, but the absolute density of first-year carry-over seeds was close to the mean density from the Idaho study (2600 seeds per $m^2$). Second-year carry-over in the denuded area at Whiterocks averaged 7.4% of the 1-year-old seeds present. Using the mean first-year carry-over from the

 

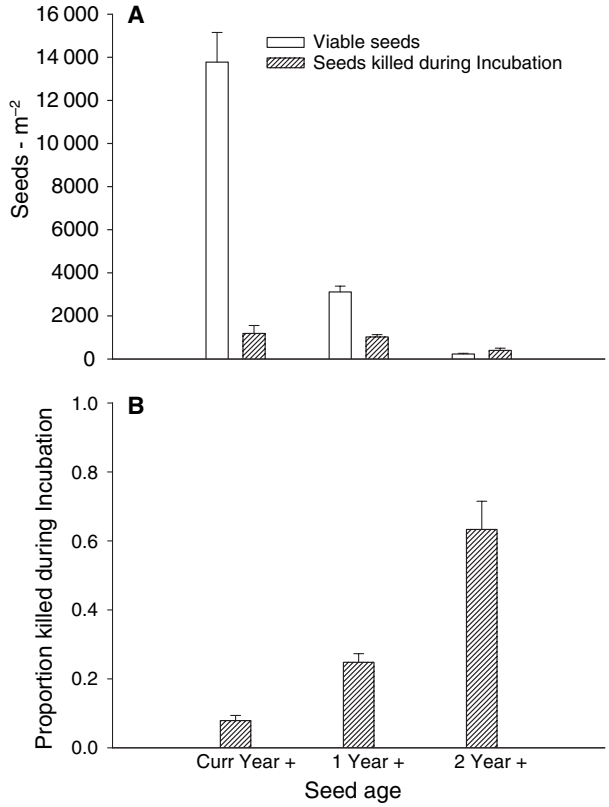


**Fig. 4** (A) Density of viable *Bromus tectorum* seeds and of seeds killed by *Pyrenophora semeniperda* in incubation, for seeds of three ages at the Whiterocks study site in Skull Valley, Utah, (B) proportion of apparently viable seeds in the seedbank that were killed by *P. semeniperda* during incubation. Current-year-plus seeds represent all seeds in the seedbank after dispersal of current-year seeds in 1999, one-year-plus seeds represent all the seeds in the seedbank in the summer of 1999 in a nearby area where grasshoppers prevented all current-year seed production, and 2-year-plus seeds represent all seeds in the seedbank in the latter area the following spring, after germination was complete but before dispersal of current-year seeds. (Error bars represent standard error of the mean).

Idaho study (12.4%) as an estimate of first-year carry-over at Whiterocks in 1999, successful second-year carry-over would represent 0.124% × 0.074% or 0.9% of the original seedbank.

The proportion of seeds that were killed during incubation by *P. semeniperda* increased dramatically with seed age (Fig. 4). Absolute numbers at the site with current-year seed and the site with only seed of at least 1 year old were essentially identical, suggesting again that current-year seeds in the autumn seedbank rarely, if ever, develop the disease at inoculum levels that occur naturally in field seedbeds. Because most of the seeds were current-year seeds, the killed seed proportion was only 0.075. In the seedbank containing only seeds of at least 1 year old, this proportion increased to 0.248, while in the seedbank containing only seeds of at least 2 years old, almost two thirds (0.633) of the seeds were killed during incubation.

## Discussion

By any estimate, *B. tectorum* populations produce prodigious quantities of seeds in a typical year. Mean autumn seedbank density in our study equated to more than 20 000 seeds per m$^2$, and densities ranged from 8000 to 31 000 seeds per m$^2$. These values are similar to those reported by other workers (Young *et al.*, 1969; Hull & Hansen, 1974; Humphries & Schupp, 2001). Late spring seedbank densities and first-year seedbank carry-over percentages in our study also fell within the range of previously reported values (Chepil, 1946; Hulbert, 1955; Young *et al.*, 1969; Hull & Hansen, 1974; Wicks, 1997).

Our data support earlier conclusions that *B. tectorum* forms seedbanks that persist for at least a year in habitats where autumn precipitation is limiting, but that seedbank persistence beyond 3 years is unlikely. One possible exception to this generalization is found in arable systems, where deep burial can result in persistence of a small fraction of *B. tectorum* seeds for up to 5 years (Wicks *et al.*, 1971). Enforced dormancy under the anoxic conditions of deep burial has been reported for many weedy species (Baskin & Baskin, 1998).

