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Apparent Competition with Bromus tectorum Through Pyrenophora semeniperda Reduces Establishment of Native Grasses

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Apparent competition with *Bromus tectorum* through *Pyrenophora semeniperda* reduces establishment of native grasses

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

Apparent competition with *Bromus tectorum* through *Pyrenophora semeniperda* reduces establishment of native grasses

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Contributing to the success of *Bromus tectorum* in the Intermountain West may be a mechanism called apparent competition, which occurs when one species increases the pressure of a consumer on a second species. This indirect interaction has been documented only a few times in invasive plant systems, and never in a fungal pathosystem. We examined the effects of the invasive annual *Bromus tectorum* and predation by the seed pathogen *Pyrenophora semeniperda* on seedling emergence and survival for two native grasses (*Pseudoroegneria spicata* and *Elymus elymoides*), by manipulating *B. tectorum* densities and *P. semeniperda* inoculum loads in randomized plots. Identical field studies were conducted in Skull Valley, Utah (xeric site) and Sprague, Washington (mesic site). The addition of inoculum decreased emergence of native grass seedlings at both sites and increased the amount of unemerged native seeds that were killed by *P. semeniperda*. Higher densities of *B. tectorum* decreased native grass survival at the mesic site and increased survival at the xeric site probably due to the beneficial effects of *B. tectorum* litter on soil moisture. At both sites, there were more *B. tectorum* seeds found in the seed banks in plots with high *B. tectorum* densities than in low-density plots. This indicates an increase in available prey for *P. semeniperda*. There was a much lower level of infection in *B. tectorum* seed bank seeds at the mesic site than at the xeric site. The high level of ungerminated native seeds killed by background levels of *P. semeniperda,* combined with the increase in available prey for the fungus in high-density *B. tectorum* plots, shows that apparent competition may play a role, along with direct competition, in the success of *B. tectorum*. This interaction is important to consider when dealing with control of *B. tectorum*.

Keywords: Apparent competition, *Bromus tectorum*, *Elymus elymoides*, *Pseudoroegneria spicata*, *Pyrenophora semeniperda*

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INTRODUCTION

Apparent competition, the phenomenon of a multi-host predator or pathogen decreasing the abundance of one host in the presence of another, has been demonstrated in several systems. It is present in marine environments with multi-host parasites that attack two different species of isopods (Grosholz 1992). It has also been well documented in tropical forest food webs involving herbivorous insects and their parasites (Morris et al. 2004). Apparent competition has been demonstrated in plant communities as well, between sand dune grasses with shared nematode parasites, where it plays a role in community succession (Van der Putten and Peters 1997). Multiple studies have shown that the presence of *Avena fatua* in California grasslands increases the rate of barley and cereal yellow dwarf virus infections in nearby native bunchgrasses through apparent competition (Malmstrom et al. 2005a, Malmstrom et al. 2005b, Malmstrom et al. 2006). Although the majority of studies on apparent competition deal with situations where the mediating consumer is native, it can also occur with exotic consumers (Sessions and Kelly 2002, Rand and Louda 2004). Furthermore, a reduction or elimination of one host species is most likely to occur when the other host species is invasive (Meiners 2007).

Apparent competition has yet to be demonstrated in a fungal pathosystem (Malmstrom et al. 2005a). The interactions between the seed pathogen *Pyrenophora semeniperda* and the invasive grass *Bromus tectorum* may create a situation of apparent competition (indirect competition). In this case, we hypothesized that the large amount of seed produced by *B. tectorum* (300 seeds per plant, or as high as 50,000 seeds per m^2) creates a substantial resource reservoir for *P. semeniperda*, greatly increasing *P. semeniperda* inoculum loads (Mack and Pyke 1983, Smith et al. 2008). This increase in inoculum has potential negative effects on the nearby native grasses. These also act as hosts to the fungus but do not sustain its population as *B.*

tectorum does, due to a comparatively lower seed production by these natives. Although establishment failure of the natives may be due to direct competition with *B. tectorum*, another factor that has been overlooked is the possible apparent competition involving *P. semeniperda*. Further, pathogen spillover, (when a high pathogen load in a population of one species [e.g., *B. tectorum*] increases the pathogen load in a population of another species [e.g., a native grass]) has been demonstrated with *B. tectorum* and *P. semeniperda*, which supports the possibility of apparent competition (Beckstead et al. 2010).

Since its arrival from Eurasia in the late 1800's, the invasive annual grass *Bromus tectorum* L., has played a major role in the ecosystems of western North America (Mack 1981, Mack and Pyke 1983, Whitson and Koch 1998, Chambers et al. 2007). The ability of *B. tectorum* to germinate in a wide variety of environmental conditions, and to quickly establish an extensive root system allows it to spread into many habitats including deserts, forests, and shrub-steppe (Thill et al. 1984, Pierson and Mack 1990, Humphrey and Schupp 2001). Although it generally only dominates an area after a disturbance event (such as overgrazing or fire), once *B. tectorum* becomes the dominant species it can remain as such indefinitely (Harris 1967, Thill et al. 1984). Seeds mature in early summer and experience dry-afterripening through the summer and fall, which causes a loss of dormancy (Bauer et al. 1998). With sufficient precipitation, *B. tectorum* seeds germinate in the fall, and seedlings survive through winter in a semi-dormant state. These fall-germinated seedlings will become the plants with the highest seed production (compared to spring-germinated seedlings) the following summer (Mack and Pyke 1984). Once established, *B. tectorum* is a strong competitor with native grasses, forbs, and shrubs (Thill et al. 1984, Knapp 1996, Booth et al. 2003, Humphrey and Schupp 2004). *Bromus tectorum* is highly flammable and quickly reestablishes after fire, forming a near-continuous monoculture.

Pyrenophora semeniperda (Brittlebank and Adam) Shoemaker is a multi-host, or generalist, fungus that possibly uses toxic cytochalasins to attack the seeds of over 35 genera of grasses, including *B. tectorum* (Evidente et al. 2002, Campbell et al. 2003). The pathogen was originally studied due to its infection of cereal grains (Campbell and Medd 2003). However, studies have shown that *P. semeniperda* can kill tens of thousands of seeds of *B. tectorum* per square meter (Meyer et al. 2007). It may have traveled with *B. tectorum* from Eurasia to North and South America and is currently known from deserts, grasslands, and cereal-growing regions of South America, Australia, South Africa, New Zealand, Turkey, Greece, and Egypt (Meyer et al. 2008, Stewart et al. 2009). Capable of completing its entire life cycle on seeds, *P. semeniperda* is common in *B. tectorum* soil seed banks and can cause high mortality of dormant seeds in the field (Meyer et al. 2007). It is easily identifiable from its large black stromata that protrude out of the seed. From the stromata, asexual conidial spores are produced that infect other seeds. These asexual spores germinate on the seed coat, then penetrate and produce mycelium inside the seed. Sporulation is initiated while the seed is being consumed and new stromata are produced (Beckstead et al. 2007). The sexual stage of *P. semeniperda* is rarely observed (Paul 1969).

