Chapter 7
Community Ecology of Fungal Pathogens on *Bromus tectorum*

Susan E. Meyer, Julie Beckstead, and JanaLynn Pearce

Abstract *Bromus tectorum* L. (cheatgrass or downy brome) presents a rich resource for soil microorganisms because of its abundant production of biomass, seeds, and surface litter. Many of these organisms are opportunistic saprophytes, but several fungal species regularly found in *B. tectorum* stands function as facultative or obligate pathogens. These organisms interact dynamically with abiotic factors such as interannual variation in weather, with other soil microorganisms, with their hosts, and with each other to create spatially and temporally varying patterns of endemic or epidemic disease. Five principal soilborne pathogens, *Ustilago bullata* Berk. (head smut pathogen), *Tilletia bromi* (Brockm.) Nannf. (chestnut bunt pathogen), *Pyrenophora semeniperda* (Brittlebank & Adams) Shoemaker (black fingers of death pathogen), *Fusarium* Link sp. n. (*Fusarium* seed rot pathogen), and a new species in the Rutstroemiaceae (bleach blonde syndrome pathogen), are known to have sometimes major impacts on *B. tectorum* seed bank dynamics, seedling emergence, and seed production. These pathogens exhibit niche specialization, so that they are rarely in direct competition. They sometimes interact to increase the total impact on *B. tectorum* stand structure, which can result in stand failure or "die-off." Die-offs represent areas where *B. tectorum* has been controlled by natural processes, suggesting that these areas might be suitable targets for restoration. Naturally occurring fungal pathogens that can have a strong negative impact on *B. tectorum* success have also been considered as candidate organisms for *B. tectorum* biocontrol using an augmentative mycoherbicidal strategy.

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7.1 Introduction

Monocultures of exotic annuals in the *Bromus* genus represent a valuable resource for many pathogens, which in turn can have strong impacts on stand dynamics, although stands are remarkably well buffered from these impacts in most years. Most of the information we have on stand dynamics and almost all the information on the effects of fungal pathogens come from work with *B. tectorum*, and this information forms the basis for most of the discussion that follows. What we know about other annual bromes largely confirms that they have similar life histories and seed bank dynamics. For *Bromus tectorum* L. (downy brome or cheatgrass), very high seed production, efficient seed dispersal, and the ability to form short-persistent (1–2 years) seed banks contribute to the buffering of disease impacts (Meyer et al. 2007a; Smith et al. 2008). Productivity varies dramatically from year to year due to variation in precipitation, but years without seed production are rare. The seeds of *B. tectorum* would seem to present a major resource for granivores, but native rodents do not prefer them, and even harvester ants and birds apparently consume only a small fraction of the crop (Connolly et al. 2014). There is no indication of secondary metabolites produced by the plant or its endophytes that would deter granivores, although this has not been specifically examined. Fall seed bank densities are generally similar to seed production estimates, suggesting that few seeds are removed. Densities range from 5000 to as high as 50,000 seeds m^{-2}, which is approximately equivalent to a grain yield of up to 1500 kg ha^{-1} (Meyer et al. 2007a; Smith et al. 2008). However, this surfeit of seeds does not go to waste. A diverse community of soilborne pathogens is ready to take advantage of this resource as soon as the first rains arrive following dispersal. *Bromus tectorum* seeds are dormant in early summer at dispersal and lose dormancy through dry afterripening under dry summer conditions (Christensen et al. 1996). They are poised to germinate rapidly with the first substantial rains of autumn, but if rainfall is intermittent or insufficient to trigger complete germination, or if precipitation arrives late in the fall, ungerminated seeds may enter secondary dormancy (Allen et al. 2010). These dormant seeds comprise the persistent seed bank. Seedling emergence can take place any time from late summer through early spring depending on precipitation patterns. Regardless of emergence time, the plants bolt and set seed in spring, that is, they exhibit a facultative winter annual life cycle. The seeds, more accurately referred to as florets containing single caryopses, are quite large, weighing an average of 3 mg. As with most grasses, the primary storage compound is starch.

Plant ecologists and range managers working in *B. tectorum*-dominated systems have long been aware of the presence of fungal pathogens in *B. tectorum* stands and

have speculated about their importance in regulating stand dynamics. Piemeisel (1938) was among the first to document *B. tectorum* successional processes. He observed that stands of overwintered plants sometimes suffered high mortality in the very early spring, a phenomenon he called "winterkill." He suggested that perhaps *Microdochium nivale* (Fr.) Samuels & Hallett, then called *Fusarium nivale* (Fr.) Sorauer (pink snow mold), might be the causal organism but also considered that abiotic (weather) factors could be responsible. Klemmedson and Smith (1964) reported that pink snow mold was a common pathogen on *B. tectorum* in the inland Pacific Northwest and also that many other pathogens had been reported from this host (Sprague 1953 in Klemmedson and Smith 1964). They also specifically reported on the occurrence of head smut epidemics caused by *Ustilago bullata* (head smut pathogen) that resulted in smutting of >95 % of the individuals and consequent succession to perennial plants.

Piemeisel (1951) also described a phenomenon that he called cyclic succession in *B. tectorum* monocultures on abandoned cropland in southern Idaho. He observed that as *B. tectorum* stands became more and more dense in the years following initial establishment, they ultimately reached a "degenerate" state in which seed production was prevented and stand loss ensued. *Bromus tectorum* would then reestablish on the newly vacant site at low density, and the cycle would repeat itself. He credited this effect to increasing intraspecific competition, but it seems plausible that plant pathogens associated with the heavy litter and crowded conditions of "degenerate" stands could have played a role. This process is very similar to the "die-off" or stand failure in *B. tectorum* monocultures documented in recent years (Baughman and Meyer 2013; Meyer et al. 2014a). The die-off phenomenon is therefore not new, even though it has only recently come to the attention of land managers. The term "die-off" refers to the complete lack of a current-year stand on a site previously occupied by a *B. tectorum* monoculture, in other words, establishment failure (Baughman and Meyer 2013). It is usually the result of mass mortality of germinating seeds or preemergent seedlings, though loss of the persistent seed bank or failure of seed bank replenishment through lack of seed production the previous year can also be involved. All of the processes involved with die-offs can potentially be mediated through fungal pathogens.

The objective of our research with *B. tectorum* fungal pathogens is to understand how they interact with abiotic factors, with the soil microbial community, with their hosts, and with each other to impact stand dynamics in *B. tectorum* and specifically to cause stand failure. We hope to use this knowledge as a restoration tool to create *B. tectorum* stand failure, either through in situ manipulation of the factors controlling disease levels or through inoculum augmentation. The objective would be to temporarily reduce *B. tectorum* competition in the context of restoration seeding. The more recent information presented here is based largely on our own research. We present our data and current understanding of these pathogens in a historical sequence, with the best known species presented first, followed by less well-studied and more recently discovered organisms.

7.2 Principal Pathogens on *Bromus tectorum*

7.2.1 Ustilago bullata *(Head Smut Pathogen)*

The head smut disease of *B. tectorum*, caused by the cosmopolitan basidiomycete pathogen *U. bullata*, is the *B. tectorum* disease most familiar to land managers, ranchers, and recreationists. It is macroscopically visible and conspicuous late in the spring as smutted flowering heads on diseased plants that are interspersed in *B. tectorum* stands among healthy plants in seed (Fig. 7.1a). Under epidemic

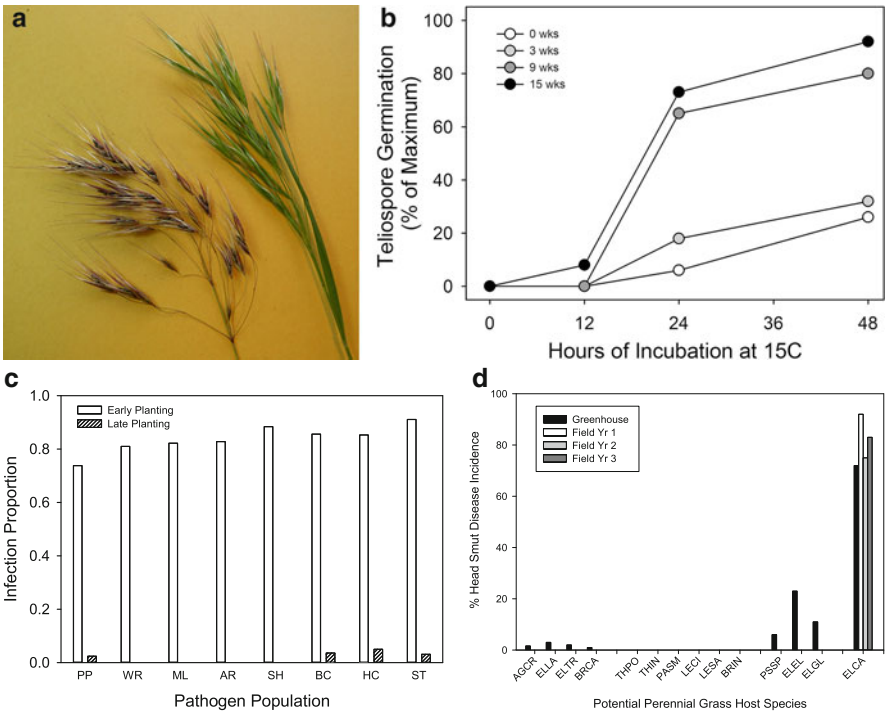

Fig. 7.1 (**a**) Normal *Bromus tectorum* inflorescence (*right*) and smutted inflorescence infected with *Ustilago bullata* (*left*). (**b**) Dormancy loss during 15 weeks in dry storage at 30 °C for a representative *U. bullata* teliospore collection as measured by germination time courses during incubation at 15 °C on PDA for 48 h (each data point represents proportion of teliospores germinated out of 100 examined, corrected for viability; the data points are independent. The experiment was repeated in time for multiple teliospore collections with similar results). (**c**) Field disease incidence following inoculation with teliospores of eight *U. bullata* populations and planting either in early fall or late fall (from Boguena et al. 2007). (**d**) Disease incidence on 12 perennial grass species after inoculation with pathogen strains from *B. tectorum*. (Group 1: AGCR *Agropyron cristatum*, ELLA *Elymus lanceolatus*, ELTR *Elymus trachycaulus*, BRCA *Bromus carinatus*. Group 2: THPO *Thinopyrum ponticum*, THIN *Thinopyrum intermedium*, PASM *Pascopyrum smithii*, LECI *Leymus cinereus*, LESA *Leymus salinus*, BRIN *Bromus inermis*. Group 3: PSSP *Pseudoroegneria spicata*, ELEL *Elymus elymoides*, ELGL *Elymus glaucus*. Group 4: ELCA *Elymus canadensis*)

conditions, its black, soot-like spores can collect as a visible powder on the boots and pant-legs of people who walk through a heavily diseased stand.

