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The influence of soil moisture on losses of buried seeds to fungi

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Abstract

Although soil fungi are likely to be a major cause of mortality for buried seeds, few ecological studies have examined the role these pathogens play in natural systems. In particular, few studies have investigated whether losses of seeds to soil fungi are habitat-dependent. We used fungicide treatments to investigate whether losses of buried seeds of four grasses (*Bromus inermis*, *Danthonia spicata*, *Glyceria striata*, and *Poa pratensis*) to soil fungi differed among meadows differing in soil moisture. We also applied water to some treatments, to determine whether this increased losses of seeds to fungi. For all four grasses, fungicide additions improved one or more measures of seed viability, though this effect was small. For *Danthonia* and *Glyceria*, fungicide was less likely to improve viability in dry meadows than in wet and/or mesic meadows. Adding water reduced some measures of viability of seeds of *Danthonia* and *Poa* in dry meadows, but fungicide partly counteracted these negative effects, suggesting that adding water reduced performance by increasing fungal attack. These results indicate that fungi represent a hazard for buried seeds of these species, particularly in wetter soils, and potentially may contribute to the reduction of populations of vulnerable species in wetter sites.

Keywords: Grasses, Old fields, Pathogens, Plant disease, Seed banks, Soil fungi

1. Introduction

A majority of mortality in many plants occurs at the seed stage, and a large component of this mortality often occurs within the seed bank (Harper 1977; Roberts 1981; Cavers 1983; Leck et al. 1989; Chambers and MacMahon 1994). Despite this, few ecological field studies have addressed the effects of pathogens on seed banks (Kremer 1993; Chambers and MacMahon 1994; Baskin & Baskin 1998, Thompson 2000, Gilbert 2002), though studies of seedlings and young plants have indicated that fungal pathogens can have important impacts at these stages (e.g., Augspurger 1983; Packer and Clay 2000). The few studies which have specifically considered buried seeds tend to suggest that mortality caused by fungi may be important at this stage as well. For example, Kirkpatrick and Bazzaz (1979) found that seeds of four early successional annuals were colonized by numerous fungal species, and that fungal isolates negatively affected seed germination and seedling development. Similarly, Crist and Friesse (1993) studied five shrub-steppe species in Wyoming and found evidence that fungal pathogens were responsible for up to 35% of seed mortality. Finally, studies using fungicides have demonstrated that reducing fungal populations significantly increases seed survival in such disparate habitats as northern Australian shrublands (Lonsdale 1993), Panamanian rainforest (Dalling et al. 1998), English grasslands (Leishman et al. 2000), and Ontario old fields (Blaney and Kotanen 2001, 2002).

One of the most important factors influencing attack by fungal pathogens may be the availability of water. Moisture can influence the distribution and spread of many pathogens, as well as affecting the development, longevity, germination, and infectiveness of fungal spores (Agrios 1997). While some fungal diseases, such as dry rot of beans (caused by *Fusarium solani*), prefer drier environments (Agrios 1997), the occurrence of many diseases is favored by heavy rainfall, dew, or extended periods of high relative humidity (Royle and Butler 1986). For

example, such soil-borne "damping-off" pathogens as *Pythium* and *Phytophthora* have long been known to be favoured by high humidity and wet, poorly drained soils (Roth and Riker 1943; Weber 1973; Rotem 1978).

Variation among habitats in soil moisture may result in spatial variation in losses to seed pathogens. In an old field study conducted in southern Ontario, Blaney and Kotanen (2001) found that fungal mortality of seeds was significantly higher in wetland sites than in upland sites. Studies by Augspurger (1983) and Augspurger and Kelly (1984) also suggest that moist, shady microenvironments can increase losses of germinating seeds. Patterns such as these may have profound importance for the distribution of plant species, but few other studies of natural systems have considered interactions between spatial variation in physical conditions and losses of seeds to fungi.

The goal of this study was to investigate differences in seed mortality among habitats of varying soil moisture and to determine whether these differences were due to variation in attack by fungi. Our principal question was:

1) Are losses of seeds to fungi greater in wetter habitats?