If apparent competition is a factor contributing to the success of *B. tectorum* in these sites, we expect to see an increase in levels of disease and a decrease in emergence of native seeds (measured as seed death) in control plots compared with fungicide-treated plots, regardless of presence or absence of *B. tectorum*. Alternatively, if apparent competition is not a factor contributing to the success of B. tectorum, we expect to see no impact of fungicide on the emergence of native seedlings. Additionally, we expect that native grasses have reduced emergence and survival when grown in direct competition with *B. tectorum*. To investigate the possible contribution of *P. semeniperda* to interactions between *B. tectorum* and native grasses, we measured its effects on the emergence and survival of *Elymus elymoides* and *Pseudoroegneria spicata* at both mesic and xeric locations.

MATERIALS AND METHODS

Study design and treatments

Identical field studies were conducted at two locations: Whiterocks, a xeric site located in Skull Valley, Utah (40° 19.680'N 112° 46.680'W elevation 1446 m, average annual precipitation of 15.25 cm), and Packard Creek, a mesic site located southeast of Sprague, Washington (47° 5.981'N 117° 49.862'W elevation 550 m, average annual precipitation 35.55 cm). Precipitation information was generated using PRISM Climate Group website (http://prismclimate.org, accessed November 2010). Both sites are vegetated with near-monocultures of *Bromus tectorum*. The experiment was a split plot design: +, - glyphosate herbicide treatment in May 2009 was the main plot into which a 3x2x2 factorial treatment design was established: three *Pyrenophora semeniperda* inoculum load treatments (high = 45g/ft^2 *P. semeniperda* added inoculum, medium = the control level of *P. semeniperda* naturally occurring on the site, and low = fungicide treatment to reduce *P. semeniperda*) x two irrigation treatments (with or without water addition following planting) x two native species seeded into each site (*Pseudoroegneria spicata* (bluebunch wheatgrass) and*, Elymus elymoides* (squirreltail)). For each treatment combination, 10 block replications were included, resulting in a total of 240 plots that measured 0.093m^2 each. The herbicide treatment resulted in half of the plots having a very low level of *B. tectorum* plants the following fall and spring and the other half an unmanipulated and higher level of *B. tectorum* during the fall and spring.

Bulk inoculum for field use was produced by seeding conidia of a representative pathogen strain into potato dextrose broth (PDB) that had been autoclaved in large-batch (10 liter) glass fermenters. Resulting mycelium was grown for 2-3 days at room temperature in aerated submerged culture. The mycelial mass was concentrated by centrifuging for two minutes at 3750 rpm to make it clump, then combined with fresh PDB prior to mixing with a sterilized inert carrier (Agsorb calcined montmorillonite clay). Enough PDB was added to saturate the Agsorb. The resulting carrier material was dried slowly under ambient temperature conditions for 24-48 hours under a combination of both cool white and ultraviolet lights which encouraged conidial sporulation on the carrier. Following sporulation, the inoculum material was further dried in tissue-covered containers in a warm greenhouse. This material was then pressed through a #16 sieve to break it into granular form. The bulk inoculum was then weighed into vials (45g/ vial) for hand application in the field. Based on results of preliminary laboratory trials we determined that 45g/m^2 is a high enough level to affect germination and emergence of both native grasses included in the study. In fungicide treated plots, fenbuconazole was mixed with water and applied to the plots at a rate of 0.1185mL fenbuconazole/m^2. This rate was found to be effective for *Pyrenophora semeniperda* control in a previous field trial (unpublished data).

Water was added to irrigated plots on days 0, 7 and 14 from seed sowing to ensure sufficient soil moisture for seedling establishment in the possible absence of fall precipitation. For the irrigation treatment, each plot was slowly watered with 2.54cm of water over a 20-30 minute period by poking holes in water bags suspended over plots on a wire frame. This rate allowed the water to seep into the ground without running off of the plots.

Both native grass species used in this study are common in the areas around the study locations and are susceptible to attack by *P. semeniperda* (Beckstead et al. 2010). *Pseudoroegneria spicata* seeds were collected from the south-facing slope of the mouth of Rock

Canyon near Provo, Utah in July of 2009. *Elymus elymoides* seeds were collected near Sunshine Canyon west of Cedar Fort, Utah in June of 2009. Both populations were collected by hand and cleaned with a blower to remove debris. Seeds were after-ripened at room temperature for three months to encourage dormancy loss. In order to track the fate of individual seeds through the duration of the study, native seeds were glued to bamboo toothpicks with Elmer's Washable School Glue (Elmer's Products, Inc., Columbus OH) using a technique described by Leger et al. (2009). Toothpicks with attached seeds were inserted into the ground until the seed was completely covered, leaving the majority of the toothpick above ground. Toothpicks were arranged into a grid pattern to evenly space toothpicks 6.35cm apart in four rows of five seeds as they were planted. The experiment was installed at each site in September of 2009.

Field data collection

Emergence and survival of each individual seedling was recorded approximately every two weeks throughout the fall and spring. Emerged seedlings were marked by placing colored paperclips around both the new seedling and its attached toothpick. This made seedlings easier to distinguish from surrounding vegetation as they grew. Each time plots were read, previously emerged seedlings were scored as dead or alive. Fall data collection was terminated once nighttime temperatures consistently dropped below freezing and no more seedling emergence was likely. The last dates for scoring of fall emergence were December 3 (Whiterocks) and November 8 (Packard Creek). Data collection began again the following spring once emergence in these locations was observed (March 11 at Whiterocks and April 4 at Packard Creek) and stopped once emergence had ended due to drying soils (March 24 at Whiterocks and May 19 at Packard Creek). This was considered the end of spring emergence. All non-emerged seeds were then collected.

Unemerged seeds were scored and categorized as either germinated, or infected with *Pyrenophora semeniperda* (detected by the protrusion of distinct black stromata). Seeds that did not fall into either of these categories were incubated at 20°C for three weeks between two water-saturated germination blotters (Anchor Paper, St. Paul MN) within 10-cm plastic Petri dishes. The dishes were scored weekly to determine if any had germinated or become infected. Seeds in either of these categories were counted and removed from the dishes. At the end of three weeks all remaining seeds were cut and those with white, firm embryos were scored as viable (Meyer et al. 2010).

Soil seed bank samples

In order to measure the impact of treatments on the *B. tectorum* seed bank, samples were taken in June of 2010 from half of the plots (all *Pseudoroegneria spicata* plots). Samples were taken by pushing a steel can (6cm diameter x 4 cm high) into the soil until flush with the surface. A trowel was used to remove the can along with the intact soil core. Samples were transported to the laboratory where they were sifted through screens (1.79mm) to retrieve all seeds within the seed bank. The number of *Bromus tectorum* seeds with visible fungal stromata and all apparently viable (intact and firm) seeds were counted. Seeds that were apparently viable were incubated as described earlier and scored each week for germination and infection. At the end of the four-week incubation period all remaining seeds were cut to determine viability as previously described.