7.2.1.1 *Ustilago bullata* Life Cycle

The head smut pathogen is an obligate biotroph, which means it must grow inside the tissues of a living host plant in order to complete its life cycle (Fischer and Holton 1957). This cycle includes spore dispersal, germination, infection of emerging coleoptiles (grass seedling leaves) of the host plant, and systemic growth inside the host. This is followed by preemption of the flowering physiology of the host, so that the florets, instead of containing seeds, are modified into smut bullae for the production and delivery of teliospores (pathogen propagules). Many people assume that the head smut pathogen attacks the inflorescence directly, but this is not the case. The head smut pathogen is not a flower-infecting pathogen but instead is a systemic, seedling-infecting fungus that lives inside vegetative tissues until the plant flowers. Conditions during seed germination and very early seedling growth are therefore the major environmental determinants of disease levels each year.

Teliospores are the dispersal stage of the pathogen. They are released after rainfall causes the bullae to expand and rupture. Most are probably dispersed in raindrops onto the soil immediately adjacent to the smutted plant, though they can also be dispersed by wind or animals. The spores are usually dormant at the time of dispersal. They lose dormancy over the summer under hot, dry conditions in a pattern that parallels dormancy loss in seeds of the host (Fig. 7.1b). This coevolved pattern ensures that smut spores and seeds will germinate synchronously. The spores as well as the seeds are highly germinable in early autumn when temperatures are still warm. If seed germination-triggering rains arrive early, disease levels can be very high. This pathogen has minimal ability to infect at cooler temperatures. If autumn rains arrive late, disease levels will generally be low even if inoculum levels were high (Fig. 7.1c; Boguena et al. 2007). The window of infection is narrow, from the time the coleoptile (seedling leaf) begins to emerge from the seed until the seedling tissue becomes hardened and resistant to penetration. The pathogen can increase its chances of infection during this narrow window by producing free-living, yeast-like sporidia. Individual teliospores are dikaryotic (i.e., they contain two nuclei per spore). The teliospores undergo nuclear fusion, meiosis, and germination, after which a period of asexual multiplication begins. In this way one teliospore can potentially produce hundreds of the sporidial cells that can take part in mating. Two sporidial cells of opposite mating type must fuse to form the infection hypha (mycelial strand), which again contains two nuclei per cell. The infection hypha is not capable of free-living growth. It must encounter a susceptible host coleoptile and penetrate before it exhausts its limited resources.

Smut teliospores do not form persistent spore banks, so that the occurrence of disease is dependent on the presence in the seed bank of spores produced the previous season or, in the longer term, on aerial or seedborne spore dispersal. In years following *B. tectorum* stand failure, which prevents production and dispersal of smut teliospores along with seeds, the incidence of head smut disease on plants that establish from the persistent seed bank is essentially zero.

Once safely inside a plant, the fungus almost always survives to reproduce. The mycelium lives in the crown of the plant from the time of hyphal penetration until bolting commences in mid-spring, then follows the elongating flowering shoots upward and takes over the flowering physiology of the plant, preventing seed formation. The teliospores mature in the resulting bullae, completing the life cycle of the fungus. Plant pathogens with this mode of attack have been called sterilizing or castrating fungi, as opposed to pathogens that cause outright plant death. For an annual plant like *B. tectorum*, the outcome is essentially the same, namely, termination of the host life cycle.

7.2.1.2 Host Range

Ustilago bullata has one of the widest host ranges of any smut fungus, infecting several genera of cool-season annual grass weeds and forage grasses, including *Bromus*, *Agropyron*, and *Elymus* (Fischer 1940; Kreizinger et al. 1947; Meiners and Fischer 1953). The pathogen exhibits a high degree of host specialization, in spite of being an apparent generalist. Specific pathogen races are virulent on only a small subset of the host range of the species as a whole, most often on a single species and sometimes only on specific genotypes within a species.

We have examined the host range of *U. bullata* strains isolated from *B. tectorum* as part of an evaluation of nontarget effects associated with the possible use of this organism for *B. tectorum* biocontrol. We tested six strains at very high inoculum loads on 14 species of perennial grasses (32 plants per species per strain) in the genera *Agropyron*, *Pseudoroegneria*, *Thinopyrum*, *Pascopyrum*, *Leymus*, *Elymus*, and *Bromus* in a series of greenhouse and field inoculation trials. *Bromus tectorum* controls were 100 % diseased in every test. Pathogen strains showed similar patterns of disease and were combined for data presentation.

Nontarget species generally fell into four susceptibility categories (Fig. 7.1d). Ten species were clearly nonhosts with respect to pathogen strains from *B. tectorum*, exhibiting very low disease levels the first year in the greenhouse (Group 1) or, for those that failed to flower in the greenhouse the first year, during three subsequent years after out-planting (Group 2). These included the four introduced forage grasses in the test as well as the native species *Leymus cinereus* (Scribn. & Merr.) Á. Löve (basin wildrye), *Leymus salinus* (M.E. Jones) Á. Löve (salina wildrye), *Pascopyrum smithii* (Rydb.) Á. Löve (western wheatgrass), *Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould (thickspike wheatgrass), *Elymus trachycaulus* (Link) Gould ex Shinners (slender wheatgrass), and *Bromus carinatus* Hook. & Arn (mountain brome). Three native species, *Elymus glaucus* Buckley (blue wildrye), *Elymus elymoides* (Raf.) Swezey (squirreltail), and *Pseudoroegneria spicata* (Pursh) Á. Löve (bluebunch wheatgrass), showed low to moderate levels of disease (6–23 %) the first time they flowered in the greenhouse, but when these smutted individuals were outplanted, they flowered normally and did not exhibit the disease in three subsequent years (Group 3). These species were consistently able to outgrow the disease and are

probably not true hosts for strains from *B. tectorum*. Only one perennial species, the native *Elymus canadensis* L. (Canada wildrye; Group 4), showed disease at moderate to high levels (70–90 %) for multiple years following inoculation.

The fact that head smut pathogen strains from *B. tectorum* are largely avirulent on native grasses makes it unlikely that these strains originated in the North American range but instead arrived along with *B. tectorum* seeds from the native Eurasian range. Thus, while *B. tectorum*-infecting strains are very common and widely distributed in western North America, they are probably not truly native, even though *U. bullata* clearly includes native strains on native grasses (Fischer 1940).

We also examined susceptibility of *B. tectorum* and two other introduced annual bromes to several head smut races from different brome species in an unreplicated pilot inoculation experiment with 12 plants per treatment combination. *Bromus tectorum* was quite susceptible to a smut race from a conspecific host population (82 % disease incidence) and somewhat susceptible to races from the exotic annual brome species *Bromus arvensis* L. (field or Japanese brome; syn. *B. japonicus* Thunb.; 58 % disease incidence) and *Bromus sterilis* L. (poverty brome; 30 % disease incidence). It was completely resistant to smut populations from *Bromus diandrus* Roth (ripgut brome) and *Bromus rubens* L. (red brome). *Bromus diandrus* and *B. rubens* were each completely resistant to smut populations from the other four annual bromes but susceptible to smut populations from conspecific hosts (*B. diandrus*, 100 % disease incidence; *B. rubens*, 91 % disease incidence). All three annual species tested were also completely resistant to a smut population from the native perennial *B. carinatus*. This study provides preliminary evidence for race-specific resistance against head smut races from other *Bromus* species in all three introduced annual brome species tested.

7.2.1.3 *Ustilago bullata* Distribution, Epidemiology, and Genetics

Head smut disease is ubiquitous and common throughout the western North American range of *B. tectorum*, and it is almost always possible to find smutted plants in a population. Epidemic levels of disease (>30 % smutted tillers) are encountered sporadically, usually but not always at more mesic sites with reliable fall precipitation and early *B. tectorum* emergence. We carried out a five-state survey of *B. tectorum* diseases at 32 sites in 2005, using a point-intercept method with 40 placements of a ten-pin sampling frame in each population. We recorded a mean head smut disease incidence of 12 % (range 1–69 %). A similar survey at 45 sites in 2006 yielded a mean head smut disease incidence of 16 % (range 0–51 %).

We followed the course of a head smut epidemic at a disturbed sagebrush steppe site in the foothills above Boise, Idaho, over a 4-year period (1999–2003; Meyer et al. 2010a). At the height of this epidemic, over 95 % of the *B. tectorum* population

was smutted. The epidemic resulted in near extinction of *B. tectorum* over several hectares and consequently local extinction of the head smut pathogen as well. The site became dominated by *Poa bulbosa* L. (bulbous bluegrass), which was a minor constituent of the vegetation at the beginning of the epidemic.