This would imply that fungicide additions should improve seed survival more in wet sites than in dry sites (a fungicide x habitat interaction).

Greater losses to fungi in wetter sites might reflect differences in soil biota driven by redox, soil organic content, or other factors as well as moisture *per se*. Consequently, we also asked a second question:

2) Does experimentally increasing soil moisture within a habitat increase losses of seeds to fungi?

This would imply that adding water to a site should decrease seed survival unless fungicide also was added (a fungicide x water interaction).

2. Materials and methods

2.1. Study area

This experiment was conducted in old field habitats located throughout the 348 ha Koffler Scientific Reserve at Joker's Hill, Regional Municipality of York, Ontario (44°02' N, 79°31' W) (<http://www.erin.utoronto.ca/~w3pkota/jh.html>). Three habitat types were chosen and six replicates of each were used. The three habitat types were drawn from old fields representing varying degrees of soil moisture: dry meadow, mesic meadow (intermediate wetness), and wet meadow. Each habitat was defined by indicator plant species, which also were the principal foci of this study. The xeric grass, *Danthonia spicata*, was used to identify dry upland meadows. The wetland grass, *Glyceria striata*, was used to identify wet meadows. Finally, the mesic grasses, *Bromus inermis* and *Poa pratensis*, were used to identify mesic meadows. Nomenclature follows Gleason and Cronquist (1991).

2.2. Experimental procedure

Mature seeds of *Bromus inermis*, *Poa pratensis*, *Danthonia spicata*, and *Glyceria striata* were collected from naturally occurring plant populations at Joker's Hill in July 1999. *Danthonia* produces both terminal and cleistogeme flowers (Darbyshire and Cayouette 1989); only seeds from terminal flowers were used in our study. Twenty filled seeds of each species were placed between two pieces of nylon stocking and held together by a plastic slide mount (Polaroid 35mm Slide Mounts, Polaroid Corp., Cambridge, MA, USA). This method was used so that seeds could be suspended in close contact with the soil, while still allowing the recovery of every seed.

Once the seeds were incorporated into the slide mounts (144 slide mounts/species), an initial fungicide treatment was applied. Half of the slide mounts were dipped in a 1:100 w/v solution of 75% Captan (Maestro 75DF, Zeneca Corp., Stoney Creek, ON, Canada), and half were dipped in water as a control. This concentration was recommended by the manufacturer for

dipping bulbs and tubers. Captan is a non-systemic heterocyclic nitrogen fungicide used against a wide range of Oomycetes, Ascomycetes, and Basidiomycetes (Sharville 1961; Torgeson 1969; Neergaard 1977) and is noted as being particularly effective against seed-rotting organisms (Neergaard 1977), such as damping-off diseases (Maude 1996). Captan has been shown to have mild effects on endomycorrhizal fungi and both positive and negative effects on ectomycorrhizae, depending on species (Trappe et al. 1984; Vyas 1988). The effect of Captan on the germination of the four grasses was tested and resulted in germination rates similar to the controls (Fisher's Exact Tests; $N=60$ seeds / species; $p > 0.7$ for each species).

In August 1999 the assembled slide mounts were set into the soil at each field site. A hole was dug large enough to sink a 10cm x 10cm square pot into the ground. The removed soil was placed back into the pot and two assembled slide mounts were placed vertically within the pot. This procedure allowed seeds to be suspended approximately 2-4cm below the soil surface; a representative depth for seeds in the soil seed bank (Leck et al. 1989; Baskin and Baskin 1998). Each of the 18 sites comprised a 4 x 4 grid containing 16 pots (4 species x 4 treatments) spaced 1m apart.

Following the establishment of these sites, additional treatments were applied in the field. Four factorial treatment combinations were used: 1) control, 2) water addition, 3) fungicide addition, 4) water addition + fungicide addition. For the water treatments, the soil in each pot was saturated once every two weeks. The fungicide treatments were re-applied once a month by adding 5mL of a 4gL^{-1} solution of Captan to the soil surface of each pot. For those pots that did not receive a fungicide treatment, 5mL of water was added as a control. After November 21, the application of treatments was suspended due to winter snowfall; water and fungicide treatments were resumed May 5. Some pots were disturbed, presumably by mammals or frost heave; pots and slide mounts were placed back into the ground if no visible damage had occurred.

