Plant Soil (2018) 422:155–168
DOI 10.1007/s11104-017-3420-9

REGULAR ARTICLE

Does dormancy protect seeds against attack by the pathogenic fungus *Fusarium tricinctum* in a semiarid grassland of Northwest China?

Tao Chen · Zhibiao Nan · Xingxu Zhang · Fujiang Hou · Michael Christensen · Carol Baskin

Received: 1 April 2017 / Accepted: 8 September 2017 / Published online: 20 September 2017

Abstract
Aims Soil fungal pathogens can result in the failure of seedling establishment, but the effects of fungicide applications on seed/seedling survival have differed among studies. We assumed that the variation may relate to seed dormancy/germination characteristics and hypothesized that nondormant germinating seeds are more likely to be killed by fungal pathogens than dormant seeds.
Methods Dormant and nondormant seeds of *Stipa bungeana* and *Lespedeza davurica* were inoculated with a pathogenic fungus *Fusarium tricinctum* under laboratory and field conditions. The outcomes of seed/seedling fate and other parameters were evaluated.
Results In the laboratory, nondormant seeds inoculated with *F. tricinctum* developed white tufts of mycelium on the radicles of germinating seeds causing them to quickly die, but dormant seeds remained intact. In contrast, in the field inoculation with *F. tricinctum* did not cause higher mortality of nondormant than dormant seeds but resulted in higher percentages of seedling death before they emerged from soil than the controls.
Conclusions Our results suggest that dormancy protects seeds from being attacked by some pathogens by preventing germination, but the protection is lost once germination has commenced. Further study involving various plant species with more seeds is needed to assess the generality of this pathogen-seed interaction hypothesis.

Keywords Dormancy · Fungal pathogens · Grasslands · Pathogen-seed interactions · Seed burial

Responsible Editor: Philippe Simoneau

T. Chen · Z. Nan (✉) · X. Zhang · F. Hou
State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agricultural Science and Technology, Lanzhou University, PO Box 61, Lanzhou, Gansu 730020, China
e-mail: zhibiao@lzu.edu.cn

M. Christensen
Retired scientist of AgResearch, Grasslands Research Centre, Palmerston North, New Zealand

C. Baskin
Department of Biology, University of Kentucky, Lexington, KY 40506, USA

C. Baskin
Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA

Introduction

In plants, seed dormancy is defined as an innate constraint on germination under conditions that would otherwise be favourable for germination, i.e. after seeds become nondormant (Baskin and Baskin 2004; Footitt et al. 2011). In contrast, the lack of dormancy (nondormancy) enables seeds to start germinating as soon as conditions become favorable for germination and thereby to maximize the growing season and minimize germination risk (Willis et al. 2014). Baskin and Baskin (2004) have proposed a comprehensive

classification system that includes five classes of seed dormancy: physiological, morphological, morphophysiological, physical and combinational. This system is hierarchically with five classes being further divided into several levels and then into types. Regardless of class or degree of dormancy, the essential role of seed dormancy is assumed to be similar, i.e. regulating the timing of germination, and bet-hedging against unpredictably variable environments (Donohue et al. 2005; Poisot et al. 2011).

For many plant species, the stage of transition from seed to established seedling is very sensitive to environmental change and represents a major bottleneck to plant recruitment (Dalgleish et al. 2010; Francisco et al. 2004), since seeds in this stage are exposed to a wide variety of biotic and abiotic soil factors that can cause high levels of seed/seedling death (Chambers and MacMahon 1994; Forget 2007). Soil fungal pathogens are considered to be one of the key factors resulting in the death of seeds/seedlings during this stage (Beckstead et al. 2010; Gómez-Aparicio et al. 2012). Thus, seeds are frequently treated with fungicides to protect them against fungal pathogens at planting. However, the effects of fungicide applications on seed/seedling survival have differed greatly among studies. For example, a study by Dalling et al. (1998) showed that the addition of fungicide decreased seed mortality by 39 and 47% for the two pioneer tropical tree species *Cecropia insignis* and *Miconia argentea*, respectively. In another study, however, fungicide application deceased seed mortality by only 0.1–0.2% for the two *Trifolium* species in Australian pasture (Jansen and Ison 1995). Also, complicating the effects of fungicide applications to seeds as revealed in previous studies was that the effects on seed mortality were species-specific. For example, fungicide addition did not affect seed mortality for *Danthonia spicata* following a 12-month burial, but it significantly reduced seed mortality by 43% for *Bromus inermis* (Schafer and Kotanen 2003).

One possible explanation for the variation of the effects of fungicide applications to seed/seedling survival relates to differences in dormancy characteristics of the seeds. For example, in semiarid regions of western North America, the establishment failure of the invasive winter annual grass *Bromus tectorum* was suspected of being caused by soil-borne pathogens (Baughman and Meyer 2013; Meyer et al. 2014). However, dormant *B. tectorum* seeds were present at similar densities in the seed banks of die-off and adjacent non-die-off soils, suggesting that fungal pathogens resulting in seed/seedling death were unlikely to affect dormant seeds (Baughman and Meyer 2013). It has been proposed that at the molecular and physiological level, nondormant seeds are less resistant to the attack by pathogens than dormant seeds (Bolingue et al. 2010; Fuerst et al. 2011). The relationship between seed dormancy/germination and pathogen-caused mortality has recently received theoretical consideration, but there are few empirical studies available to test these model predictions (Dalling et al. 2011). Although some studies regarding pathogen-seed interactions have involved seeds of crop species (Nelson 2004) or weed species (Gómez et al. 2013), few studies involving wild plants to date have been performed to explore whether seed germination increases the risks of seeds being attacked by fungal pathogens.

In semiarid grasslands of northwestern China, *Lespedeza davurica* (Laxm.) Schindl. (Fabaceae) and *Stipa bungeana* Trin. (Poaceae) are two of the most preferred species for grazing animals and also play a key role in reducing soil loss because of their highly developed root systems (Cheng et al. 2011). *Lespedeza davurica* is a perennial semi-prostrate legume that flowers in August with the seeds becoming mature and starting to disperse in late September. Each pod produces a single seed, approximately 1.9–2.2 mm in length and 1.2–1.5 mm in width (Chen et al. 2017a). Seeds of *L. davurica* have strong physical dormancy (i.e. the seed coat is water-impermeable), and dormancy can be broken by damaging/scarifying the seed coat. *Stipa bungeana* is a perennial grass that flowers in early May, and seeds ripen and are dispersed in late June. The dispersal unit of *S. bungeana* is an awned caryopsis tightly enclosed by the palea and lemma (collectively the hulls), and hereafter the dispersal unit of *S. bungeana* will be referred to as a seed (Hu et al. 2014). Seeds are 4.5–5.2 mm in length and 0.5–0.7 mm in width. Newly harvested seeds of *S. bungeana* have high levels of physiological dormancy, and dry storage at room temperature for a few months can accelerate the loss of dormancy (Hu et al. 2014).

Members of the genus *Fusarium* have a wide host range and are important crop pathogens worldwide (Booth 1971). The disease caused by *Fusarium* on wild plants are relatively less well-known. *Fusarium tricinctum* commonly occurs in many plants of the semiarid grasslands of northwest China, and can infect different organs of the host, such as seeds and roots

(Chen et al. 2017a; Chen et al. 2017b). In the present study, an isolate of *F. tricinctum*, LDR04 (FT-LDR04, Genbank accession KX008375), obtained from root tissues of *L. davurica*, was selected as the inoculum. A previous trial had shown that this isolate can cause severe symptoms on developing seedlings of *L. davurica* and *S. bungeana* (submitted by Chen et al.). We hypothesized that nondormant germinating seeds are more likely to be killed by fungal pathogens than dormant seeds. This is because the protective structures enclosing the seeds are ruptured when seeds started to germinate, providing access for pathogen attack (Dalling et al. 2011). To test the hypothesis, physically dormant seeds of *L. davurica* and physiologically dormant seeds of *S. bungeana* were selected to perform inoculation trials with the pathogenic fungus *F. tricinctum* under laboratory and field-based conditions. By testing the hypothesis, we expected to further our mechanistic understanding of the spermosphere interactions between seeds and their pathogens.

