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Abstract: The generalist fungal pathogen Pyrenophora semeniperda occurs primarily in cheatgrass (Bromus tectorum) seed banks, where it causes high mortality. We investigated the relationship between this pathogen and its cheatgrass host in the context of fire, asking whether burning would facilitate host escape from the pathogen or increase host vulnerability. We used a series of laboratory and field experiments to address the ability of host seeds and pathogen life stages to survive fire. First, we determined the thermal death point (TDP50; temperature causing 50% mortality) of seeds and pathogen propagules at two time intervals using a muffle furnace. We then measured peak fire temperatures in prescribed burns at sites in Utah and Washington, and quantified seed and fungal propagule survival using pre- and post-burn seed bank sampling and inoculum bioassays. Last, we investigated the survival of both seeds and pathogen after wildfires. We found that radiant heat generated by both prescribed and wild cheatgrass monoculture fires was generally not sufficient to kill either host seeds or pathogen propagules; most mortality was apparently due to direct consumption by flames. The 5-minute mean TDP50 was 164ºC for pathogen propagules and 148ºC for host seeds, indicating that the pathogen is more likely to survive fire than the seeds. Peak fire temperature at the surface in the prescribed burns averaged 130ºC. Fire directly consumed 85-98% of the viable seed bank, but prescribed burns and wildfires generally did not lead to dramatic reductions in pathogen inoculum loads. We conclude that the net effect of fire on this pathosystem is not large. Rapid post-burn recovery of both host and associated pathogen populations is the predicted outcome. Post-fire management of residual cheatgrass seed banks should be facilitated by the persistent presence of this seed bank pathogen.

Rebuttal Letter

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Fire effects on the cheatgrass seed bank pathogen *Pyrenophora semeniperda*

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ABSTRACT

The generalist fungal pathogen *Pyrenophora semeniperda* occurs primarily in cheatgrass (*Bromus tectorum*) seed banks, where it causes high mortality. We investigated the relationship between this pathogen and its cheatgrass host in the context of fire, asking whether burning would facilitate host escape from the pathogen or increase host vulnerability. We used a series of laboratory and field experiments to address the ability of host seeds and pathogen life stages to survive fire. First, we determined the thermal death point (TDP_{50}; temperature causing 50% mortality) of seeds and pathogen propagules at two time intervals using a muffle furnace. We then measured peak fire temperatures in prescribed burns at sites in Utah and Washington, and quantified seed and fungal propagule survival using pre- and post-burn seed bank sampling and inoculum bioassays. Last, we investigated the survival of both seeds and pathogen after wildfires. We found that radiant heat generated by both prescribed and wild cheatgrass monoculture fires was generally not sufficient to kill either host seeds or pathogen propagules; most mortality was apparently due to direct consumption by flames. The 5-minute mean TDP_{50} was 164ºC for pathogen propagules and 148ºC for host seeds, indicating that the pathogen is more likely to survive fire than the seeds. Peak fire temperature at the surface in the prescribed burns averaged 130ºC. Fire directly consumed 85-98% of the viable seed bank, but prescribed burns and wildfires generally did not lead to dramatic reductions in pathogen inoculum loads. We conclude that the net effect of fire on this pathosystem is not large. Rapid post-burn recovery of both host and associated pathogen populations is the predicted outcome. Post-fire management of residual cheatgrass seed banks should be facilitated by the persistent presence of this seed bank pathogen.

KEY WORDS

biological invasion, *Bromus tectorum*, natural enemies, plant-microbial interactions, *Pyrenophora semeniperda*, thermal death point

INTRODUCTION

The cheatgrass (*Bromus tectorum* L.) invasion of vast areas of the Intermountain West is uniquely intertwined with fire. Cheatgrass has increased the frequency and size of wildfires, and these fires have in turn allowed cheatgrass to expand its dominance (Whisenant 1990; D'Antonio and Vitousek 1992; Rotenberry and Knick 1997; Chambers et al. 2007). Cheatgrass typically completes its life cycle as a winter annual. It produces highly flammable standing dead biomass in early summer following seed production, greatly increasing the likelihood of subsequent fire (Blank et al. 2006). Following burning, these wildlands often persist as cheatgrass-dominated areas, threatening the existence of many native plants and animals (Baker 2006; Humple and Holmes 2006; Keeley 2006; Larrucea and Brussard 2008). Even prescribed shrubland burns that aim to increase forage for livestock often result in an expansion in annual grass invasion, especially on more arid sites in the shrub steppe (Keeley 2006).

A key component of the cheatgrass-fire cycle is the fact that a fraction of the cheatgrass seed bank can survive fire (Humphrey and Schupp 2001; Young et al. 1976), thus allowing for its recovery following fire and its eventual domination of burned sites. Several factors can influence the number of cheatgrass seeds surviving a fire. Seed mortality is greatest with fires that burn while seeds are still attached to the plant, especially just before seed shatter in the summer (Brooks 2002). Higher seed mortality has also been reported for fires that burn at higher temperatures (Brooks 2002), either due to exceptionally high density of standing dead biomass

(Humphrey and Schupp 2001) or to the presence of woody plants (Brooks 2002). Although most factors influencing the number of cheatgrass seeds surviving fires are abiotic (as listed above), biotic factors may also affect cheatgrass ability to recover following fire.

Cheatgrass-associated fire can potentially have profound effects on the soil microbial community. This may be important for understanding the cheatgrass-fire cycle because changes in plant-microbe interactions, both positive and negative, may influence native species recovery following fire. If burning reduces plant-suppressive microbes or pathogens that provide biotic resistance to cheatgrass, or reduces beneficial mycorrhizae that facilitate native species establishment, then the post-fire period can provide a window that favors cheatgrass expansion over native species establishment. The positive plant-microbe interactions in cheatgrass-invaded shrub steppe, specifically those involving mycorrhizae, have been examined by Rowe et al. (2007). The effects of fire on negative plant-microbe interactions in the shrub steppe remain largely unexplored. However, Kinter et al. (2007) found that rush skeletonweed (*Chondrilla juncea*) seeds had higher emergence in field-burned cheatgrass-invaded soils than in unburned soils, suggesting that perhaps soil organisms with a negative impact on rush skeletonweed may themselves be negatively impacted by fire. The soil microbes in the Kinter et al. (2007) study were not identified.

An important soil microbial organism of cheatgrass-invaded sites is the ascomycete seed bank pathogen *Pyrenophora semeniperda* (Brittlebank and Adam) Shoemaker (anamorph *Drechslera campanulata* (Lév.) B. Sutton). Seed infection is usually initiated by asexually produced spores (conidia), which germinate on the surface, penetrate the coverings, and then ramify as mycelium inside the seed (Beckstead et al. 2007). As the seed is consumed, the fungus initiates sporulation by producing elongate black stromata (i.e., sporocarps) that protrude through

the seed coverings. These stromata then produce another generation of conidial spores that are released into the seed bank. *Pyrenophora semeniperda* frequently kills large numbers of cheatgrass seeds in field seed banks. Meyer et al. (2007) obtained densities of field-killed cheatgrass seeds with distinctive *P. semeniperda* stromata as high as 20,000 seeds per m^2 at cold desert sites in Utah and Idaho. *Pyrenophora semeniperda* usually has greater impacts on cheatgrass seed banks at drier sites, where the secondarily dormant seeds in the spring seed bank that are its primary host are likely to be present in higher densities (Beckstead et al. 2007). Soil seed banks dominated by cheatgrass maintain much higher inoculum levels of this generalist seed pathogen than native grass seed banks (Beckstead et al. 2010).

