Epidemiology / Épidémiologie

A seed bank pathogen causes seedborne disease: *Pyrenophora semeniperda* on undispersed grass seeds in western North America

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Abstract: The generalist pathogen *Pyrenophora semeniperda* is abundant in seed banks of the exotic winter annual grass *Bromus tectorum* in semiarid western North America and is also found in the seed banks of co-occurring native grasses. In this study, we examined natural incidence of disease caused by this pathogen on undispersed host seeds, that is, seeds that were never directly exposed to inoculum in the seed bank. We determined experimentally that at least 90% of undispersed *B. tectorum* seeds exhibiting the disease were likely infected after maturity by conidia borne superficially on the seed-covering structures. The fraction of undispersed seeds exhibiting disease under optimum conditions for infection in the laboratory varied from 0% to 22% for three grass species. Relatively high disease incidence on undispersed seeds for this dry-sporulating fungus was significantly correlated with low mean annual rainfall, dry conditions during the conidial dispersal period in early summer, and high concentrations of soilborne inoculum as evidenced by the presence of high densities of killed seeds bearing fungal stromata in the seed bank. These three variables explained 66% of the variation in seedborne disease incidence for *B. tectorum* seed collections made in 2005. By dispersing conidia onto host seeds while seeds are still on the plant, this pathogen achieves the potential for targeted dispersal along with seeds of its host. This may be particularly advantageous for *P. semeniperda*, which has large conidia (phragmospores) that may not be efficiently dispersed beyond the grass canopy boundary layer.

Key words: conidia, *Bromus tectorum*, *Drechslera campanulata*, *Elymus elymoides*, *Leymus cinereus*, *Pyrenophora semeniperda*, spore dispersal.

Résumé : L'agent pathogène opportuniste *Pyrenophora semeniperda* abonde dans les banques de semences de variétés exotiques de brome non alternatif *Bromus tectorum* des régions semi-arides de l'ouest de l'Amérique du Nord ainsi que dans les banques de semences de graminées indigènes environnantes. Au cours de cette étude, nous avons examiné l'incidence naturelle de la maladie causée par cet agent pathogène chez des semences hôtes non dispersées, c'est-à-dire chez des semences qui n'avaient jamais été directement exposées à l'inoculum dans une banque de semences. Nous avons déterminé expérimentalement qu'au moins 90 % des semences non disséminées de *B. tectorum* qui affichaient des signes de la maladie avaient de toute évidence été infectées, après leur murissement, par des conidies qu'elles portaient à la surface des structures qui les recouvrent. La fraction des semences non disséminées qui affichait des signes de maladie à la suite d'exposition dans des conditions contrôlées optimales variait de 0 % à 22 %, et ce, pour trois espèces de graminées. Une incidence relativement élevé de la maladie chez les semences non disséminées, causée par ce champignon sporulé à sec, a été significativement corrélée avec la faible précipitation moyenne annuelle, les conditions sèches durant la période de dispersion des conidies en début d'été et les fortes concentrations d'inoculum terricole, comme le prouvent les forts taux de semences mortes de la banque, semences portant des stromates fongiques. Ces trois variables expliquaient 66 % de la variation de l'incidence de la maladie transmise par les semences de *B. tectorum* ramassées en 2005. En disséminant des conidies sur les graines hôtes pendant que celles-ci sont encore attachées au plant mère, l'agent pathogène atteint son potentiel de dispersion le plus élevé lorsque les graines sont elles-mêmes dispersées. Ceci peut être particulièrement avantageux pour *P. semeniperda* qui possède de larges conidies (phragmospores) qui autrement risqueraient de ne jamais franchir la couche limite formée par la couverture herbeuse.

Mots-clés : conidies, *Bromus tectorum*, *Drechslera campanulata*, *Elymus elymoides*, *Leymus cinereus*, *Pyrenophora semeniperda*, dispersion des spores.

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Introduction

Spore dispersal is an important component of the life cycle for many fungi, serving the multiple roles of increasing population extent, establishing new populations, reducing intraspecific competition, and increasing the chances for genetic recombination through increased potential encounters between products of asexual reproduction by different genotypes (Dix and Webster 1995). The most common mode of spore dispersal is by wind. Ballistic ejection, gravity, water, and animals are often listed as secondary mechanisms for which particular fungal taxa may be specifically adapted (Ingold 1971). One mode of dispersal that is rarely included in such lists is dispersal by plants, that is, on or inside the seeds of plants, which are themselves subject to dispersal by various agents. The importance of seedborne inoculum in the transport and transmission of disease is well established for many pathogens of crops (Maude 1996). In some cases, these pathogens grow systemically in the plant and arrive at the seed by an internal route (Grazia-Garza et al. 1999). Biological invasions of fungal pathogens involving long-distance transport with crop seeds have been well documented (Elmer 2001).

