

Cheatgrass facilitates spillover of a seed bank pathogen onto native grass species

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Summary

1. Pathogen spillover occurs when disease levels for a given population are driven by transmission from a reservoir species that carries a high pathogen load. Pathogen spillover is widely documented in crop systems, but has been little studied in natural plant communities.

2. The abundant seed production of weedy species may create a scenario where spillover of a generalist seed pathogen onto less abundant seeds of native hosts is possible. The invasive annual weed cheatgrass (*Bromus tectorum*) is a potential reservoir species for *Pyrenophora semeniperda*, a multiple-host fungal seed pathogen that naturally occurs in the semi-arid western United States.

3. To investigate potential community-level consequences of spillover by this pathogen in plant communities invaded by cheatgrass, we first used artificial inoculation studies to determine the relative susceptibility of seeds of cheatgrass and five co-occurring native grasses to *P. semeniperda*. Secondly, we quantified the pathogen reservoir (density of pathogen-killed seeds) in the spring seed bank for cheatgrass monoculture, cheatgrass-invaded native grass, and uninvaded native grass patches. Thirdly, potential pathogen spillover onto co-occurring native grasses was quantified by planting native grass seeds into field-collected seed-zone samples from each vegetation patch type and scoring subsequent seed mortality.

4. All species tested were susceptible to infection by *P. semeniperda*, but their vulnerability to seed death varied as a function of germination time and degree of susceptibility.

5. Seed bank samples from cheatgrass-dominated patches contained seed densities over four times higher than samples from uninvaded native grass patches, and *P. semeniperda*-killed seeds were also present at much higher densities, indicating that cheatgrass can function as a reservoir for *P. semeniperda*. Native seeds planted into seed-zone samples from cheatgrass-dominated patches were more likely to be killed by *P. semeniperda* than those planted into samples from uninvaded native patches. Seed mortality also varied across years, sites and host species.

6. *Synthesis*. Pathogen spillover onto native seeds is likely to operate within seed banks of semi-arid communities invaded by cheatgrass, and perhaps other weeds, and may have broad consequences for community structure. Our findings also demonstrate the ecological significance of multiple-host pathosystems that operate at the seed stage.

Key-words: apparent competition, *Bromus tectorum*, *Drechslera campanulata*, multiple-host pathogen, pathogen spillover, *Pyrenophora semeniperda*, seed pathogen

Introduction

Attack by pathogens can have ecological consequences for plants at many scales, such as the individual, population and community scale, although the latter is the least studied. Community-level consequences of disease in natural plant communities can drive facilitation in succession (Van der Putten, Van

Dijk & Peters 1993), maintain species diversity in tropical rain forest (i.e. Janzen–Connell hypothesis; Clark & Clark 1984) and promote exotic invasion via negative feedback loops (Callaway *et al.* 2004). The pathogens involved in the above community-level interactions are often restricted to a narrow host range and target the seedling or reproductive stage. Although these studies are useful in providing an understanding of the consequences of disease in natural plant communities, they do not address the dynamic and complex consequences of disease

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caused by multiple-host pathogens, nor do they address pathogens that target the critical seed stage.

Historically, disease theory has focused on pathogens that are restricted to single hosts (Grenfell & Dobson 1995), but today biologists realize that multiple-host pathogens are important players in disease epidemics that can lead to novel dynamics and consequences (Dobson 2004; Bradley, Gilbert & Martiny 2008). One potential consequence of disease caused by multiple-host pathogens is the 'spillover effect' or 'pathogen spillover' (Power & Mitchell 2004). Pathogen spillover occurs when disease levels for a population of a given species are driven by transmission from a reservoir species that carries a high pathogen load. Even though both species are susceptible hosts, one host species is able to support high pathogen loads and thereby cause indirect disease-mediated consequences for the co-occurring host species. Spillover effects have been documented to occur between domesticated and wild animals (e.g. canine distemper in African wild dogs and domesticated dogs; Daszak & Cunningham 2000) and between crops and wild plants (e.g. maize dwarf mosaic virus from johnsongrass to corn; King & Hagood 2003). These effects are largely unexplored in natural plant communities (for exceptions, see Power & Mitchell 2004; Malmstrom *et al.* 2005). Pathogen spillover can result in 'apparent competition' between shared hosts, where the negative impact of the reservoir species, mediated indirectly through the shared pathogen, can make it appear as if direct competition were taking place (Power & Mitchell 2004).

Little is known about seed pathogens in natural communities, even though these pathogens are assumed to be key contributors to disease-related mortality in plant populations (Chambers & MacMahon 1994; Gilbert 2002). Studies have documented that seed mortality from pathogens increased following consumption by bird frugivores (Vander Kloet & Crossland 1996) but decreased following consumption by monkeys (Lambert 2001). More generally, seed pathogens can greatly reduce the seed bank in a range of ecosystems (Crist & Friese 1993; Dalling, Swaine & Garwood 1998; Schafer & Kotanen 2004).

Consequences of seed pathogen spillovers are well understood in crop settings. For example, a group of weedy species acted as an inoculum reservoir for a fungal seed pathogen that reduced germination of soybeans (Mengistu & Reddy 2005). Several cropping practices have been implemented to reduce pathogen spillover; these include rotation to a non-host crop (Wisler & Norris 2005) and selective biological controls that are plant growth promoters that protect the crop, but not the alternative host, from the pathogen (Larkin, Hopkins & Martin 1996; Mazzola *et al.* 2002).

Given the wide range of studies that document spillover of seed pathogens in agricultural settings, coupled with the importance of pathogens in mediating seed bank size for many species in natural communities, it seems likely that this complex interaction not only exists in natural communities but also is important to community dynamics. If a seed pathogen in a natural system causes high levels of disease in the seed bank of a preferred or abundant host, resulting in high pathogen inocu-

lum loads, then could this preferred or abundant host act as a reservoir and cause high levels of disease on the seeds of less preferred or less abundant co-occurring alternative hosts? To address this question, we studied a multiple-host pathosystem that is common in shrub-steppe and semi-arid grassland communities in western North America. The players in this interaction include the fungal seed pathogen *Pyrenophora semeniperda* (anamorph *Drechslera campanulata*), the invasive winter annual cheatgrass (*Bromus tectorum*), and several co-occurring native perennial grasses (*Achnatherum hymenoides*, *Elymus elymoides*, *Hesperostipa comata*, *Poa secunda* and *Pseudoroegneria spicata*). This natural system is ideal for the exploration of seed pathogen spillover interactions. *Pyrenophora semeniperda* is a generalist that infects multiple hosts, primarily grass species (Medd 1992), and that targets the seed stage (Beckstead *et al.* 2007).