Statistical Analysis

Experimental data for each study site were analyzed using mixed model analysis of variance (ANOVA) with SAS Proc Mixed (SAS 9.2, 2007). Experimental designs were parallel at the two sites, with fixed effects that included cheatgrass competition, native species planted, watering treatment, and inoculum treatment. The model used was a split-plot design with cheatgrass competition as the main plot and with a randomized factorial combination of the other three treatments as subplots. Block interactions appropriate to this split-plot design were used as random effects in each model.

Emergence and survival response variables in the mixed model ANOVA for each study site included proportions of seeds emerging in fall, in spring and in total, and proportion of emerged seedlings that survived through the spring. The fate of seeds that did not produce emerged plants was included as proportion of seeds that germinated and proportion of seeds killed by the pathogen. Because the data for unemerged seeds were obtained only from seeds that did not produce seedlings and also remained attached to their toothpicks (less than half) at the end of the experiment, sample sizes within experimental units were highly variable. However, it was possible to calculate the proportional variables based on remaining seeds for each experimental unit, so that the design remained balanced. Proportional variables were arcsine square root transformed prior to analysis to improve homogeneity of variance. Means separations from an LSMEANS statement for each ANOVA were examined for each main effect and first-order interaction that was significant ($P<0.05$) in ANOVA.

Densities of pathogen-killed and viable *Bromus tectorum* seeds in seed bank samples were also examined using mixed model ANOVA for each study site as described above. To improve normality and homogeneity of variance, these dependent variables were log-transformed, while the derived variable proportion of total seeds killed by the pathogen was arcsine square root transformed prior to analysis. Means separations were performed as described earlier.

RESULTS

Emergence

The addition of inoculum resulted in lower emergence of natives at the Whiterocks location. Fungicide had no effect (Fig. 1, Table 1). Emergence of *Pseudoroegneria spicata* was always decreased more by the inoculum treatment regardless of water or cheatgrass treatments. There was no difference for emergence with *Elymus elymoides*.

The absence of cheatgrass also resulted in lower emergence of natives at the Whiterocks (Fig. 1, Table 1). For *P. spicata*, inoculum lowered emergence more in cheatgrass plots than non-cheatgrass plots. Cheatgrass treatments did not affect emergence and had no significant interaction with inoculum effects in plots with *E. elymoides* (Fig. 1). The addition of water greatly increased emergence in the fall and also overall emergence. Most spring emergence was in the non-watered plots (Fig. 2).

At Packard Creek the addition of inoculum resulted in lower emergence than either fungicide or control treatments. There was lower emergence in the cheatgrass plots as well (Fig. 1 and Table 1). The trend of decreasing emergence from fungicide to control to inoculum treatments was greater in non-cheatgrass plots than cheatgrass plots, especially for *P. spicata* (Fig. 1). Despite the trend, the fungicide treatment was never significantly different than the control. Water increased emergence in the fall (Fig. 2).

Survival

At Whiterocks both native grasses experienced higher survival in the cheatgrass plots than the non-cheatgrass plots, although the difference in survival was greater for *E. elymoides* (Fig. 3 and Table 2). As expected, both species also had increased survival in the watered plots compared to the non-watered plots (Fig. 3). The presence of cheatgrass and water additions decreased survival of native grasses at Packard Creek (Fig. 3 and Table 2).

Unemerged seeds

Inoculum addition and presence of cheatgrass at Whiterocks both increased the proportion of unemerged native seeds that were killed by *Pyrenophora semeniperda* (Fig. 4 and Table 3). Inoculum also decreased the proportion of native seeds that germinated but failed to emerge when compared to both the fungicide treatment and control (Fig. 4 and Table 4). There were more ungerminated seeds of *P. spicata* killed by *P. semeniperda* than seeds of *E. elymoides* (Fig. 4 and Table 3). There was increased seed mortality due to *Pyrenophora semeniperda* in cheatgrass plots compared to non-cheatgrass plots in both species (Fig. 4 and Table 3). Water addition resulted in an increase in seed mortality in both species (Fig. 5 and Table 3).

Inoculum and cheatgrass also increased the proportion of unemerged native seeds killed by *Pyrenophora semeniperda* at Packard Creek. Here *P. spicata* also experienced greater seed mortality due to the fungus than did *E. elymoides* (Fig. 4 and Table 3). *Pyrenophora semeniperda* killed more unemerged seed in cheatgrass plots than in non-cheatgrass plots (Fig. 4 and Table 3). Water addition increased the amount of seeds killed by the disease and the proportion of germinated but unemerged seeds in both species (Fig. 5 and Table 4).

Soil seed bank samples

At both sites there were more *B. tectorum* seeds in the carryover seed bank at the end of the experiment in cheatgrass plots than in non-cheatgrass plots (Fig. 6 and Tables 5 and 7). At Whiterocks, a smaller proportion of the seeds in the cheatgrass plots were killed by *Pyrenophora semeniperda* compared to the non-cheatgrass plots (Fig. 7 and Table 6). Inoculum increased the amount of killed seeds compared to the fungicide treatment and control (Fig. 6 and Table 7).

Inoculum also increased the proportion of seeds killed, an effect that was greater in non-cheatgrass plots (Fig. 7 and Table 6).

At Packard Creek the proportion of killed native seeds was greater in the non-cheatgrass plots (Fig. 7 and Table 6). Compared to the control, the inoculum treatment increased the amount of killed seeds, and was strongest in the cheatgrass plots (Fig. 6 and Table 7). Although the proportion of seeds killed at each site was similar, the density of seeds killed was much higher at Whiterocks because the overall seed density was higher (Fig. 6).

DISCUSSION

At both the Packard Creek and Whiterocks sites, the addition of *P. semeniperda* inoculum reduced the emergence of native grasses and increased seed mortality. Further, *P. semeniperda* killed more native seeds in both the high inoculum and cheatgrass treatment plots when compared to fungicide or control plots. Because *P. semeniperda* is a seed pathogen, apparent competition is expected to become evident at the seedling emergence stage. These results, though they do not prove the existence of apparent competition, does give evidence for the possibility of it playing an important part of the overall competition between *B. tectorum* and native grass species.

Previous studies have shown that *B. tectorum* can act as a reservoir species (i.e. capable of carrying a high predator load) for *P. semeniperda* due to high seed (prey) production (Beckstead et al. 2010). Our seed bank sample results support this idea, as *P. semeniperda* abundance was positively correlated with *B. tectorum* seed density (fig. 6). This study illustrates that *B. tectorum* has a negative effect on native grasses at the pre-emergence stage, by increasing the presence of *P. semeniperda*. These findings support previous studies on apparent competition that show that when an abundant prey species (such as *B. tectorum*) leads to high predator abundance (such as *P. semeniperda*) it can result in the severe reduction or elimination of the less abundant prey species, in this case native grasses. Specifically this occurred with *Pseudoroegneria spicata*, which is the more susceptible of the two native species (MacArthur 1970, Holt and Lawton 1994, Beckstead et al. 2010). This severe reduction of the secondary host species is most likely to be the case when the host species supporting the higher amount of predator pressure is an exotic (Meiners 2007, Orrock et al. 2008).