Another case where seedborne spore dispersal might be important is for long-distance dispersal of pathogens that reside in soil, particularly seed bank pathogens. It is difficult for microbial spores in the soil to become entrained in the wind because of boundary layer effects, especially if they are relatively large (Allen et al. 2002). Dispersing beyond the boundary layer created by a dense grass canopy is especially difficult because of turbulence effects, though intermittent strong winds can sometimes override these effects (Aylor 1999). By dispersing spores short distances directly from the soil onto undispersed host seeds within this boundary layer, that is, onto seeds while they are still on the plant, these pathogens could take advantage of host dispersal ability to achieve their own long-distance dispersal, with the added advantage of arriving at a new location already in the company of a suitable host seed.

In spite of their potential importance to the population biology of plants, seed bank pathogens are poorly studied, largely because of the intrinsic difficulty of identifying and quantifying the pathogens involved (Gilbert 2002). The generalist grass seed bank pathogen *Pyrenophora semeniperda* (Brittlebank and Adam) Shoemaker (anamorph *Drechslera campanulata* (Lév.) B. Sutton) produces distinctive, macroscopic stromata on diseased seeds, making it a tractable organism for developing a model of how seed bank pathosystems operate in nature (Beckstead et al. 2007; Meyer et al. 2007a, 2007b). *Pyrenophora semeniperda* carries out its entire life cycle on living seeds and is believed to be an obligate biotroph in nature (Medd et al. 2003; Paul 1969). It sometimes forms leaf spots on seedling leaves, but is not known to sporulate from these leaf spots (Campbell and Medd 2003). It is rarely seen in the perfect state, but is commonly encountered as the anamorph during seed testing of cereal grains and other grasses, at least in North and South America and in Australia. It has not yet been reported from Eurasia (Yonow et al. 2004). It has a very wide host range among the grasses and is occasionally reported from dicot seeds (Medd 1992). Sampling of in situ seed banks, especially of introduced annual grasses in semiarid and arid habitats, has revealed the importance of *P. semeniperda* as a seed bank pathogen in western North America (Meyer et al. 2007a, 2007b). Tens of thousands of *P. semeniperda*-killed seeds per square metre have been found in the seed banks of the exotic winter annual *Bromus tectorum* L. (cheatgrass). This species can produce as many as 50 000 viable seeds per square metre annually (S.E. Meyer, unpublished data).

The biology of *P. semeniperda* has received considerable study in recent years because of its potential as a mycoherbicide for annual grass weeds (Medd et al. 2003; Medd and Campbell 2005). It has been considered a weak pathogen because it often fails to kill infected seeds, which may develop into normal seedlings in spite of the presence of pathogen stromata protruding through the seed coverings (Campbell and Medd 2003). Most of this previous work was with fast-germinating crop seeds such as wheat. Direct inoculation of mature seeds caused development of disease but did not result in seed or seedling mortality. Medd and Campbell (2005) established that seed mortality of target weeds could be increased by inoculating seeds with pathogen conidia while still in early developmental stages on the plant. This resulted in "floral infection," where conidial germ tubes penetrated into the seed covering structures during development. The now deeply seedborne pathogen became latent as the seeds experienced maturation drying, and the mycelium commenced regrowth and caused disease upon subsequent imbibition in inoculation trials. Penetration of the endosperm and embryo did not take place until the mature seeds were imbibed; thus, the pathogen was not as deeply seedborne as those organisms that infect the developing seed systemically or penetrate deeper into these interior tissues from a point of infection on the ovary surface (Singh and Mathur 2004). Medd and Campbell (2005) postulated that floral infection was the primary infection mode for this pathogen.

In contrast, we have found that the fate of seeds infected by *P. semeniperda* is largely a function of their germination rate, with slow-germinating or dormant seeds suffering high mortality following direct inoculation onto mature seeds in the laboratory (Beckstead et al. 2007). We have also established that mature, dormant seeds of the annual grass weed *B. tectorum* can suffer high mortality in field seed banks after exposure to soilborne inoculum (Meyer et al. 2007a, 2007b). The importance of floral infection, or seedborne disease in general, has not been evaluated in natural host populations. In this study, we examined factors that regulate incidence of disease caused by *P. semeniperda* on undispersed host seeds of three grass species in semiarid western North America. This begins to address the role of seedborne spore dispersal in the evolutionary ecology of this fungus.

The objectives of our study were as follows: (i) to determine whether infection by *P. semeniperda* on undispersed seeds of the host grass *B. tectorum* is due to deeply seedborne inoculum (i.e., floral infection; Medd and Campbell 2005) or superficially seedborne inoculum (i.e., surface contamination; Singh and Mathur 2004); (ii) to quantify incidence of natural seedborne infection caused by *P. semeniperda* on undispersed seeds of three host grasses; and (iii) to relate incidence of seedborne infection to soilborne

inoculum concentration and to environmental factors. Because of the long dew periods required for successful floral infection (Medd and Campbell 2005), we hypothesized that this mode of pathogen transmission would be rare at the semiarid study site where we collected seed samples. The lack of any special adaptations in the fungus for propelling conidia onto host plant infructescences also led us to expect disease incidence on undispersed seeds to be relatively low overall. We hypothesized that higher soilborne inoculum loads and drier weather during conidial production and seed ripening would be associated with higher incidence of seedborne disease for this dry-sporulating pathogen (Aylor and Flesch 2001). Because the abundance of *P. semeniperda* in soil seed banks is generally higher at more xeric sites, we also expected higher incidence of seedborne disease at more xeric sites.