Materials and methods

Study area

The study site is located in the Tianshui Grassland Research Station of Lanzhou University, Huan County, Gansu Province (37.12°N, 106.82°E, 1650 m above sea level). The area is a typical semiarid ecosystem and is sensitive to grazing disturbance (Christensen et al. 2004). The mean annual temperature is about 7.1 °C, and mean annual rainfall is approximately 360 mm with more than 80% occurring from June to September. Dominant plant species in the area are *Artemisia capillaris* Thunberg (Asteraceae), *S. bungeana*, and *L. davurica*.

Seed collection

In the middle of June and October in 2014 and 2015, mature seeds of *S. bungeana* and *L. davurica* were collected by hand from plants growing on roadsides near the grassland station, respectively. Harvested seeds were taken to the laboratory where they were cleaned and allowed to dry at room temperature for one week (RH, 20–35%; 18–25 °C). Each cleaned seed lot was stored in water-impermeable packaging at 20 °C. Seed lots of *S. bungeana* harvested in 2014 (one-year old) and 2015 (newly harvested), and the seed lot of *L. davurica* harvested in 2014 were chosen for this study.

Seed germination and viability test

The aim of this trial was to determine whether the initial germination and viability of seeds differed among seed lots. For *L. davurica*, 400 seeds were randomly selected, after which half of them were gently scarified using fine sandpaper, and the remainder was kept intact. For *S. bungeana*, 200 seeds that were either one-year old or newly harvested were randomly selected. Fifty seeds of each lot were placed in each of four 12-cm-diameter Petri dishes containing two sheets of filter paper moistened with 8-ml distilled water and incubated in darkness at 20 °C. To obtain darkness, all Petri dishes were covered with two layers of aluminum foil, and seeds were monitored daily for germination for 14 days under a LED green safe light (520 nm ± 610 nm, Sanpai, Shanghai, China) (Hu et al. 2013). Seeds were considered to have germinated when the radicle was more than 1 mm (Hu et al. 2013).

Viability of nongerminated seeds was determined at the end of the germination trial. Seeds of *S. bungeana* were soaked in distilled water for 12 h, after which hulls and half of the endosperm were removed from each seed. The remaining embryo-containing part of each seed was soaked in 1% tetrazolium phosphate-buffer solution for 6–8 h at 30 °C in the dark. Seeds with embryos that stained red were considered to be viable and those with nonstained embryos nonviable (Hu et al. 2014). For *L. davurica*, nongerminated seeds were scarified with a scalpel and incubated for a further 14 days at 20 °C to determine if seeds would germinate.

A laboratory-based inoculation trial

The aim of this trial was to determine whether *F. tricinctum* can infect dormant and/or nondormant seeds, and if so does it cause germinating seeds to die in the laboratoy condition. As the status of dormancy for a particular seed was unknown, in the laboratory trial we artificially treated seeds to obtain dormant and nondormant seeds as follows. For *L. davurica*, 400 seeds of *L. davurica* were randomly selected, half of which was gently scarified using fine sandpaper, and the remainder was kept intact. For *S. bungeana*, 200 newly harvested and 200 one-year-old seeds each were selected randomly. All seeds were surface-sterilized in 75% ethanol for

1 min, 0.1% sodium hypochlorite for 5 min, rinsed in sterilized water, and placed in Petri dishes containing two sheets of sterilized filter paper (moistened with sterilized water) and incubated in darkness at 20 °C. Seeds were examined using a stereo dissecting microscope every day during incubation. A large fraction of scarified seeds of *L. davurica* and one-year-old seeds of *S. bungeana* had started to germinate on the second and fourth days, respectively, and these seeds were considered to be nondormant. Seeds with the radicle less than 1 mm (still regarded as a seed) were immediately picked out of the dishes using sterile tweezers and prepared to be inoculated with *F. tricinctum* in the laboratory. On the fifteenth day, most of the nonscarified seeds of *L. davurica* (newly harvested seeds of *S. bungeana*) had not germinated (considered to be dormant), and they were picked out for the laboratory inoculation as well.

Five nondormant or dormant seeds of each species were placed in a line across the center of each Petri dish containing 2% water agar. Five 3-mm diam. Mycelial discs of FT-LDR04, cultured on potato dextrose agar (PDA) at 20 °C for 7 days, were placed on the water agar close to the seeds (Christensen et al. 1988). The Petri dishes containing nondormant or dormant seeds without fungal inoculation served as controls. This trial involved 2 plant species × 2 dormancy status × 2 inoculation treatments × 4 replicates, for a total of 32 Petri dishes. All Petri dishes were sealed with Parafilm (Pechiney Plastic Packaging, Chicago) and stacks of four replicates of each treatment combination were bound together and placed on edge in darkness at 20 °C with the rows of seeds aligned horizontally to ensure that seedling roots would grow across the surface of the agar.

Seven days later, seed/seedling survival was determined, and disease symptoms were observed using a stereo dissecting microscope. The viability of nongerminated seeds was tested as described above for each species following surface-sterilized in 75% ethanol for 1 min, 0.1% sodium hypochlorite for 5 min, followed by three rinses in sterilized water.

A field-based inoculation trial

The aim of this trial was to determine whether *F. tricinctum* can cause dormant and/or nondormant seeds to die, and to affect seedling establishment under variable field conditions. The field inoculation trial was performed in the three 0.5 ha plots (regarded as three replicates) at the grassland station where sheep grazing had been prevented since 2001 (see Chen et al. 2017b). Within each plot, five 1.5 m × 1.0 m subplots (2 m away from the edge of plot) along a diagonal line were established equidistantly to improve the accuracy of our measurements.

Inoculum was prepared by adding 3 mm diam. Mycelial discs of FT-LDR04, cultured on PDA at 20 °C for 7 days, to 250-ml glass flasks containing 100 g of moist autoclaved millet (*Panicum miliaceum*) seeds (Nan 1995). After growing for 3 weeks, the millet inoculum was mixed thoroughly using a stirring rod. Conidial loadings were estimated according to the method of Brownbridge et al. (2012) with the following modifications. One gram of the millet inoculum was added to a sterile 50-ml plastic tube. After soaking in sterile water for 30 min, the tubes were shaken on a wrist shaker set at the maximum speed for 10 min. A serial dilution was prepared, and the number of conidia was recorded with a haemocytometer. The final number was approximately 6.70×10^6 conidia per gram of millet inoculum.

At the beginning of July 2015, 24 holes (6 holes per line × 4 lines) within each subplot were excavated using a 10-cm-diameter auger. The depth of each hole was 10 cm, and the distance between holes was 20 cm. Each of these holes allowed us to later insert a plastic pot (9 cm in height and 9 cm in diameter). The soil removed from each subplot was passed through a 0.3-mm sieve to remove debris and any seeds of *L. davurica* and *S. bungeana*. To minimize side effects from other soil organisms, approximately 3.6 kg of the sieved soil from each subplot was placed in a sealable plastic bag and fumigated by adding 45-ml propylene oxide (Gallandt et al. 2004). The seals were broken after 48 h, and the soil samples were ventilated for 48 h before being placed into plastic pots.