In mid-April 2000, one slide mount was recovered from each pot and brought back to the lab (8 month trial); the remaining slide mount similarly was recovered late in August 2000 (12 month trial). The 8 month trial estimates mortality between fall seed dispersal and spring germination; the 12 month trial estimates annual losses. Each slide mount was opened and each of the seeds it contained was inspected under a dissecting microscope. The fate of nearly all of the seeds (99.3%) could be determined as germinated, ungerminated but dead (soft to touch), or intact (hard seed).

All intact seeds from each slide mount (up to 20 seeds) were used for further tests of germinability. Seeds were placed on top of potting soil in cell packs (40mm x 47mm x 55mm deep) and grown for 10 weeks in a seed germinator (12 hour graded 5°C-25°C, light/dark cycle; 90% humidity). As seeds germinated, seedlings were counted, removed, and recorded. These methods provide a minimum estimate of viability, since dormant seeds may not germinate; however, viable seeds of the species used all exhibited high germinability.

2.3. Analyses

The proportion of seeds falling into each survival category was normalized by square root arcsin transformation prior to analyses (Kirk 1982). Each species was analyzed separately, as they were expected *a priori* to vary in their intrinsic germination biology. The analyses used were standard split-plot factorial ANOVA designs (Kirk 1982) with field site used as a random blocking factor and nested inside habitat type. Type III sums of squares were used throughout. Degrees of freedom vary because not all experimental replicates were successfully recovered, and in some cases because too many seeds died or germinated in the field to permit further tests. Only fully recovered sites were analyzed, and missing data were not interpolated. This approach was adopted in order to avoid the assumptions and analytical problems associated with unbalanced designs and interpolation, especially as multiple pots were lost from many of the sites

affected; reanalysis with the entire dataset did not alter most results, and did not generally improve significance.

3. Results

3.1. Overall trends

With all treatments combined, total germination rates varied among plant species. *Bromus inermis* had the highest germination in both the 8 and 12 month trials (90.2% and 89.5%) and *Poa pratensis* had the lowest (55.8% and 45.2%) (Figs. 1-4). A majority of the germination of *Bromus inermis* occurred in the field, while the other three grasses had higher germination in the lab.

3.2. Species-specific results

Most tests of treatment effects were nonsignificant, and for the minority that were significant, effect sizes generally were small. As well, degrees of freedom were reduced in many tests because of damage to experimental sites, as noted in the *Methods* section; this was especially a problem for the 12 month trials, which often suffered greatly reduced power as a result. Nonetheless, all species showed at least some significant responses to the experimental treatments. In particular, for all species, fungicide addition improved recovery in at least some tests.

3.2.1. Bromus inermis

Seeds of this species germinated rapidly in the field (Fig. 1), resulting in the death of many seedlings below ground. Only one significant treatment effect was detected (Table 1): the proportion of dead seeds was reduced by fungicide addition in the 12 month trial (+fungicide: 6.3%, -fungicide: 11.1%). No significant habitat effect, water addition effect, or interactions were detected (Table 1). Too few ungerminated seeds remained to allow the standard ANOVAs of

germination in the lab; however, analyses using all of the available data failed to detect any significant effects ($P>0.05$).

