Evidence for resistance polymorphism in the *Bromus tectorum – Ustilago bullata* pathosystem: implications for biocontrol

S.E. Meyer, D.L. Nelson, and S. Clement

Abstract: *Bromus tectorum* L. (cheatgrass or downy brome) is an important exotic weed in natural ecosystems as well as in winter cereal cropland in semiarid western North America. The systemic, seedling-infecting head smut pathogen *Ustilago bullata* Berk. commonly infects cheatgrass stands, often at epidemic levels. We examined factors controlling *U. bullata* infection levels in greenhouse studies with parental lines of four *B. tectorum* populations from contrasting habitats and *U. bullata* bulk teliospore collections from within the four populations. The *U. bullata* infection process appeared to have broad environmental tolerances, so that it was relatively simple to develop a protocol for obtaining high infection percentages in susceptible lines. *Bromus tectorum* populations generally showed highest infection levels when inoculated with locally collected *U. bullata* teliospores. This effect was most marked for the warm desert population, which was completely resistant to *U. bullata* collected from other areas, but 100% susceptible to locally collected inoculum. Two of the four populations showed major differences in susceptibility among parental lines, with the differences most pronounced when nonlocal inoculum was used. In preliminary trials with paired monosporidial isolates, two paired isolates infected all nine inbred lines to levels near 100%, while a third paired isolate was pathogenic on only five of the nine lines. These results demonstrate resistance polymorphism both among and within *B. tectorum* populations. This polymorphism may be important in developing strategies for the use of *U. bullata* as a biocontrol agent for *B. tectorum*.

Key words: cheatgrass, disease, downy brome, pathogen races, smut, virulence.

Résumé : Le *Bromus tectorum* L. (brome des toits) est une mauvaise herbe introduite, importante tant dans les écosystèmes naturels que dans les champs de céréales d'hiver des zones semi-arides de l'ouest de l'Amérique du Nord. L'*Ustilago bullata* Berk., un agent du charbon de l'épi qui attaque les plantules, infecte régulièrement les populations de brome des toits, souvent à un stade épidémique. Nous avons étudié les facteurs qui déterminent les niveaux d'infection par l'*U. bullata* dans des études en serre avec les lignées parentales de quatre populations de *B. tectorum* provenant d'habitats variés et de groupes de téliospores récoltées en vrac à l'intérieur des quatre populations. Le processus d'infection par l'*U. bullata* semble avoir une marge de tolérance environnementale importante, ce qui fait qu'il a été relativement simple de développer un protocole pour obtenir un pourcentage élevé d'infection des lignées sensibles. En général, les populations de *B. tectorum* montraient les niveaux d'infection les plus élevés lorsque inoculées avec les téliospores d'*U. bullata* récoltées localement. Cet effet était plus spécialement observé pour les populations de désert chaud qui étaient entièrement résistantes aux *U. bullata* d'autres zones, mais 100% sensibles à l'inoculum récolté localement. Deux des quatre populations ont montré des différences majeures de sensibilité entre les lignées parentales, les différences les plus marquées étant observées lorsque l'inoculum non local était utilisé. Dans des essais préliminaires avec des isolats monospores jumelés, deux isolats jumelés ont infecté les neuf lignées autofécondées à des niveaux approchant les 100%, alors qu'un troisième isolat jumelé n'était pathogène que sur cinq des neuf lignées. Ces résultats démontrent un polymorphisme de la résistance entre et à l'intérieur des populations de *B. tectorum*. Ce polymorphisme peut être important lors du développement de stratégies visant à utiliser l'*U. bullata* comme agent de lutte biologique contre le *B. tectorum*.

Mots clés : brome des toits, maladie, races pathogènes, charbon, virulence.