In the middle of July (rain was forecast for the following three days, ensuring that the pots would quickly become hydrated), the plastic pots were placed into the holes in the field in a completely randomized design. Hence, this field trial involved 2 plant species × 2 dormant status × 3 inoculation treatments × 3 replicates × 5 subplots × 2 sampling dates, for a total of 360 pots. Approximately 150 g of fumigated soil was added to each plastic pot, the wall of which contained hundreds of about 0.3-mm holes punctured by a flamed needle. Three grams of FT-LDR04 inoculum was spread uniformly at the 5–6 cm level of the 9 cm high pots, onto which was sown 20 seeds and covered with the remaining of fumigated inoculum-free soil. A 0.3-mm nylon

mesh was fixed on the top of each pot to prevent entry of other seeds. For *L. davurica*, half of the seeds were gently scarified using fine sandpaper and the remainder was kept intact. Prior to sowing, all seeds of *L. davurica* and *S. bungeana* were surface-sterilized in 75% ethanol for 1 min, 0.1% sodium hypochlorite for 5 min and rinsed with sterilized water. The fumigated soil alone and fumigated soil +3 g sterilized millet seeds served as controls. Each pot was numbered and marked with a colour-coded plastic tag to enable identification upon retrieval.

On August 13th and September 16th, 180 pots were retrieved after burial for 4 weeks and 8 weeks, respectively. The number of seedlings successfully emerged from the soil surface per pot was recorded. After that, soil in each pot was removed, carefully loosened and then passed through a 0.3-mm sterilized sieve. Seeds that retained within the 0.3-mm mesh were picked out using sterile tweezers and examined under a stereo dissecting microscope. Seeds were classified as dead (soft to the touch), intact (hard to the touch) and germinated (the radicle >1 mm) (Schafer and Kotanen 2003). The germinated seeds were further divided into: seedlings dead before emerging from soil (DBE), living but remaining in soil (LRS), living and emerging from soil (LES). The number of seeds and/or seedlings in each pot falling into each category was recorded. Half the intact nongerminated seeds of each species were washed free of soil and tested for viability as described above. The remaining intact seeds, dead seeds and dead and living seedlings were temporarily stored at 4 °C for subsequent fungal isolation (see below).

Isolation of fungi

Dead/living seeds/seedlings belonging to each category were washed free of soil using tap water, surface-sterilized in 75% ethanol for 30 s, in 0.1% sodium hypochlorite for 1 min, and rinsed with sterile water. Seedlings were cut into 2–3 mm-long segments. Ten seeds/seedling segments were plated on each Petri dish with PDA (containing 200 mg/L of both penicillin and streptomycin), yielding a total of 288 dishes (2 plant species × 2 dormant status × 3 inoculation treatments × 4 isolation origins (i.e. dead and/or living seeds/seedlings used for isolation) × 2 sampling dates × 3 replicates). All dishes were sealed with Parafilm and placed in a growth chamber for 10 days in the dark at 20 °C. The number of seeds and/or seedling segments colonized by fungi was recorded from the third day after incubation. The proportion of each type of fungus isolated was determined at the end of the evaluation period. *Fusarium tricinctum* and other isolates were identified by colony characteristics and also by the examination of spores using a compound microscope.

Calculations and statistical analysis

Proportion of seeds falling into each category was calculated as the number of seeds in a particular category divided by the total number of buried seeds. Proportion of seedlings falling into each category was calculated as the number of seedlings in a particular category divided by the number of germinated seeds. The isolation frequency of *F. tricinctum* was calculated as the number of seeds or seedling segments that had *F. tricinctum* divided by the total number of seeds or seedling segments incubated. Each species was analyzed separately, as it was expected to vary in its intrinsic germination biology. The proportions of seeds/seedlings falling into each category, and isolation frequency of *Fusarium tricinctum* were analyzed with factorial ANOVAs in a generalized linear model. The differences of individual comparisons were tested using Tukey's tests or independent *t*-tests at $p < 0.05$. Prior to data analyses, all data were checked for normality and homogeneity. Proportional data were arcsine square-root transformed to meet the assumptions for parametric testing (Kirk 1982) and converted to percentage values for data presentation. Means are reported with their standard errors. All data analyses were performed using GenStat version17.1 (VSN International Ltd., UK).

Results

Seed germination and viability

The germination percentage of scarified seeds of *L. davurica* and one-year-old seeds of *S. bungeana* was significantly higher than that of the nonscarified and newly-harvested seeds of each species (Table 1). Initial seed viability did not differ significantly between

Table 1 Percentages of initial germination and viability of seed lots of *Lespedeza davurica* and *Stipa bungeana* used in the experiments

Plant species	Seed lot	Germination (%)	Viability (%)
L. davurica	Scarified	95.0 ± 4.2***	100
	Nonscarified	6.0 ± 2.8	100
S. bungeana	One-year old	78.5 ± 4.4***	83.9 ± 3.1
	Newly harvested	7.5 ± 1.9	86.5 ± 1.9

Data are represented as the mean of four replicates with standard errors. *** indicates significant differences between seed lots of each species at $p < 0.001$ (independent samples *t*-tests)

the scarified and nonscarified seeds for *L. davurica* nor between the one-year-old and newly-harvested seeds for *S. bungeana* (Table 1).

Laboratory-based inoculation trial

In the in vitro study, the nondormant seeds continued to germinate, and the radicles extended along the surface of the water agar upon which seeds had been placed; the dormant seeds did not germinate (Fig. 1, Table 2). The pink mycelium of *F. tricinctum* accumulated prolifically on the exterior of nondormant and dormant seeds (Fig. 1c–h), and formed a white tuft of mycelium on the developing radicles of germinating seeds (Fig. 1c and g). Subsequent observation using a stereo dissecting microscope showed that the radicles of all nondormant seeds exposed to *F. tricinctum* were stunted and rotten (Table 2). By contrast, the dormant seeds remained intact (firm to the touch) after exposure to *F. tricinctum*, although they were covered by the pink mycelium of *F. tricinctum* (Fig. 1d and h). Subsequent viability testing showed that the dormant seeds were viable (Table 2).

Field-based inoculation trial

The percentage of germination of scarified seeds of *L. davurica* and one-year-old seeds of *S. bungeana* was significantly higher than that of the nonscarified and newly-harvested seeds of each species, respectively (Fig. 2a and b, Table 3). In contrast, a higher percentage of nonscarified than scarified seeds of *L. davurica* and newly harvested than one-year-old seeds of *S. bungeana* remained intact in the field (Fig. 2c and d, Table 3). The percentage of field germination of both species was enhanced significantly by the length of burial time (Fig. 2a and b, Table 3). The percentage of seeds found dead/missing in the