Cheatgrass is an important host for *P. semeniperda* (Beckstead *et al.* 2007; Meyer *et al.* 2007) and, as an annual plant, it is dependent on seeds for population maintenance. It is a prolific seed producer (Smith, Meyer & Anderson 2008), and this high seed production, on the order of tens of thousands of seeds $\text{m}^{-2} \text{year}^{-1}$, results in high densities of seeds in the soil. Once seeds are dispersed, they can either germinate or carry over to the subsequent year as a persistent seed bank. The seeds are dormant when dispersed in early summer, lose dormancy through dry after-ripening over the summer, and are poised to germinate quickly in response to autumn rains. The pathogen has limited ability to kill these rapidly germinating seeds. The process of infection and disease development is essentially a race, such that, a rapidly germinating seed can escape disease and sometimes infection (Beckstead *et al.* 2007). Autumn rains are, however, often insufficient to trigger complete germination, especially in drier environments, and most of the seeds that do not germinate in the autumn re-enter dormancy under winter conditions. It is these slow-germinating, dormant carry-over seeds in the spring seed bank that are the primary target of *P. semeniperda*. Meyer *et al.* (2007) found densities of field-killed *B. tectorum* seeds with *P. semeniperda* stromata to range from 5000 to 20 000 seeds m^{-2} at cold desert sites in Utah and Idaho, USA. *Pyrenophora semeniperda* can also be dispersed with seeds of its host (Meyer *et al.* 2008), potentially allowing this pathogen to disperse over long distances along with the seeds.

Cheatgrass is a highly invasive species, which has succeeded in dominating tens of millions of hectares in drier parts of the Intermountain West (Billings 1991). The co-occurring perennial grass species in these invaded communities have not only had to deal with direct competition from cheatgrass but also most certainly with the seed pathogen *P. semeniperda*. The impressive invasion by cheatgrass, the documentation of high levels of *P. semeniperda* in cheatgrass seed banks (i.e. a potential reservoir), the ability of *P. semeniperda* to disperse over long distances with its cheatgrass host and thus encounter co-occurring hosts, and the wide host range of *P. semeniperda* all indicate that pathogen spillover from a cheatgrass reservoir to co-occurring native species is a real possibility. We predict that cheatgrass seed banks will be larger and will support

higher levels of *P. semeniperda* than native grass seed banks, and that native seeds dispersed into cheatgrass-invaded soils will sustain greater pathogen-caused mortality than seeds dispersed into uninvaded native soils. Given that not all hosts are equal, we predict that native species whose seeds germinate more slowly will fall prey to the pathogen more frequently than species whose seeds germinate more quickly.

We investigated these predictions for two semi-arid plant communities invaded by cheatgrass in western North America. First, using an artificial inoculation study in the laboratory, we determined the relative degree to which the seeds of cheatgrass and five co-occurring native grasses were susceptible to *P. semeniperda* infection and likely to experience pathogen-caused mortality in the field. Additionally, we investigated whether patterns of susceptibility and seed mortality were influenced by mean germination time (measured as days to 50% germination). Secondly, we quantified the pathogen reservoir (density of pathogen-killed seeds) in the spring (carryover) seed bank for cheatgrass-monoculture patches, cheatgrass-invaded native grass patches and uninvaded native grass patches, including evaluation of the *in situ* seed bank for each species. Thirdly, potential pathogen spillover onto co-occurring native grasses was quantified by planting native grass seeds into field-collected samples of the seed zone (i.e. including intact litter and underlying surface soil) from each vegetation patch type and scoring subsequent seed mortality.

Materials and methods

DETERMINING SUSCEPTIBILITY OF POTENTIAL CO-OCCURRING HOSTS

Seeds of five native grass species that co-occur with *B. tectorum* at the study sites were artificially inoculated with *P. semeniperda* in the laboratory, along with dormant and non-dormant seeds of *B. tectorum*. The native species, previously mentioned, included: *A. hymenoides* (Indian ricegrass), *E. elymoides* (bottlebrush squirreltail), *H. comata* (needle and thread grass), *P. secunda* (Sandberg bluegrass) and *P. spicata* (bluebunch wheatgrass). All seed lots were collected from populations in the Intermountain West and were highly (> 95%) viable. Seeds were stored at room temperature to encourage dormancy loss prior to initiation of the experiment, with the exception of dormant *B. tectorum* seeds, which were stored at -18°C to retain dormancy.

We used inoculum obtained from two *P. semeniperda* isolates, one each from Whiterocks, Tooele County, Utah, and Tenmile Creek, Box Elder County, Utah. We obtained conidial inoculum by first culturing surface-sterilized stromata from killed seeds found in cheatgrass seed bank samples from each site on V8 agar (Beckstead *et al.* 2007). Then we transferred stromata produced on V8 agar to MAM (modified alphacell medium) agar, which promotes direct production of conidia on the mycelial surface (Campbell, Medd & Brown 1996). Conidia were harvested by rinsing the plates with sterile water, filtering the conidial suspension through a 25- μm sieve and air-drying the filtrate. To inoculate seeds of each species, two replications of 50 seeds were placed in 4-mL glass vials with an amount of dry conidial inoculum sufficient to saturate the seed surface (all species received 0.0025 g of inoculum, except for *P. secunda* seeds, which received 0.001 g of inoculum due to reduced weight). It should be noted that these experiments were conducted using very high pathogen inoculum

loads; field inoculum loads would be expected to be orders of magnitude lower. To ensure complete coverage, vials were vibrated for 1 min with a modified sander. Inoculated seeds were then placed in plastic Petri dishes (100 $\times$ 15 mm) on two germination blotters (Anchor Paper, St Paul, MN, USA) saturated with water. Petri dishes were randomly stacked in plastic bags closed with rubber bands to retard water loss and incubated for 6 weeks at 20°C under 12-h diurnal photoperiod for the first 2 weeks followed by no lights for the remaining 4 weeks. Dishes were rewetted as needed. Infection (as indicated by the presence of fungal stromata) and seed mortality (as indicated by the presence of fungal stromata on ungerminated seeds) were measured weekly and compared with uninoculated controls (two replications of 50 seeds). Germination (radicle > 1 mm) was measured at 2, 4, 7, 11, 14, 21, 28 and 42 days. To aid in interpretation, mean germination time (days to 50% germination) was calculated. Shorter mean germination times result in faster rates of germination. Germinated seeds were retained for the full 42 days in the dishes with their coleoptiles clipped to evaluate infection levels on germinated seeds, as evidenced by the appearance of pathogen stromata. On day 42, the remaining ungerminated seeds were examined for stromata of *P. semeniperda* and scored as viable and dormant (seed viability determined by cut test; Ooi, Aulk & Whelan 2004), killed by the pathogen, or non-viable (initially unfilled/non-viable or killed during incubation by a different pathogen).