The primary question addressed in our study is how fire impacts survival of *P. semeniperda* and seeds of its cheatgrass host. If fire completely destroys the pathogen, then cheatgrass seeds surviving the fire would be able to establish free of this natural enemy. Conversely, if the pathogen has high post-burn survival, then cheatgrass recovery from the seed bank could be negatively impacted. We investigated this question and these hypotheses in a series of laboratory and field studies. First, we quantified the thermal death point (temperature at which 50% of the individuals experience death) for three life stages of *P. semeniperda* (i.e., conidia, mycelium, and stromata) and for cheatgrass seeds. Second, we measured the temperature of prescribed cheatgrass fires in the field and compared field temperatures with the thermal death points. Third, we determined the ability of *P. semeniperda* propagules and cheatgrass seeds to survive both prescribed fires and natural wildfires.

METHODS

Thermal Death Point Determination

To determine the thermal death point (TDP_{50}, temperature causing 50% mortality) for cheatgrass seeds and for three life stages of the pathogen, we subjected them to a range of temperatures in a muffle furnace (20 (control), 65, 100, 125, 150, 200 and 300°C) for 5 or 15 minutes (to simulate fast vs. slow-burning fires), and performed subsequent survivability assessment. First, to obtain the three life stages of the pathogen, we used conidial inoculum obtained from a *P. semeniperda* isolate from Tenmile Creek, Box Elder County, Utah USA (see Beckstead et al. 2010 for inoculum production methods). We inoculated groups of 15 healthy, viable cheatgrass seeds with 0.001 g of dry conidial inoculum by placing seeds and inoculum together in glass vials (4 ml) and shaking vigorously for one minute using a hand-held shaker. For the conidial stage, inoculated seeds were placed immediately into the heat treatment. To produce the mycelial and stromatal life stages, inoculated seeds were incubated for 4 and 14 days, respectively, in individual Petri dishes (100 x 15 mm) on moist germination blotters (Anchor Paper, St. Paul, MN, USA) in a dark 20°C incubator. The seeds with mycelium or stromata were then allowed to air-dry prior to heat treatment. Fifteen replicate seeds in individual Petri dishes for each fungal stage treatment (uninoculated, with conidia, with mycelium, and with stromata) were subjected to each heat treatment.

To assess pathogen survival following the heat treatment, an uninfected host seed from the same seed collection was placed on each side of the inoculated, heat-treated seed, as a 'bait' for the pathogen. The dishes were incubated at 20°C in the dark for 4 weeks and scored for development of stromata on at least one bait seed, indicating survival of the pathogen to

infection. For the seed survival assessment, uninoculated, non-dormant heat-treated seeds were incubated as described above and scored for germination.

The effect of temperature treatment on survival of pathogen life stages and cheatgrass seeds was analyzed using logistic regression (LOGIT; JMP, Version 8.0.1, SAS Institute Inc., Cary, NC, USA). Model optimal predictability was determined by Receiver Operating Characteristic (ROC) curve area and was satisfactory for each analysis (DeLong et al. 1988). Inverse prediction set at 50% was used to calculate TDP_{50}. This experiment included 15 replicated seeds for each of the pathogen life stages and cheatgrass seeds subjected to each of seven heat treatments at two time intervals, for a total of 840 experimental units.

Prescribed Burn Studies

We utilized sites at Whiterocks in Skull Valley, Utah USA (40°32’ N 112°77’ W, 1450 m elevation) and at Haven Flats on the Hanford Reach National Monument in Washington USA (46°39’ N, 119°40’ W, 96 m elevation) for prescribed burn studies. These studies combined evaluation of peak fire temperatures using pyrometers, a bioassay technique for assessment of inoculum loads of *P. semeniperda,* and direct examination of pre-burn and post-burn seed banks (Beckstead et al. 2010). The design included twenty burned plots (0.10 m^2) at each site. Each plot was sampled before and after burning for the inoculum load and seed bank studies; peak fire temperatures were measured on each plot during the burn.

The prescribed burns were applied differently at the two sites, but both took place in near-monocultures of cheatgrass (>85 % cover). At the Haven Flat site, the plots were burned as part of a prescribed fire (743 m^2) carried out in conjunction with associated field inoculation studies (P. Allen, unpublished data). The fire crew used a backing-fire technique to burn into the

wind, resulting in a low rate of spread (8 chains per hour). Pre-burn sampling took place on April 19, 2008, the burn took place on June 8, 2008, and post-burn sampling took place on June 12, 2008. The twenty 0.10 m^2-sampling plots were randomly chosen along five randomly chosen transects; plots sampled were a minimum of 2 m apart.

At Whiterocks, we were unable to carry out our large-scale prescribed burn in a timely manner. Instead, individual plots were burned using a burn-barrel technique (Korfmacher et al. 2003), which is appropriate for field studies where precise knowledge of timing and magnitude of temperature changes are not needed. We utilized a steel burn-barrel 1 m in diameter, which enclosed the sampling plot (0.10 m^2) with at least a 20-cm buffer from the edge of the barrel and which included slots around the bottom to permit air intake during the burn. Conditions during the burn were hot, windy, and dry, resulting in very rapid fuel consumption after ignition within each barrel placement, with a flame duration of <30 seconds. The pre-burn samples were taken on May 20, 2008, the burn took place on July 8, 2008, and the post-burn samples were taken on July 8, 2008. The 1-m burn-barrel plots were laid out at 1.5 m between-plot intervals along two transects spaced 2 m apart.

Peak Fire Temperature Measurement. At the center of each of twenty sampling plots (0.10 m^2) at a given site, we installed pyrometers at four vertical positions to measure relative peak fire temperature: 5 cm above the soil surface, at the soil surface, and at 1 cm and at 3 cm below the soil surface. Pyrometers were constructed using industrial temperature-indicating lacquer paints (Tempilaq, Tempil, Inc., South Plainfield, NJ, USA) applied to thin copper oval tags (90 mm x 19 mm x 0.125 mm; National Band and Tag Co., Newport, KY, USA) using methods similar to those of Wally et al. (2006). The tags were painted with eight Tempilaq paints melting at 52, 56, 79, 93, 121, 177, 232, and 288ºC, painted in 10 mm streaks with 5 mm

between streaks. The temperatures selected encompass the expected range of cheatgrass-associated fires and mirror those measured by Brooks (2002). After drying, the painted surface was covered with another copper tag and secured with wire through the tag eyelet and a staple. Pyrometers were exhumed on the same date that inoculum bioassay samples were taken and evaluated visually to estimate peak burn temperature.

We analyzed the peak fire temperature experiment as a mixed model ANOVA with block nested within site as the random effect (Proc Mixed; SAS Version 8.1, SAS Institute Inc., Cary, NC, USA). Site and pyrometer position were considered fixed effects. The response variable (peak fire temperature) was transformed to improve homogeneity of variance. This experiment included 20 replicated plots at each of four pyrometer positions at each of two field sites for a total of 160 experimental units.

Pathogen Inoculum Bioassay. The two inoculum bioassay sample points (i.e., pre- and post-burn) in each plot were consistently located relative to pyrometer placement. We took samples by pounding a steel ring (10 cm diameter and 2.5 cm deep) into the soil until it was flush with the surface. A mason's trowel was then used to lift the ring with its surface litter and underlying soil layer intact (i.e., an intact sample of the seed zone). Twenty ring samples, one from each sampling point, were then placed in Petri dishes (15 × 100 mm), bound with rubber bands and transported to the laboratory. This procedure was carried out both before and after burning, for a total of 40 ring samples from each site.