Materials and methods

Inoculation experiments with potentially infested vs. disinfested seeds

Bulk seed collections from at least 100 individuals were made at the Whiterocks study site in western Utah in early summer 2006 and 2007. Seeds were maintained in primary dormancy in a freezer until initiation of the experiment in November 2007. Four hundred filled florets (hereafter referred to as seeds) of each lot were then hand selected. Half of the selected seeds from each lot were subjected to disinfestation (surface sterilization) following a protocol similar to that of Beckstead et al. (2007) to kill superficially borne conidia. The seeds of each lot were placed in a tea strainer and suspended for 2 min in 70% ethyl alcohol, 2 min in 10% chlorine bleach, then for an additional 2 min in 70% ethyl alcohol. They were then rinsed thoroughly in sterile distilled water. Disinfested and potentially infested control seeds were counted into five replications of 40 seeds each and placed onto two blue germination blotters (Anchor Paper, St. Paul, Minn.) wetted with tap water inside 15 mm × 100 mm disposable plastic Petri dishes and incubated at 20 °C. Seeds were scored for germination and for appearance of the characteristic fungal stromata at 2, 3, and 4 weeks. Very few seeds (<2%) germinated; these were held in the dishes post-germination and evaluated with ungerminated seeds for the appearance of stromata. At the end of 4 weeks, ungerminated seeds without stromata were evaluated for viability using a cut test, which consists of examining longitudinally bisected seeds (AOSA 2007). Seeds with a firm endosperm and an intact, uniformly colored embryo were scored as viable. The viability of the remaining seeds was 100%. The proportion of initially viable seeds that developed stromata during incubation was the response variable in a two-factor fixed effect analysis of variance for a completely randomized design, with seed lot and disinfestation treatment as the class variables. Proportional data were arcsine square root transformed to improve homogeneity of variance prior to analysis and converted to percentage values for data presentation.

Germination experiments with naturally inoculated seeds

Bulk seed collections were made in the summer of 1992 from 23 populations of *B. tectorum*, four populations of *Elymus elymoides* (Raf.) Swezey, and four populations of *Leymus cinereus* (Scribn. & Merr.) A. Löve in Utah, Idaho, and Nevada from a range of habitats for each species (Beckstead et al. 1995; Meyer et al. 1995, 1997). Each collection included seeds from at least 30 individuals. Seeds were cleaned by hand-rubbing and blowing in a forced-air blower and stored in paper sacks under laboratory conditions until initiation of experiments. Experiments were initiated within two weeks of collection, while seeds were still in a state of maximum primary dormancy. Seeds were subjected to incubation at four alternating temperature regimes: 5/15 °C, 10/20 °C, 15/25 °C, and 20/30 °C (12/12 h), with cool-white fluorescent light during the higher temperature period each day. For each treatment, a collection was represented by four replications of 25 seeds placed in 15 mm × 100 mm plastic Petri dishes on the surface of two blue germination blotters saturated with tap water. The dishes were stacked in plastic bags to conserve moisture before placement in the incubation chambers and were watered as needed during the course of the experiment. Germinated seeds (radicle protrusion >1 mm) were counted and removed after 1, 2, 4, 7, 11, 14, 21, and 28 days of incubation. On day 28, the remaining ungerminated seeds were examined for stromata of *P. semeniperda* and scored as dormant, diseased, or initially nonviable (due to causes other than this disease). The proportion of initially viable seeds showing fungal stromata was then calculated for each replication as (diseased seeds)/(germinated seeds + dormant seeds + diseased seeds). This methodology could have underestimated disease incidence because germinated seeds may have been discarded before they could exhibit disease. Because the seeds were in a state of primary dormancy and only germinated slowly if at all, this source of error should not be large. We assumed in these calculations that seeds manifesting stromata during incubation were initially viable because when these seed lots were tested after dormancy was lost, seeds almost always germinated normally. We also assumed that seeds that exhibited stromata prior to germinating were killed by the pathogen. In experiments with artificially inoculated seeds, those that exhibited stromata prior to germinating were always nonviable as assessed by a post-incubation cut test, which revealed the extensive damage caused by the pathogen (Beckstead et al. 2007).

We carried out similar germination experiments and evaluations of disease incidence with additional *B. tectorum* and *E. elymoides* seed collections made in 1993, 1994, and 1995, each year from a slightly different subset of populations included in the original 1992 study (Bair et al. 2006; Bauer et al. 1998; Beckstead et al. 1996; Meyer et al. 2000). Seeds of *B. tectorum* and *E. elymoides* were collected at the Whiterocks study site in each of the four years.