The effect of species and inoculum treatment on the proportion of seeds infected and the proportion of seeds killed was analysed using analysis of variance (ANOVA) for completely randomized designs (PROC GLM; SAS 2000). The two inoculum sources did not vary significantly, thus inoculum was pooled for data analysis (i.e. total of four replicates of 50 seeds each). The response variables (proportions) were arcsine square-root transformed to improve homogeneity of variance prior to analysis and predicted model residuals were checked for normal distributions. The Duncan multiple-range test ($P < 0.05$) was used for means separations.

QUANTIFYING PATHOGEN RESERVOIRS AND *IN SITU* SEED BANKS

Seed bank samples were obtained from cheatgrass-dominated and native grass-dominated vegetation patch types at two semi-arid sites in North America. At the Davis Mountain site in western Utah (located on USDA Bureau of Land Management land 14 km south of English Village, Dugway Army Proving Grounds; $40^{\circ}7' \text{N}$, $112^{\circ}40' \text{W}$; 1550 m a.s.l. elevation), samples were collected in 2006 and 2007 from an invaded shadscale (*Atriplex confertifolia*)-bunchgrass community. Sampled patch types in this community included cheatgrass monocultures, cheatgrass-invaded native grass patches and uninvaded native grass patches. The native grass patches contained both *P. secunda* and *E. elymoides*. At the Saddle Mountain study site, located on Hanford Reach National Monument in central Washington (USDI Fish and Wildlife Service; $46^{\circ}47' \text{N}$, $119^{\circ}27' \text{W}$; 582 m a.s.l. elevation), samples were collected only in 2007 from an invaded bunchgrass community. Sampled patch types in this community included cheatgrass-monoculture patches and uninvaded *A. hymenoides*, *H. comata* and *P. spicata* patches.

To measure carryover seed banks and disease incidence in native grass and *B. tectorum* patches, we collected all seed bank samples in late spring (May), after germination was complete but prior to dispersal of current-year seed. For each vegetation patch type, 20 vegetation patches were randomly selected and one randomly located soil seed bank sample was collected. Samples were taken by inverting a steel can (6 cm in diameter and 4 cm deep), pressing it into the soil,

and then using a trowel to lift the can with its soil core intact. Samples were usually dry at collection; moist samples were allowed to air-dry before processing. The samples were screened to remove loose soil, then processed by hand within 2 weeks of collection to remove, identify, and quantify apparently viable (intact) seeds and also any field-killed seeds with protruding stomata of *P. semeniperda*. Apparently viable seeds were then incubated for 4 weeks at 20 °C using the protocols described above to determine the number of seeds that were viable or killed by the pathogen, as determined by germination or positive post-incubation viability evaluation or the appearance of stomata on ungerminated seeds, respectively. Seeds exhibiting pathogen stomata within the first 7 days of incubation were considered to have been killed in the field; those exhibiting stomata later in the incubation period were counted as viable when collected, as infection probably took place during incubation. Conidia of this fungus can reside on the seed coat and infect seeds when placed in conditions conducive for germination (Meyer *et al.* 2008).

The effects of patch type (both sites) and year (western Utah site only) on total seed density (viable plus pathogen-killed seeds) and on density of pathogen-killed seeds were analysed using ANOVA for a completely randomized design (PROC ANOVA; SAS 2000). This analysis was performed by study site for each species and also for all species pooled. The response variables (count data) were square-root transformed to improve homogeneity of variance prior to analysis.

QUANTIFYING INOCULUM LOADS AND POTENTIAL PATHOGEN SPILLOVER

To assess potential pathogen spillover onto co-occurring native species and to quantify *P. semeniperda* inoculum loads in the soil seed banks of different patch types, we set up experiments using seed-zone samples from the two study sites where seed bank sampling took place. The experiments differed slightly in design due to the availability of different patch types at the two sites. At the Davis Mountain site in western Utah, dormant seeds of cheatgrass and seeds of the two native grass species (*P. secunda* and *E. elymoides*) were planted into seed-zone samples from each of three vegetation patch types (cheatgrass monoculture (> 95% cover of cheatgrass), cheatgrass-invaded native grass patches (40–70% cover of cheatgrass) and uninvaded native grass patches (< 1% cover of cheatgrass)). At the Saddle Mountain site in central Washington, dormant seeds of cheatgrass were planted into seed-zone samples from each of the three native grass (*A. hymenoides*, *H. comata* and *P. spicata*) patch types and from the cheatgrass-monoculture patch type. Each of the three native grass species was planted only into seed-zone samples from its own patch type and from the cheatgrass-monoculture patch type. Seeds for the experiments were field-collected from wild populations in the Intermountain West. All seed lots were of high quality (> 95% viability) and had been stored at room temperature with the exception of the cheatgrass seeds, which had been stored at –18 °C to maintain dormancy.

In 2006 (Utah site) and 2007 (both sites), we extracted 20 field-collected samples of the seed zone (surface soil with intact litter) from random locations within each patch type. In the perennial patch types, samples were obtained from within the crowns of individual plants. Samples were taken in spring (May), at the same time as seed bank sampling. We obtained seed-zone samples by pounding a metal ring (10 cm diameter and 2.5 cm deep) into the soil until it was flush with the soil-litter surface. A mason's towel was then inserted below the ring and used to lift the ring with its surface litter and underlying soil layer intact. Rings containing seed-zone samples were then placed in Petri dishes (15 × 100 mm), bound with rubber bands and trans-

ported to the laboratory. Most samples were dry at collection; moist samples were allowed to air-dry.