Inoculum bioassays were conducted by planting dormant cheatgrass seeds into the seed-zone rings and monitoring *P. semeniperda*-caused disease. Within three weeks of field collection, each seed-zone ring was planted with twenty-five surface-sterilized and safranin-dyed

dormant cheatgrass seeds (dyed to distinguish them from *in situ* seeds; see Beckstead et al. 2010 for methods). Bioassay rings were incubated for 4 weeks at 20ºC without lights; rings were randomized weekly and watered as needed. Coleoptiles of any emerged seedlings were kept clipped to 2-5 cm. On day 28, all safranin-dyed seeds were exhumed and examined for germination and for presence of disease (*P. semeniperda* stromata). Seeds were classified as germinated without stromata, germinated with stromata, ungerminated without stromata, and ungerminated with stromata. All seeds that developed stromata, whether they germinated or not, were presumed to have been infected by the pathogen (Beckstead et al. 2007). All ungerminated seeds lacking fungal stromata were checked for viability using a cut test (Ooi et al. 2004). There was little or no loss of viability due to causes other than *P. semeniperda.*

We analyzed the inoculum bioassay experiment as a mixed model ANOVA with block nested within site as the random effect in SAS Proc Mixed as described earlier. Site and burn treatment were fixed effects. The response variable (proportion of seeds infected) was transformed to improve homogeneity of variance. This experiment contained 20 replicated field-collected seed-zone sample (each planted with 25 seeds) for each of the burn treatments (pre- and post-burn) at each of two study sites, for a total of 80 experimental units.

Seed Bank Quantification. We also monitored the effects of the prescribed burn treatments described earlier on cheatgrass seed banks. Pre- and post-burn seed bank samples were collected from the same plots on the same dates as the bioassay ring samples. Seed bank samples were collected using a steel can 6 cm in diameter and 4 cm deep, which was inverted and pressed into the soil until flush with the soil surface, then lifted out with a mason trowel and its contents emptied into a labeled paper sack. Samples were air-dried, screened, and hand-processed to remove all apparently viable and field-killed cheatgrass seeds (see Beckstead et al.

2010 for methods). Apparently viable seeds were incubated for 4 weeks at 20°C and scored as germinated, viable but dormant, killed in incubation by the pathogen, or nonviable/unfilled. From this incubation test, we determined the following response variables: viable seed density (i.e., living, free of disease, the sum of germinated and dormant seeds), field-killed seed density (i.e., recently killed by *P. semeniperda* in the field and exhibiting stromata), and incubation-killed seeds (i.e., seeds obtained from field that were pre-infected with *P. semeniperda* and that developed disease signs in incubation).

For Whiterocks, we included a third sample data set in the seed bank analysis. These samples were taken from a closely adjacent area (within 50 m of the burn study) as part of a different study. Samples were taken from 20 blocks in an unburned area on August 27, 2008, and processed and evaluated as described above. The reason for including this third data set was to obtain an estimate of seed survival after burning. Because initial sampling on the burn plots took place before seed dispersal, but both burning and post-burn sampling were delayed until after seed dispersal, sampling from an adjacent unburned area was necessary to obtain an estimate of seed rain prior to the burn. Field-killed seed densities were expected to be the same for pre-burn and unburned plots, as these are not likely to change during summer; the only variable expected to change between pre-burn (and pre-seed-dispersal) plots and unburned plots was viable seed density.

We had a similar problem estimating seed survival after burning at Haven Flats, but we did not have a seed bank data set from an unburned area for comparison purposes. Instead, we used a data set for cheatgrass density on burned vs. unburned plots obtained as part of an adjacent field inoculation study (P. Allen, unpublished data) to indirectly estimate seed survival after burning. The difference in plant density on burned and unburned plots was assumed to be

directly related to the variable of interest, namely the difference in seed density with and without burning. These data were obtained the spring following the same prescribed burn described above for Haven Flats. Fifteen 0.10 m^2 uninoculated control plots from adjacent unburned and burned areas at the Haven Flats site were randomly chosen. Each of these plots had received approximately 250 supplemental seeds the previous fall (i.e., post-burn). Although these seed additions complicate the purpose of the present study, we were still able to detect the relative effect of prescribed burning on seed survival, as indicated by post-burn plant density. Both burned and unburned plots received the same seed additions, and these additions were not sufficient to swamp out the effect of burn treatment on the seed bank. To estimate seed survival, density of individual plants was obtained by hand-counting in the field, when cheatgrass was at the early seed production stage. We also counted individual tillers, and biomass per plot was measured by collecting aboveground plant shoots, drying them at 60°C for 72 hours, and weighing. From these measures we calculated plant density, tillers per plant, biomass per plant, and biomass per plot for each of the 30 plots.

The analyses for the effect of prescribed burning on the seed bank were different for Whiterocks and Haven Flats. We chose to exclude the seed bank data from Haven Flats because of problems with sampling dates described above. The Whiterocks seed bank data were analyzed using ANOVA in SAS for a completely randomized design with burn treatment (pre-burn, post-burn, and unburned) as the fixed effect. The experiment could not be considered blocked because the unburned treatment was not included in the original block design. Response variables were viable seed density, field-killed seed density, and density of incubation-killed seeds as described earlier; these were log-transformed prior to analysis. We included twenty replications in each of the three burn treatments for a total of 60 experimental units.

For the Haven Flats site, the effect of the prescribed burn treatment on plant density, tillers per plant, biomass per plant, and biomass per plot were analyzed using a completely randomized ANOVA design in SAS as described above. The response variables were log-transformed to improve homogeneity of variance prior to analysis. We included 15 replicates for each of the two burn treatments for a total of 30 experimental units.

Wildfire Seed Bank Studies

We took advantage of natural August 2007 burns in cheatgrass monocultures on West Mountain in northern Utah (40.13851 N 111.80478 W, 1390 m elevation) and at Rattlesnake Mountain on the Hanford Reach National Monument in Washington (46.22573 N 83.30382 W, 732 m elevation) to evaluate the effect of wildfire on both cheatgrass seed banks and *P. semeniperda* abundance. Sampling took place within one month of each burn and prior to any cheatgrass autumn germination. On the West Mountain burn, we selected a site where an elongate island of unburned cheatgrass remained in the center of a burned area. This island apparently remained unburned because of wind shifts during the fire and not because of any obvious topographical or soil difference. At 5-m intervals along the boundary of the unburned area, we sampled at a distance of 2 m from the edge of the burn on both the burned and unburned sides. Ten seed bank samples each from burned and unburned areas were obtained. Seed bank samples were taken as described earlier. A similar protocol was followed at the Rattlesnake Mountain site, except the burned and unburned areas were located on opposite sides of a dirt road, 20 samples each from burned and unburned areas were obtained, and a small hand shovel was used instead of a can (although area sampled was roughly the same). Soil seed bank samples were processed and evaluated as described above.

Data sets from the two sites were combined for analysis of variance (ANOVA) for a randomized design. We used SAS Proc GLM because of unequal replication between sites. Fixed main effects specified in the ANOVA were site (West Mountain vs. Rattlesnake Mountain) and burn treatment (burned vs. unburned). The response variables were density of viable seeds, density of field-killed seeds and density of incubation-killed seeds; these were log-transformed for analysis to increase homogeneity of variance. This experiment included either 10 (West Mountain) or 20 (Rattlesnake Mountain) replicated field-collected seed bank samples for each of the burn treatments for a total of 60 experimental units.