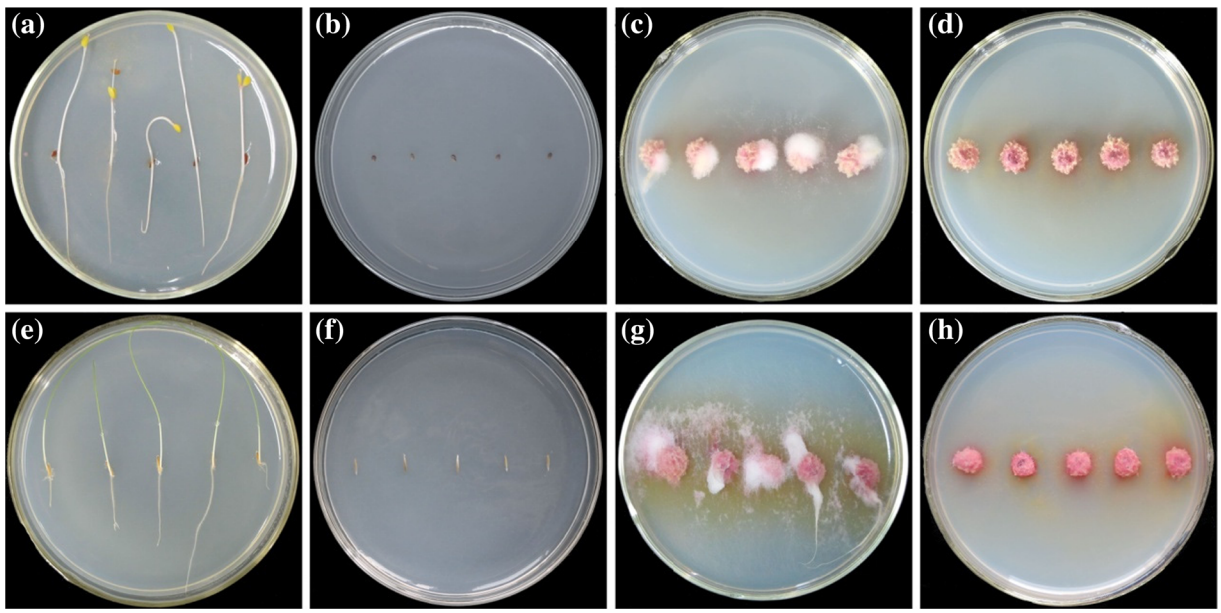

Fig. 1 Effects of inoculation with *Fusarium tricinctum* on dormant and nondormant seeds of *Lespedeza davurica* (**a**, **b**, **c**, and **d**) and *Stipa bungeana* (**e**, **f**, **g**, and **h**) cultured on water agar at 20 °C for one week. **a** and (**e**) refer to nondormant seeds without inoculation; (**b**) and (**f**) refer to dormant seeds without inoculation; (**c**) and (**g**) refer to nondormant seeds inoculated with *F. tricinctum*; (**d**) and (**h**) refer to dormant seeds inoculated with *F. tricinctum*

Table 2 Effects of inoculation with *Fusarium tricinctum* or without inoculation (control) on seed germination, seed/seedling mortality, and seed viability of dormant and nondormant seeds of *Lespedeza davurica* and *Stipa bungeana* that were cultured on water agar at 20 °C for one week

Plant species	Dormancy status	Treatment	Seed germination (%)	Seed/seedling mortality (%)	Seed viability (%)
L. davurica	Nondormant	Control	100	0	–
		Inoculation	100	100	–
	Dormant	Control	0	0	100
		Inoculation	0	0	100
S. bungeana	Nondormant	Control	100	0	–
		Inoculation	100	100	–
	Dormant	Control	0	0	100
		Inoculation	0	0	100

Data are represented as the mean value of four replicates

"-" indicates that all seeds had germinated

field was not affected by the status of dormancy, the treatment of inoculation and burial time (Fig. 2e–h, Table 3). The viability of intact seeds of *S. bungeana* ranged from 78% to 84%, and that of *L. davurica* ranged from 95% to 100%, but it did not differ significantly between the status of dormancy, inoculation treatment, or burial time (Table 3).

The percentage of DBE of both species in the soil inoculated with *F. tricinctum* was significantly higher than that in the two control treatments but did not vary with the length of burial time and dormancy status (Fig. 3a and b, Table 4). In contrast, inoculation with *F. tricinctum* caused a lower percentage of LRS for both species, as compared with the two controls (Fig. 3c and d, Table 4). There was an interactive effect of dormancy status and burial time on percentage of LRS for *L. davurica* (Table 4). Nonscarified seeds of *L. davurica* buried for 8 weeks in the field had a lower percentage of LRS than those buried for 4 weeks (Fig. 3c). For *S. bungeana*, the newly harvested seeds had a higher percentage of LRS than the one-year-old seeds (Fig. 3d, Table 4). Scarified seeds of *L. davurica* and one-year-old seeds of *S. bungeana* exposed to *F. tricinctum* had a lower percentage of LES than the two controls (Fig. 3e and f, Table 4). There was an interactive effect of burial time and dormancy status on LES for *L. davurica* (Table 4). The nonscarified seeds of *L. davurica* buried for 8 weeks had a higher percentage of LES than those buried for 4 weeks (Fig. 3e, Table 4). For *S. bungeana*, the one-year-old seeds had a higher percentage of LES than the newly harvested seeds (Fig. 3f, Table 4). No seedlings were found dead after they successfully emerged from the soil surface in any treatment during the period of field burial.

Isolation of fusarium tricinctum

In total, 8 and 6 fungal species, including *F. tricinctum*, were isolated from dead/living seeds and/or seedlings of *L. davurica* and *S. bungeana*, respectively (data were not shown). Overall, seeds/seedlings of both species exposed to the *F. tricinctum* inoculation had a higher isolation frequency of *F. tricinctum*, as compared to the two controls (Figs. 4 and 5, Table 5). There was an interactive effect of origin of isolation and inoculation treatment on the isolation frequency of *F. tricinctum* (Table 5). The frequency of isolation of *F. tricinctum* from dead seedlings was commonly highest among all isolation origins for the two species (Figs. 4 and 5), except for the scarified seeds of *L. davurica* in which the highest frequency of *F. tricinctum* occurred in dead seeds (Fig. 4b and d). The conidia of *F. tricinctum* that formed in colonies growing on PDA were consistent in shape and size with those of FT-LDR04.

Discussion

In this study, we performed both laboratory- and field-based trials to test the hypothesis that nondormant germinating seeds are more likely to be killed by the pathogenic fungus *F. tricinctum* than dormant seeds. The hypothesis was well supported by the findings from