Head smut epidemics are a result of a complex interplay of factors that make their occurrence difficult to predict. These include inoculum loads resulting from previous-year disease, weather patterns, and the genetic composition of both host and pathogen populations, which can sometimes respond dramatically to short-term selection (Meyer et al. 2010a). In addition to host specialization at the species level, *U. bullata* is also divided into a complex series of pathogen races both among and within populations on *B. tectorum* (Meyer et al. 2001, 2005, 2010a). The patterns of virulence in the pathogen and corresponding resistance or susceptibility in the host generally follow the gene-for-gene model that has been demonstrated in many plant–pathogen interactions that exhibit race-specific resistance (Crute et al. 1997). In a gene-for-gene system, the gene product of a single locus in a pathogen race can be recognized by a complementary gene product of a single locus (a resistance locus) in a host resistance phenotype, resulting in host resistance to this particular pathogen race. If the pathogen has an allele at that locus that does not make the elicitor gene product, or if the host lacks the allele at the complementary resistance locus enabling recognition of the elicitor, then the host exhibits susceptibility to this race. The smuts and bunts are among the few systemic pathogens for which race-specific host resistance has been documented (Crute et al. 1997).

While several resistance loci have been identified in *B. tectorum* that have corresponding avirulence loci in the head smut pathogen, most *B. tectorum* lineages are susceptible to most head smut races, making the function of resistance genes difficult to discern (Meyer et al. 2001, 2005, 2010a). A notable exception is the dominant *B. tectorum* genotype in the Mojave Desert population at Potosi Pass in southern Nevada, which is completely resistant to all head smut pathogen races from Great Basin populations. It is attacked by a unique co-occurring race of the pathogen (Meyer et al. 2005). This unique Mojave Desert race can infect Great Basin *B. tectorum* lineages in greenhouse inoculation trials but has not been found in populations north of the Mojave Desert (Meyer et al. 2005).

7.2.2 Tilletia bromi *(Chestnut Bunt Pathogen)*

Tilletia bromi (chestnut bunt pathogen), like the head smut pathogen, is a basidiomycete seedling-infecting, systemic smut fungus that prevents seed set in infected plants (Duran and Fischer 1961). It is not as common as *U. bullata* and occurs over a much narrower range of environmental conditions. It also produces symptoms that are much less conspicuous than the smutting caused by *U. bullata*, and its presence in *B. tectorum* populations usually goes unnoticed (Fig. 7.2a). However, epidemic levels of chestnut bunt disease can occur and can have a strong negative impact on seed production.

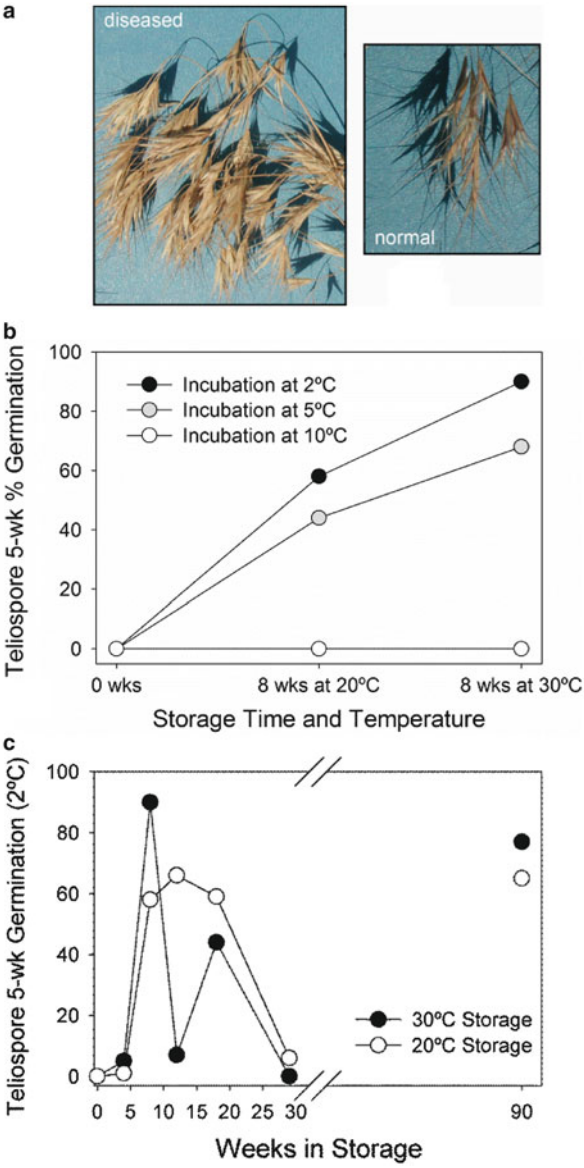

Fig. 7.2 (**a**) Normal *Bromus tectorum* inflorescence (*right*) and diseased inflorescence infected with *Tilletia bromi* (*left*), (**b**) Final germination percentages after 5 weeks on PDA for a representative *T. bromi* teliospore collection tested at three incubation temperatures when recently harvested and after 8 wks of dry storage at 20 and 30 °C, (**c**) Cyclic changes in *T. bromi* teliospore germination percentage at an incubation temperature of 2 °C during time in storage for 90 wks at constant temperatures of 20 and 30 °C. (No data are available for the period 30–90 weeks but the 90-wk data point is provided to show that at least one more cycle of dormancy release ensued. For (**b**) and (**c**), each data point represents proportion of teliospores germinated out of 100 examined, corrected for viability; the data points are independent. Each experiment was repeated in time for a different spore lot with similar results)

7.2.2.1 *Tilletia bromi* Life Cycle

As with head smut, the teliospores of this pathogen are produced in bullae, which are modified ovaries in the host inflorescence. In this case the bullae (sometimes referred to as "bunts") are also produced in florets within the spikelet that do not normally produce seeds, giving the spikelet a "chevron" appearance that is absent in normally developed spikelets. It requires a trained eye to see this difference in the field (Fig. 7.2a). These bullet-like bullae do not rupture on the plant, but remain in the litter after the plants are pushed over by winter storms, where they eventually disintegrate, releasing the chestnut-colored teliospores. The teliospores appear to be long-lived in the surface litter, forming a persistent spore bank. This is advantageous because conditions for infection are not met every year.

The requirements for chestnut bunt teliospore germination contrast strongly with those for head smut spore germination (Meiners and Waldher 1959). The spores do not germinate at all at temperatures above 5 °C, and they germinate best at the near-freezing temperature found under persistent winter snow cover (Fig. 7.2b). We have found that chestnut bunt teliospores are dormant at maturity within the bullae and initially lose dormancy during dry storage in much the same fashion as head smut spores, as long as the requirement for low temperature germination is met. The spores do not necessarily remain nondormant, however, but instead appear to be capable of reentering dormancy, even under constant temperature conditions in dry storage, and then to once again become nondormant in a cyclic pattern (Fig. 7.2c). The period of the cycle appears to be related to temperature, with storage at 30 °C giving a more rapid cycling pattern than storage at 20 °C. This ability to cycle between the dormant and nondormant states is probably related to the ability of the teliospores to form persistent spore banks, but much remains to be learned about this process and how it operates under field conditions.

As in the head smut pathogen, both the chestnut bunt vegetative mycelium inside the plant and the teliospores are dikaryotic, and nuclear fusion and meiosis take place prior to spore germination. This is immediately followed by mating to produce the dikaryotic secondary basidiospores that are the infective units in this fungus (Duran and Fischer 1961).

The ecology of *T. bromi* is very similar to that of its close relative *T. controversa*, the causal agent of dwarf bunt of winter wheat (Meiners 1958; Mathre 1996). Infection takes place in winter, underneath snow cover, after seedling emergence from the soil. The spores are not seedborne, and inoculating seeds directly does not result in disease. Instead, the spores must germinate on the surface of the litter and form secondary basidiospores, which must then intercept the seedling coleoptile after emergence. High levels of disease are confined to years when snow remains on the ground for extended periods.

Once the seedling has been penetrated and infected, fungal mycelium resides systemically in the seedling, then in the crown of the vegetative plant. It grows upward with the bolting flowering stalks in spring and preempts the flowering

physiology of the plant, prevents seed set, and produces the "bunts" that contain pathogen teliospores.

7.2.2.2 *Tilletia bromi* Host Range

Tilletia bromi is a pathogen of worldwide distribution that infects members of the grass genera *Bromus*, *Festuca*, *Ventenata*, and some species of *Vulpia* (Castlebury et al. 2005). A closely related species, *Tilletia fusca* Ellis & Everh., is known to infect only two native North American *Vulpia* species (Boyd and Carris 1997, 1998). Within *T. bromi*, there are at least two major pathotypes that show strong host specialization and that may be distinct species. In Washington state, one pathotype infects *B. tectorum* while the other infects *B. arvensis*. Even in intermixed populations of the two hosts, the pathotypes are strongly genetically differentiated, indicating a high degree of host specialization (Pimentel et al. 2000). This finding supports earlier work on host specialization in this group of fungi (Hoffmann and Meiners 1971). It is not known whether pathogen races from *B. tectorum* can infect closely related species that are also known hosts for this pathogen, e.g., *B. sterilis*, or whether race-specific resistance against this pathogen occurs within *B. tectorum*, as we have demonstrated for *U. bullata*. Detailed work on host range in this group has been largely precluded by the technical difficulties associated with experimental inoculation trials.