RESULTS

Thermal Death Point Determination

The ability of the seed pathogen *P. semeniperda* to survive high temperature and subsequently infect adjacent host seeds decreased with increasing temperature and varied among life stages after the 5-minute heat treatment (Figure 1a; temperature main effect: Chi-square = 225.27, df = 1, $P < 0.0001$; life stage main effect: Chi-square = 6.71, df = 2, $P = 0.03$). The conidial stage experienced 50% mortality at a lower temperature than the mycelial and stromatal stages (conidial stage TDP_{50} = 141.36ºC, mycelial stage TDP_{50} = 174.57ºC, stromatal stage TDP_{50} = 178.34ºC).

After the 15-minute heat treatment, pathogen survival was again significantly affected by temperature, but the difference between life stages was only marginally significant (Figure 1b; temperature main effect: Chi-square = 227.87; df = 1; $P < 0.0001$; life stage main effect: Chi-square = 5.52; df = 2; $P = 0.06$). Mean thermal death point across life stages was 14% lower for

the longer-duration heat treatment (15-minute overall TDP_{50} = 141.60ºC and 5-minute overall TDP_{50} = 164.21ºC), indicating lower survival after a longer heat treatment period.

The ability of cheatgrass seeds to survive high temperature also decreased with increasing temperature and showed a similar pattern after 5- and 15-minute heat treatment durations (Figure 1c; 5-minute treatment: Chi-square = 126.32, df = 1, $P < 0.0001$; 15-minute treatment: Chi-square = 129.78, df = 1, $P < 0.0001$). The TDP_{50} for cheatgrass seeds was 10% lower than the mean TDP_{50} for the pathogen at each of the two treatment durations, indicating that the seeds would be killed at a lower temperature than the pathogen (for seeds: TDP_{50} at 5-minute interval = 147.64ºC and TDP_{50} at 15-minute interval = 127.33ºC).

Prescribed Burn Studies

Peak Fire Temperature Measurement. Prescribed burn peak fire temperature varied with height above or below the soil surface. The highest flame temperatures were measured at and above the soil surface, averaging around 144ºC, whereas fire temperatures were lower below the soil surface, averaging around 63ºC (Figure 2; pyrometer position main effect: df = 3, 152; $F = 74.44$; $P < 0.0001$). There was no significant difference in overall peak fire temperature between the two sites (Figure 2; site main effect: df = 1, 152; $F = 1.67$; $P = 0.20$). There was however a significant site x pyrometer position treatment interaction (df = 3, 152; $F = 5.92$; $P = 0.0008$), indicating that soil properties and/or aboveground litter were different between sites, resulting in different peak fire temperature patterns. The fire burned substantially hotter at and above the soil surface at Haven Flats, whereas subsurface temperatures during the burn were slightly higher at Whiterocks.

Pathogen Inoculum Load Bioassay. Laboratory experiments with field-collected seed-zone samples from pre-burn and post-burn plots at the two sites demonstrated significant differences between sites in the fraction of planted cheatgrass seeds infected by *P. semeniperda* (Figure 3). Mean disease incidence was 25% for Whiterocks Utah but only 13% for Haven Flats (site main effect: df = 1,76, $F = 23.48$, $P < 0.0001$). Mean disease incidence did not vary significantly between burn treatments, indicating that pathogen inoculum was generally able to survive fire (burn treatment main effect: df = 1,76; $F = 0.14$; $P = 0.71$). There was a significant site x burn treatment interaction, however (df = 1,76; $F = 10.57$; $P = 0.002$). This interaction was significant because samples at the Haven Flats site demonstrated a decrease in seed infection following the burn, whereas samples from the Whiterocks site actually showed an increase in post-burn seed infection.

Seed Bank Quantification. The prescribed burn treatment at the Whiterocks site had a very large impact on viable seed density in the cheatgrass seed bank (Figure 4a; df = 2,57; $F = 30.90$; $P < 0.0001$). Fire drastically reduced the cheatgrass seed bank; only an estimated 12% survived the burn (mean of 30,400 seeds/m^2 in the unburned plots vs. 3600 seeds/m^2 in the post-burn plots). The majority (97%) of the seed bank present at the time of the burn was from current-year seed production, as the carryover present prior to both seed shatter and burning was only 800 seeds/m^2. Fire had a similar impact on field-killed seed density (Figure 4b; df = 2,57; $F = 15.68$; $P < 0.0001$). Post-burn density of only 1400 field-killed seeds/m^2, compared to 7650 and 8940 killed seeds/m^2 in the pre-burned and unburned plots, respectively, or an average burn survival of 17% (Figure 4b). As expected, field-killed seed density did not differ significantly between the unburned area and the pre-burn plots, evidence that these plots represented two sets of samples from essentially the same population (Figure 4b). In contrast to viable and field-

killed seed density, the density of seeds killed in incubation did not differ significantly among burn treatments (Figure 4c; d.f. = 2, 57, F = 0.82, $P = 0.44$), and for samples from the same plots, the means were very similar (pre-burn 850 vs. post-burn 810 seeds/m^2). The density was somewhat higher in the unburned plots, but because of high variance this difference was not significant. Incubation-killed seeds are seeds that were already infected by the pathogen at the time of seed bank drying in late spring, so that the ability of the pathogen to grow out of these seeds following burning indicates that the mycelial stage inside the host seed was able to survive the fire.

Prescribed burning at the Haven Flats site also had a large impact on several cheatgrass density and biomass measures taken the following spring. Fire reduced plant density by 96% (Figure 5a; df = 1,21; $F = 372.18$; $P < 0.0001$). However, the plants on burned plots had 3x as many tillers as plants on unburned plots (Figure 5b; df = 1,21; $F = 114.61$; $P < 0.0001$). Although density varied greatly between burn treatments, biomass per plot did not differ significantly (Figure 5c; df = 1,28; $F = 1.11$; $P = 0.30$). Individual plants on burned plots were 20x larger on average than plants on unburned plots (Figure 5d; df = 1,21; $F = 311.06$; $P < 0.0001$).

Wildfire Seed Bank Studies

In the wildfire seed bank studies, density of viable cheatgrass seeds differed between sites and as a function of wildfire (Figure 6a; Table 1). The West Mountain, Utah site had 1.4x as many viable seeds per m^2 as the Rattlesnake Mountain, Washington site, though both sites had viable seed densities of over 10,000/m^2 in the unburned treatment. Wildfire dramatically reduced viable seed density at both sites; the Rattlesnake Mountain site had a 98% reduction, while the

West Mountain site had an 85% reduction. This difference in seed survival (2 vs. 15%) was reflected in a significant site by burn interaction (Table 1).

The potential inoculum load for *P. semeniperda* measured as the density of field-killed cheatgrass seeds with stromata varied significantly between sites; the West Mountain site had a field-killed seed density 20x higher than the Rattlesnake Mountain site (Figure 6b; Table 1). Overall, fire reduced the density of field-killed seeds from 890 to 680 killed seeds/m^2, a difference that was only marginally significant. The ability of the pathogen to survive fire at the mycelial stage, internally in seeds, was once again indicated by the lack of any significant difference between burn treatments in density of incubation-killed seeds. The West Mountain site had much higher densities of incubation-killed seeds overall (1190 vs. 130/m^2), a result similar to the result for field-killed seeds (Figure 6c).

DISCUSSION

The hypothesis that fire creates a window for cheatgrass expansion by eliminating the natural enemy *P. semeniperda* was not supported. We also found that fires in cheatgrass monocultures destroyed most of the current-year seed production through direct flame consumption, but that fires rarely burned hot enough to kill seeds via radiant heat. In the laboratory experiment, seeds had to be exposed to temperatures greater than 150ºC for 5 minutes to result in high mortality. In the prescribed burn experiments seeds at the soil surface or above (i.e., in the litter layer) did experience temperatures around 150ºC (duration less than 5 minutes) and would also experience direct flame consumption. However, seeds 1 cm below the soil surface only experienced fire temperature less than 70ºC; not hot enough to kill seeds via radiant heat. Similarly, *P. semeniperda* inoculum on or in seeds or in the litter may be consumed directly by fire along with

host seeds, but if not directly consumed, the pathogen frequently survives. Although both cheatgrass seeds and *P. semeniperda* can survive fire, they do not survive to the same degree; the pathogen exhibited higher post-burn survival than cheatgrass seeds (e.g., Figure 1, Figure 6).