Within 2 weeks of field collection, the seed-zone samples were planted with surface-sterilized (1 min in 70% ethanol, 1 min in 0.0525% sodium hypochlorite) and safranin-dyed seeds of cheatgrass and native grass species. The safranin dyed the seeds bright pink and allowed for planted seeds to be easily distinguished from seed bank seeds. Preliminary experiments determined that the dye had no direct effect on germination or fungal infection. For each study site, 25 sterilized and dyed seeds of each species were planted into each of ten seed-zone samples from each patch type. Each seed-zone sample was planted with a single species, with the exception of *P. secunda* and *E. elymoides*, which were planted together. Seed-zone samples were kept on Petri dish bottoms (tops were removed), watered to saturation with deionized water, randomly placed on trays and incubated for 2 weeks at 15/25 °C under a 12-h diurnal photoperiod and then for two more weeks at 20 °C without lights. Rings were rewetted as needed. The coleoptiles of all emerged seedlings were clipped to 2 cm. On day 28, all dyed seeds were exhumed and examined for germination and presence of *P. semeniperda* stomata. Seeds were scored as germinated without stomata, germinated with stomata, ungerminated without stomata, or ungerminated with stomata. Ungerminated seeds that developed stomata were presumed to have been killed by the pathogen (supported by Koch's postulate experiments; Beckstead *et al.* 2007). All ungerminated seeds lacking fungal stomata were checked for viability using a cut test at the end of the incubation period (Ooi, Aulk & Whelan 2004). There was little or no loss of viability due to causes other than *P. semeniperda* during these experiments.

We analysed data from the two experimental sites separately. For the Davis Mountain study site in Utah, differences in the fraction of seeds killed by the pathogen were analysed using ANOVA, with year, vegetation patch type and species planted as fixed effects (PROC GLM; SAS 2000). The Saddle Mountain site in Washington lacked year as a fixed effect. To improve homogeneity of variance, proportional response variables were arcsine square-root transformed prior to analysis.

To aid in interpretation of the seed bank and pathogen spillover experiments, we obtained mean monthly and yearly precipitation data from nearby NOAA (National Oceanic and Atmospheric Administration) reporting stations. We used data from the Dugway, Utah, NOAA station as an estimate for yearly weather variation at the Davis Mountain site and data from the Priest Rapids Dam, Washington, NOAA station as an estimate for the Saddle Mountain site. Because relevant data for a few months were missing from the Dugway NOAA precipitation record, we substituted estimates generated by the Prism climate simulation model (<http://www.prism.oregonstate.edu/>).

Results

DETERMINING SUSCEPTIBILITY OF POTENTIAL CO-OCCURRING HOSTS

Laboratory inoculation trials with *P. semeniperda* showed that all species included in our field studies were susceptible to pathogen infection and are potential hosts under natural conditions (Fig. 1; species main effect for infection; d.f. = 6, 24; $F = 29.49$; $P \leq 0.0001$; species main effect for pathogen-caused mortality; d.f. = 6, 23; $F = 54.67$; $P \leq 0.0001$). There were no significant differences between the two inoculum

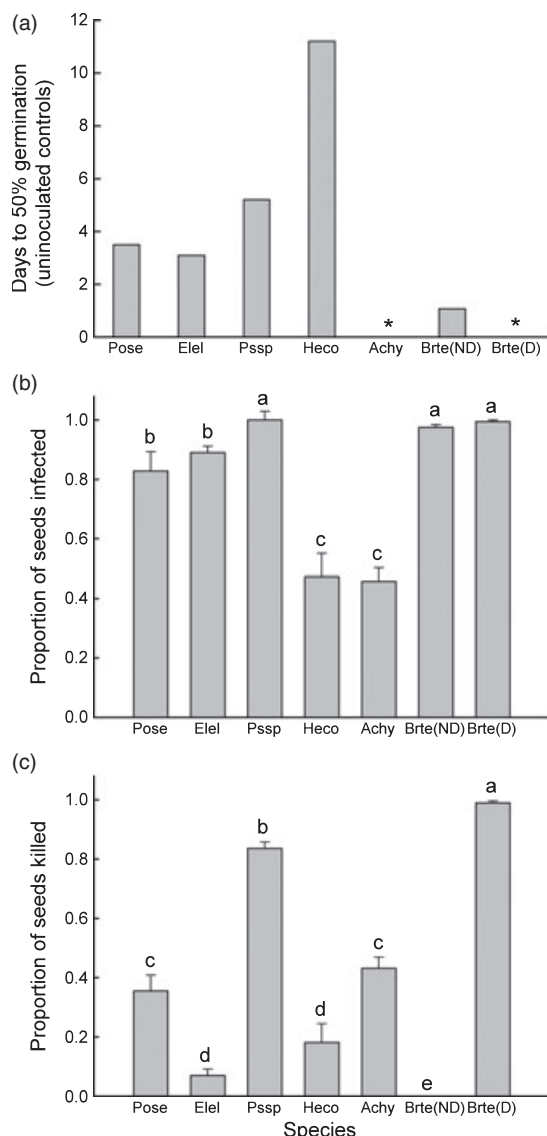


Fig. 1. (a) Days to 50% germination for uninoculated controls, (b) proportion of seeds infected following *Pyrenophora semeniperda* laboratory inoculations and (c) proportion of seeds killed following *P. semeniperda* laboratory inoculations. Data represent means of inoculations with two isolates (mean $\pm$ 1 SE). Species included *Poa secunda* (Pose), *Elymus elymoides* (Ellel), *Pseudoroegneria spicata* (Pssp), *Hesperostipa comata* (Heco), *Achnatherum hymenoides* (Achy) and *Bromus tectorum* (Brte); ND, non-dormant seeds; D, dormant seeds. * < 50% germination within 28 days. Within each panel, bars capped by the same letter are not significantly different at the $P < 0.05$ level according to a Duncan multiple-range test.

sources in infection or mortality; mean values are presented. Pathogen-caused seed mortality in these experiments varied as a function of germination rate and host susceptibility. Fifty percent of non-dormant seeds of *B. tectorum* germinated in a day and exhibited no pathogen-caused mortality, despite their very high susceptibility, as evidenced by the ability of the pathogen to infect and sporulate on a high percentage of germinated seeds. In contrast, dormant *B. tectorum* seeds, which were also highly susceptible, suffered complete mortality when

inoculated and only a very low percentage germinated in the control.