High *B. tectorum* had opposite effects on native seedling emergence at the two locations. At Packard Creek, the more mesic site, emergence was decreased in high cheatgrass plots, whereas at Whiterocks, the more xeric site, seedling emergence was greater in high cheatgrass plots. We believe the reason for this apparent discrepancy is water availability. Low cheatgrass plots were created by the application of herbicide in the spring, resulting in a loss of standing litter on those plots by the time the experiments were installed the following fall. It has been previously demonstrated that the presence or absence of litter can greatly affect soil moisture levels in sites dominated by cheatgrass by limiting evaporation (Chambers et al. 2007). At Packard Creek, frequent fall and spring precipitation (as recorded in PRISM, http://www.prism.oregonstate.edu/) indicates that soil moisture was not likely a limiting factor to emergence in low or high cheatgrass plots. High precipitation at this site also resulted in very dense litter covering the high cheatgrass plots which has been demonstrated to delay the emergence of *B. tectorum* seedlings (Pierson and Mack 1990). We believe that the reason for lower emergence in the high cheatgrass plots at Packard Creek was that the thick layers of *B. tectorum* litter present in these plots may have been prevented seedlings from growing through the thick litter because of light obstruction, thus making them difficult to detect before they died. In contrast, at Whiterocks, where soil moisture is expected to be a limiting factor, the existence of litter (which was much less thick than at Packard Creek) in the high cheatgrass plots may have increased soil moisture by slowing down the drying of the soil after a precipitation event. At this site, soil moisture was a limiting factor to both emergence and survival of native species, which is why the removal of that litter decreased native species in both of these categories (Beckstead and Augspurger 2004, Smith et al. 2008).

The addition of water increased emergence at both sites, although the effect was much greater at Whiterocks where there was no fall precipitation. Almost all of the fall emergence at Whiterocks was in the watered plots, and the majority of the spring emergence was in the unwatered plots. In contrast, seasonal emergence was not divided between water treatments at Packard Creek, where there was substantial fall precipitation. However, added water still resulted in an increase in total fall emergence at this site.

Direct competition between *B. tectorum* and native grasses begins when resources (mainly soil moisture) become limiting (after the seedling stage) and is well documented (Mack 1981, Mack and Pyke 1983, Humphrey and Schupp 2004, Chambers et al. 2007). Survival therefore, and not emergence, is where direct competition becomes evident. Due to the differences in developmental stages that these two types of competition deal with they are not mutually exclusive interactions. It is very probable that both are affecting the relationship between *B. tectorum* and native grasses (Holt 1977, Meiners 2007).

B. tectorum outcompetes both *P. spicata* and *E. elymoides* seedlings for soil moisture (Harris 1977, Booth et al. 2003). This was the case at Packard Creek where high cheatgrass decreased the survival of the two native grasses. The opposite was true at Whiterocks due to the differences in litter as discussed previously. The facultative effects of standing litter on soil moisture in this xeric environment were greater than the negative effects of competition between

B. tectorum and the native grasses, resulting in greater survival of natives in the cheatgrass plots compared to the non-cheatgrass plots.

Although not proven, apparent competition may be a force in the ability of *B. tectorum* to dominate over native grasses. The further understanding of this interaction may aid in the general goal of restoring invaded areas and protecting other areas from invasion by *B. tectorum*. Furthermore, this study suggests the possibility that other seed pathosystems may also experience apparent competition and are worth investigating.

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TABLES

Table 1. Results of analysis of variance contrasting differences in the mean number of emerged native seedlings in each treatment group. "Cheatgrass" indicates the difference between high and low cheatgrass levels, "water" indicates the difference between added water and no water treatments, "inoculum" indicates the difference between the added inoculum, control, and added fungicide treatments, and "species" represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	9	15.12	0.0037
water	1	9	329.55	<.0001
inoculum	2	18	6.71	0.0066
species	1	9	5.05	0.0512
water*cheatgrass	1	9	1.47	0.2569
water*inoculum	2	18	0.28	0.7585
water*species	1	9	0.08	0.7825
cheatgrass*inoculum	2	18	2.19	0.1410
cheatgrass*species	1	9	7.55	0.0226
inoculum*species	2	18	10.39	0.0010
Packard Creek				
cheatgrass	1	8	34.07	0.0004
water	1	8	13.82	0.0059
inoculum	2	18	5.04	0.0183
species	1	8	5.62	0.0452
water*cheatgrass	1	8	1.79	0.2183
water*inoculum	2	18	0.13	0.8818
water*species	1	8	0.51	0.4969
cheatgrass*inoculum	2	18	0.85	0.4432
cheatgrass*species	1	8	12.11	0.0083
inoculum*species	2	18	0.29	0.7528

Table 2. Results of analysis of variance contrasting differences in the mean number of native seedling that survived through the spring in each treatment. “Cheatgrass” indicates the difference between high and low cheatgrass levels, “water” indicates the difference between added water and no water treatments, “inoculum” indicates the difference between the added inoculum, control, and added fungicide treatments, and “species” represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	2	38.21	0.0252
water	1	2	50.32	0.0193
inoculum	2	3	5.34	0.1028
species	1	2	0.02	0.9017
water*cheatgrass	1	2	1.23	0.3830
water*inoculum	2	3	0.85	0.5104
water*species	1	2	5.83	0.1372
cheatgrass*inoculum	2	5	1.35	0.3390
cheatgrass*species	1	2	0	0.9674
inoculum*species	2	3	0.95	0.4803
Packard Creek				
cheatgrass	1	8	10.73	0.0113
water	1	8	0.03	0.8718
inoculum	2	15	0.07	0.9326
species	1	8	0.31	0.5933
water*inoculum	2	15	0.02	0.9761
water*species	1	8	4.01	0.0801
water*species	1	8	1.47	0.2593
cheatgrass*inoculum	2	15	1.51	0.2525
cheatgrass*species	1	8	2.6	0.1456
inoculum*species	2	17	0.15	0.8658

Table 3. Results of analysis of variance contrasting differences in the mean number of unemerged native seedlings killed by *Pyrenophora semeniperda* in each treatment. “Cheatgrass” indicates the difference between high and low cheatgrass levels, “water” indicates the difference between added water and no water treatments, “inoculum” indicates the difference between the added inoculum, control, and added fungicide treatments, and “species” represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	9	22.36	0.0011
water	1	9	8.09	0.0193
inoculum	2	18	17.93	<.0001
species	1	9	72.15	<.0001
water*inoculum	2	18	0.25	0.7812
water*species	1	9	0.35	0.5677
water*species	1	9	0.89	0.3704
cheatgrass*inoculum	2	18	1.23	0.3156
cheatgrass*species	1	9	0.81	0.3905
inoculum*species	2	18	0.09	0.9158
Packard Creek				
cheatgrass	1	9	6.38	0.0324
water	1	9	5.93	0.0377
inoculum	2	18	4.41	0.0276
species	1	9	6.06	0.0360
water*inoculum	2	18	0.65	0.5343
water*species	1	9	3.87	0.0806
water*species	1	9	0.26	0.6255
cheatgrass*inoculum	2	18	1.03	0.3783
cheatgrass*species	1	9	2.10	0.1809
inoculum*species	2	18	2.58	0.1037