Comparison of the thermal death point (TDP_{50}) for pathogen life stages with fire temperatures measured during prescribed burns explains why radiant heat during the cheatgrass burns apparently had little impact on pathogen viability. Laboratory measurements of TDP_{50} for the pathogen predicted that temperatures above 164ºC for 5 minutes were needed for mortality, whereas predicted peak fire temperature for seed mortality was 148ºC for 5 minutes. TDP_{50} was thus higher for the pathogen, indicating that hotter fires would be needed to kill the pathogen than host seeds. The mycelial and stromatal stages of the fungus survived high temperatures better than the conidial stage. Field-measured peak aboveground fire temperatures ranged from about 120ºC to 170ºC, suggesting that particularly the hotter fire at Haven Flats could have killed some seeds and pathogen propagules via radiant heat. These temperatures are slightly higher than those reported for annual grassland burns in the arid western Mojave Desert (Brooks 2002) but similar to those reported for grassland fires of the Canadian prairies (Bailey and Anderson 1980). However, it is likely that peak temperatures during cheatgrass fires usually last only a few seconds, and only rarely as long as five minutes (Stinson and Wright 1969; M. Brooks, personal communication, February 2010). This suggests that TDP_{50} values for peak temperatures at the short durations experienced in field burns would be even higher, further explaining the low mortality from radiant heat in our field studies. Our data do predict that somewhat hotter fires, as when shrublands first burn (Bailey and Anderson 1980) or when there is exceptionally heavy standing litter (Humphrey and Schupp 2001) could eliminate this pathogen and create a

423 temporary “enemy-free” zone. However, under these hot fire conditions our data predict that it
424 is likely that most if not all cheatgrass seeds would be killed as well.

425 In general, we saw higher seed mortality than pathogen mortality as a consequence of
426 fire, particularly when the pathogen was in the mycelial life stage within seeds. This could be
427 partly due to the superior ability of the pathogen to survive radiant heat. It could also be related
428 to the vertical position of healthy viable seeds versus infected or killed seeds in the seed bank.
429 Most of the viable seeds were recently dispersed and probably located on the surface of the litter,
430 where they would be more likely to be directly consumed by fire. In contrast, infected and killed
431 seeds were carryover seeds that would be likely to be buried deeper in the litter or located at the
432 interface with mineral soil, where they would be less likely to be consumed directly by fire and
433 also less likely to experience lethal doses of radiant heat.

434 From the seed pathogen perspective, prescribed fires and wildfires did not lead to
435 dramatic reductions in infection or inoculum loads; the one exception was the Haven Flats
436 prescribed burn where surface fire temperatures were high, reducing infection by 56%. At
437 Whiterocks, there was no evidence for an inoculum decrease; mortality in the seed bioassay test
438 was actually greater in the post-burn samples. The reason for this is unknown, but it could be
439 related to differences in the phenology of conidial production at the two sites. Cheatgrass at
440 other sites on the Hanford Reach National Monument, where Haven Flats is located, generally
441 have advanced phenology relative to Whiterocks, because the springs are warmer and drier there
442 (Beckstead et al. 2010). It is likely that this relative difference in phenology is present for
443 pathogen conidial production as well. Most infection in seed bioassays is probably due to
444 conidial inoculum. If conidial production had not yet taken place at the time of pre-burn
445 sampling at Whiterocks, this could have reduced infection in the inoculum bioassay test relative

446 to post-burn levels, which in turn were not much impacted by the burn itself because of cooler
447 surface fire temperatures.

448 Integrating our laboratory thermal death point measurements with the field-measured fire
449 intensities, it is clear that annual grassland fires in our study, whether prescribed burns or
450 wildfires, were not hot enough to eliminate *P. semeniperda* from the seed zone. Although spores
451 have been found to be fire-resistant fungal propagules primarily for mycorrhizae (Baar et al.
452 1999; Pattinson et al. 1999; Bruns et al. 2002), other studies support our findings of resistant
453 mycelium embedded within plant tissues. For example, pathogenic fungi such as *Phellinus*
454 *weirii* (Dickman and Cook 1989) and *Phanerochaete raduloides* (Penttilä and Kotiranta 1996)
455 appear to persist as latent mycelium and to spread within living trees after fire. Sporocarps, such
456 as stromata, are often thought to protect fungi during fire (Pattinson et al. 1999), and this idea
457 was also supported by our study. In several of the studies examining fungal life stages and fire,
458 it was found that newly dispersed fungal spores moved on to burned sites and were the primary
459 source of fungal colonization (Wicklow 1975; Johannesson et al. 2001; Bruns et al. 2002).
460 While this process is also likely to occur with *P. semeniperda*, it cannot explain the high level of
461 inoculum found post-burn at Whiterocks, because the samples were taken immediately after
462 burning, so that dispersal onto the site could not yet have taken place.

463 We found that both prescribed fires and wildfire had a dramatic impact on the cheatgrass
464 seed bank. In our quantification of pre- and post-burn viable seed densities, only 2-15% of the
465 seed bank survived fire, values similar to those from prior studies (3 to 20%; Young and Evans
466 1976; Humphrey and Schupp 2001). However, evidence from Haven Flats indicated that
467 cheatgrass was able to compensate for a large reduction in plant density the spring following fire
468 with much larger biomass per individual in burned versus unburned plots. These findings are

similar to those for a closely related species, *Bromus rubens.* Brooks (2002) found that biomass per *B. rubens* individual was 5-100x larger in burned than unburned plots, depending on burn treatment and microhabitat, showing the tremendous phenotypic plasticity in response to resource availability. Density and biomass of annual bromes can increase or decrease in the first few years post-fire (Brooks 2002). However, eventually *Bromus* dominates burned sites, and it does so across a wide range of community types throughout the Mojave Desert and Great Basin (Young et al. 1976; Hunter 1991).

IMPLICATIONS

The pathogen *P. semeniperda* can destroy a large fraction of the cheatgrass seed bank, up to 50% of the seeds produced each year (Meyer et al. 2007). The demonstrated ability of this pathogen to survive fire means that pathogen-caused mortality will continue unabated, whether or not a site burns. High mortality from this pathogen appears to have minimal demographic consequences for its annual grass host, however. These minimal consequences result both from prolific host seed production (up to 30,000 seeds/m^2; Smith et al. 2008) and because rapid germination of the first seedling cohort each year virtually guarantees that numerous host seeds escape from the pathogen and establish as seedlings that produce seeds the following spring (Beckstead et al. 2007). This assures the continued existence of both host and pathogen through time regardless of the frequency of burning. Our studies predict rapid post-burn recovery of both host and associated pathogen populations after fire. Post-fire management of residual cheatgrass seed banks may be facilitated by the persistent presence of this seed bank pathogen. Pathogen attack has the potential to reduce viable cheatgrass seed density, which will likely already be quite low following fire, to levels that could permit post-burn establishment of seeded species.