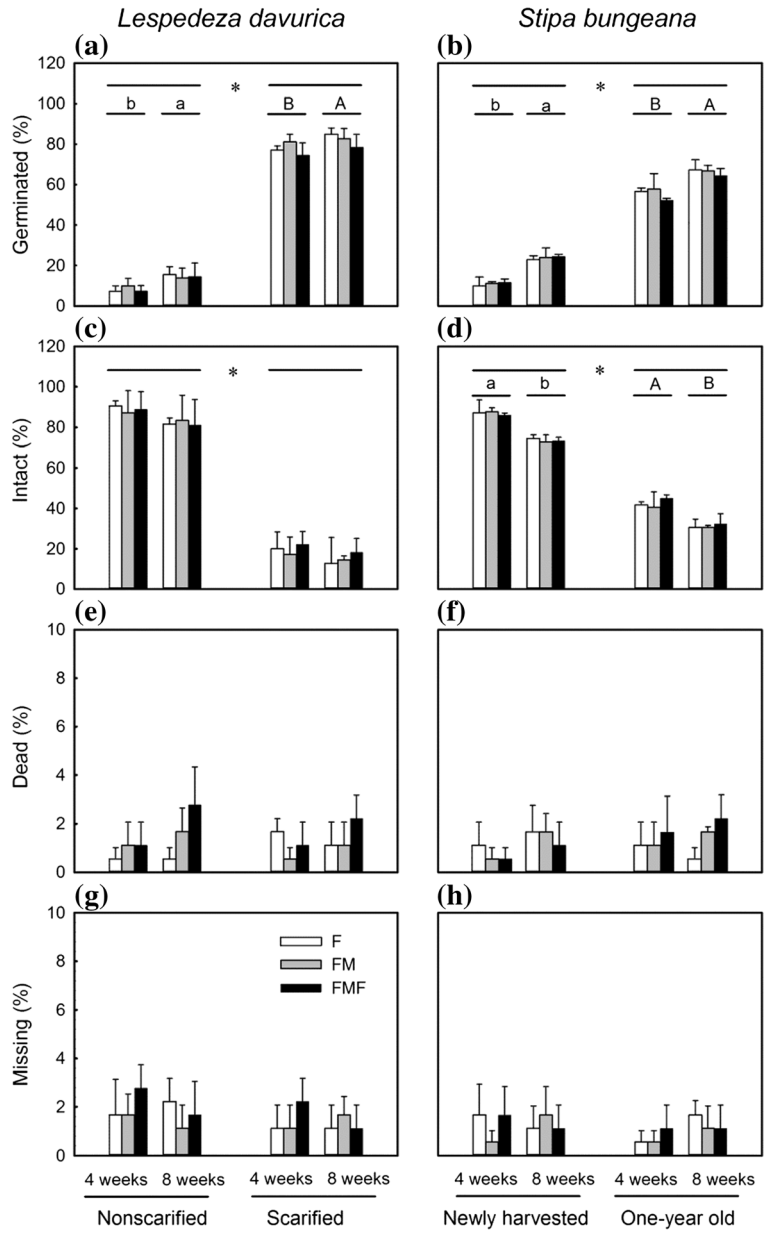

Fig. 2 Percentages of *Lespedeza davurica* (**a**, **c**, **e**, and **g**) and *Stipa bungeana* (**b**, **d**, **f**, and **h**) seeds found germinated, intact, dead, and missing after exposure to different soil treatments; fumigated soil alone (F), fumigated soil + millet (FM), and fumigated soil + millet + *Fusarium tricinctum* inoculum (FMF) buried in the field for 4 and 8 weeks. Dormant seeds of *L. davurica* (nonscarified) and of *S. bungeana* (newly harvested) and nondormant seeds of *L. davurica* (scarified) and of *S. bungeana* (one-year old) were used in the burial experiment. Data are represented as the mean values (n = 3) and the bars indicate standard errors. Within each panel, different lowercase and uppercase letters indicate significant differences between the lengths of burial time for dormant and nondormant seeds, respectively, and * indicates significant differences between the status of dormancy (dormancy vs nondormancy) at $p < 0.05$ (independent samples *t*-tests)

the laboratory experiment. In the in vitro trial, dormant seeds of both plant species remained intact after exposure to *F. tricinctum*, although they were covered by the pink mycelium of *F. tricinctum* (Fig. 1d and h). By contrast, nondormant germinating seeds were quickly colonized by the pink mycelium of *F. tricinctum*, and a white tuft of mycelium accumulated on the developing radicles (Fig. 1c and g). The radicles from germinating seeds were stunted and rotten following exposure to *F. tricinctum*, suggesting that germination results in seeds being killed by fungal pathogens. In contrast, the field-based trial did not support the hypothesis since the inoculation of soil with *F. tricinctum* did not result in higher mortality of nondormant than dormant seeds (Fig. 2e and f). However, we found that the inoculation with *F. tricinctum* resulted in higher percentages of seedling death before they emerged from the soil as compared to the controls (Fig. 3a and b). These findings suggest that in the field *F. tricinctum* would be unlikely to cause seeds to die at the early stages of germinating

Table 3 Results of three-way ANOVA performed individually for seeds of *Lespedeza davurica* and *Stipa bungeana* found germinated (GS), dead (DS), intact (IS), and missing (MS) during the field burial, and viability (VS) of intact seeds exhumed following subsequent testing in the laboratory

Factor	df	GS†	IS†	DS†	MS†	VS‡
L. davurica						
Duration of Burial (DB)	1	4.65*	3.88	1.19	0.39	0.67
Status of dormancy (SD)	1	453.74***	312.47***	0.15	0.82	2.96
Treatment of inoculation (IT)	2	0.62	0.21	0.95	0.36	0.28
DB × SD	1	0.25	0.16	0.05	0.11	0.40
DB × IT	2	0.33	0.30	1.03	1.36	0.16
SD × IT	2	0.22	0.28	1.75	0.29	0.89
DB × SD × IT	2	0.01	0.05	0.12	0.80	1.15
S. bungeana						
Duration of Burial (DB)	1	98.16***	96.16***	2.85	0.40	0.07
Status of dormancy (SD)	1	871.04***	987.39***	0.38	0.11	1.22
Treatment of inoculation (IT)	2	0.43	0.18	0.10	0.37	0.80
DB × SD	1	3.15	3.64	1.03	0.40	0.27
DB × IT	2	0.13	0.01	0.80	0.26	0.67
SD × IT	2	1.77	0.90	2.04	0.07	1.41
DB × SD × IT	2	0.13	0.34	0.34	0.83	0.51

"Duration of burial" (DB) refers to the length of time (4 or 8 weeks) that seeds were buried in soil; "status of dormancy" (SD) refers to nondormancy (seeds of *L. davurica* were scarified and seeds of *S. bungeana* were one-year old) and dormancy (seeds of *L. davurica* were nonscarified and seeds of *S. bungeana* were newly harvested); "treatment of inoculation" (IT) refers to the type of medium (*Fusarium tricinctum* inoculum + millet + fumigated soil, millet + fumigated soil, or fumigated soil alone) to which seeds were exposed. Data are represented as F-values and the asterisk indicates significant *p*-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

† Data arcsin$\sqrt{x}$ transformed

‡ Data arcsin$\sqrt{x + 0.1}$ transformed before analysis

but can infect seedlings and cause them to die prior to their emergence from soil.

It has been suggested that the impermeable seed coat of physically dormant seeds, as well as the embryo-enclosing structures of physiologically dormant seeds, provide an effective barrier against fungal access to seed resources (Dalling et al. 2011). In our study, seeds of *L. davurica* had strong physical dormancy and newly harvested seeds of *S. bungeana* had high levels of physiological dormancy. For *L. davurica*, it seems that the nonruptured seed coat can effectively prevent the seed being attacked by *F. tricinctum* as the mycelium of *F. tricinctum* can not penetrate the water-impermeable testa. However, the embryo-enclosing structures of *S. bungeana*, such as the palea, lemma and fruit/seed coat are water-permeable, but dormant seeds of this species were not attacked by *F. tricinctum*, suggesting that water-impermeability is not necessary to prevent *F. tricinctum* from attacking the seeds. It has been proposed that hyphal growth is directed by the presence and quantity of specific exudates released during seed germination (Campbell and Medd 2003; Gow 1994), particularly for *Fusarium* species (Nelson 2004). Also, in a previous study investigating the mode of *F. tricinctum* infection on seeds of *B. tectorum*, it is noted that the exudates triggering the fungal attack were released from the floret attachment scar as it represented the most vulnerable location for pathogen attack on rapidly germinating seeds (Franke et al. 2014). In our study, the mycelium of *F. tricinctum* accumulated on both dormant or germinating seeds under benign environmental conditions in the laboratory, but the recognition of seed exudates emanating from germinating seeds allow hyphae to grow towrad the resources of the germinating seeds. Additional studies are needed to determine how seed exudates trigger the pathogen-