3.2.2. *Danthonia spicata*

In the 8 month trial, results for *Danthonia* differed significantly among habitats for death of seeds in the field (dry: 4.8%, mesic: 7.6%, wet: 10.2%) and germination in the field (dry: 30.9%, mesic: 8.1%, wet: 1.0%) (Table 2; Fig. 2). In addition, during the 8 month trial, a significant fungicide x water interaction influenced the death of seeds in the field (+ water + fungicide: 3.4%, + water - fungicide: 9.7%, - water + fungicide: 7.8%, -water - fungicide: 8.9%). A three-way interaction demonstrated that this effect was primarily the result of responses in dry meadows, where adding water increased death rates unless fungicide also was supplied (Table 2; Fig. 2). In the 12 month trial, a fungicide x habitat interaction occurred in which the addition of fungicide decreased the death of seeds in mesic (+fungicide: 7.6%, -fungicide: 27.9%) and wet meadows (+fungicide: 17.6%, -fungicide: 27.2%) but not in dry meadows (+fungicide: 12.9%, -fungicide: 13.1%), where it was low with or without fungicide treatments (Table 2; Fig. 2). Effects of water addition alone, as well as all other interactions, were not significant (Table 2).

3.2.3. *Glyceria striata*

For *Glyceria* in the 8 month trial, the addition of fungicide significantly reduced the death of seeds in the field (+fungicide: 3.9%, -fungicide: 6.4%) (Table 3; Fig. 3). In addition, during the 8 month trial, a fungicide x habitat interaction occurred for death of seeds in the field (Table 3; Fig. 3): the addition of fungicide reduced death of seeds in mesic meadows (+fungicide: 0.9%, -fungicide: 7.9%), more than in dry (+fungicide: 3.2%, -fungicide: 4.6%) or wet meadows (+fungicide: 7.5%, -fungicide: 6.7%). In the 12 month trial, a fungicide x habitat interaction also occurred; however, here the addition of fungicide reduced the germination in the lab of intact seeds from dry meadows (+fungicide: 42.4%, -fungicide: 72.5%) and mesic meadows

(+fungicide: 85.9%, -fungicide: 86.6%), while increasing germination of intact seeds from wet meadows (+fungicide: 80.6%, -fungicide: 72.1%) (Table 3; Fig. 3). One possible explanation is a negative effect of fungicide on performance which was outweighed by control of pathogens in wet meadows, though we found little evidence of this elsewhere in our data. Effects of water addition, as well as all other interactions, were not significant (Table 3).

3.2.4. *Poa pratensis*

Poa exhibited significant responses to treatments only at the 8 month trial; this probably reflects the small sample sizes available after 12 months. Field germination was low, but still differed significantly among habitats: germination was greater in dry meadows (7.1%) than in mesic meadows (0.6%) and wet meadows (0.7%) (Table 4; Fig. 4). With the addition of fungicide, *Poa pratensis* enjoyed reduced death of seeds (+fungicide: 10.5%, -fungicide: 20.9%) (Table 4; Fig. 4). A water x habitat interaction occurred in which the addition of water increased the death of seeds in dry meadows (+water: 16.4%, -water: 11.8%), but decreased death of seeds in mesic meadows (+water = 13.5%, -water = 16.7%) and wet meadows (+water: 17.3%, -water: 21.6%) (Table 4; Fig. 4). In addition, a three-way interaction between fungicide, water, and habitat occurred for seeds germinated in the field, though values were low and need to be treated with caution (Table 4): germination in dry sites was reduced by water additions unless fungicide was added (Fig. 4), suggesting that seeds in dry meadows benefit from increased moisture only when they are not killed by soil fungi. Effects of water addition alone, as well as all other interactions, were not significant (Table 4).

4. Discussion

4.1. Are losses of seeds to fungi greater in wetter habitats?

In natural habitats, fungal pathogens can have significant impacts on the survival of both growing plants (Augspurger and Kelly 1984; Stephenson 1986; Bever 1994; Packer and Clay

2000) and buried seeds (Crist and Friese 1993; Dalling et al. 1998; Leishman et al. 2000; Blaney and Kotanen 2001, 2002). It has long been known that agricultural plants are more susceptible to disease in some environments than others (Manners 1982; Agrios 1997; Agarwal and Sinclair 1997), but few studies have documented differences among natural habitats in the impacts of plant diseases. In our study, the majority of treatment effects were small or nonsignificant, suggesting the impacts of seed-destroying fungi were weak in comparison with other potential sources of mortality such as inappropriate germination, drought, seed predators, and competition with established vegetation. Nonetheless, we found evidence that attack by fungal seed pathogens caused measurable losses of buried seeds in all four of our experimental grasses, as shown by increased viability after treatment with fungicide in some or all habitats. For two of these grasses, *Danthonia spicata* and *Glyceria striata*, fungicide was less likely to improve viability in dry meadows than in wet and/or mesic meadows, suggesting the impacts of fungal pathogens differed among habitats.