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584

585 Figure captions

586 Figure 1. Thermal death curves showing the effect of radiant heat after 5- and 15-minute heat
587 treatments on survival of *Pyrenophora semeniperda* life stages (a, b) and cheatgrass (*Bromus*
588 *tectorum*) seeds (c) (n = 15 seeds). Thermal death points (TDP_{50}; temperature at which 50% of
589 the individuals experience death) are indicated for the average of all pathogen life stages after 5
590 minutes (a; TDP_{50} = 164°C), for the average of all pathogen life stages after 15 minutes (b;
591 TDP_{50} = 142°C), and for cheatgrass seeds after 5 and 15 minutes (c; TDP_{50} = 148°C and 127°C,
592 respectively).

593 Figure 2. The peak fire temperatures measured during prescribed burns using pyrometers with
594 temperature-indicating paints placed at positions above, at, or below the soil surface at the Haven
595 Flats, Washington and Whiterocks, Utah study sites (mean + 1 SE). Data represent twenty
596 replicated pyrometers for each site by pyrometer position combination.

597 Figure 3. The percentage of cheatgrass (*Bromus tectorum*) seeds infected by the seed pathogen
598 *Pyrenophora semeniperda* after planting into field-collected seed-zone samples collected from
599 twenty burn plots prior to and after burning at each of two prescribed burn sites: Haven Flats,
600 Washington and Whiterocks, Utah (mean + 1 SE). Each replicate included twenty-five planted
601 seeds.

602 Figure 4. Seed bank density of (a) viable seeds (i.e., living, free of disease), (b) field-killed seeds
603 (i.e., recently killed by *Pyrenophora semeniperda* in the field and exhibiting stromata), and (c)
604 incubation-killed seeds (i.e., seeds obtained from field that are pre-infected with *P. semeniperda*
605 and that develop disease signs in incubation) for cheatgrass (*Bromus tectorum*) at Whiterocks,
606 Utah in each of three burn treatments (pre-burn, post-burn, and adjacent unburned; mean + 1

SE). Bars capped by the same letter are not significantly different at the $P = 0.05$ level according to a Duncan multiple range test. Data represent twenty-replicated soil seed bank samples obtained from each burn treatment.

Figure 5. Effects of a prescribed burn at Haven Flats, Washington on cheatgrass (*Bromus tectorum*): (a) density, (b) biomass per unit area, (c) tillers per plant, and (d) biomass per plant (mean + SE). Data represent means from fifteen plots per burn treatment sampled in late spring following prescribed fire the previous summer.

Figure 6. Effects of wildfires at Rattlesnake Mountain, Washington and West Mountain, Utah on cheatgrass (*Bromus tectorum*) seed bank (a) viable seed density (i.e., living, free of disease), (b) field-killed seed density (i.e., recently killed by *Pyrenophora semeniperda* in the field and exhibiting stromata), and (c) density of seeds killed in incubation (i.e., seeds obtained from field that are pre-infected with *P. semeniperda* and that develop disease signs in incubation); mean + 1 SE. Data represent ten (West Mountain) or twenty (Rattlesnake Mountain) soil samples collected from each treatment combination.

Table 1. ANOVA results for the effects of site and wildfire burn treatments on seed bank components, including cheatgrass (*Bromus tectorum)* viable seed density (i.e., living, free of disease), field-killed seed density (i.e., recently killed by *Pyrenophora semeniperda* in the field and exhibiting stromata), and density of seeds killed in incubation (i.e., seeds obtained from field that are pre-infected with *P. semeniperda* and that develop disease signs in incubation).

		Viable seed density		Field-killed seed density		Incubation-killed seed density	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*
Site	1, 56	14.21	0.0004	65.50	0.0001	35.64	0.0001
Burn	1, 56	86.67	0.0001	3.75	0.0578	0.13	0.7214
Site x burn	1, 56	7.63	0.0078	0.05	0.8225	0.04	0.8519

Figure 1

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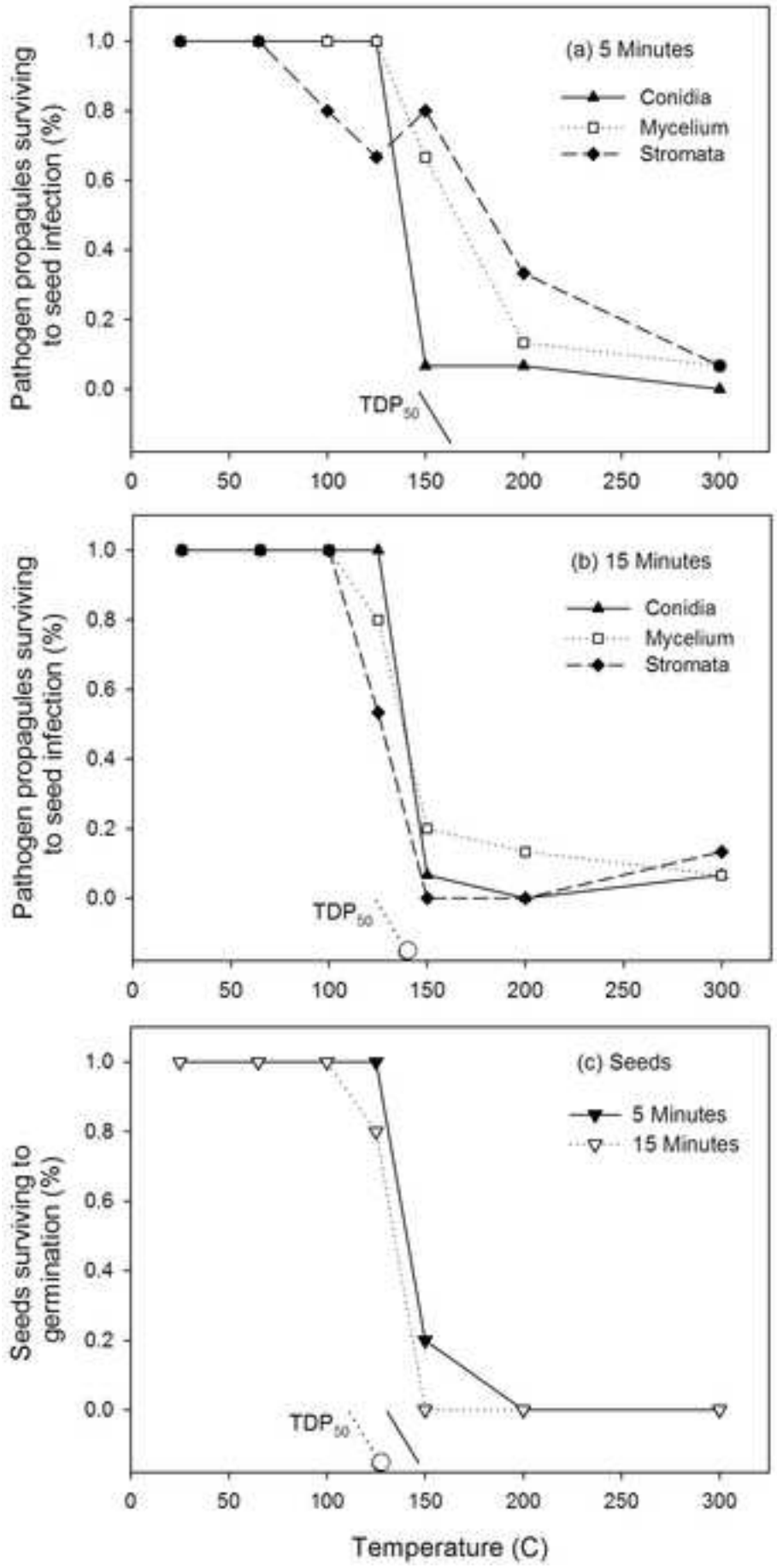