To examine the relationship between habitat and disease incidence, we regressed log (mean disease incidence) for each collection (averaged across incubation temperatures) on mean annual precipitation at the collection site. We included collections of all three species and all four years for a total of 47 collections. Mean annual precipitation data

were obtained from nearby NOAA (National Oceanic and Atmospheric Administration) reporting stations or SNOTEL (Natural Resources Conservation Service Snow Pack Telemetry) stations for each collection site. Collection sites were chosen to represent a range of habitats for each species with multiple representation for habitats where each species was most common. This resulted in more collections from low elevation sites than high elevation sites.

To examine the relationship between disease incidence and precipitation during flowering and seed ripening, we used four years of disease incidence data for the *B. tectorum* and *E. elymoides* populations at the Whiterocks study site, located in xeric salt desert habitat in west-central Utah. *Bromus tectorum* produces flowers and fruits approximately one month earlier than *E. elymoides* in this environment. Seeds of *B. tectorum* usually reach full maturity in early to mid-June and those of *E. elymoides* in early to mid-July. If seedborne disease were due to floral infection during prolonged dew periods, we would expect a positive relationship between precipitation during flowering and subsequent disease incidence. To test for this, we performed analysis of covariance (ANCOVA) with species as the class variable, precipitation during flowering as the continuous variable, and disease incidence on undispersed seeds as the response variable. We used April precipitation data for the flowering period for *B. tectorum* and May precipitation data for the flowering period for *E. elymoides*. Conversely, if seedborne disease were due to dispersal of conidia onto mature, undispersed seeds, we would expect a negative correlation between precipitation data during seed ripening and disease incidence because conidia are more likely to disperse into the air from dry soil (Aylor and Flesch 2001; Allen et al. 2002). We used May precipitation data for the ripening period for *B. tectorum* and June precipitation data for the ripening period for *E. elymoides*. Monthly precipitation totals from the Dugway, Utah NOAA reporting station (mean annual precipitation 199 mm), located approximately 15 km southwest of the study site, were used for these analyses. The exact timing of anthesis each year for each species was not recorded.

Seed bank inoculum loads and disease incidence on undispersed seeds

In 2005, we initiated a new study examining *P. semeniperda* disease incidence on *B. tectorum* across a broad range of habitats. At each of 28 study sites, located in Utah, Colorado, Idaho, and Washington, we collected 10 in situ seed bank samples in late spring from random points in a *B. tectorum* monoculture using a soil sampling can measuring 6 cm in diameter and 4 cm deep (see Meyer et al. 2007a for details on methods). The seed bank samples were air-dried if necessary and stored for several weeks to allow viable *B. tectorum* seeds to lose dormancy (Bauer et al. 1998). The samples were then processed individually by hand to remove and quantify all apparently viable, ungerminated *B. tectorum* seeds as well as all ungerminated seeds bearing the stromata of the pathogen and assumed to be dead. Apparently viable seeds were then incubated at 20 °C for two weeks as described above and scored as germinable, dormant, nonviable, or killed by the pathogen, as evidenced by the development of stromata on ungerminated seeds during incubation. For the present analysis, only the densities of field-killed seeds with stromata were used, as these seeds were considered the primary source of any conidial inoculum likely to be dispersed out of the seed bank and onto undispersed seeds.

To evaluate incidence of seedborne disease, we collected mature seeds from 20 randomly selected individuals in each of the 28 populations. Seeds harvested from plants at each field site were stored in a cold room at 2 °C for 5–9 weeks (depending on collection date) to maintain primary dormancy until the evaluation of seedborne disease incidence could be initiated. Ten seeds from each of 20 individual plants obtained at each site were incubated in individual Petri dishes at 20 °C for 4 weeks and evaluated for the presence of stromata on ungerminated seeds as described earlier. Seeds were highly dormant; there was very little germination during this test. The few germinated seeds were counted and discarded. Disease incidence for each dish was calculated as described earlier, and the values were averaged to obtain mean seedborne disease incidence for each population.

Mean annual precipitation and current-year precipitation data during the ripening period (May) for each site were estimated from nearby weather stations as described for the earlier study. For the statistical analysis, we performed stepwise multiple regression with disease incidence on undispersed seeds as the response variable. The three independent predictor variables were density of killed seeds with stromata in the seed bank, mean annual precipitation at the site, and May precipitation at the site for the year of sample collection. These three independent variables were not significantly correlated with each other in the 2005 data set.

Results

Inoculation experiments with potentially infested vs. disinfested seeds

Disease incidence on undispersed dormant seeds collected at Whiterocks in 2006 and 2007 varied between years (Fig. 1). This difference was significant, with a mean disease incidence on control seeds of 20.5% for 2006 but only 4.5% for 2007 (year main effect: $F_{[1,16]} = 25.48$, $P = 0.0009$). Disease incidence was reduced on average by 90% (from 12.5% to 1.2%, averaged across collection years) with surface disinfestation, indicating that most disease was caused by conidia borne superficially on the surface of the seed coverings (disinfestation treatment main effect: $F_{[1,16]} = 54.86$, $P < 0.0001$). This supported our hypothesis that floral infection would not be a common occurrence at this xeric site and that disease was most likely caused by conidia dispersed onto the seeds from the seed bank immediately before seed dispersal. The year by disinfestation treatment interaction was also significant ($F_{[1,16]} = 5.18$, $P = 0.037$), as indicated by the greater proportional decrease following disinfestation in the year with higher overall disease incidence. There was no disease on disinfested seeds in the 2007 seedlot, whereas 2.5% of disinfested seeds in the 2006 seed lot developed disease.