Danthonia experienced less death and greater germination in its usual habitat (dry meadows) than in other habitat types, but the addition of fungicide reduced this difference at the 12 month trial by decreasing the death of seeds in mesic and wet meadows. Treatment with fungicide also improved the recovery of viable seeds of the wetland grass, *Glyceria*. After 8 months, the greatest improvement occurred for the recovery of seeds from mesic meadows, where *Glyceria* does not normally occur, rather than from wet meadows; however, after 12 months, fungicide treatment increased lab germination of seeds from wet meadows relative to seeds from dry meadows. For these two species, fungal mortality is a lesser risk in relatively dry soils than moister mesic and/or wet habitats, as reported for a variety of species by Augspurger (1983), Augspurger and Kelly (1984), and Blaney and Kotanen (2001).

The remaining two species also suffered losses to fungi, but these losses were not clearly greater in wetter habitats. *Poa pratensis* experienced the highest levels of mortality in the field of all four grasses, and the addition of fungicide resulted in reduced death of seeds after 8 months. The effects of fungicide did vary among habitats, but very weakly, and in an ambiguous manner: the positive effects of fungicide on germination were strongest in dry habitats, but only when they were made artificially wet by adding water. *Bromus inermis* also showed some positive responses to fungicide addition, but no differences among habitats. This may be due to the rapid germination of *Bromus inermis* in the field: few seeds remained in the seed bank long enough to experience a significant risk of mortality, regardless of habitat.

Factors other than habitat characteristics may affect pathogen populations. For example, plants may experience a decrease in survival and recruitment over time as the soil beneath them accumulates fungal pathogens (Van der Putten et al. 1993; Bever 1994; Van der Putten and Peters 1997; Mills and Bever 1998). Such negative feedbacks may help to maintain diversity in plant communities by preventing the dominance of competitively superior species (Janzen 1970; Connell 1971); although results have been mixed (Connell 1978; Hubbell 1980; Clark and Clark 1984), much recent research has supported variants of this hypothesis (Dobson and Crawley 1994; Harms et al. 2000; Howe and Miriti 2000; Olff et al. 2000). The results of our study do not clearly support this model. For *Bromus* and *Poa*, attack by fungal pathogens did not seem to differ consistently among habitats. *Glyceria* suffered from fungal pathogens in all three habitats, but the most negative effects occurred both in mesic meadows, where it does not typically occur, and in hydric meadows, where it does. Finally, *Danthonia* experienced less fungal attack in the dry meadows it typically occupies than in mesic or wet meadows. Consequently, the simplest interpretation of our results is that when fungal mortality differed among habitats, it was driven by soil characteristics, not by the composition of the plant community.

4.2. Does experimentally increasing soil moisture within a habitat increase losses of seeds to fungi?

Among-habitat variation in fungal populations may reflect physical factors other than soil moisture. For example, wetter soils often differ from drier soils in characteristics such as pH, redox, and organic content; these factors and many others may influence the abundance of soil fungi (Bruehl 1987; Dix and Webster 1995; Agrios 1997). If these factors are more important than water availability, then adding water to a site may not necessarily increase rates of fungal attack. For example, Leishman et al. (2000) used water and fungicide additions in an English grassland to demonstrate that higher simulated summer rainfall (plus winter warming) did not increase losses of seeds to fungi. In our study, two species did provide some evidence that water addition increased losses to soil fungi, albeit at a single sampling period. For *Danthonia*, adding water increased the death of seeds in dry meadows unless fungicide also was added. For *Poa*, water addition increased the death of seeds in dry meadows, but decreased death of seeds in mesic and wet meadows; reasons for this difference are unclear. Adding fungicide partly counteracted the negative effects of water on *Poa* in dry habitats, as reflected in increased germination, though values were very low and must be treated with caution. This effect probably occurred because seeds were more likely to germinate successfully in response to increased moisture when they are protected from soil fungi. These examples provide evidence that adding water increases losses to fungal pathogens in dry habitats, though effect size in our experiments generally was small. In wetter habitats, sufficient water evidently is present to maintain fungal activity, and so water additions had little incremental effect.