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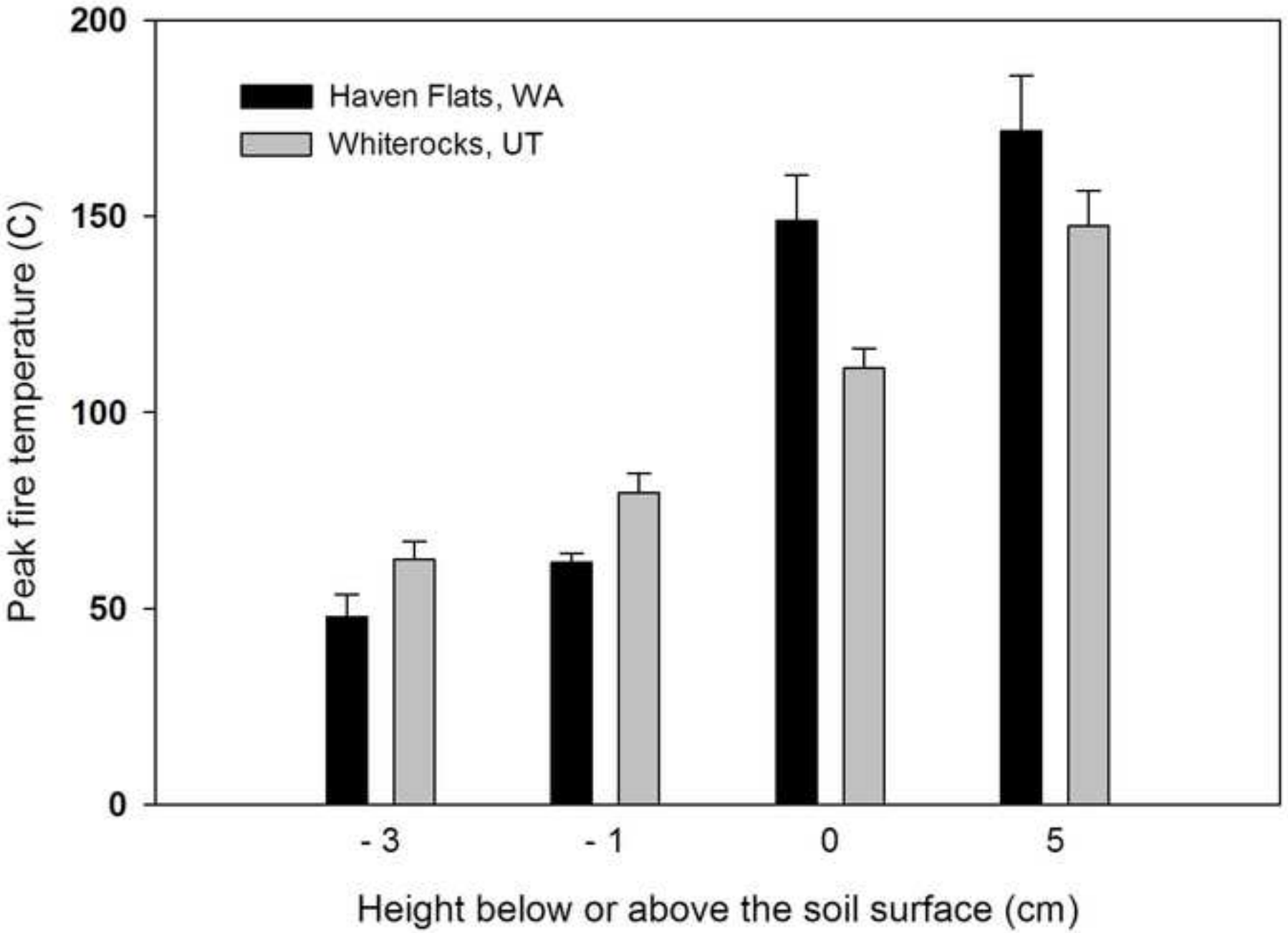

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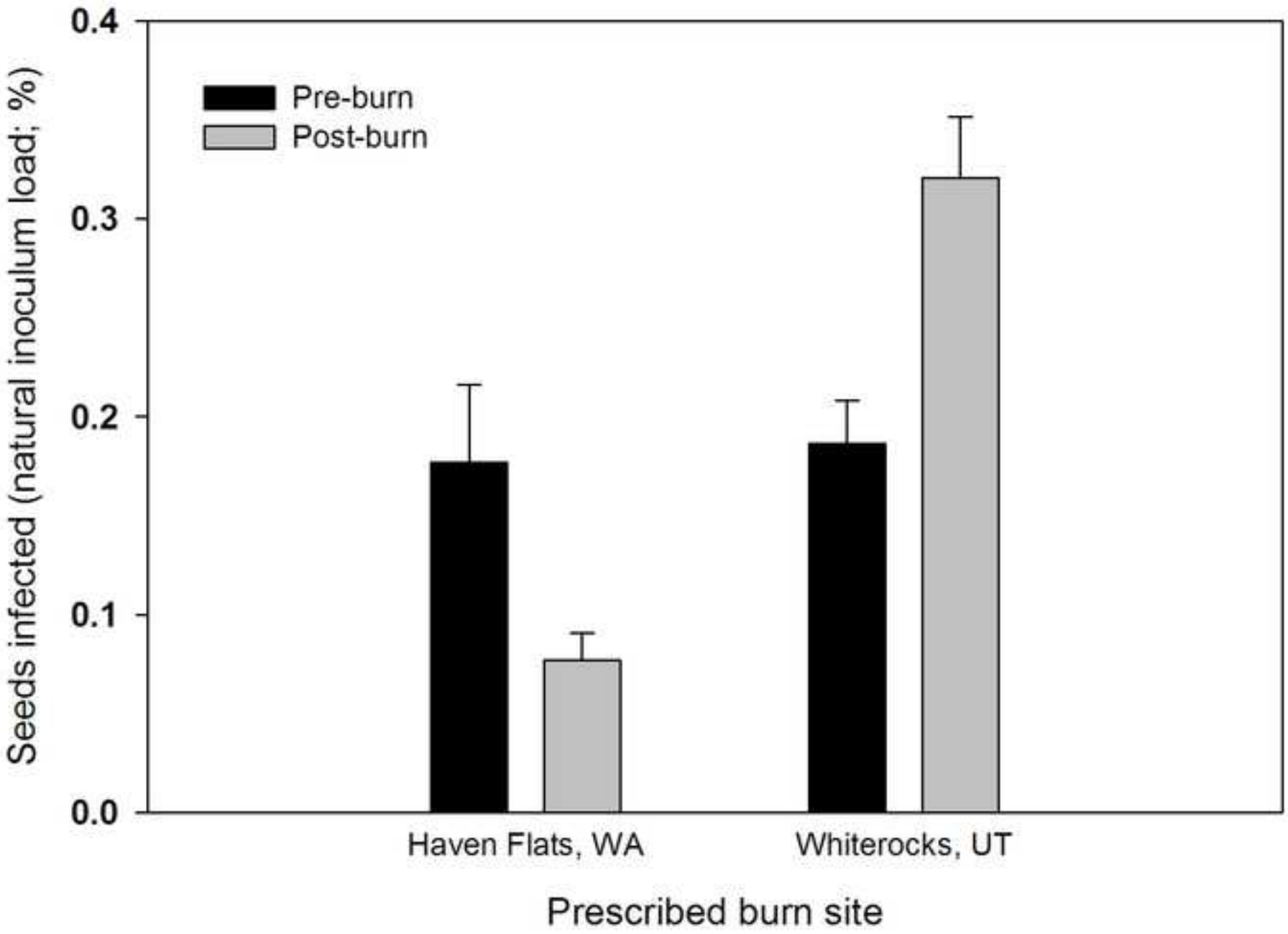