7.2.2.3 *Tilletia bromi* Distribution and Epidemiology

The chestnut bunt pathogen is widely distributed on *B. tectorum* throughout the Intermountain Region, but its occurrence is sporadic. Many populations contain no sign of this organism, and only occasionally is it detected at epidemic levels. In *B. tectorum* disease surveys in 2005 and 2006, described earlier for head smut disease, chestnut bunt disease incidence averaged 8.3 and 6.0 %, respectively, at 32 and 45 survey sites. In 2005, the disease was epidemic (>20 % incidence) at five sites, four of which were in upper foothill or montane environments likely to have winter snow in most years. Similarly, in 2006, the disease was epidemic at six sites, all of which were in the upper foothill or montane zone. Conversely, we never found any sign of the disease at six Mojave Desert sites. These findings support the earlier conclusion that snow cover in winter is essential for the development of even moderate levels of chestnut bunt disease. But because the spores are likely long-lived in soil, even a single successful infection year can leave a legacy of spores that can cause occasional bunted plants even in suboptimal environments. The fact that this pathogen has no obvious means of spore dispersal also makes it likely that the disease is absent in many environments favorable for its development. This could account for its apparently sporadic occurrence even in montane environments.

7.2.3 Pyrenophora semeniperda *(Black Fingers of Death Pathogen)*

Pyrenophora semeniperda is a well-known generalist ascomycete seed pathogen found throughout the temperate regions of the world (Medd et al. 2003; Stewart et al. 2009). It was dubbed "black fingers of death" because of its conspicuous black, fingerlike fruiting structures (stromata) that protrude from the surface of killed seeds (Fig. 7.3a). It has been regarded as only a weak pathogen that causes little or no damage to cereal crops, and it received little study until Richard Medd and colleagues in Australia initiated studies of the potential of this organism for grass weed biocontrol in wheat (Medd et al. 2003; Campbell and Medd 2003; Medd and Campbell 2005). An exception was the early study by Kreitlow and Bleak (1964). They studied the natural occurrence of the disease on native and introduced grasses at wildland sites in northern Utah using bait seed experiments and also performed greenhouse studies of host susceptibility.

7.2.3.1 *Pyrenophora semeniperda* Life Cycle

The asexual state of this fungus is by far the most frequently encountered state, although sexual structures (perithecia) have been found on a few seeds from *B. tectorum* field seed bank samples. In contrast, seed bank samples may contain literally hundreds of killed seeds with the protruding stromata that produce asexual spores (conidia; Meyer et al. 2007a). There have been conflicting reports on the life cycle of this organism. Campbell and Medd (2003), working with wheat seeds, found that direct conidial inoculation of mature seeds resulted in infection but that the seedlings easily outgrew the fungus and suffered no long-term consequences. They concluded that the fungus must infect during flowering and be internally seedborne in order to cause seed death.

We determined early in our study of this organism that seed germination rate was the key factor in determining whether or not *P. semeniperda* infection would result in seed mortality, a phenomenon we called the "race for survival" (Beckstead et al. 2007). *Bromus tectorum* seed germination rate is a function of dormancy status. When mature nondormant seeds are inoculated with the pathogen, most escape through very rapid germination. When mature seeds are inoculated in the dormant state, most are killed. We determined that infection levels are as high on nondormant seeds as on dormant seeds and that pathogen development can take place on nondormant seeds that have successfully germinated and produced seedlings. Campbell and Medd (2003) worked with the rapidly germinating seeds of wheat and found that the pathogen could cause infection, but not mortality. Because they did not test the pathogen on slow-germinating seeds, they concluded that it had limited ability to kill mature seeds.

Medd and Campbell (2005) were able to infect developing seeds in the inflorescence of annual grass weeds such as ripgut brome (*B. diandrus*) with *P. semeniperda* using

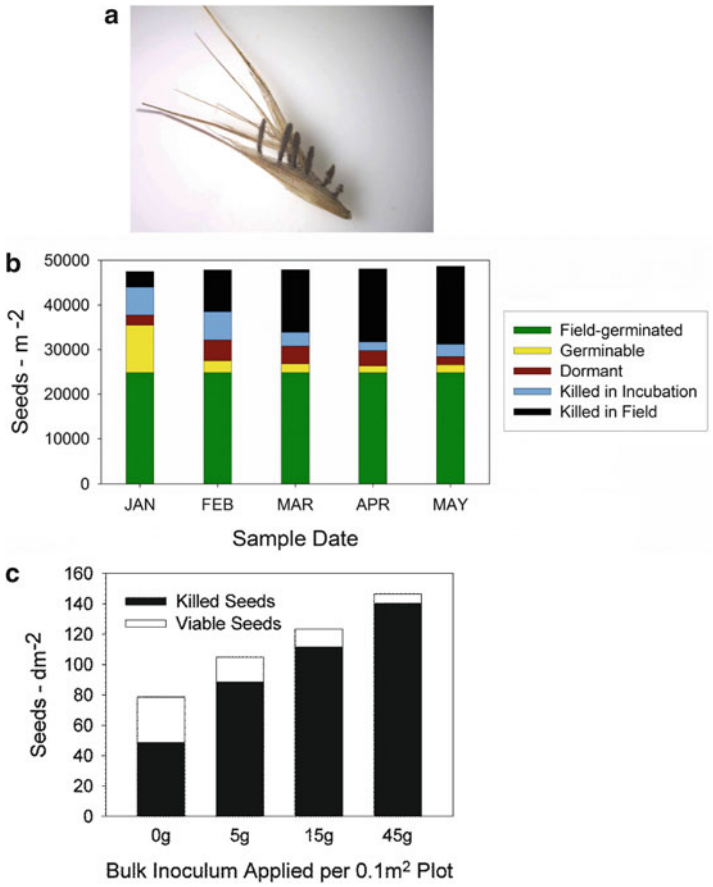

Fig. 7.3 (**a**) *Bromus tectorum* seed killed by *Pyrenophora semeniperda*, showing the protruding stromata that engendered the moniker 'black fingers of death', (**b**) *Bromus tectorum* seed density in different categories as measured monthly during a field seed bank study in 2005–2006 at the Whiterocks exclosure in Skull Valley, Utah. (For each date 20 seed bank samples were collected and processed as described for the cheatgrass disease survey), (**c**) Densities of viable and killed seeds in the seed bank at Haven Flats on the Hanford Reach National Monument in spring 2010 after application of *P. semeniperda* inoculum at three levels the previous fall ($n = 10$ for controls and $n = 40$ for inoculated plots, i.e., mean of four pathogen strains at each inoculum level. Data were obtained from seed bank samples collected from each plot as described earlier)

an aerial inoculum spray method. Wallace (1959) also succeeded in producing infection and subsequent mortality as mature seeds in wheat and oats using this method. In both studies, extended dew periods were required in order for successful floret infection to occur, calling into question whether this process would ever occur naturally in the semiarid environments where these annual grass weeds are a problem. We approached this question indirectly by examining *P. semeniperda* disease

levels on undispersed seeds of *B. tectorum* and the native grass *E. elymoides* and examining the correlation with weather during seed maturation, when floret infection on the plant would have to take place (Meyer et al. 2008a). We found that the highest levels of disease were significantly associated with the driest conditions rather than the wettest conditions during flowering and that disease levels on undispersed seeds were positively correlated with current inoculum levels in the soil. We also found that the conidia on undispersed seeds were not deeply seedborne, as would be the case for floral infection, but instead were superficially borne on the floret bracts, as evidenced by elimination of the disease with surface sterilization. We concluded that floral infection is highly unlikely in nature, at least in ecosystems where *B. tectorum* is prevalent, and that aerial dispersal of spores from the seed bank into the seed heads under dry conditions accounted for the occurrence of the disease on undispersed seeds. This also provides a mechanism for the conidia of this otherwise soilborne pathogen to experience targeted dispersal along with the seeds of its host (Meyer et al. 2008a).

Both the asexual and sexual stages of the life cycle of *P. semeniperda* are reported to occur exclusively on seeds (Shoemaker 1966; Paul 1969). This pathogen has been reported to cause a foliar disease called ring spot on young wheat plants, but this disease is of no economic importance, and the fungus has never been reported to sporulate on living leaves (Campbell and Medd 2003). Most members of the genus *Pyrenophora* are foliar pathogens, and some of them, e.g., *P. tritici-repentis* (Died.) Drechsler, causal agent of tan spot of wheat, cause serious damage on cereal crops. It appears that *P. semeniperda* retains some residual ability to cause leaf spots even though it is dependent on host seeds for the completion of its life cycle. Interestingly, *P. semeniperda* produces in liquid culture some of the same toxic compounds (spirocyclic lactams) as *P. tritici-repentis*, but it does not produce these compounds in solid culture on seeds, suggesting that they are not necessary for seed pathogenesis (Masi et al. 2014b).

We have some evidence that *P. semeniperda* can occur as an endophyte in *B. tectorum* plants, presumably by growing into the seedling following infection of a rapidly germinating seed (Beckstead et al. 2012). There is no evidence that the disease is vertically transmitted through seeds, however. The infected litter could potentially act as an inoculum source in early summer soon after production, but it loses its effectiveness prior to contact with the seed bank.

7.2.3.2 *Pyrenophora semeniperda* Host Range

As mentioned earlier, *P. semeniperda* has a very wide host range among the grasses, with >36 genera reported as hosts (Medd et al. 2003). The species has also been reported occasionally from dicot seeds. Most reports have been made in the context of laboratory tests of seed quality. Prior to our work, there were no published reports of this organism in soil seed banks, explaining why it was regarded as relatively uncommon.