Fig. 1. Disease percentages on dormant, undispersed seeds of *Bromus tectorum* with and without surface-disinfestation for collections made at the Whiterocks study site in western Utah in June 2006 and June 2007. Standard error bars are shown for each mean.

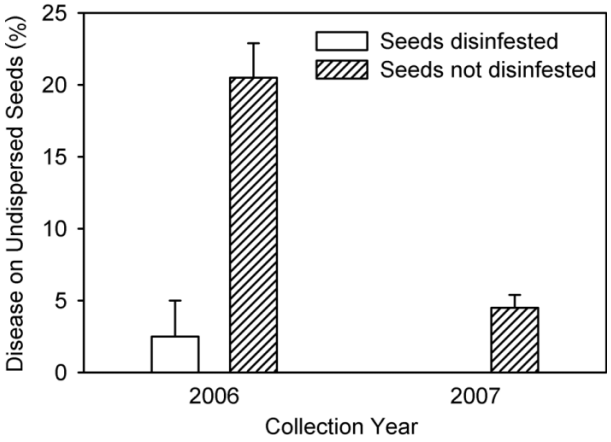
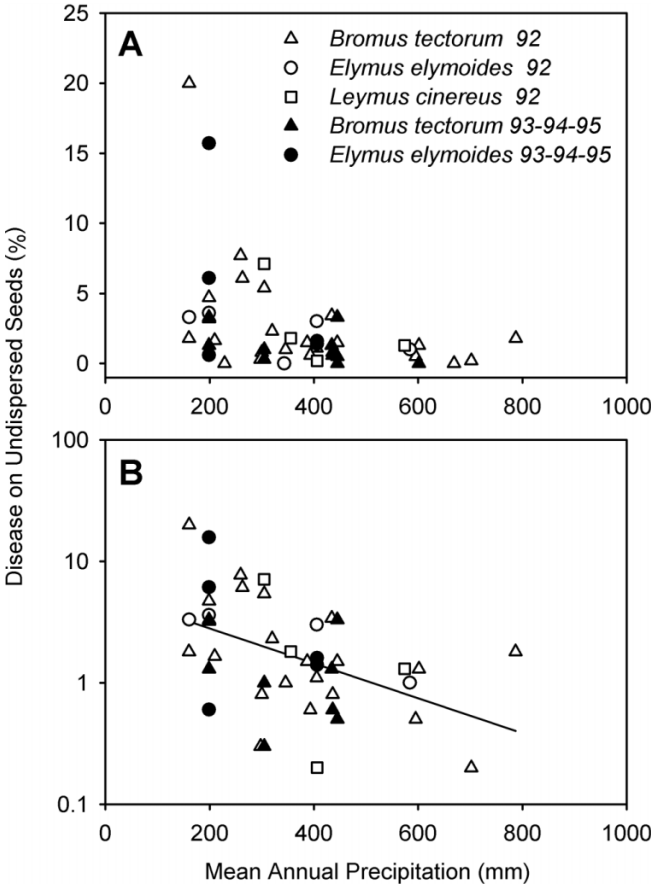


Fig. 2. (A) The relationship between disease percentage on undispersed seeds of three grass species and mean annual precipitation at the collection site for 47 seed collections belonging to three species made from 1992–1995. (B) $\log_{10}$ (disease incidence) regressed on mean annual precipitation for 47 seed collections shown in (A) ($\log_{10}$ (disease incidence) = –0.00144 (mean annual precipitation) + 0.735; R^2 = 0.218, df = 45, P < 0.001).