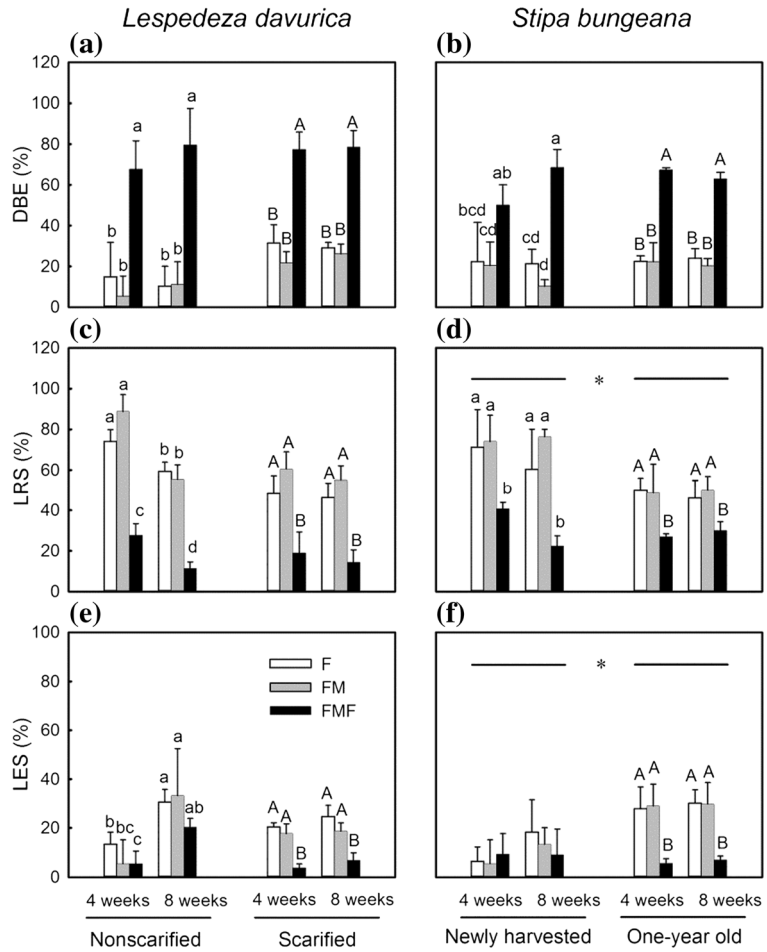

Fig. 3 Percentages of *Lespedeza davurica* (**a**, **c**, and **e**) and *Stipa bungeana* (**b**, **d**, and **f**) seedlings found dead before emerging from soil (DBE), living but remaining in soil (LRS), living and emerging from soil (LES) after exposure to different treatments; fumigated soil alone (F), fumigated soil + millet (F), and fumigated soil + millet + *Fusarium tricinctum* inoculum (FMF) buried in the field for 4 and 8 weeks. Dormant seeds of *L. davurica* (nonscarified) and of *S. bungeana* (newly harvested) and nondormant seeds of *L. davurica* (scarified) and of *S. bungeana* (one-year old) were used in the burial experiment. Data are represented as the mean values ($n = 3$) and the bars indicate standard errors. Within each panel, different lowercase and uppercase letters indicate significant differences among inoculation treatments for dormant and nondormant seeds, respectively at $p < 0.05$ (Tukey's tests). * significant differences between the status of dormancy (dormancy vs nondormancy) at $p < 0.05$ (independent samples *t*-tests)

seed interaction and fungal access to the germinating seeds.

Although our study verified that germinating seeds are more likely to be killed by fungal pathogens than dormant seeds, other mechanisms that explain the pathogen-seed interactions have been reported. For example, Beckstead et al. (2007) hypothesized that fast-germinating seeds would be more likely to escape seed death than slow-germinating seeds by mobilizing seeds resources, which appears to contradict our present hypothesis. This race-for-survival hypothesis was tested for the North American seed pathogen *Pyrenophora semeniperda* on seeds of the annual grass *B. tectorum*, an invasive plant in the western USA. In that study, recently harvested seeds of *B. tectorum* germinated slowly as a result of physiological dormancy and usually were killed by the pathogen *P. semeniperda*, whereas older, after-ripened (nondormant) seeds germinated rapidly and often escaped (Beckstead et al. 2007). The variations between our study and that of Beckstead et al. (2007) may be explained by the use of different host and pathogen species, and differences in the environmental conditions of the two field sites, which are key elements in determining the incidence of a disease (Agrios 1988).

Our laboratory and field inoculation studies indicate that germination increases the risk of seeds/seedlings being attacked by fungal pathogens, but there are several concerns that have to be acknowledged. One major concern relates to the variation in seed mortality in the laboratory and field experiments. The approaches of scarifying seeds of *L. davurica* and using after-ripened seeds of *S. bungeana* and in direct contact with *F. tricinctum* inoculum were optimized to promote the fungal attack on seeds. Also, soil fumigation was expected to maximize the opportunity of pathogen attack as this procedure of sterilization may

Table 4 Results of three-way ANOVA performed individually for *Lespedeza davurica* and *Stipa bungeana* seedlings found dead before emerging from soil (DBE), living but remaining in soil (LRS), living and emerging from soil (LES) following the field burial

Factor	df	*L. davurica*			*S. bungeana*		
		DBE	LRS	LES	DBE	LRS	LES
Duration of Burial (DB)	1	0.63	10.09^{**}	22.56^{***}	0.08	1.70	2.70
Status of dormancy (SD)	1	3.19	2.63	0.35	2.47	18.39^{***}	14.28^{***}
Treatment of inoculation (IT)	2	44.27^{***}	39.32^{***}	9.84^{**}	51.52^{***}	28.95^{***}	5.55^{**}
DB × SD	1	0.37	5.58^{*}	12.57^{**}	0.44	1.85	1.47
DB × IT	2	0.49	1.21	0.69	1.16	0.71	0.53
SD × IT	2	1.74	1.47	4.08^{*}	0.05	2.51	3.55^{*}
DB × SD × IT	2	0.24	0.97	1.48	1.45	0.79	0.64

Data arcsin$\sqrt{x}$ transformed before analysis

"Duration of burial" (DB) refers to the length of time that seeds were buried in soil (4 or 8 weeks); "status of dormancy" (SD) refers to nondormancy (seeds of *L. davurica* were scarified and seeds of *S. bungeana* were one-year old) and dormancy (seeds of *L. davurica* were nonscarified and seeds of *S. bungeana* were newly harvested); "treatment of inoculation" (IT) refers to the type of medium (*Fusarium tricinctum* inoculum + millet + fumigated soil, millet + fumigated soil, or fumigated soil alone) to which seeds were exposed. Data are represented as F-values and the asterisk indicates significant p-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

remove soil microbes that would compete with the inoculated *F. tricinctum* (Dalling et al. 2011). However, in the field, seed mortality of both plant species was very low, regardless of dormancy status and inoculation treatment, suggesting the resiliency of seeds/seedlings in soil.