Generalist pathogens can exhibit differential success on different hosts, resulting in complex host range patterns. Several factors operate to reduce realized host range relative to potential host range, particularly under field conditions. We explored factors influencing host range of *P. semeniperda* by first measuring potential host range in laboratory experiments at high inoculum loads with 26 grass species, including the primary host *Bromus tectorum*, and developing models to predict susceptibility and tolerance based on host traits, including germination speed, seed hardness, seed size, and phylogenetic relationships (Beckstead et al. 2014). Susceptibility was defined by the level of infection whether seeds survived or were killed, whereas tolerance was defined by the ability of infected seeds to survive. All species tested were at least somewhat susceptible to the pathogen at high inoculum loads, but both infection and mortality varied widely. Species more closely related to the original host (*B. tectorum*) were more susceptible to infection, whereas species with slower seed germination were less tolerant and therefore more likely to suffer mortality. We also examined the effect of inoculum load on host susceptibility and tolerance to *P. semeniperda* in laboratory experiments (Beckstead et al. 2014). Both infection and mortality were sharply reduced as inoculum load was reduced. Intermediate loads had major negative impacts on dormant *B. tectorum* seeds but generally minimal effects on native grass species.

We also searched for this pathogen in the seed banks of co-occurring native grasses and determined that *P. semeniperda* rarely exploits the seeds of native hosts under field conditions (Beckstead et al. 2010, 2014). This marked reduction in realized host range relative to potential host range suggests that laboratory host range studies are potentially a poor predictor of either the current or possible future realized host range for wildland plant pathogens. Subsequent theoretical and field experimental studies on this pathosystem have supported the conclusion that *P. semeniperda* poses low risk to native grass species even when they are planted directly into seed beds with high inoculum loads (Mordecai 2013; Meyer et al. 2014b).

7.2.3.3 *Pyrenophora semeniperda* Distribution and Epidemiology

Medd et al. (2003) reported that *P. semeniperda* was definitely known from Australia, North and South America, and South Africa, with one report from Egypt, and Stewart et al. (2009) found it in Turkey and Greece. It is probable that seed bank studies in drier temperate regions of the world where annual bromes are important members of the flora would reveal a wider distribution.

We examined the distribution of *P. semeniperda* in the Intermountain Region as part of the *B. tectorum* disease survey described earlier. Disease incidence was measured as density of *P. semeniperda*-killed seeds in the soil seed bank. Ten spring seed bank samples were collected at each site. Killed seeds with visible stromata were counted, and apparently viable seeds were allowed to lose dormancy, then incubated and scored as viable, nonviable due to other causes, or killed by *P. semeniperda* (Meyer et al. 2007a). The mean killed seed density was similar each year:

ca. 3500 killed seeds m^2 in 2005 and 3900 killed seeds m^{-2} in 2006, with values ranging from 0 to as high as 20,000 killed seeds m^{-2}. In the 2005 data set, there was a significant trend for a larger proportion of potential carryover seeds to be killed at sites with higher seed densities in the potential carryover seed bank, namely, at drier sites (Meyer et al. 2007b). This pathogen is thus more important at sites with less reliable autumn rainfall and a higher probability that seeds will enter secondary dormancy and become part of the potential carryover seed bank. In essence, when large numbers of seeds fail to germinate in the first germination-triggering storms and subsequently become secondarily dormant, most of these seeds are killed by the pathogen. At more mesic sites, where the potential carryover seed bank is small, the pathogen is present only at low levels, and most of the small number of seeds that remain ungerminated can escape mortality. Even though the pathogen can sporulate on germinated seeds that go on to form seedlings, its fitness is clearly increased by causing seed mortality.

Seed bank studies with more frequent sampling dates were carried out in 2005–2006 at the Whiterocks study site in Skull Valley, Utah, the location of many of our published studies on this pathosystem (e.g., Beckstead et al. 2007, 2012; Meyer et al. 2007a, 2014b), permitting us to examine these patterns in more detail (Fig. 7.3b). The fall of 2005 was extremely dry, so that the first germination-triggering rainfall event took place during a warm period just before New Year's Day. Approximately half of the 48,000 seeds m^{-2} in the seed bank germinated during this storm and about half of the remaining seeds were still germinable. These remaining seeds rapidly entered dormancy under winter conditions and became prey to attack by *P. semeniperda*. By the end of spring, the pathogen had killed 76 % of the potential carryover seed bank in the field, and another 12 % were likely already infected, as they developed pathogen stromata in subsequent incubation, for a total of 88 % mortality of the potential carryover seed bank and 42 % mortality of the previous-year seed crop.

The demographic consequences of the high 2005–2006 seed mortality for *B. tectorum* were very likely negligible. Germinated *B. tectorum* seeds can successfully establish a stand and produce a new crop of seeds regardless of the impact of *P. semeniperda* on ungerminated seeds. It is only following years of stand failure that *P. semeniperda* becomes potentially important to *B. tectorum* demographics, because in those years, the stand must reestablish from the in situ carryover seed bank, and the density of viable seeds remaining in the seed bank becomes a major factor limiting stand density. In effect, the carryover seed bank only serves as an insurance policy in the event of stand failure, and in most years, stand failure does not occur. The pathogen exploits excess seed production but leaves the *B. tectorum* population largely unharmed and able to produce large quantities of seeds to support pathogen success in subsequent years. *Bromus tectorum* would likely form much larger carryover seed banks in the absence of *P. semeniperda* in the dry environments that favor seed bank carryover, but in spite of this, destruction by the pathogen of a major fraction of the seed crop each year poses little threat to *B. tectorum* persistence.

Most studies support the idea that the main target of this pathogen is dormant seeds, but we have also encountered pathogen strains that can kill fast-germinating, nondormant seeds (Meyer et al. 2010b). We first thought that faster-growing pathogen strains would be more likely to kill fast-germinating seeds, but in fact the opposite proved to be true. The strains that caused the highest mortality on fast-germinating seeds were the slowest-growing strains. This apparent contradiction could be due to the high cost of producing toxins that could quickly disable a germinating seed. This fungus produces large quantities of cytochalasin B, a toxin that prevents cell division following mitosis (Evidente et al. 2002), making this toxin a likely candidate. We measured cytochalasin B production in a series of pathogen strains with different growth rates and obtained a significant negative correlation between cytochalasin B production and mycelial growth rate (Masi et al. 2013). This resource trade-off between mycelial growth and toxin production was later demonstrated more conclusively (Meyer et al. 2015).

Field evidence for pathogen-caused nondormant seed mortality comes from inoculum addition experiments in which the density of killed plus viable seeds in the carryover seed bank was much increased with inoculum addition, as well as the proportion of seeds killed (Fig. 7.3c). This implies that the pathogen at augmented inoculum loads killed seeds that would otherwise not have carried over, i.e., nondormant seeds.

Another mechanism that could explain how *P. semeniperda* kills nondormant seeds is through water stress associated with intermittent small autumn precipitation events. Mortality of nondormant seeds was greatly increased if they were first incubated postinoculation at water potentials that suppressed radicle emergence but permitted pathogen development prior to incubation in free water (Finch et al. 2013). This could explain the mortality of seeds that should otherwise have been fast-germinating and able to escape in field seed banks.

7.2.3.4 *Pyrenophora semeniperda* Genetics

We recently published a genome assembly for *P. semeniperda*, opening the door for comparative genomic studies with other species of *Pyrenophora* for which sequenced genomes are available (Soliai et al. 2014). This could be especially helpful in elucidating the evolutionary origin and function of the phytotoxins that it produces (Evidente et al. 2002; Masi et al. 2013, 2014a, b). We also sequenced the ITS region (internal transcribed spacer sequence from ribosomal DNA) of a total of 417 strains from 20 of the pathogen populations in the *B. tectorum* disease survey described earlier (Boose et al. 2011). Genetic analysis revealed high diversity, with 12 different ITS haplotypes, but very little population structure. Most of the variation (>80 %) was accounted for by within-population variance. There was weak but significant differentiation between northern (Washington and Idaho) and southern (Utah and Colorado) population groups, and the northern group had significantly higher gene diversity than the southern group. Overall, these results suggest that the

P. semeniperda populations on *B. tectorum* did not originate from local populations of native grass hosts. The relationship between genetic and geographic distance was only weakly supported ($r=0.146$, $P=0.053$). It seems more likely that the populations on *B. tectorum* traveled as seedborne inoculum from the Eurasian range and also accompanied *B. tectorum* during its subsequent expansion throughout the West. The strains isolated from seeds collected in Turkey and Greece belonged to ITS haplotypes also found in Intermountain populations, supporting a Eurasian origin for the pathogen, at least for populations on *B. tectorum* (Stewart et al. 2009). The pathogen is so rare in native seed banks that we have been unable to unequivocally identify strains originating from native grasses. It is possible that all the strains in North America were introduced along with their exotic annual grass hosts.

7.2.4 Fusarium *sp. n. (*Fusarium *Seed Rot Pathogen)*

As mentioned earlier, stand failure is a widespread phenomenon in *B. tectorum* dominated ecosystems that is commonly referred to as "die-off" (Baughman and Meyer 2013). As part of studies to understand the causes of stand failure, we planted *B. tectorum* bait seeds into die-off soils in both field and greenhouse experiments and isolated putative causal organisms from killed seeds (Meyer et al. 2014a). We detected an array of fungal organisms that could potentially be seed or preemergent seedling pathogens, but *Fusarium* was by far the most commonly isolated. We therefore initiated studies to investigate whether *Fusarium* could be a die-off causal organism (Meyer et al. 2014a).

Members of the ascomycete genus *Fusarium* are ubiquitous in soils worldwide and include many important pathogens of cultivated plants, particularly vegetables and winter cereal crops (Nelson et al. 1981). The occurrence of *Fusarium* species in natural ecosystems is also frequently reported, but its role in the microbial ecology of these ecosystems is much less well documented (e.g., Walsh et al. 2010).

Molecular-genetic characterization of isolates from *B. tectorum* die-off soils using both ITS and TEF (translation elongation factor) sequence data determined that they belonged to the *Fusarium tricinctum* species complex (O'Donnell et al. 2013). The strains in our study represent one or more undescribed biological species within this complex, but a more exhaustive multilocus molecular-genetic analysis will be required to clarify their status.