Germination experiments with naturally inoculated seeds

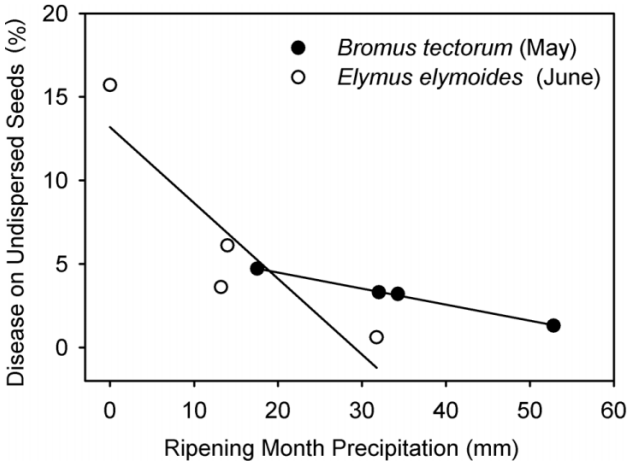
Disease incidence in the 1992–1995 multiple-species survey varied from 0% to 22%, with most seed lots showing low or very low disease incidence (Fig. 2). Seeds collected from mesic environments (mean annual precipitation >500 mm) always showed very low disease incidence (<2%). Collections from the most xeric sites (mean annual precipitation <200 mm) were the only collections with disease incidence >10%, and all collections with disease incidence >5% were from xeric sites (mean annual precipitation <300 mm). Many collections from xeric sites and most collections from semiarid sites (mean annual precipitation 300–500 mm) also exhibited low or very low disease incidence. The disease incidence response to mean annual precipitation appeared to be negatively exponential, though this apparent relationship hinged largely on a few outlier points. To test the significance of the relationship, we log-transformed the dependent variable prior to regression analysis (Fig. 2A). In the resulting linear regression, log (disease incidence) was significantly negatively related to mean annual precipitation at the collection site, although the R^2 value was low, accounting for only 22% of the variance (Fig. 2B).

In the ANCOVA for mean disease incidence as a function of species and flowering month precipitation for *B. tectorum* and *E. elymoides* at Whiterocks in 1992–1995, there were no significant effects (data not shown). This indicates that rainy periods during flowering were not associated with increased incidence of seedborne disease for either species, making floral infection an unlikely explanation for seedborne disease. In contrast, in the ANCOVA for mean disease incidence as a function of species and ripening month precipitation, the precipitation variable was significant ($F_{[1,4]}$ = 14.67, P = 0.0186) and had a negative slope, indicating an increase in disease incidence with decreasing ripening month precipitation (Fig. 3). This result is consistent with the hypothesis that disease is caused by conidia that disperse short distances within the canopy from dry soil onto the seeds during the last phases of seed ripening. The species by precipitation interaction was marginally significant ($F_{[1,4]}$ = 6.18, P = 0.0677). This interaction is displayed in Figure 3 as a steeper negative slope for *E. elymoides* than for *B. tectorum,* probably because *E. elymoides* experienced a year with an exceptionally dry ripening month followed by high disease incidence, while *B. tectorum* did not. The two species graphs taken together suggest a negative nonlinear disease incidence response to ripening month precipitation. The difference between species (disease incidence averaged across years) was not significant.

Seed bank inoculum loads and disease incidence on undispersed seeds

In results from the 2005 disease survey of 28 sites, disease incidence on undispersed seeds was not significantly correlated with density of killed seeds with stromata in the seed bank unless the three outliers from xeric low elevation sites in southeastern Utah were excluded from the analysis (Fig. 4A). These sites had rather low killed-seed densities

Fig. 3. The relationship between disease percentage on dormant, undispersed seeds of two grass species and precipitation during the seed ripening month at the Whiterocks study site in western Utah in 1992–1995. The seed ripening month was May for *Bromus tectorum* and June for *Elymus elymoides*. Statistics from analysis of covariance with species as the class variable and ripening month precipitation as the continuous variable were as follows: overall $F_{[3,4]} = 7.67$, $P = 0.039$; ripening month precipitation $F_{[1,4]} = 14.67$, $P = 0.019$; species $F_{[1,3]} = 0.53$, n.s. ($P > 0.1$); and species × ripening month precipitation $F_{[1,1]} = 6.18$, $P = 0.068$.



but relatively high disease incidence on undispersed seeds. The negative correlation between mean annual precipitation at the site and disease incidence on undispersed seeds was significant but only explained 45% of the variation in disease incidence (Fig. 4B). A significant negative correlation was also obtained with current-year ripening month (May) precipitation (Fig. 4C). The three southeastern Utah outliers anchored the lower end of both the mean annual precipitation regression and the May precipitation regression, suggesting that combining the variables in multiple regression analysis might result in a better model fit than any one of the independent variables when fit individually. Stepwise regression showed that the three-variable model gave the best fit to the data, with all three variables making significant contributions and 66% of the variation in disease incidence explained (Table 1). These results supported our hypothesis that higher concentrations of soilborne inoculum, more extreme site aridity, and drier weather during seed ripening would be positively associated with higher *P. semeniperda* disease incidence on undispersed seeds of *B. tectorum*.

Discussion

At least two lines of evidence from this study discount the importance of deeply seedborne inoculum and floral infection of *P. semeniperda* as a source of seedborne disease under natural conditions. First, disease incidence was reduced from relatively high values to near zero by surface disinfestation of the seeds, strongly suggesting that the inoculum was not deeply seedborne. Beckstead et al. (2007) obtained a similar result with surface disinfestation as a treatment to reduce natural inoculum loads in artificial inoculation experiments; disease incidence on disinfested control seeds averaged 0.5%. Second, the long dew periods during flowering that are needed for experimental induction

Fig. 4. The independent effects of three variables on disease percentage on dormant, undispersed seeds of *Bromus tectorum* collected from 28 sites in summer 2005. (A) Density of seed bank seeds with pathogen stromata at the seed collection site. (B) Mean annual precipitation at the seed collection site. (C) Current-year ripening month (May 2005) precipitation at the seed collection site. Outliers are three sites in southeastern Utah that were excluded from the regression in (A) but included in the regressions in (B) and (C).