Table 4. Results of analysis of variance contrasting differences in the mean number of germinated but unemerged native seedlings in each treatment. "Cheatgrass" indicates the difference between high and low cheatgrass levels, "water" indicates the difference between added water and no water treatments, "inoculum" indicates the difference between the added inoculum, control, and added fungicide treatments, and "species" represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	9	0.60	0.4579
water	1	9	3.15	0.1098
inoculum	2	18	5.25	0.0160
species	1	9	26.33	0.0006
water*inoculum	2	18	0.36	0.7036
water*species	1	9	0.01	0.9389
water*species	1	9	5.10	0.0503
cheatgrass*inoculum	2	18	1.44	0.2629
cheatgrass*species	1	9	1.60	0.2370
inoculum*species	2	18	0.04	0.9632
Packard Creek				
cheatgrass	1	9	2.59	0.1418
water	1	9	5.76	0.0399
inoculum	2	18	1.37	0.2789
species	1	9	3.29	0.1030
water*inoculum	2	18	2.19	0.1411
water*species	1	9	3.08	0.1132
water*species	1	9	0.90	0.3669
cheatgrass*inoculum	2	18	0.10	0.9014
cheatgrass*species	1	9	2.29	0.1648
inoculum*species	2	18	0.33	0.7255

Table 5. Results of analysis of variance contrasting differences in the mean total number of Bromus tectorum seeds, from the seed bank, killed by *Pyrenophora semeniperda* in each treatment. "Cheatgrass" indicates the difference between high and low cheatgrass levels, "water" indicates the difference between added water and no water treatments, "inoculum" indicates the difference between the added inoculum, control, and added fungicide treatments, and "species" represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	18	19.38	0.0003
water	1	90	0.02	0.8823
inoculum	2	90	0.81	0.4480
water*inoculum	2	90	0.33	0.7213
water*species	1	90	0.18	0.6750
cheatgrass*inoculum	2	90	1.18	0.3109
Packard Creek				
cheatgrass	1	18	5.94	0.0254
water	1	70	0.04	0.8393
inoculum	2	70	5.14	0.0083
water*inoculum	2	70	0.83	0.4413
water*species	1	70	1.48	0.2279
cheatgrass*inoculum	2	70	1.09	0.3404

Table 6. Results of analysis of variance contrasting differences in the mean proportion of *Bromus tectorum* seeds, from the seed bank, killed by *Pyrenophora semeniperda* in each treatment. "Cheatgrass" indicates the difference between high and low cheatgrass levels, "water" indicates the difference between added water and no water treatments, "inoculum" indicates the difference between the added inoculum, control, and added fungicide treatments, and "species" represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	18	12.91	0.0021
water	1	89	0.03	0.8574
inoculum	2	89	3.63	0.0306
water*inoculum	2	89	0.02	0.9775
water*species	1	89	0.02	0.897
cheatgrass*inoculum	2	89	1.08	0.3433
Packard Creek				
cheatgrass	1	18	14.17	0.0014
water	1	70	0.09	0.768
inoculum	2	70	1.84	0.1668
water*inoculum	2	70	0.18	0.8378
water*species	1	70	0.03	0.8703
cheatgrass*inoculum	2	70	5.62	0.0054

Table 7. Results of analysis of variance contrasting differences in the mean total number of viable *Bromus tectorum* seeds from the seed bank. "Cheatgrass" indicates the difference between high and low cheatgrass levels, "water" indicates the difference between added water and no water treatments, "inoculum" indicates the difference between the added inoculum, control, and added fungicide treatments, and "species" represents the result difference between the two native species (*Elymus elymoides*, and *Pseudoregneria spicata*).

Treatment	Num DF	Den DF	F Value	Pr > F
Whiterocks				
cheatgrass	1	18	45.55	<.0001
water	1	90	0	0.9493
inoculum	2	90	4.14	0.0190
water*inoculum	2	90	0.13	0.8772
water*species	1	90	0.01	0.9121
cheatgrass*inoculum	2	90	1.23	0.2974
Packard Creek				
cheatgrass	1	18	16.66	0.0007
water	1	70	0.06	0.8043
inoculum	2	70	0.82	0.4463
water*inoculum	2	70	1.76	0.1796
water*species	1	70	0.01	0.9112
cheatgrass*inoculum	2	70	3.76	0.0280