Species of *Fusarium* have been reported to be pathogenic on seeds or newly germinated seedlings and to cause diseases referred to as "seed rot" that can result in emergence failure (Slykhuis 1947) or, in the case of the seeds of plant-parasitic plants, host penetration failure (Sauerborn et al. 1996; Muller-Stover et al. 2009). We have demonstrated conclusively that *Fusarium* strains isolated from diseased *B. tectorum* seeds are pathogenic on *B. tectorum* seeds and are capable of causing sometimes high mortality, especially under conditions of intermittent water stress (Meyer et al. 2014a). However, the role of *Fusarium* in *B. tectorum* stand failure in the field has not yet been conclusively demonstrated.

7.2.4.1 *Fusarium* Seed Rot Life Cycle

The asexual life cycle of *Fusarium* begins with conidia (asexually produced spores; Fig. 7.4a). Two types of conidia, macroconidia and microconidia, are normally produced, although some of the strains from *B. tectorum* rarely if ever produce

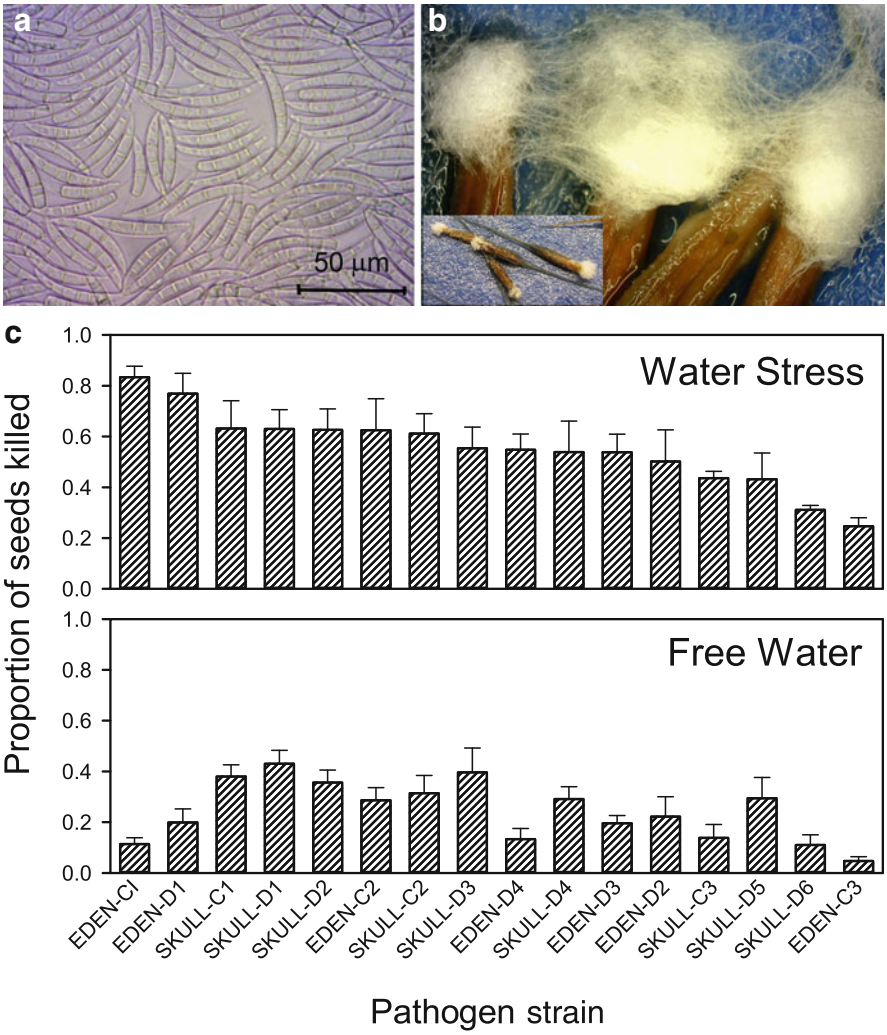

Fig. 7.4 (**a**) Macroconidia produced by a *Fusarium* strain from a die-off soil. (**b**) The development of abundant mycelium on killed *Bromus tectorum* seeds that had been inoculated with *Fusarium* macroconidia, incubated for 7 days at −1.5 MPa, and then transferred to free water (inset shows the development of infection cushions or "white tufts" over the point of incipient radicle emergence during incubation at low water potential). (**c**) *Bromus tectorum* seed mortality caused by 16 strains of *Fusarium* when inoculated seeds were incubated for 7 days at −1.5 MPa prior to transfer to water (*upper panel*) and when inoculated seeds were placed directly into free water (*lower panel*) at 25 °C (From Meyer et al. 2014a)

macroconidia and some sporulate only weakly in culture. It is likely that most of these strains also produce chlamydospores, which are thick-walled resting spores that can persist in the soil for longer periods of time, but we have not yet observed these in culture (Leslie and Summerell 2006).

We have recently elucidated the asexual life cycle of a *Fusarium* strain from *B. tectorum* as it is expressed during pathogenesis on seeds in the laboratory using scanning electron microscopy (Franke et al. 2014). Inoculated nondormant host seeds were held under water stress (−1.5 MPa) to retard germination and to provide *Fusarium*, which can germinate and grow at this water potential, the opportunity to achieve infection. Conidia germinated within a few hours, and the resulting hyphae grew rapidly toward the point of impending radicle emergence, apparently in response to a chemical cue produced during germination. The pathogen formed a conspicuous infection cushion within 48 h, and penetration and seed mortality followed soon after transfer to free water (Franke et al. 2014).

Baughman and Meyer (2013) produced circumstantial evidence that the pathogen responsible for *B. tectorum* emergence failure during a "die-off" affected only germinating seeds. They found that densities of dormant *B. tectorum* seeds in the persistent seed bank following a die-off were the same in the seed banks of recent die-off areas and in adjacent areas that had supported full *B. tectorum* stands. Interestingly, *Fusarium* strains isolated from *B. tectorum* seeds are largely unable to initiate pathogenesis on dormant seeds. This is apparently because of the lack of a chemical cue from the germinating seed to direct mycelial growth. *Fusarium* does not form an infection cushion on dormant seeds and has very limited ability to attack directly through the floret coverings (Franke et al. 2014).

If *Fusarium*, which is ubiquitous in the soils of both die-offs and intact *B. tectorum* stands, is a die-off causal organism, then stand recovery following a die-off must involve *Fusarium* suppression. There is evidence from many studies that fungal spore germination and hyphal growth can often be suppressed in field soil, a phenomenon referred to as "fungistasis" (Lockwood 1977; Garbeva et al. 2011). This suppression is usually alleviated in autoclaved soil, indicating that it has a biological cause. Many studies point to the role of soil microorganisms, specifically bacteria, in causing fungistasis, either through direct competition for nutrients, even to the point of "robbing" the spores of their own nutrients, or through the action of volatile compounds that inhibit fungal activity. Soil amendments that increase the level of available labile carbon, the organic compounds that most soil heterotrophs use as an energy source, tend to alleviate fungistasis and allow pathogenic fungi to resume activity in the soil (Bonanomi et al. 2013). These soil amendments could either make labile carbon temporarily non-limiting, or they could provide the pathogen with the energy to produce its own defensive compounds (Garbeva et al. 2011). Studies on the role of fungistasis in mediating *B. tectorum* stand failure and recovery have been initiated.

Fusarium species are capable of producing a host of secondary metabolites, many of which have phytotoxic, mycotoxic, or antibiotic activity (O'Donnell et al. 2013). It has been recently demonstrated in a very interesting study that *Fusarium*

toxin production is upregulated in the presence of common soil bacteria such as *Bacillus subtilis* (Ola et al. 2013). In these experiments, toxin production was increased by an order of magnitude in co-culture with this bacterium. This strongly suggests that the fungus makes this toxic compound at least partly as a defense response against microorganisms. The role of toxic secondary metabolites produced by *Fusarium* in alleviating fungistasis in the soil and thus in regulating the cycle of disease represented by periodic *B. tectorum* stand failure is a topic for further investigation.

7.2.4.2 *Fusarium* Seed Rot Host Range

Fusarium species generally exhibit wide host range, but many species are made up of series of *formae speciales* that are highly host specific (e.g., *F. solani*; O'Donnell 2000). Strains isolated from *B. tectorum*-infested soils have caused mortality on seeds of the native perennial grasses *Pseudoroegneria spicata* and *E. elymoides* as well as *B. tectorum* in both field and laboratory studies (Meyer et al. 2014b). It is apparent that these strains are not strictly host specific and can cause disease on multiple cool-season grass species. There is still much to be learned about genetic variation in this group and its role in host specificity. The strains from *B. tectorum* soils may represent a series of closely related species with somewhat different host ranges.

7.2.4.3 *Fusarium* Seed Rot Distribution and Epidemiology

In surveys using bait seed experiments at *B. tectorum*-infested sites in northern Nevada, western Utah, and central Washington, all *Fusarium* strains identified to date belong to the *F. tricinctum* species group (O'Donnell et al. 2013; Meyer et al. 2014a). This suggests that it is widely distributed and common in the Western United States and is likely the primary *Fusarium* taxon present in *B. tectorum* monocultures throughout the Intermountain Region.