There are several considerations that could explain the low mortality of seeds in the field trial. Firstly, the

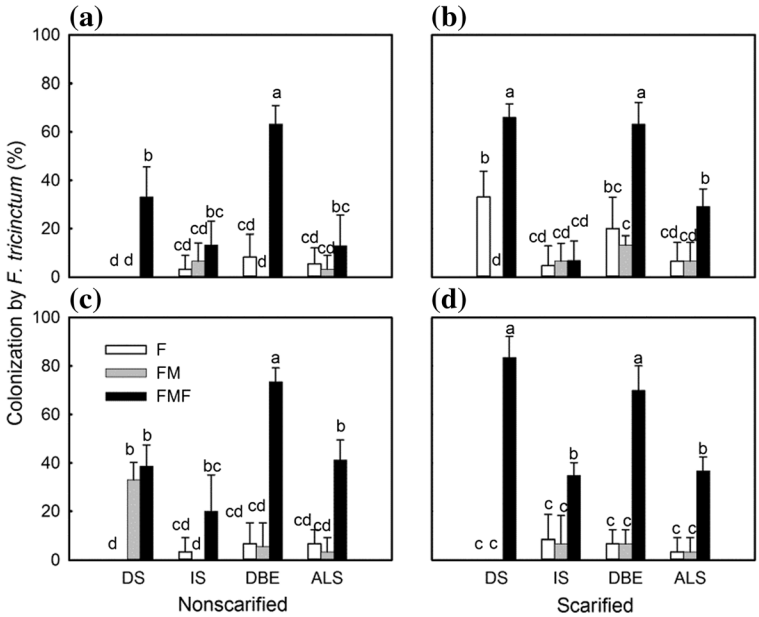

Fig. 4 Percentages of *Fusarium tricinctum* isolated from the dead seeds (DS), intact seeds (IS), dead seedlings before emerging from soil (DBE), all living seedlings (ALS) after nonscarified or scarified seeds of *Lespedeza davurica* were buried in the field for 4 weeks (**a**, **b**) or 8 weeks (**c**, **d**) following exposure to different soil treatments; fumigated soil alone (F), fumigated soil + millet (FM), and fumigated soil + millet + *Furarium tricinctum* inoculum (FMF). Data are represented as the mean values ($n = 3$) and the bars indicate standard errors. Within each panel, different lowercase letters indicate significant differences at $p < 0.05$ (Tukey's tests)

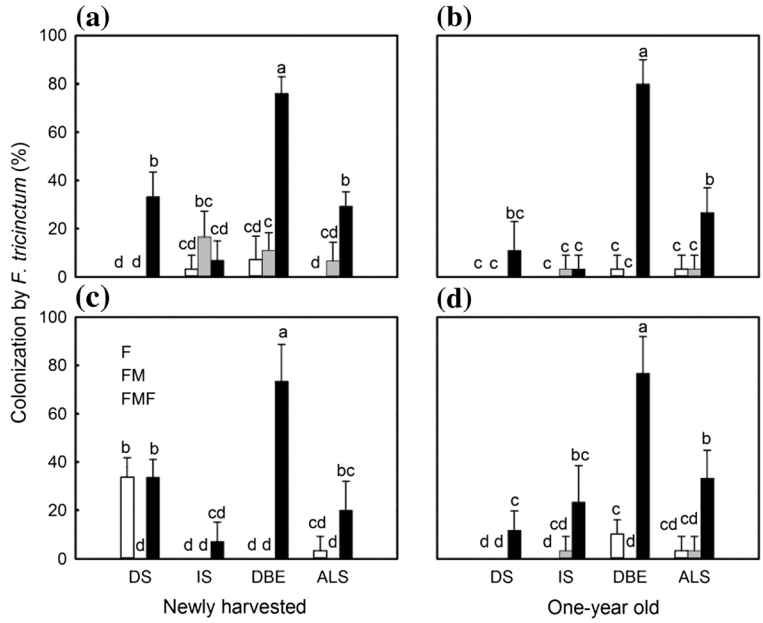

Fig. 5 Percentages of *Fusarium tricinctum* isolated from the dead seeds (DS), intact seeds (IS), dead seedlings before emerging from soil (DBE), all living seedlings (ALS) after newly harvested and one-year old seeds of *Stipa bungeana* were buried in the field for 4 weeks (**a**, **b**) or 8 weeks (**c**, **d**) following exposure to different soil treatments; fumigated soil alone (F), fumigated soil + millet (FM), and fumigated soil + millet + *Furarium tricinctum* inoculum (FMF). Data are represented as the mean values (n = 3) and the bars indicate standard errors. Within each panel, different lowercase letters indicate significant differences at $p < 0.05$ (Tukey's tests)

density of inoculum is an important factor determining the outcome of pathogen-seed interactions (Keim et al. 2014). In our study, seeds in the soil were likely exposed to a lower density of inoculum than those on water agar in Petri dishes, and hence they were less likely to be attacked by the pathogenic fungus. Secondly, a small fraction of scarified seeds of *L. davurica* and one-year-old seeds of *S. bungeana* in the field trial was still dormant, which might bias the difference in seed/seedling mortality between dormant and nondormant seeds. Thirdly, the number of seeds/replicates in the laboratory-based trial was smaller than that in the field-based trial. Thus, there is a chance that more susceptible seeds were selected for the laboratory than for the field trial. Furthermore, it is possible that interactions of abiotic and biotic conditions in the field that can promote dormancy-break/germination, such as fluctuating temperatures (Footitt et al. 2011), and wet-dry cycling (Hoyle et al. 2008), or other biotic factors (Chee-Sanford et al. 2009) might have confounded the effects observed in the laboratory. In the semiarid grasslands of northwest China, water availability is rare and sporadical (Zeng et al. 2010), and this may greatly suppress the germination of pathogen spores, resulting in the rate of fungal growth in soils being much slower than in Petri dishes.

Another important concern relates to the length of burial time in the field experiment. Our field trial only lasted for two months, which was relatively shorter than many other burial experiments (Dalling et al. 1998; Schafer and Kotanen 2003). Thus, the 2-month period may be insufficient for *F. tricinctum* to be able to kill seeds. In addition, a small proportion of experimental seeds was not retrieved from the soil-containing pots after following field burials, probably due to the error of hand recovering or predation by soil insects that entered the pot from outer environments. Also, as for the pots inoculated with *F. tricinctum*, some germinated seedlings may have decayed following attack by the pathogen, making it impossible for us to find them. However, these did not invalidate our results as the proportion of missing seeds/seedlings did not differ among treatments.

To our knowledge, this is the first study to have performed both laboratory and field inoculation trials

Table 5 Results of multiple-way ANOVA for *Lespedeza davurica* and *Stipa bungeana* seeds/seedlings colonized by *Fusarium tricinctum* after field burial

Factor	df	Frequency of *F. tricinctum*	
		L. davurica	*S. bungeana*
Duration of burial (DB)	1	0.06	0.82
Status of dormancy (SD)	1	3.23	2.25
Origin of isolation (OI)	3	6.27^{**}	7.65^{**}
Treatment of inoculation (IT)	2	65.71^{***}	81.33^{***}
DB × SD	1	2.61	2.35
DB × OI	3	0.14	0.05
DB × IT	2	0.60	3.33
SD × OI	3	1.28	1.26
SD × IT	2	0.75	1.60
OI × IT	6	4.16^{*}	7.75^{**}
DB × SD × OI	3	1.59	0.36
DB × SD × IT	2	0.24	1.66
DB × OI × IT	6	0.87	2.12
SD × OI × IT	6	1.95	2.81
DB × SD × OI × IT	6	0.06	1.65

Data arcsin$\sqrt{x}$ transformed before analysis

"Duration of burial" (DB) refers to the length of time that seeds were buried in soil (4 or 8 weeks); "status of dormancy" (SD) refers to nondormancy (seeds of *L. davurica* were scarified and seeds of *S. bungeana* were one-year old) and dormancy (seeds of *L. davurica* were nonscarified and seeds of *S. bungeana* were newly harvested); "origin of isolation" (OI) refers to the dead/living seeds/seedlings used for the isolation of *F. tricinctum*; "treatment of inoculation" (IT) refers to the type of medium (*Fusarium tricinctum* inoculum + millet + fumigated soil, millet + fumigated soil, or fumigated soil alone) to which seeds were exposed. Data are represented as F-values and the asterisk indicates significant p-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

to investigate the relationship between seed dormancy/germination and pathogen-caused mortality. The laboratory trial showed that germination results in seeds being attacked by the pathogenic fungus *F. tricinctum*. However, in the field *F. tricinctum* would be unlikely to cause seeds to die at the early stages of germinating but can infect seedlings and cause them to die prior to their emergence from soil. Our study strongly indicates that dormancy contributes to a seed's resistance to *F. tricinctum* attack by preventing germination, but the effectiveness of dormancy to protect seeds from pathogens is greatly reduced following the onset of germination. This study provides further insights into our understanding of the interaction between seeds and fungal pathogens in field conditions.