Two native grass species with dormant or slow-germinating seeds, *H. comata* and *A. hymenoides*, were much less susceptible to the pathogen, showing infection levels of < 50% and even lower levels of mortality (Fig. 1). The native grass species *P. secunda* and *E. elymoides* showed the opposite pattern; they were highly susceptible (i.e. high pathogen infection), but suffered relatively little mortality because of their fast germination. The regional dominant *P. spicata* was even more highly susceptible, and this, coupled with its slightly slower germination rate, caused it to suffer considerable mortality.

QUANTIFYING PATHOGEN RESERVOIRS AND *IN SITU* SEED BANKS

At Davis Mountain in Utah, total seed densities were significantly different among seed bank samples collected from different vegetation patch types (Fig. 2a,b; patch type main effect; $n = 60$; d.f. = 2, 54; $F = 30.04$; $P \leq 0.0001$). Total seed density differences were largely driven by the abundance of cheatgrass seeds (Fig. 2a,b; patch type main effect; $n = 60$; d.f. = 2, 54; $F = 78.42$; $P \leq 0.0001$). Cheatgrass seed density in the cheatgrass-monoculture patch type was nearly four times higher than in the cheatgrass-invaded patch type and 45 times higher than in the uninvaded-native patch type; these seed densities (112.1, 29.4 and 2.5 seeds dm^{-2} , respectively) were significantly different from each other. The native species *P. secunda* and *E. elymoides* also showed significant differences in seed density among patch types (patch type main effect; d.f. = 2, 54; $F = 33.29$; $P \leq 0.0001$ and d.f. = 2, 54; $F = 10.55$; $P = 0.0001$, respectively; Fig. 2a). Native grass seeds were detected only in the cheatgrass-invaded and uninvaded native patch types; the primary native seed encountered was *P. secunda*.

Most seeds encountered in seed bank samples at Davis Mountain in western Utah were seeds killed by the pathogen (82% of all viable plus pathogen-killed seeds; Fig. 2c,d). Pathogen-killed seed densities at Davis Mountain mirrored overall seed bank patterns. Cheatgrass was the dominant host, and cheatgrass killed-seed densities varied significantly among patches (patch type main effect; $n = 60$; d.f. = 2, 54; $F = 51.42$; $P \leq 0.0001$). Cheatgrass monocultures contained 80% more pathogen-killed cheatgrass seeds than the cheatgrass-invaded patch type and 97% more than the uninvaded native patch type (98.2, 19.6 and 2.5 seeds dm^{-2} , respectively). Native species also showed significant differences among patch types in density of pathogen-killed seeds (Fig. 2c,d; patch type main effect; $n = 60$; d.f. = 2, 54; $F = 19.81$; $P \leq 0.0001$ and $n = 60$; d.f. = 2, 54; $F = 14.07$; $P \leq 0.0001$, for *P. secunda* and *E. elymoides*, respectively). *Poa secunda* had 44% more pathogen-killed seeds in the uninvaded native patch type than in the cheatgrass-invaded patch type, a pattern that mirrored its total seed bank densities. A few pathogen-killed seeds of *E. elymoides* were found in the cheatgrass-invaded patch type but not in the other patch types.

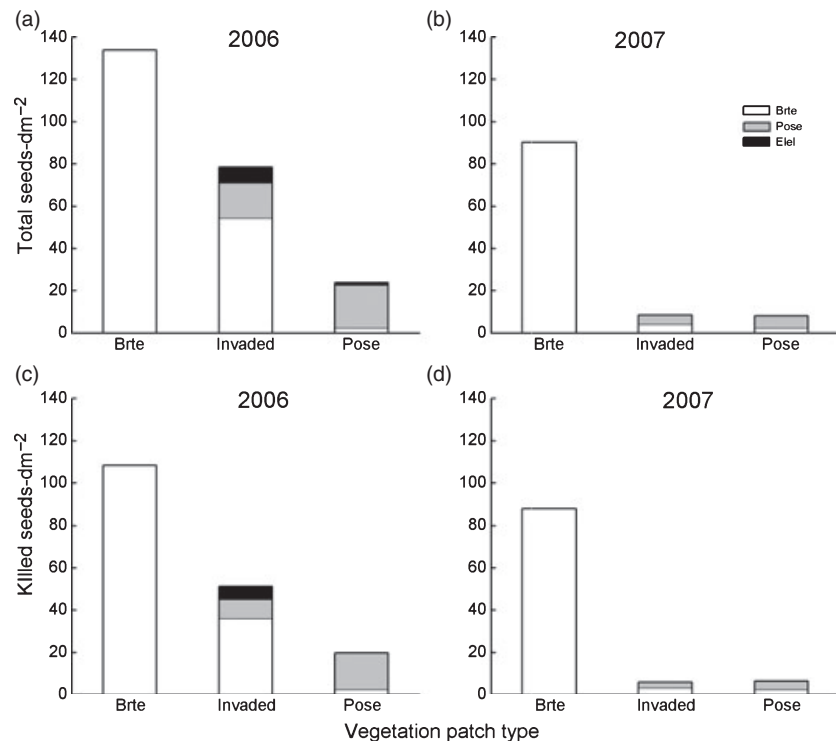


Fig. 2. Late spring seed bank density at the Davis Mountain, Utah, study site for total seeds (a,b) and pathogen-killed seeds (c,d) of *Bromus tectorum* (Brte), *Poa secunda* (Pose) and *Elymus elymoides* (Elel), as a function of vegetation patch type: cheatgrass monoculture (Brte), cheatgrass-invaded native grass (Invaded) and uninvaded native grass (Pose), and year: 2006 (a,c) and 2007 (b,d).

Both seed bank densities and pathogen-killed seed densities were much higher in 2006 than in 2007 (year main effect: d.f. = 1, 54, $F = 31.05$, $P \leq 0.0001$ and d.f. = 1, 54, $F = 12.68$, $P = 0.0008$, respectively). These differences were also driven by the abundance of cheatgrass seeds, which made up about four-fifths of the total seed bank each year (Fig. 2; total cheatgrass seed density year main effect: d.f. = 1, 54; $F = 16.72$; $P = 0.0001$; pathogen-killed seed density year main effect: d.f. = 1, 54; $F = 5.42$; $P = 0.02$). The relative densities of native grass seeds varied even more dramatically between years (Fig. 2). The *P. secunda* seed bank contained four times as many seeds and pathogen-killed seeds in 2006 as in 2007 (year main effect: d.f. = 1, 54; $F = 22.89$; $P \leq 0.0001$ and d.f. = 1, 54; $F = 15.44$; $P = 0.0002$, respectively). *Elymus elymoides* seeds, including pathogen-killed seeds, were found only in 2006 (year main effect: d.f. = 1, 54; $F = 18.52$; $P \leq 0.0001$ and d.f. = 1, 54; $F = 14.07$; $P = 0.0004$, respectively).