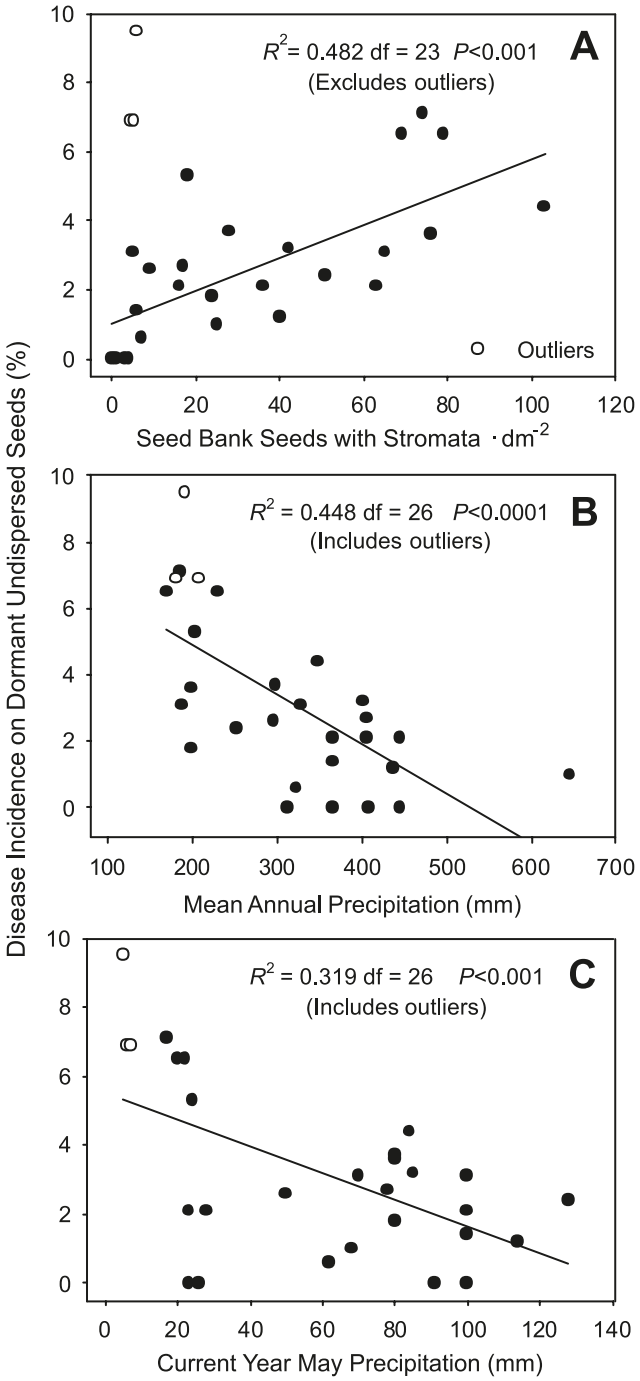


Table 1. Multiple regression analysis for the effects of three independent variables on disease incidence percentage on dormant, undispersed *Bromus tectorum* seeds collected at 28 sites in 2005.

Parameter	Parameter estimate	SE	*t* value	*P*-value
Y-intercept	7.58	1.042	7028	<0.0001
Seed bank seeds with stromata × dm^{-2}	0.0252	0.0110	2.3	0.0302
Current-year May precipitation	–0.0329	0.0088	–3.72	0.0011
Mean annual precipitation	–0.0102	0.0029	–3.46	0.0020

Note: Overall regression statistics: $R^2 = 0.663$, $F_{[3,24]} = 15.75$, $P < 0.0001$.

of floral infection (Medd and Campbell 2005) were rarely encountered under field conditions and were not positively associated with increased incidence of seedborne disease, at least at the Whiterocks study site during the period 1992–1995. Although Medd and Campbell (2005) showed that it is possible to induce deeply seedborne disease with artificial inoculation, it appears that this transmission mode is probably rare in nature. Once mature seeds are infected, however, they can readily become bearers of latent infection. For example, infected seeds can undergo repeated cycles of dehydration under both laboratory and field seed bank conditions and then be quickly killed by the pathogen when conditions once again become conducive for its growth and sporulation (S.E. Meyer, unpublished data).

The hypothesis that seedborne disease incidence would be higher at drier sites was also supported by two independent data sets. The negative exponential relationship between mean annual precipitation at the seed collection site and disease incidence on undispersed seeds in the multiple-species survey data set was significant but not particularly strong, accounting for only 22% of the variation in disease incidence. As we have seen, there are several other factors that can affect the amount of conidial inoculum that disperses onto undispersed seeds, so the relatively poor fit of the one-factor model is not surprising. In the 2005 *B. tectorum* disease survey data set, this hypothesis was supported in both simple and multiple regression analysis.