In a laboratory pathogenicity test with 16 strains isolated from killed *B. tectorum* seeds, wide variation in virulence was observed (Fig. 7.4c, Meyer et al. 2014a). Mortality was generally higher (27–83 %) when nondormant seeds were held at low water potential during the initial stages of infection as described earlier. However, some strains caused mortality of >40 % even in free water, while the least virulent strain caused <10 % mortality under this condition. As these strains are likely capable of living saprophytically in the soil, high virulence may not be a prerequisite for long-term survival, but it is likely that strains capable of causing seed mortality under field conditions have a selective advantage in terms of resources available for sporulation. There may be a continuum in this group such that some strains are almost exclusively saprophytes whereas others exhibit a strongly pathogenic life history strategy.

7.2.5 *Rutstroemiaceae sp. n. (Bleach Blonde Pathogen)*

A newly discovered ascomycete pathogen that may be quite important on *B. tectorum* has been identified using molecular-genetic techniques as a relative of *Sclerotinia homoeocarpa* Bennett, the dollar spot pathogen of turfgrass (Franke et al. 2014). We first observed the symptoms of the disease caused by this pathogen many years ago but were uncertain whether the syndrome was caused by abiotic stress or pathogen activity.

Unlike the pathogens described earlier, this organism apparently infects the crowns of already established seedlings or young plants. The plants survive to bolting, but the spikelets in the inflorescences abort prior to the completion of seed filling, leaving the plants sterile. These sterile plants are stunted in comparison with healthy plants, and they turn straw colored while healthy plants are still at the green or purple stages of ripening. These wispy, straw-colored heads with unfilled seeds are symptoms of the bleach blonde syndrome (Fig. 7.5a).

We thought this syndrome might be caused by some pathogen that causes similar symptoms on winter cereals, e.g., *Gaeumannomyces graminis* (Sacc.) Arx & Olivier (the causal agent of take-all disease) or *Fusarium culmorum* (W.G. Sm.) Sacc. (the causal agent of dryland foot rot). These pathogens produce clear disease signs on the lower stems and roots, whereas bleach blonde plants have no readily visible disease signs. Isolations from the crowns of diseased plants consistently yielded an organism that grouped within the family Rutstroemiaceae based on its ITS sequence but was not a perfect match for any known species, indicating that it likely represents an undescribed taxon. More intensive molecular-genetic work will be necessary to typify and name this new organism.

We have demonstrated in greenhouse pathogenicity tests that the organism isolated from the crowns of diseased *B. tectorum* plants in the field is definitely the causal organism responsible for the bleach blonde syndrome (J. Pearce, unpublished data). Disease incidence in the pathogenicity test was 18 %. The pathogen was readily re-isolated from diseased plants, which had significantly lower total biomass, floret number per tiller, and mass per floret than healthy plants (Fig. 7.5b). They closely resembled bleach blonde-affected plants observed in the field.

7.2.5.1 Bleach Blonde Pathogen Life Cycle

Members of the family Rutstroemiaceae generally do not produce asexual spores but instead produce asexual resting structures called sclerotia or stromata. They are potentially capable of sexual reproduction, but the fleshy cup mushrooms that are formed are seen much more often in species of mesic environments. The bleach blonde pathogen is soilborne and apparently infects the host plants after seedlings are established, either through the roots or directly into the crown. There seems to be no movement of the fungus within the bolting flowering stalk, and unlike the smuts and bunts, it does not produce spores or other reproductive structures in the

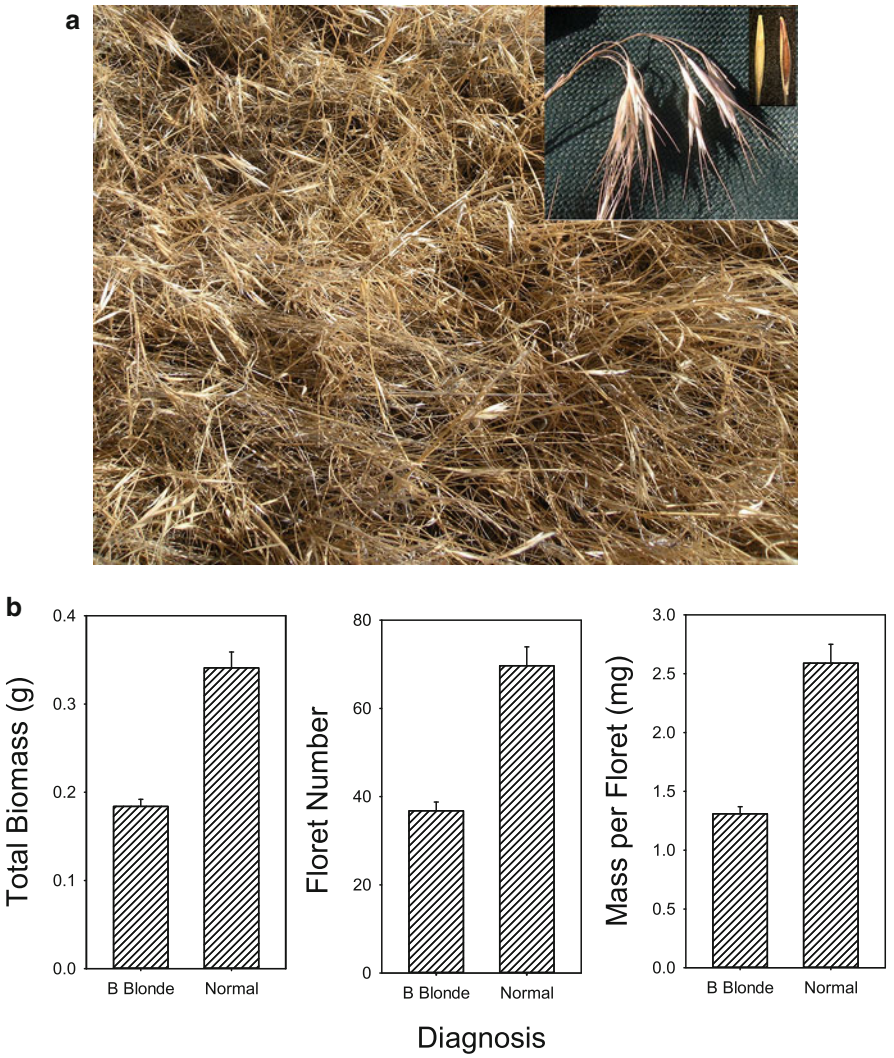

Fig. 7.5 (**a**) A patch of *Bromus tectorum* monoculture that suffered epidemic disease caused by the bleach blonde pathogen (Rutstroemiaceae sp. n.). Inset shows a close-up of a sterile inflorescence from a diseased plant and a comparison of a sterile floret (*left*) with a normal filled floret (*right*). (**b**) Effect of disease caused by the bleach blonde pathogen on whole plant biomass, number of florets per tiller, and mean mass per floret in a greenhouse pathogenicity test. (Florets with a mean mass of <1.5 mg are nonviable)

inflorescences. These inflorescences abort because disease development at the base of the plant apparently blocks the vascular tissue and causes water stress in the flowering shoots, in a manner similar to a vascular wilt disease. If surface-sterilized stem bases of diseased plants are incubated in the laboratory, the irregularly shaped black stromata characteristic of the family Rutstroemiaceae are produced within the

crown and stem tissue. Similar stromatal structures are also produced in culture. In related organisms, the stromata can persist in the soil for many years. They resume active growth only in the presence of host root exudates (e.g., *Stromatinia gladioli*, a pathogen of cultivated gladiolus; Jeves and Coley-Smith 1980).

7.2.5.2 Bleach Blonde Syndrome Pathogen Host Range

The host range of this newly discovered pathogen is completely unknown. We have casually observed individuals of *B. rubens* and *B. diandrus* with apparently the same disease syndrome, but these plants were not critically examined. In a preliminary greenhouse pathogenicity test, we observed no bleach blonde disease on inoculated plants of *B. arvensis*. We are currently engaged in tests to determine whether root exudates of different potential hosts can release pathogen stromata from their dormant state, as a next step in examining bleach blonde pathogen host range.

7.2.5.3 Bleach Blonde Pathogen Distribution and Epidemiology

We have detected individuals with bleach blonde syndrome in many populations of *B. tectorum*, but we do not yet have any quantitative data on its distribution. We have observed the disease at epidemic levels a few times, usually in association with known die-off areas. We know from the greenhouse pathogenicity test that this organism has no effect on seeds or seedling emergence; its association with *B. tectorum* stand failure must therefore be indirect. At epidemic levels, the disease occurs in patches that can be recognized by the fine texture and short stature of the diseased tillers, which have a tendency to collapse during the summer, forming a deep mat of tangled stems (Fig. 7.5b). At epidemic levels, this pathogen has a major effect on seed production. In plots established in 2012 at the Whiterocks Exclosure in Skull Valley, Utah, this disease resulted in an estimated 60–80 % reduction in seed rain (J. Pearce, unpublished data).