The twofold difference between years in the size of the carry-over seed bank at the Davis Mountain site (Fig. 2a,b) was likely due to the differences in precipitation patterns in the period preceding sampling each year (Fig. 3). This site is quite arid (mean annual precipitation 197 mm). Weather records show that the 2004–05 growing season (September 2004–May 2005) was a period of exceptionally high precipitation, and seed production of cheatgrass as well as native grasses was correspondingly high (Fig. 3; S. E. Meyer, unpubl. data). In contrast, the autumn of 2005 was very dry, and precipitation in winter and spring was not much above average, resulting in a smaller seed crop in 2006. In addition, the dry autumn of 2005 delayed germination until midwinter, which promoted seed carryover into spring 2006, whereas precipitation during the autumn of 2006 was well above average and triggered a large flush of

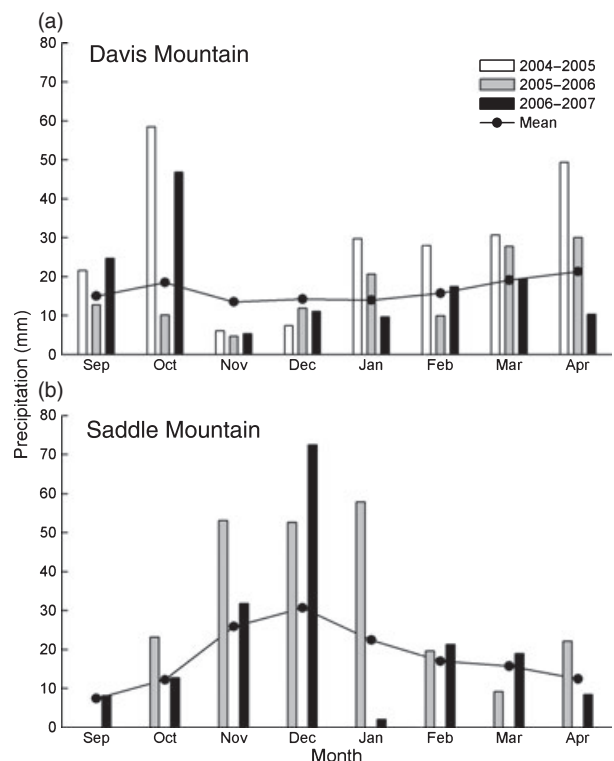


Fig. 3. Monthly precipitation (mm) totals from autumn through spring for NOAA reporting stations near (a) Davis Mountain, Utah, and (b) Saddle Mountain, Washington, study sites for years immediately prior to and during the study, as well as the long-term monthly means for each site.

autumn germination, further reducing potential carryover into spring 2007 (S. E. Meyer, unpubl. data). Absolute pathogen-killed seed densities were also about twice as high in

2007, but the fraction of seeds killed in 2007 (94%) appeared to be higher than in 2006 (76%).

The seed bank and pathogen-caused mortality patterns at the Saddle Mountain site in central Washington were somewhat similar to those at the western Utah Davis Mountain site, except that total seed densities were lower overall and native seed densities were much lower. The total density of seeds and density of pathogen-killed seeds were significantly different among patch types, with the great majority of both total and pathogen-killed seeds found in cheatgrass-monoculture patches (d.f. = 3, 36; $F = 18.22$; $P \leq 0.0001$ and d.f. = 3, 36; $F = 15.68$; $P \leq 0.0001$, respectively; Fig. 4). Even more than in the Utah site, these differences were driven by the abundance of cheatgrass seeds, which made up 96% of all seeds encountered. Consequently, cheatgrass total seed and killed-seed densities showed a similar distribution among patch types (d.f. = 3, 36; $F = 19.83$; $P \leq 0.0001$; d.f. = 3, 36; $F = 15.68$; $P \leq 0.0001$, respectively). Very few native seeds were encountered in seed bank samples, and no native seeds that had been killed by the pathogen were found. No significant differences among patch types were detectable for any native species.

The relatively low carryover cheatgrass seed densities at the Saddle Mountain central Washington study site were probably due to the fact that, even though this site is quite arid (mean

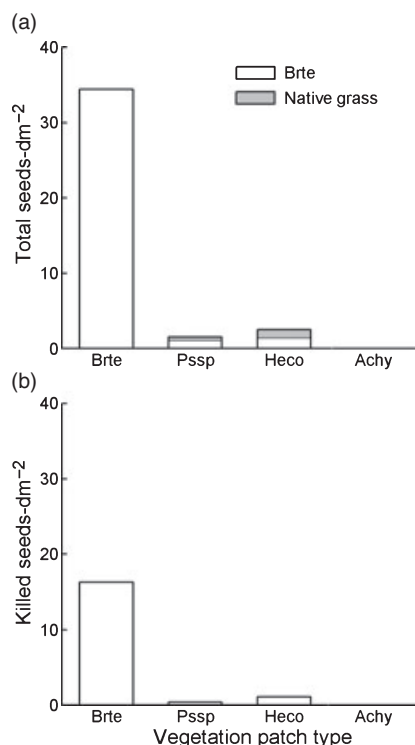


Fig. 4. Late spring seed bank density in 2007 at the Saddle Mountain, Washington, study site for (a) total seeds and (b) pathogen-killed seeds, in vegetation patch types dominated by *Bromus tectorum* (Brte) or each of three native grass species: *Pseudoroegneria spicata* (Pssp), *Hesperostipa comata* (Heco) and *Achnatherum hymenoides* (Achy). Seeds of each native species were found only in each species' own patch type, if at all, while *B. tectorum* seeds were found in multiple patch types.

annual precipitation 177 mm), it has mild winters and fairly reliable midwinter moisture, promoting not only winter germination and relatively large seed crops but also low carryover in most years (Fig. 3). This was true in 2006–07, when over 70 mm of December precipitation probably triggered germination that depleted the pool of potential carryover seeds.