Our data supported the hypothesis that seed carry-over would be negatively correlated with autumn precipitation and therefore the probability of autumn germination. The viable seed carry-over percentages in our study are a measure not only of seeds that escaped germination in the previous growing season, but also of seeds that escaped infection by *P. semeniperda* (as well as other unmeasured causes of mortality such as seed predation). The net result of this interplay was lower successful seedbank carry-over at the drier site, although a higher fraction would be expected to remain ungerminated in early spring at this site. This supports our hypothesis that the risk of *P. semeniperda*-caused mortality is higher at the drier site. This conclusion is supported by the fact that three times as many apparently viable seeds from the drier site were killed during incubation and there were almost five times as many field-killed seeds with pathogen stromata in seedbank samples from the drier site. Neither of these values gives a direct measure of pathogen-induced mortality, however. The seeds that are killed during incubation probably represent only a fraction of those that are killed by this pathogen each spring. We have no estimate of the time of death for field-killed seeds with stromata in seedbank samples. These numbers can be taken to indicate generally higher levels of disease at the drier site, but they cannot be used as a direct measure of seed

mortality in a given spring. A much more detailed seedbank study, tracking the fate of seeds over short time intervals through the spring, would be necessary to measure pathogen-caused mortality directly. The increased proportion of pathogen-killed seeds at the drier site, in the carry-over seedbank, and in older seeds in the Whiterocks study all point to infection in the spring seedbank as the primary mode of seed infection for this pathogen on *B. tectorum*.

Mortality risk clearly increased with seed age in the Whiterocks study, probably because disease risk is cumulative over time, i.e. the longer a seed sits in the seedbank, the higher the probability that the conditions conducive to infection will eventually occur. The pathogen may be an important factor in limiting seedbank carry-over of *B. tectorum* in wildland settings to 2 or possibly 3 years.

The higher incidence of *P. semeniperda*-caused mortality at the drier site in this study is supported by data from other studies. In a comparison of disease incidence on *B. tectorum* seeds at an arid shadscale site and a mesic mountain brush site, Beckstead *et al*. (2006) found that both seedbank carry-over and disease incidence were extremely low at the mountain brush site, where complete autumn seedling emergence occurs most years. This trend for increased disease levels at drier sites was supported in a disease survey carried out at 32 sites across a range of habitats in 2005 (SE Meyer, J Beckstead & PS Allen unpubl. obs.). In an earlier study, higher levels of infection occurred when perennial forage grass seeds were planted into sagebrush communities than when they were planted into more mesic mountain brush communities; the disease appeared to be completely absent from montane aspen–conifer communities (Kreitlow & Bleak, 1964). These perennial grasses, which have slower mean germination rates than *B. tectorum*, were killed in the autumn prior to any seedling emergence. The pathogen could have considerable impact on perennial seedling establishment under this scenario. To our knowledge, Kreitlow and Bleak (1964) are the only workers who have addressed the impacts of *P. semeniperda* on perennial grasses. These impacts will need to be considered carefully in the context of *B. tectorum* biocontrol using this pathogen in wildland settings.

In their evaluation of *P. semeniperda* as a possible biocontrol agent for annual grass weeds in winter cereal crops, Medd and Campbell (2005) proposed a methodology predicated upon the idea that infection of developing ovules is the primary mode of seed infection for this pathogen. One of the limitations of the floral inoculation method is that an extended dew period of 48 h coupled with relatively warm temperatures is needed to obtain high infection levels. Such conditions may only rarely be encountered during flowering, especially on more arid sites. Our results suggest that the idea of biocontrol with *P. semeniperda* could be extended to include seedbank inoculation, where the timing of application would be much less critical. For *B. tectorum*, field inoculation would be most likely to impact secondarily dormant carry-over seeds, whether applied to developing seeds or to mature seeds in the seedbank, because non-dormant seeds generally germinate so quickly that they can escape the deleterious effects of the pathogen (Beckstead *et al*., 2006). Use of this pathogen for control of the carry-over seedbank could be helpful as part of a programme that included other methods such as herbicide use for control of emerged seedlings or a biocontrol organism such as *Ustilago bullata* for reduction of seed set in the autumn-emerging cohort (Meyer *et al*., 2001). It is also possible that artificially elevated levels of *P. semeniperda* inoculum or inoculation with particularly virulent strains could result in death of non-dormant *B. tectorum* seeds in the field. These possibilities are currently under investigation.

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