4.3. Conclusions

Together, these results indicate that seeds of at least some species are at a greater risk of loss to fungal pathogens in wet or mesic soils than dry soils. This applies both to species of dry habitats

(*Danthonia*) and to plants of wetter sites (*Glyceria*). At the same time, desiccation is one of the primary causes of mortality of germinating seeds (Harper 1977; Leck et al. 1989; Baskin and Baskin 1998). Consequently, for seeds of many plants, the habitats that are safest in terms of physical environment may be the riskiest in terms of fungal attack.

Most of the effects that we detected were weak, and many other factors affect recruitment, including dispersal, seed predation, availability of gaps, resources, and competition (Fenner 1985, 2000). It often may be true that these other limitations outweigh the impacts of fungal seed pathogens, preventing them from translating into population-level effects. Nonetheless, our results suggest that seed-pathogenic fungi do have the potential to reduce recruitment of susceptible plants in otherwise suitable habitats.

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Table 1

Results of split-plot factorial ANOVAs for seeds of *Bromus inermis*. "Habitat" refers to the habitat to which seeds were exposed.

"Fungicide" and "water" refer to the treatments applied. Separate ANOVAs were performed for seeds found dead or germinating in the field; too few ungerminated seeds remained to allow ANOVAs of germination in the lab. Significant results ($P < 0.05$) are indicated in **bold**.

Factor	8-month trial			12-month trial		
	(error df = 13)			(error df = 8)		
	Dead		Germinated	Dead		Germinated
			(field)			(field)
	df	F	F	df	F	F
habitat	2	0.297	2.610	2	0.209	0.946
fungicide	1	2.662	2.442	1	6.223*	3.536
water	1	0.477	0.110	1	0.560	1.047
fungicide x habitat	2	0.406	0.060	2	1.029	0.252
water x habitat	2	0.020	0.290	2	0.487	0.680
fungicide x water	1	0.504	0.197	1	0.001	0.054
fungicide x water	2	0.067	0.718	2	0.552	0.221

x habitat

* = $P < 0.05$

Table 2

Results of split-plot factorial ANOVAs for seeds of *Danthonia spicata*. "Habitat" refers to the habitat to which seeds were exposed. "Fungicide" and "water" refer to the treatments applied. Separate ANOVAs were performed for seeds found dead, germinating in the field, or germinating in the lab. Significant results ($P < 0.05$) are indicated in **bold**.

Factor	<i>8-month trial</i>				<i>12-month trial</i>			
	(error df = 14†)				(error df = 10††)			
	Dead		Germinated	Germinated	Dead		Germinated	Germinated
			(field)	(lab)			(field)	(lab)
	df	F	F	F	df	F	F	F
habitat	2	4.021*	4.353*	0.953	2	0.047	1.513	3.209
fungicide	1	3.787	0.010	0.129	1	1.486	0.805	0.123
water	1	0.833	0.061	1.529	1	0.010	0.934	2.877
fungicide x habitat	2	0.511	0.380	0.263	2	6.296*	0.613	1.025
water x habitat	2	2.399	1.546	0.626	2	3.713	0.234	2.991
fungicide x water	1	5.364*	2.383	0.045	1	1.188	0.159	0.270
fungicide x water	2	4.102*	0.705	0.101	2	2.513	0.247	0.945
x habitat								

* = $P < 0.05$

† error df for lab germination = 13

†† error df for lab germination = 9

Table 3

Results of split-plot factorial ANOVAs for seeds of *Glyceria striata*. "Habitat" refers to the habitat to which seeds were exposed.