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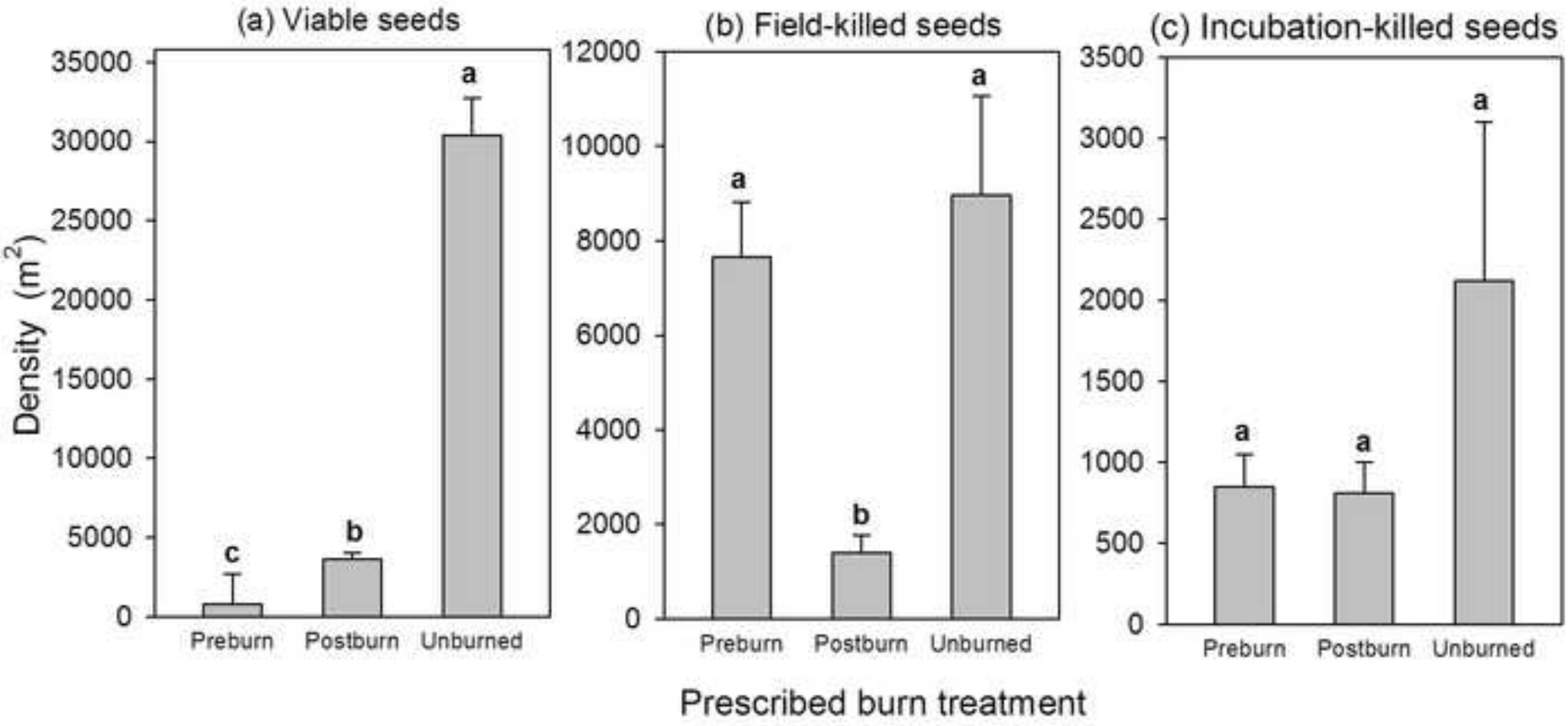

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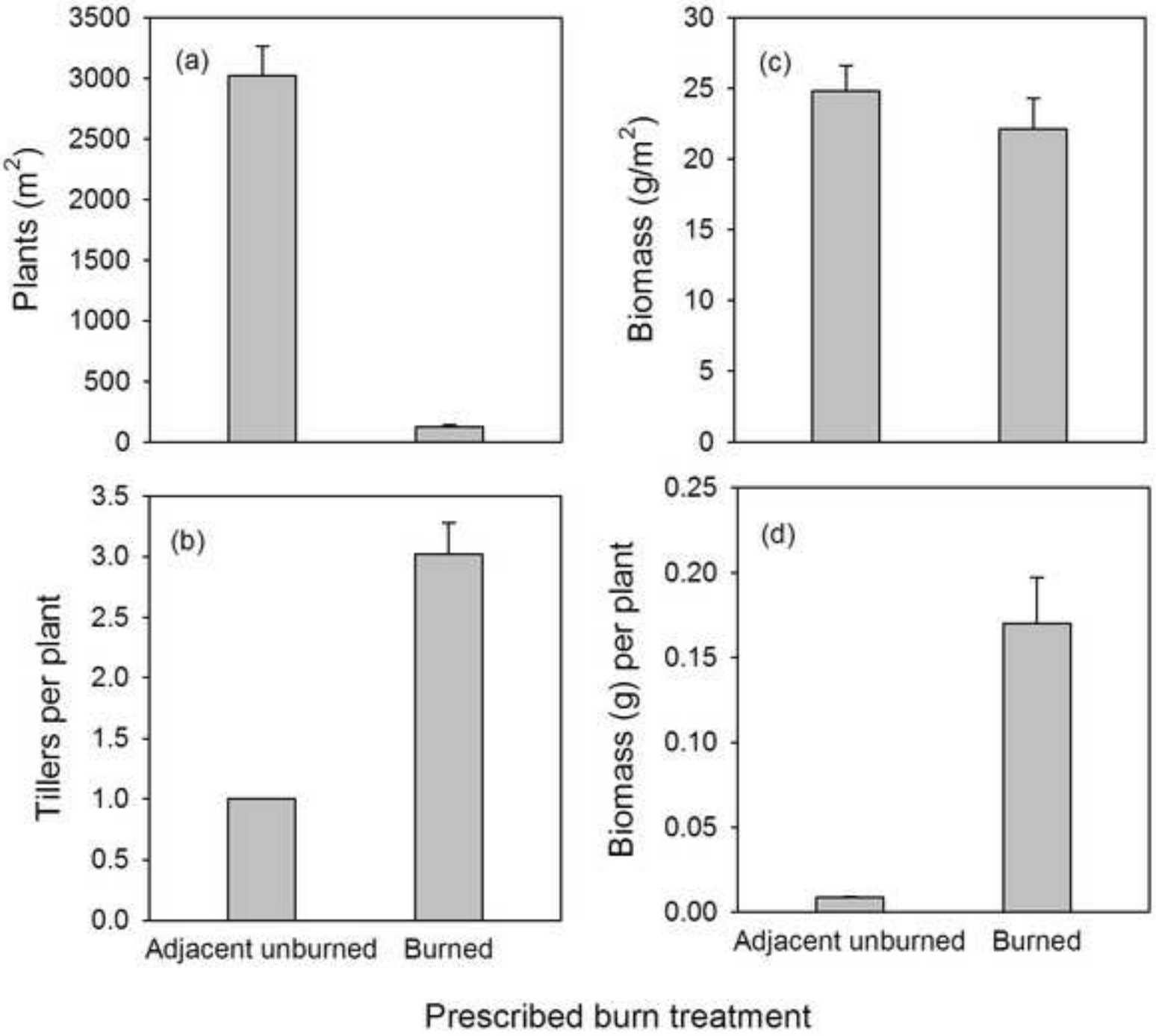

Figure 6

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