FIGURES

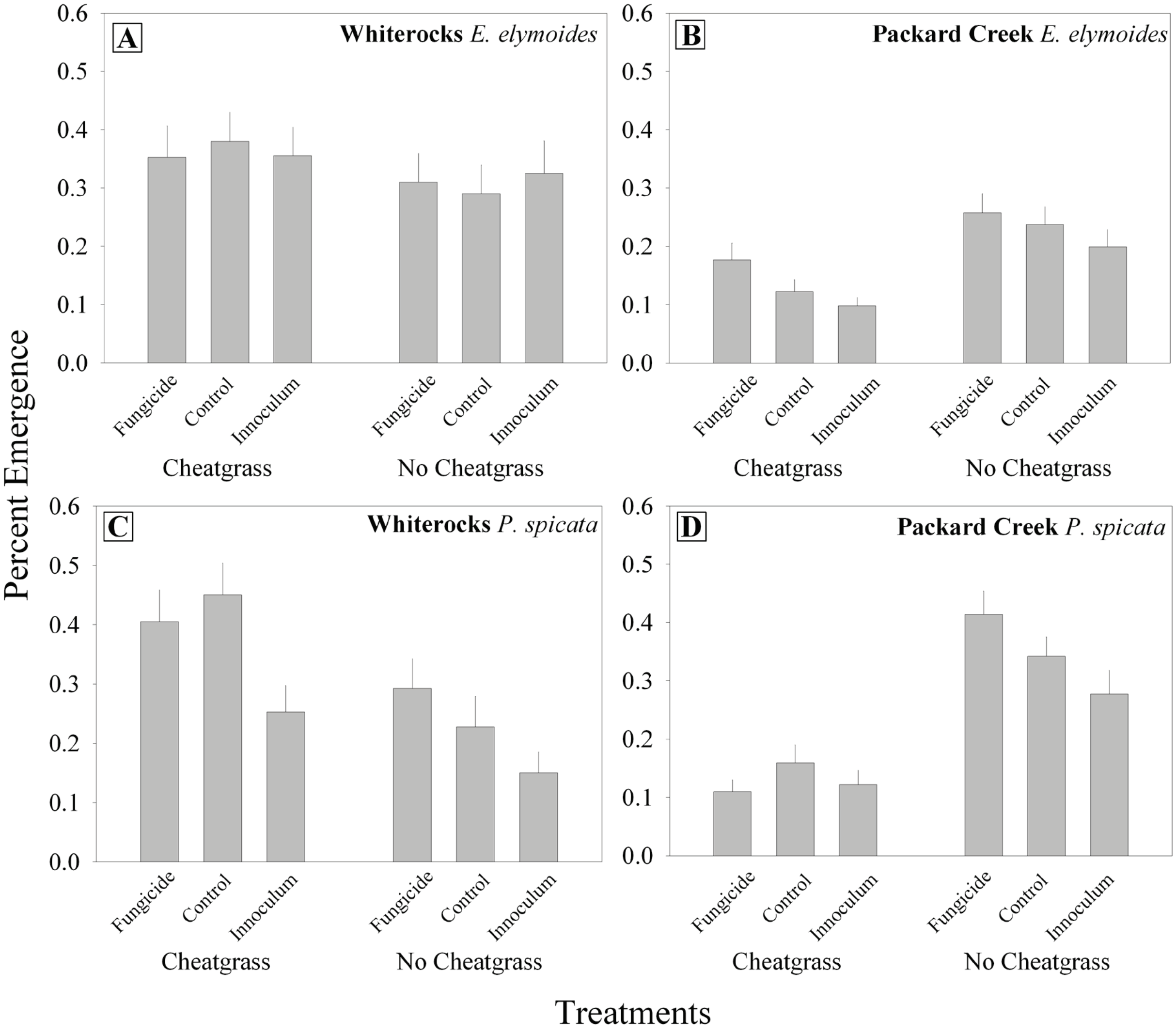

Figure 1. Effects of cheatgrass and *Pyrenophora semeniperda* inoculum levels on native seedling emergence. The control treatment is background levels of *P. semeniperda* at the site. The error bars indicate the standard error of the mean.

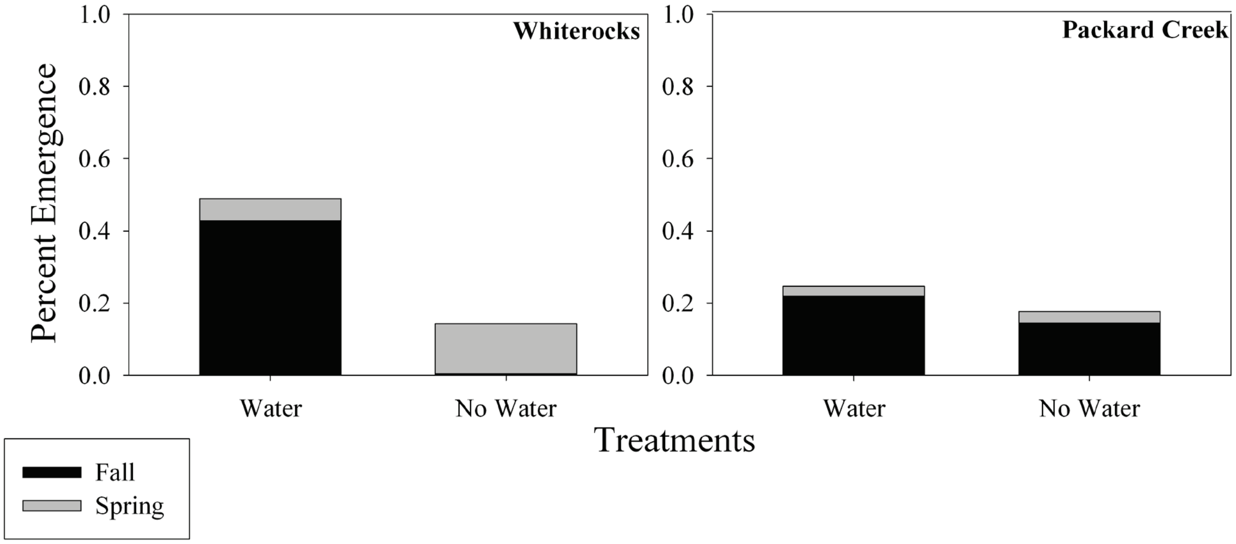

Figure 2. Effects of added water (2.54cm x 3 fall applications) on native grass seedling (*Elymus elymoides*, and *Pseudoroegneria spicata*) emergence in the fall and spring. Fall emergence data was taken every two weeks until December 5 (at Whiterocks) and November 8 (at Packard Creek). Spring emergence data was also taken every two weeks and began the following spring on March 11 (at Whitrocks) and April 4 (at Packard Creek).