QUANTIFYING INOCULUM LOADS AND POTENTIAL PATHOGEN SPILLOVER

Laboratory experiments utilizing field-collected seed-zone samples with natural inoculum loads from different vegetation patch types at Davis Mountain in western Utah demonstrated significant differences in the fraction of planted seeds killed by *P. semeniperda* in incubation (Fig. 5). We observed a major difference in disease levels in samples collected in different years, with a mean disease incidence of 8% for 2006, but only 0.5% for 2007 (year main effect: d.f. = 1, 17; $F = 258.62$; $P < 0.0001$). Disease incidence also varied significantly by patch type (patch type main effect: d.f. = 2,

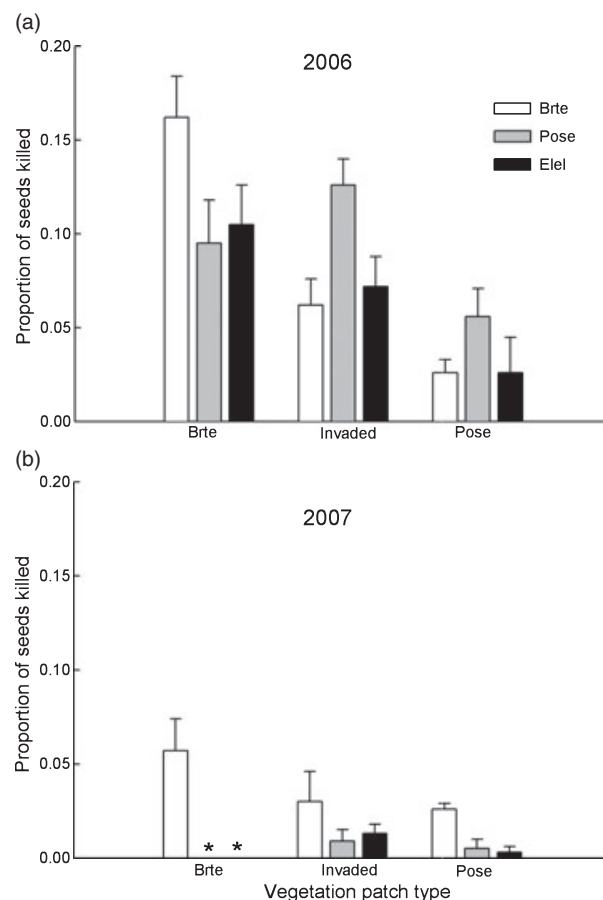


Fig. 5. Proportion of seeds killed by *Pyrenophora semeniperda* after planting into field-collected seed-zone samples collected from three vegetation patch types (Brte – *Bromus tectorum* monoculture, Invaded – *B. tectorum*-invaded native and Pose – uninvaded native *Poa secunda*) at the Davis Mountain, Utah, site in (a) 2006 and (b) 2007 (mean + 1 SE). Species planted included *Bromus tectorum* (Brte), *P. secunda* (Pose) and *Elymus elymoides* (Ellel). *Absence of any mortality.

17; $F = 21.87$; $P < 0.0001$). Disease incidence in the cheatgrass-monoculture patches was 33% greater than in the cheatgrass-invaded native patch type and 71% greater than in the uninvaded native patch type. This supported our prediction that the size of the pathogen inoculum reservoir would increase with the abundance of cheatgrass. Overall, disease incidence also varied by species planted, although this difference was not large (species main effect: d.f. = 2, 17; $F = 2.83$; $P = 0.06$; marginally significant). According to the means separation test, mean disease incidence was significantly higher for cheatgrass (5.7%) than for *E. elymoides* (3.8%), while *P. secunda* disease incidence (5.1%) was not significantly different from the other two. Pathogen-caused mortality for *B. tectorum* was greatest in samples from the cheatgrass-monoculture patch type and lowest in samples from the uninvaded native patch type. This pattern was evident across both years of the study (Fig. 5). Native *E. elymoides* showed a disease incidence pattern similar to cheatgrass in 2006, while *P. secunda* in 2006 experienced similarly high disease levels in cheatgrass-monoculture and cheatgrass-invaded patch types but lower disease levels in the uninvaded native patch type. The apparently low inoculum loads in 2007 resulted in nearly complete escape from the pathogen by the native species. These results show that disease spillover onto native grass species whose seeds disperse into cheatgrass monoculture or invaded native grass patches may occur in years when inoculum loads are high.

At the Saddle Mountain site in Washington, we found a similar pattern of variation in disease incidence as a function of vegetation patch type and species (Fig. 6). This experimental design involved three native species, with patch types limited to cheatgrass-monoculture patches and uninvaded-native

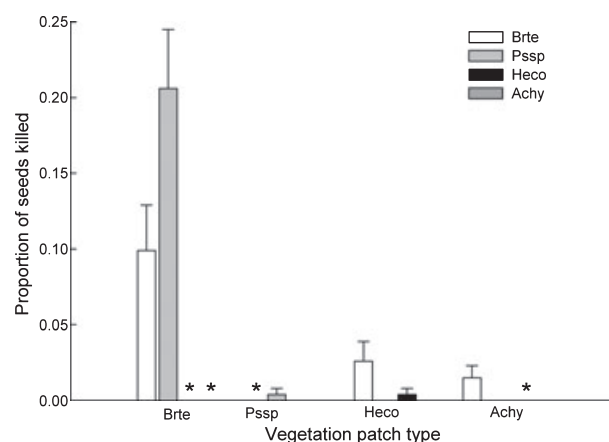


Fig. 6. Proportion of seeds of *Bromus tectorum* and three co-occurring native grasses killed by *Pyrenophora semeniperda* after planting into field-collected seed-zone samples collected from four vegetation patch types (Brte – *B. tectorum* monoculture, Pssp – uninvaded *Pseudoroegneria spicata*, Heco – uninvaded *Hesperostipa comata* and Achy – uninvaded *Achnatherum hymenoides*) at the Saddle Mountain, Washington, site in 2007 (mean + 1 SE). All four species were planted into samples from the *B. tectorum* monoculture patch type, while samples from each native grass patch type received seeds of two species only, *B. tectorum* and the grass species characteristic of that patch type. *Absence of any mortality.