The hypothesis that dry weather during conidial dispersal would increase seedborne disease incidence was also supported by two independent studies. The four-year Whiterocks study produced clear results, with ripening-month precipitation accounting for 85% of the variation in disease incidence. A similar result was obtained in the 2005 *B. tectorum* disease survey, where May precipitation was significantly negatively correlated with disease incidence both when considered singly and when included in multiple regression.

In contrast, the role of seed bank inoculum concentration in mediating disease incidence on undispersed seeds was not as well supported as expected, with relatively high seedborne disease incidence sometimes associated with apparently low concentrations of inoculum in the seed bank. One possible explanation for this is that a simple density estimate of killed seeds with stromata may not be a good index of inoculum load. We have developed a more direct bioassay for inoculum load that involves collecting intact soil surface sample cores, baiting them with dyed dormant host seeds, incubating them in the laboratory, and evaluating disease on the bait seeds. Preliminary results show a high correlation between the two methods in late spring through summer, when ungerminated conidia are expected to be present, but not later in the fall and through the winter (S.E. Meyer, unpublished data). Conidia are believed to germinate in the first storms likely to trigger seed germination and to be depleted following these storms. Seed bank samples for the present study were taken in early summer, so that killed-seed densities in these samples should be positively correlated with actual inoculum loads.

The reason for the rather low densities of killed seeds at the three southeastern Utah xeric sites (Fig. 4A) is not known, but the reason that their negative effect on regression was ameliorated by adding the other two independent variables is clear. First, these are sites with low mean annual precipitation and therefore high expected disease incidence on undispersed seeds, as was observed (Fig. 4B). Second, these sites experienced very dry May weather in 2005 (Fig. 4C). This year was unusually wet throughout the region. A series of major May storms had a significant impact, especially in the northern and western parts of the survey region, bringing up to 100 mm of rain to many xeric, low elevation survey sites. These sites tended to exhibit low disease incidence on undispersed seeds even though seed bank inoculum loads were high, thereby lowering the correlation between killed seeds in the seed bank and disease incidence on undispersed seeds. The wet May weather also apparently limited maximum disease incidence on undispersed seeds, which was much lower in this study than in the earlier survey study or than at Whiterocks in 2006 (Fig. 1 and Fig. 2). These May storms were absent from southeastern Utah even at high elevations, resulting in a lack of correlation between current-year May precipitation and mean annual precipitation overall. The result was relatively high disease incidence at the outlier sites, where seed bank inoculum loads were somewhat low but where dry May weather favored conidial dispersal.

The importance of seed-assisted dispersal for conidia of *P. semeniperda* depends on adaptations for long-distance dispersal in host seeds. Without these, the pathogen is unlikely to increase its dispersal distance by "hitch-hiking" on a seed. In the case of *B. tectorum*, long-distance dispersal by animal and human vectors is apparently very common, as evidenced by patterns of molecular genetic variation in the introduced western North American range. We have found using characteristic molecular marker fingerprints that the populations of *B. tectorum* are made up primarily of a few inbreeding lines, and most of these lines are very widely distributed across the range (Ramakrishnan et al. 2006; S.E. Meyer, unpublished data). Preliminary data from

internal transcribed spacer (ITS) sequences for ribosomal DNA in *P. semeniperda* on *B. tectorum* support a similar dispersal pattern, with considerable genetic variation but little geographic structure, as the same ITS genotypes are widely distributed throughout the range (S.E. Meyer unpublished data). This pattern is consistent with the idea of conidial dispersal onto *B. tectorum* seeds that then undergo long-distance dispersal.

We have observed that the elongate, crescent-shaped conidia of the fungus can become lodged in hairs on the outer surface of a *B. tectorum* lemma in much the same way as the similarly shaped florets of the host become lodged in animal fur. The multicellular conidia may benefit more than many fungal spores from seed-assisted dispersal because of their large size. The optimum spore size for entrainment in wind and airborne dispersal is <10 μm (Dix and Webster 1995), whereas *P. semeniperda* conidia are often ten times that length (82–146 μm; Meyer unpublished data). This places them at the large end of the size spectrum for fungal spores and makes them less likely to be dispersed beyond the boundary layer formed by a dense *B. tectorum* canopy at most sites because of the faster fall rates associated with larger size (Aylor and Flesch 2001). However, once free of the canopy boundary layer, even spores in this size range are readily entrained in the wind in the planetary boundary layer (50 m to 1 km), where most successful long-distance dispersal takes place (Isard et al. 2005; Maldonado-Ramirez et al. 2005). Determining the probability of direct long-distance conidial dispersal for *P. semeniperda* would require spore-capture studies beyond the scope of the present investigation.

Successful *P. semeniperda* conidial dispersal onto seeds followed by establishment from seedborne inoculum under field conditions has yet to be conclusively demonstrated. More detailed examination of life history, along with additional studies of population genetic structure, should further clarify the role of seed-assisted dispersal in the evolutionary ecology of this important pathogen of semiarid wildland ecosystems.

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