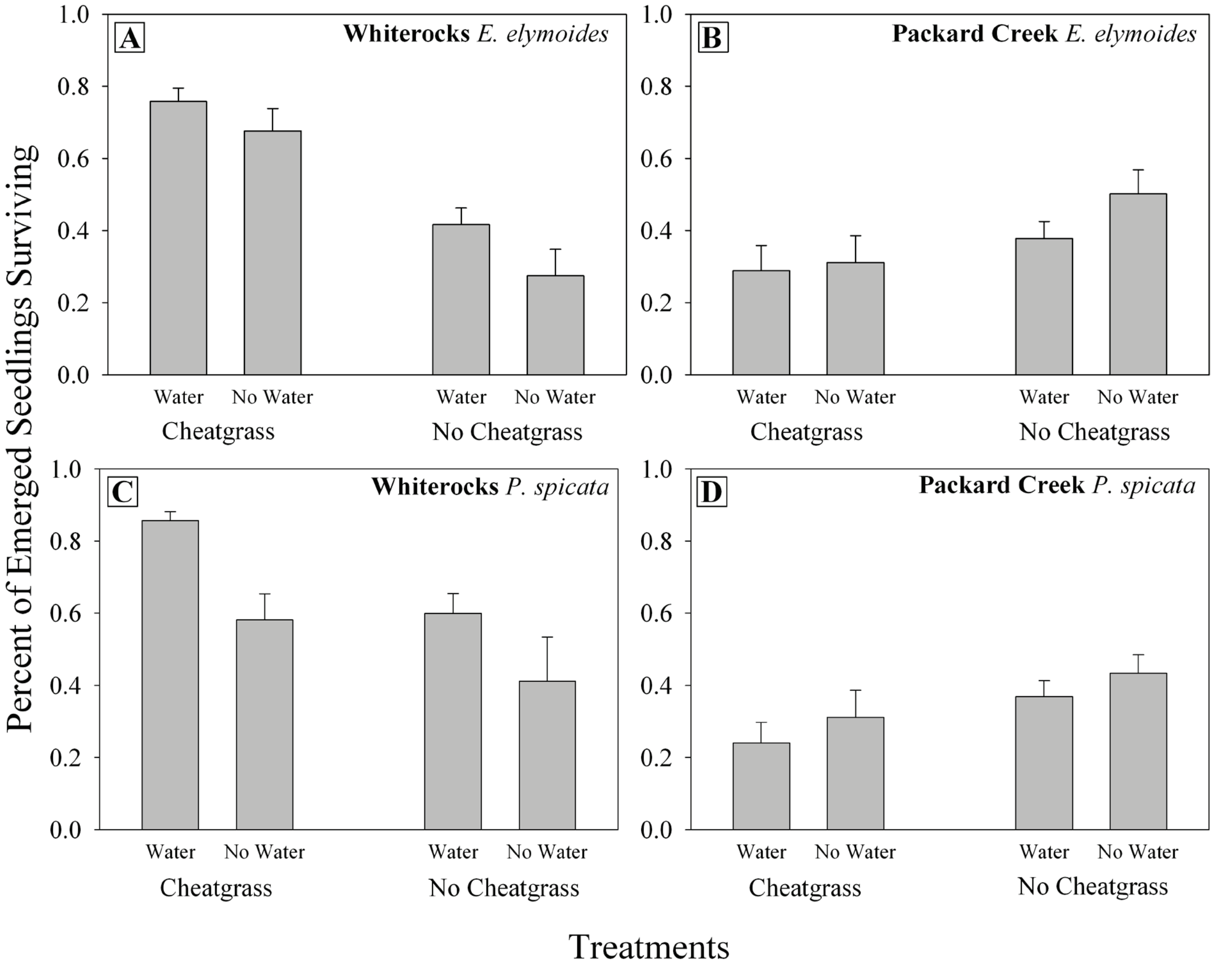

Figure 3. Effects of cheatgrass levels and added water (2.54cm x 3 fall treatments) treatments on the survival (through spring) of native grass seedlings (*Elymus elymoides* (A and B), and *Pseudoroegneria spicata* (C and D)) at Whiterocks (A and C) and Packard Creek (B and D). Error bars indicate the standard error of the mean.

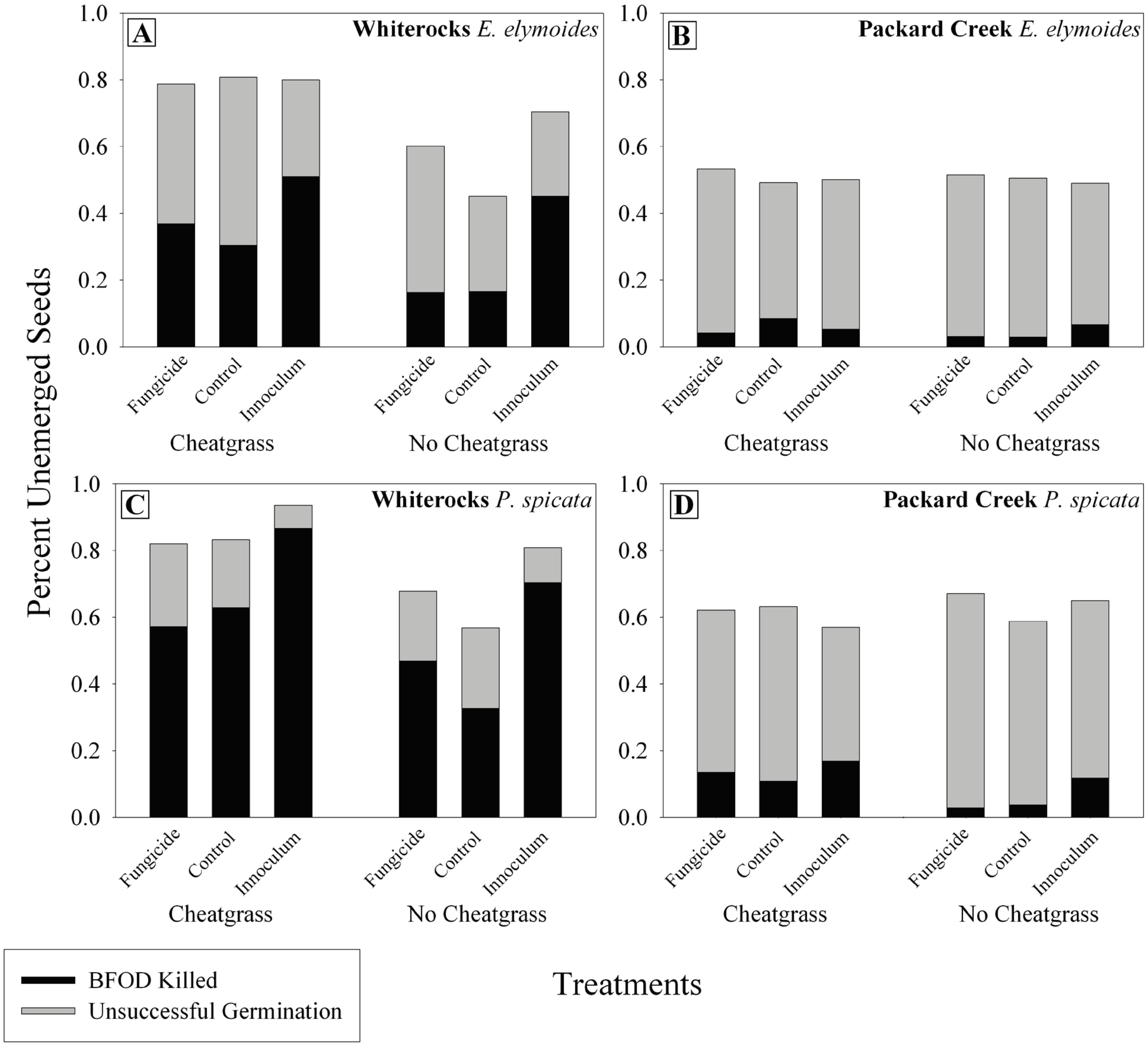

Figure 4. Effects of cheatgrass and *Pyrenophora semeniperda* levels on unemerged native seedlings (*Elymus elymoides* (A and B), and *Pseudoroegneria spicata* (C and D)) at Whiterocks (A and C) and Packard Creek (B and D). "Killed" seeds were killed by *Pyrenophora semeniperda*. "Unsuccessful germ" seeds germinated but did not emerge. The control treatment is background levels of *P. semeniperda* at the site.