patches specific to each species. Disease incidence was much lower overall than at the Davis Mountain site in Utah but was also strongly influenced by patch type (patch type main effect: d.f. = 3, 90; $F = 10.82$; $P \leq 0.0001$). Cheatgrass-monoculture patch samples generated substantially higher levels of disease on planted seeds than samples of any of the native patch types, particularly for more susceptible species. These findings parallel those from the Utah site and support our prediction that seed banks in cheatgrass monocultures contain higher levels of pathogen inoculum than seed banks in native grass patches. As for the spillover effect on native species at the Washington site, Fig. 6 shows that *P. spicata* experienced relatively high seed mortality when planted into cheatgrass-monoculture seed-zone samples but little mortality at all when planted into samples taken from beneath its own crowns. The other two native species, *H. comata* and *A. hymenoides*, almost completely escaped the pathogen in samples from all patch types at the relatively low inoculum loads present in the field samples. These results show that species that are not very susceptible to the pathogen may escape pathogen spillover, especially in a year with a low inoculum load, even though they are dormant or slow-germinating. In contrast, a highly susceptible species such as *P. spicata* may experience the deleterious effects of pathogen spillover even at a relatively low inoculum load.

Discussion

Our experiments have shown that spillover of the seed bank pathogen *P. semeniperda* from a cheatgrass reservoir to co-occurring native grass species is very likely to occur. Native seeds planted into field-collected seed-zone samples from cheatgrass patches were much more likely to suffer *P. semeniperda*-caused mortality than when planted in their own uninvaded native soils. Seed mortality for several non-reservoir species (*A. spicata*, *E. elymoides* and *P. secunda*) was determined by community context and degree of cheatgrass invasion. In one case, *P. spicata*, the presence of cheatgrass made the difference between undetectably low mortality and significant levels of disease ($> 20\%$ mortality).

A fundamental assumption of pathogen spillover is that a pathogen reservoir exists. Our data support the prediction that cheatgrass seed banks would be larger and support higher levels of *P. semeniperda* than native grass seed banks, thus enabling cheatgrass to be the pathogen reservoir for *P. semeniperda*. The seed bank in cheatgrass-dominated patches was more than four times larger than seed banks in uninvaded native grass patches in both semi-arid plant communities investigated. Likewise, the density of pathogen-killed seeds in the field was greater for cheatgrass than for all native species combined, indicating the ability of *P. semeniperda* to exploit this abundant seed source. Both abundance of seeds and levels of disease in the cheatgrass seed bank are tightly controlled by climatic conditions, specifically the timing and magnitude of precipitation events each year (Meyer *et al.* 2007; Smith, Meyer & Anderson 2008). Nevertheless, from a long-term perspective, cheatgrass seed banks are clearly an important reservoir for this pathogen. Several other studies

have found weedy species to be important pathogen inoculum reservoirs; however, most studies to date have focused on crop systems (Leyva-López *et al.* 2002; Pandey *et al.* 2005; Barreto, Vieira & Santin 2008) and few have focused on seed pathogens (but see Mengistu & Reddy 2005).

Host species were not equal in this multiple-host pathosystem. All six species tested were susceptible to infection by *P. semeniperda*, but they varied in vulnerability to seed death at high inoculum loads. We predicted that species with slow-germinating seeds would fall prey to the pathogen to a greater extent than those with rapidly germinating seeds. This prediction was supported by the fact that both dormant *B. tectorum* seeds and slow-germinating *P. spicata* seeds suffered high seed mortality (> 80%) in artificial inoculation studies, while faster-germinating *E. elymoides* and *P. secunda* seeds suffered lower mortality despite high susceptibility, and very fast-germinating non-dormant cheatgrass seeds completely escaped mortality. Studies with other seed pathosystems have reported both negative and positive relationships between infection and germination rate (Cho *et al.* 2007). Germination rate alone was not sufficient to predict *P. semeniperda*-caused mortality in our trials, however. Susceptibility to infection also varied among hosts and was important to the outcome. For example, *A. hymenoides* and *H. comata* seeds were dormant or germinated slowly, but infection levels for these species were < 50%, resulting in relatively low mortality. The presence of hard seed coverings in these species may reduce their susceptibility to infection (but see Pringle, Loayza & Terborgh 2007).

The level of susceptibility to *P. semeniperda* infection was also a key factor in the pathogen spillover interaction in the field. Native species showed wide variation in their vulnerability to seed death at inoculum levels present in field-collected samples. Species that were not susceptible to the pathogen (e.g. *H. comata* and *A. hymenoides*) almost completely escaped pathogen spillover in a year with low inoculum loads. In contrast, highly susceptible species (e.g. *P. spicata* and *E. elymoides*) experienced the deleterious effects of pathogen spillover even at relatively low field inoculum loads.

We found few seeds in native grass seed banks in this study. Native seed production varies dramatically from year to year (Fisher *et al.* 1987; Clausnitzer, Borman & Johnson 1999), and perhaps we measured seed banks following years of low seed production. Alternatively, it may be that native seeds had germinated prior to spring seed bank sampling. However, subsequent frequent sampling from these sites at multiple times throughout the year has not yielded evidence for higher seed bank levels or substantial germination (Beckstead & Meyer, unpubl. data). More extensive, long-term studies would be necessary to elucidate the native seed bank dynamics.

Seed bank pathogen spillover has the potential to influence the structure of plant communities quite differently from pathogen spillovers that impact adult plants, such as those studied by Malmstrom *et al.* (2005) and Power & Mitchell (2004). Native grass seedlings have been found to be at a competitive disadvantage in cheatgrass-dominated sites (Humphrey & Schupp 2004; Eiswerth *et al.* 2009). This study shows that the presence of cheatgrass, acting as a pathogen reservoir for

P. semeniperda, may also negatively affect native grasses indirectly at the seed stage, even before germination and emergence. Native grasses may thus be placed at a double disadvantage in terms of establishment into cheatgrass: high disease levels from the cheatgrass-generated pathogen reservoir at the seed life stage followed by direct competition at the seedling life stage for any surviving seeds. Which of these interactions is most important to community structure is unknown. It is likely that each is important, but that their relative importance may vary with environmental factors and the specific players involved (Blaney & Kotanen 2001; Gilbert 2002).

Overall, it is clear that the *B. tectorum*–*P. semeniperda* interaction has the potential to be an important force shaping natural communities in the arid Intermountain West. This study begs the question of whether there are other weeds besides *B. tectorum*, in high abundance, that are involved in seed pathogen spillover interactions that influence plant community structure. Multiple-host pathosystems may be more common and more important than we have previously thought.

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