Acknowledgements We wish to thank Craig McGill of Massey University, New Zealand for reading this manuscript. We thank Xiaowen Hu of Lanzhou University for help in the revision of this manuscript, and Chao Xia for help in the making of figures. We also acknowledge the excellent informed comments from the two referees. This research was financially supported by National Basic Research Program of China (2014CB138702), National Public Welfare Industry of Agricultural Science and Technology Special Projects (201303057) and the National Nature Science Foundation of China (31402132).

References

Agrios GN (1988) Plant pathology. Academic press, San Diego

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16

Baughman OW, Meyer SE (2013) Is *Pyrenophora semeniperda* the cause of downy brome (*Bromus tectorum*) die-offs? Invasive Plant Sci Manag 6:105–111

Beckstead J, Meyer SE, Molder CJ, Smith C (2007) A race for survival: can *Bromus tectorum* seeds escape *Pyrenophora semeniperda*-caused mortality by germinating quickly? Ann Bot 99:907–914

Beckstead J, Meyer SE, Connolly BM, Huck MB, Street LE (2010) Cheatgrass facilitates spillover of a seed bank pathogen onto native grass species. J Ecol 98:168–177

Bolingue W, Rosnoblet C, Leprince O, Vu BL, Aubry C, Buitink J (2010) The MtSNF4b subunit of the sucrose non-fermenting-related kinase complex connects after-ripening and constitutive defense responses in seeds of *Medicago truncatula*. Plant J 61:792–803

Booth C (1971) The genus *Fusarium*. Commonwealth Mycological Institute, Kew, England

Brownbridge M, Reay SD, Glare TR, Nelson TL (2012) Persistence of *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. Biol Control 61:194–200

Campbell MA, Medd RW (2003) Leaf, floret and seed infection of wheat by *Pyrenophora semeniperda*. Plant Pathol 52:437–447

Chambers JC, MacMahon JA (1994) A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. Annu Rev Ecol Syst 25:263–292

Chee-Sanford JC, Williams MM II, Davis AS, Sims GK (2009) Do microorganisms influence seed-bank dynamics? Weed Sci 54:575–587

Chen T, Christensen M, Nan Z, Hou F (2017a) Effects of grazing intensity on seed size, germination and fungal colonization of *Lespedeza davurica* in a semi-arid grassland of northwest China. J Arid Environ 144:91–97

Chen T, Christensen M, Nan Z, Hou F (2017b) The effects of different intensities of long-term grazing on the direction and

strength of plant–soil feedback in a semiarid grassland of Northwest China. Plant Soil 413:303–317

Cheng J, Cheng JM, Hu TM (2011) Distribution responses of *Lespedeza davurica* community on loess plateau to climate change. Chin J Appl Ecol 22:35–40

Christensen MJ, Falloon RE, Skipp RA (1988) A petri plate technique for testing pathogenicity of fungi to seedlings and inducing fungal sporulation. Australas Plant Pathol 17:45–47

Christensen L, Coughenour MB, Ellis JE, Chen ZZ (2004) Vulnerability of the Asian typical steppe to grazing and climate change. Clim Chang 63:351–368

Dalgleish HJ, Koons DN, Adler PB (2010) Can life-history traits predict the response of forb populations to changes in climate variability? J Ecol 98:209–217

Dalling JW, Swaine M, Garwood NC (1998) Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forest. Ecology 79:564–578

Dalling JW, Davis AS, Schutte BJ, Elizabeth Arnold A (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. J Ecol 99:89–95

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005) Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. Evolution 59:740–757

Footitt S, Douterelo Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. Proc Natl Acad Sci U S A 108:20236–20241

Forget PM (2007) Is temporal variation of seedling communities determined by environment or by seed arrival? A test in a neotropical forest. J Ecol 95:507–516

Francisco L, Josep P, Marc E (2004) Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Glob Chang Biol 10:248–258

Franke JL, Geary B, Meyer SE (2014) Identification of the infection route of a *Fusarium* seed pathogen into nondormant *Bromus tectorum* seeds. Phytopathology 104:1306–1313

Fuerst EP, Anderson JV, Kennedy AC, Gallagher RS (2011) Induction of polyphenol oxidase activity in dormant wild oat (*Avena fatua*) seeds and caryopses: a defense response to seed decay fungi. Weed Sci 59:137–144

Gallandt ER, Fuerst EP, Kennedy AC (2004) Effect of tillage, fungicide seed treatment, and soil fumigation on seed bank dynamics of wild oat (*Avena fatua*). Weed Sci 52:597–604

Gómez R, Liebman M, Munkvold G (2013) Weed seed decay in conventional and diversified cropping systems. Weed Res 54: 13–25

Gómez-Aparicio L, Ibánez B, Serrano MS, De Vita P, Avila JM, Pérez-Ramos IM, García LV, Esperanza Sánchez M, Maranón T (2012) Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. New Phytol 194:1014–1024

Gow NA (1994) Growth and guidance of the fungal hypha. Microbiology 140:3193–3205

Hoyle GL, Steadman KJ, Daws MI, Adkins SW (2008) Pre- and post-harvest influences on seed dormancy status of an Australian Goodeniaceae species, *Goodenia fasciularis*. Ann Bot 102:93–101

Hu XW, Zhou ZQ, Li TS, Wu YP, Wang YR (2013) Environmental factors controlling seed germination and seedling recruitment of *Stipa bungeana* on the loess plateau of northwestern China. Ecol Res 28:801–809

Hu XW, Wu YP, Ding XY, Zhang R, Wang YR, Baskin JM, Baskin CC (2014) Seed dormancy, seedling establishment and dynamics of the soil seed bank of *Stipa bungeana* (Poaceae) on the loess plateau of northwestern China. PLoS One 9:e112579–e112579

Jansen PI, Ison RL (1995) Factors contributing to the loss of seed from the seed-bank of *Trifolium balansae* and *Trifolium resupinatum* over summer. Aust J Ecol 20:248–256

Keim J, Mishra B, Sharma R, Ploch S, Thines M (2014) Root-associated fungi of *Arabidopsis thaliana* and *Microthlaspi perfoliatum*. Fungal Divers 66:99–111

Kirk RE (1982) Experimental design: procedures for the behavioral sciences, 2nd edn. Brooks and Cole Publishing, Monterrey, p 500

Meyer SE, Franke JL, Baughman OW, Beckstead J, Geary B (2014) Does *Fusarium*-caused seed mortality contribute to *Bromus tectorum* stand failure in the Great Basin? Weed Res 54:511–519

Nan Z (1995) Fungicide seed treatments of sainfoin control seed-borne and root-invading fungi. N Z J Agric Res 38:413–420

Nelson EB (2004) Microbial dynamics and interactions in the spermosphere. Annu Rev Phytopathol 42:211–224

Poisot T, Bever JD, Nemri A, Thrall PH, Hochberg ME (2011) A conceptual framework for the evolution of ecological specialisation. Ecol Lett 14:841–851

Schafer M, Kotanen PM (2003) The influence of soil moisture on losses of buried seeds to fungi. Acta Oecol 24:255–263

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Casas RR (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytol 203:300–309

Zeng YJ, Wang YR, Zhang JM (2010) Is reduced seed germination due to water limitation a special survival strategy used by xerophytes in arid dunes? J Arid Environ 74:508–511