7.3 Community Ecology of Pathogens on *Bromus tectorum*

The five principal pathogens on *B. tectorum* described above exhibit host relationships and infection phenologies that tend to minimize their competitive interactions. Each pathogen exhibits niche specialization by attacking at a specific stage of the *B. tectorum* life cycle (Fig. 7.6). *Pyrenophora semeniperda* usually attacks dormant seeds, either seeds in primary dormancy (if there are summer rains) or secondarily dormant seeds in the carryover seed bank. *Fusarium* usually attacks nondormant seeds in the germinable autumn seed bank. *Ustilago bullata* infects the coleoptiles of newly germinated seeds under warm autumn conditions, while *T. bromi* infects the coleoptiles of emerged seedlings under cold winter conditions. Finally, the

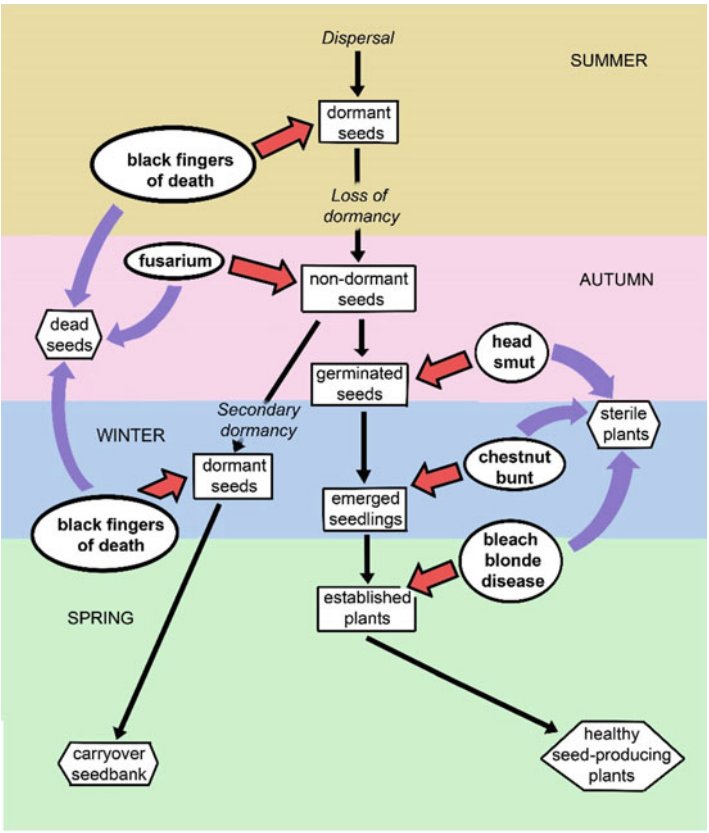

Fig. 7.6 Schematic diagram showing the relationship of each of the five pathogens discussed in the text to the stage of the *B. tectorum* life cycle when infection takes place, the season of infection, and the outcome of disease (seed death or sterility). *Black arrows* represent transitions between different *B. tectorum* life stages (*square boxes*); *red arrows* indicate life stage impacted by each of the five pathogens (*oval boxes*); *purple arrows* indicate pathogen impacts on final outcomes (*hexagonal boxes*). Pathogens are *Pyrenophora semeniperda* (black fingers of death), *Fusarium* sp. (*Fusarium* seed rot disease), *Ustilago bullata* (head smut disease), *Tilletia bromi* (chestnut bunt disease), and Rutstroemiaceae sp. n. (bleach blonde syndrome)

bleach blonde pathogen attacks the crowns of juvenile established plants. These pathogens thus clearly exhibit niche differentiation with regard to their mode of utilization of the *B. tectorum* host. There may be preemptive competition, in that host individuals killed at earlier stages in the life cycle are not available as prey for pathogens that operate at later stages. But as different weather scenarios favor infection at different life stages, opportunities for high levels of disease for each pathogen tend to be separated either in time (among years) or space (different habitats).

We do have some evidence that these pathogens can sometimes interact synergistically to increase the negative impact on *B. tectorum* stand dynamics. Specifically, epidemic levels of the bleach blonde syndrome can be associated with very high

levels of *P. semeniperda*-caused disease, resulting in greatly diminished seed production combined with minimal seed carryover (J. Pearce, unpublished data). In addition, the dense, thick litter created by the bleach blonde pathogen may create conditions conducive to the success of the *Fusarium* seed rot organism the subsequent year. In small plot studies in an area with variable levels of bleach blonde disease, we found a significant correlation between bleach blonde disease levels in the first year and stand failure the following year (J. Pearce, unpublished data). It appears that stand failure is statistically much more likely to take place in areas that have been impacted by bleach blonde disease the previous year. We hypothesize that this could be due to the nutrient composition of the bleach blonde litter, which may be high in labile carbon that can release *Fusarium* spores from fungistasis and permit the development of epidemic levels of seed rot disease (Lockwood 1977; Garbeva et al. 2011; Bonanomi et al. 2007, 2013). This hypothesis needs to be rigorously tested using multiple research approaches. If it proves to be correct, it implies that *B. tectorum* die-off occurrence could be manipulated by manipulating levels of labile carbon in the surface litter.

The fungistasis hypothesis could also explain why *B. tectorum* die-offs tend to be transient phenomena. We have found in field sowing experiments that *B. tectorum* has no difficulty establishing the year following a die-off as long as seed supply is not limiting (Meyer et al. 2013a, b). The die-off pathogen is undoubtedly still present, but perhaps the high-nutrient litter condition that permitted the epidemic does not persist, and the soil microbial community once again imposes fungistasis on *Fusarium* spores. If there are seeds in the carryover seed bank, the *B. tectorum* stand can reestablish the following year.

Our current understanding of the successional processes that sometimes cause die-offs to become more persistent focuses on the carryover seed bank and the supporting role of *P. semeniperda*. Litter dynamics once again appear to be key to this process (Beckstead et al. 2012). Without an adequate carryover seed bank to establish a stand, lack of *B. tectorum* cover the second year can result in litter loss (Smith et al. 2008). Die-off soils that lose their litter cover are often colonized by dicot weeds that are adapted to colonize bare soil, namely, *Salsola tragus* L. (prickly Russian thistle), *Sisymbrium altissimum* L. (tall tumblemustard), *Bassia scoparia* (L.) A.J. Scott Show (burningbush or ironweed), and *Ceratocephala testiculata* (Crantz) Roth (bur buttercup or curveseed butterwort). These species in turn may create litter that can promote recolonization by *B. tectorum*, but this process may take several years. The rate of *B. tectorum* recovery may also depend on whether the dicot weeds were present in the seed bank or must disperse in. Most of these are "tumbleweeds" that are effectively dispersed into the openings created by die-offs. The size of the die-off area may also be a factor in recovery rate because of increased dispersal distance for *B. tectorum* in larger die-offs.

In the course of our investigations of *B. tectorum* stand failure, we have encountered several other soilborne fungal pathogens whose impacts are still not known. Many of these, such as *Alternaria* spp., were only weakly pathogenic in laboratory tests and were subject to complete suppression in the presence of *Fusarium* (Pearce and Beckstead, unpublished data). The exception was *Epicoccum nigrum*, which

caused *B. tectorum* seed mortality under water stress in the laboratory at levels comparable to moderately pathogenic *Fusarium* strains (Poh and Saunders, unpublished data). It also suppressed *Fusarium* in co-inoculations. *Epicoccum* has also been isolated from killed seedlings and juvenile *B. tectorum* plants from the field and may be another important player in the die-off phenomenon. We have too little information to speculate further on what its role might be.

7.4 Management Implications

The main lesson to be learned from studies of *B. tectorum* disease organisms is that these pathogens exist in dynamic equilibrium with *B. tectorum* and likely have a long evolutionary history on this host. While catastrophic stand failure can occur, it is much more common for diseases to persist long term at endemic levels that fluctuate as a function of yearly weather patterns and population levels of other microorganisms, including other pathogens, in the soil. A "good" pathogen does not drive its host to local extinction because that often implies its own local extinction as well. But even when this happens, the high seed dispersal capability of *B. tectorum* means that reinvasion is just a matter of time. The pathogens find a way to reinvade as well, either on seeds or via wind or animal dispersal, or to persist in the soil.

The management goal in *B. tectorum*-infested rangelands is ultimately to replace these annual grass monocultures with perennial plant communities, preferably native communities, that offer higher resource value in terms of soil and watershed protection, biodiversity, carbon sequestration, and forage production. The study of *B. tectorum* disease epidemiology offers two possible approaches to achieve this goal. The first is to use artificially produced inoculum of a pathogen in a short-term mycoherbicidal strategy to temporarily knock down *B. tectorum* populations in the context of restoration seeding (Meyer et al. 2008b). The idea is to create disease epidemics that can provide a window for native seedlings to establish. This approach has been investigated for two of the pathogens discussed above, namely, *U. bullata* and *P. semeniperda*. For *U. bullata*, the problem of the narrow infection window and the requirement for warm temperatures during coleoptile infection would preclude its successful use in most of the environments where *B. tectorum* is a problem. For *P. semeniperda*, we have sometimes achieved complete mortality of the carryover seed bank with inoculum augmentation, but this biocontrol method must be combined with some other method for control of the current-year stand in order to be effective, greatly limiting its usefulness.

The second approach for using *B. tectorum* disease as a management tool is more promising. The idea is to take advantage of naturally occurring stand failure as an opportunity for restoration seeding or to manipulate conditions in the field to cause die-offs that can then be used as restoration opportunities. This would eliminate the need for inoculum augmentation, which is the most difficult and controversial component of mycoherbicidal biocontrol. We have already learned that die-offs are usually transient phenomena followed by a period with much reduced disease potential.

We have also learned that die-offs can create seed bed conditions conducive to the emergence and early establishment of native grasses (Baughman 2014). By developing a more detailed mechanistic understanding of the causes and consequences of die-offs, we may be able to develop a strategy for the successful restoration of *B. tectorum*-infested rangelands.

7.5 Research Needs

While we have made a great deal of progress in understanding the diseases that can affect *B. tectorum* stand dynamics in the last 15 years, much remains to be accomplished before these diseases can be successfully exploited in a management context. Specifically, we need to undertake an integrated research program aimed at understanding the full complexity of the *B. tectorum* seed bank microbial community, including direct and indirect interactions of each pathogen with other pathogens, with other microorganisms, and with microenvironmental factors such as litter dynamics and composition, all of which can affect disease incidence as well as post-die-off successional trajectories. This includes detailed studies of the systematics, ecology, and genetics of the pathogens involved, as mentioned in earlier sections. A more landscape-level approach that examines *B. tectorum* stand dynamics in space and time, especially as affected by epidemic disease and its interactions with interannual weather variation, would also be a valuable addition to the research program.

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