"Fungicide" and "water" refer to the treatments applied. Separate ANOVAs were performed for seeds found dead, germinating in the field, or germinating in the lab. Significant results ($P < 0.05$) are indicated in **bold**.

Factor	<i>8-month trial</i>				<i>12-month trial</i>			
	(error df = 14†)				(error df = 7)			
	Dead		Germinated	Germinated	Dead		Germinated	Germinated
			(field)	(lab)			(field)	(lab)
	df	F	F	F	df	F	F	F
habitat	2	1.680	0.443	0.740	2	0.897	1.665	3.488
fungicide	1	6.220*	0.873	2.929	1	0.025	0.546	0.006
water	1	0.059	1.505	0.139	1	0.006	2.377	0.826
fungicide x habitat	2	3.917*	0.975	1.031	2	0.203	1.994	8.624*
water x habitat	2	0.845	2.466	0.479	2	0.515	1.305	4.201
fungicide x water	1	0.070	0.891	1.376	1	0.425	0.042	0.374
fungicide x water	2	1.149	2.133	1.390	2	0.414	1.919	0.909
x habitat								

* = $P < 0.05$

† error df for lab germination = 12

Table 4

Results of split-plot factorial ANOVAs for seeds of *Poa pratensis*. "Habitat" refers to the habitat to which seeds were exposed.

"Fungicide" and "water" refer to the treatments applied. Separate ANOVAs were performed for seeds found dead, germinating in the field, or germinating in the lab. Significant results ($P < 0.05$) are indicated in **bold**.

Factor	<i>8-month trial</i>				<i>12-month trial</i>			
	(error df = 12)				(error df = 5)			
	Dead		Germinated	Germinated	Dead		Germinated	Germinated
			(field)	(lab)			(field)	(lab)
	df	F	F	F	df	F	F	F
habitat	2	0.895	7.764**	0.841	2	1.218	0.012	0.348
fungicide	1	11.954**	0.074	0.472	1	1.339	1.570	0.001
water	1	0.770	0.315	1.300	1	0.622	0.025	0.203
fungicide x habitat	2	0.612	0.146	1.586	2	2.243	1.468	0.212
water x habitat	2	4.249*	0.736	0.070	2	1.084	1.878	0.302
fungicide x water	1	1.019	1.093	3.681	1	1.055	0.010	0.223
fungicide x water	2	2.209	4.424*	1.230	2	0.886	1.197	0.048
x habitat								

* = $P < 0.05$; ** = $P < 0.01$

Legends of Figures

Fig. 1. Fates of seeds of *Bromus inermis* exposed to water and fungicide treatments for 8 and 12 months. Seeds were exposed in three habitat types: dry, mesic, and wet meadows. Bars indicate mean proportion ($\pm$ SE) of seeds found dead or germinating in the field, and the proportion of intact seeds germinating in the lab. At the 12 month trial, no intact seeds from dry or mesic meadows were available for laboratory germination tests of the fungicide addition treatment.

Fig. 2. Fates of seeds of *Danthonia spicata* exposed to water and fungicide treatments for 8 and 12 months. Seeds were exposed in three habitat types: dry, mesic, and wet meadows. Bars indicate mean proportion ($\pm$ SE) of seeds found dead or germinating in the field, and the proportion of intact seeds germinating in the lab.

Fig. 3. Fates of seeds of *Glyceria striata* exposed to water and fungicide treatments for 8 and 12 months. Seeds were exposed in three habitat types: dry, mesic, and wet meadows. Bars indicate mean proportion ($\pm$ SE) of seeds found dead or germinating in the field, and the proportion of intact seeds germinating in the lab.

Fig. 4. Fates of seeds of *Poa pratensis* exposed to water and fungicide treatments for 8 and 12 months. Seeds were exposed in three habitat types: dry, mesic, and wet meadows. Bars indicate mean proportion ($\pm$ SE) of seeds found dead or germinating in the field, and the proportion of intact seeds germinating in the lab.

Fig. 1

Fig. 2

Fig. 3

Fig. 4