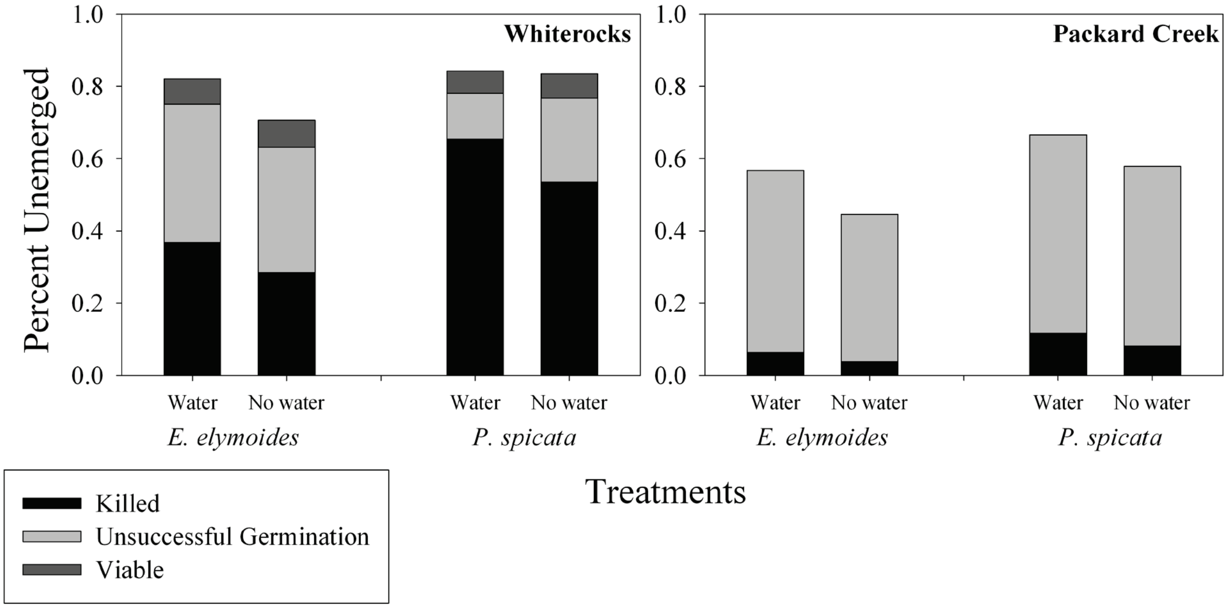

Figure 5. Effects of added water (2.54cm x 3 fall applications) on unemerged native seedlings, separated by species. ELEL represents *Elymus elymoides*, and PSSP represents *Pseudoroegneria spicata*. "Killed" seeds are those killed by *Pyrenophora semeniperda.* "Unsuccessful germ" indicates seeds that germinated but did not emerge. "Viable" seeds are those that neither died nor germinated and were still viable at the end of the study.

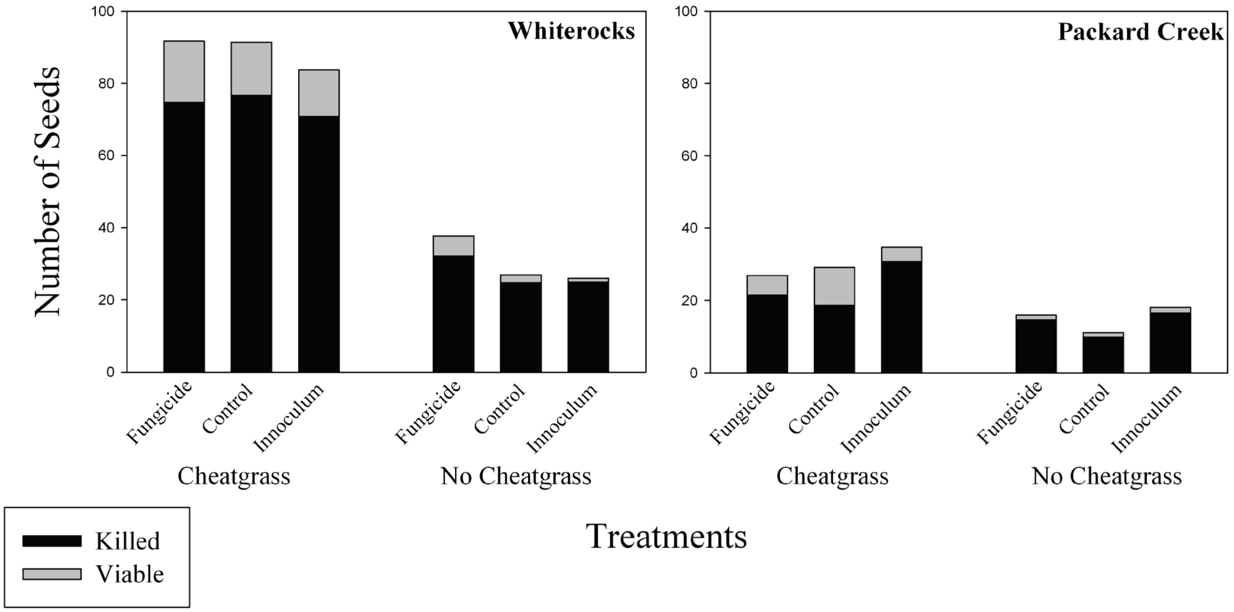

Figure 6. Effects of cheatgrass inoculum levels on the densities of *Bromus tectorum* seeds retrieved from the soil seed bank that were infected with *Pyrenophora semeniperda* or viable. “Killed” seeds are those killed by *Pyrenophora semeniperda*. “Viable” indicates those seeds that were not killed and were still viable but ungerminated by the end of the study. The control treatment is background levels of *P. semeniperda* at the site.

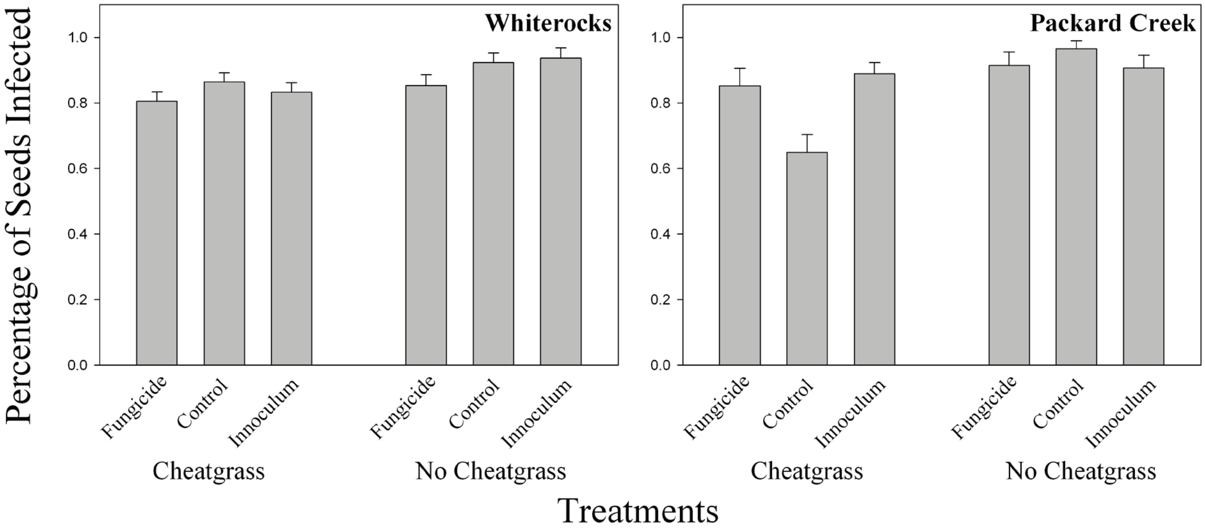

Figure 7. Effects of cheatgrass and inoculum levels on the proportion of *Pyrenophora semeniperda* infected *Bromus tectorum* seeds retrieved from the soil seed bank. Error bars indicate the standard error of the mean. The control treatment is background levels of *P. semeniperda* at the site.