Introduction

Members of the Ustilaginales are among the few systemic plant pathogens whose hosts have been shown to exhibit race-specific resistance (Burdon et al. 1996). Race-specific host resistance has been well documented for many species of *Ustilago*, including *U. hordei* (Pers.) Lagerh., *U. tritici* (Pers.) Rostr., *U. avenae* (Pers.) Rostr., *U. kolleri* Wille, and *U. nigra* Tapke, that cause diseases in small grain crops (Fischer and Holton 1957; Cherewick 1958; Holton and Halisky 1960; Halisky 1965; Christ and Person 1987; Knox et al. 1999). Fischer and colleagues (Fischer 1940; Meiners and Fischer 1953) established the existence of race-specific resistance to *Ustilago bullata* Berk., a

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pathogen with a wide host range including several genera of grasses, and provisionally defined 12 pathogenicity races based on among-species patterns of resistance and susceptibility. They also determined that different populations within species can exhibit different patterns of race-specific resistance, indicating that the races they had defined were in reality complex genetic entities (Kreizinger et al. 1947). In the study reported here, our goal was to determine whether the *U. bullata* host *Bromus tectorum* L. (cheatgrass or downy brome) exhibits race-specific resistance polymorphism, either among populations or among lines within populations of this obligately inbreeding species (McKone 1985; Novak et al. 1991).

Bromus tectorum was introduced into western North America approximately a century ago (Mack 1981). It increased rapidly in the wake of severe overgrazing on western rangelands, and represents perhaps the most significant plant invasion in the modern history of North America (D'Antonio and Vitousek 1992). Increased fire frequencies caused by the continuous fine fuel associated with high *B. tectorum* cover have resulted in dominance by this weedy annual grass over tens of millions of hectares (Whisenant 1990). Once *B. tectorum* has achieved dominance in an area, it is very difficult to reestablish native vegetation or even introduced forage grasses via direct seeding. To establish a stand of seeded species under these circumstances, some form of *B. tectorum* control is necessary (Monsen 1994). Current methods include early-season burning to destroy seed prior to shatter, postemergence tillage in fall, and herbicides such as soil sterilants (Evans et al. 1970) or preemergents (S. Monsen, personal communication). These methods generally suffer from high risk, low reliability, high cost, and disruption of remnant natives. An effective biocontrol method that could target *B. tectorum* specifically, with little or no risk to cooccurring native species, would be a valuable tool in the ongoing effort to restore native plant communities that have been supplanted by this weed. The objective would be short-term control through local prevention of seed production, to be carried out in conjunction with restoration seeding. We are examining whether *U. bullata*, a pathogen that commonly occurs on cheatgrass and often reaches epidemic proportions (Warg 1938; Stewart and Hull 1949; Fischer 1940; Mack and Pyke 1984; Gossen and Turnbull 1995) might provide such a biocontrol method.

Ustilago bullata is a systemic, seedling-infecting pathogen that proliferates in the mycelial state with few or no symptoms during vegetative growth of the host but that sporulates in the inflorescence, preventing seed set (Fischer and Holton 1957). The diploid teliospores are dispersed along with seeds of adjacent uninfected plants and remain in the soil or on the seeds until conditions are suitable for germination. They germinate to produce basidiospores through meiosis. These haploid cells are capable of yeast-like saprophytic proliferation under favorable conditions to produce large numbers of sporidia. Sporidia of opposite mating type fuse to form the obligately biotrophic dikaryon that can ultimately cause head smut disease.

We approach our study of the *U. bullata* – *B. tectorum* pathosystem by first examining the genetics of host resistance. Our hypothesis is that, if different genotypes within a population of *B. tectorum* are susceptible to different races within the cooccurring *U. bullata* populations, frequency-dependent selection (May 1990; Frank 1993; Leonard 1997) might be an important factor preventing local extinction of *B. tectorum* as a consequence of a head smut epidemic.

In frequency-dependent selection, abundance of a particular pathogen race results in scarcity of susceptible host lines, which in turn results in scarcity of the pathogen race that attacks them, which in turn is followed by abundance of those susceptible host lines. In other words, genotypes at high frequency in host and pathogen are selected against, while those at low frequency undergo positive selection. As long as there is a fitness cost to excessive pathogenicity in the pathogen, the outcome is balanced polymorphism, i.e., long-term coexistence of multiple resistance genotypes in the host and multiple races in the pathogen. If frequency-dependent selection operates in this way, it may be possible to cause local extinction of *B. tectorum* through application of *U. bullata* inoculum in which all pathogen races are abundant.

The research presented here was carried out in three phases. In the first phase, we examined environmental factors that might affect infection in susceptible host lines, to develop protocols for obtaining consistently high infection of suscepts. In the second phase, we compared greenhouse infection percentages for host lines from each of four *B. tectorum* populations, using *U. bullata* bulk inoculum collected from within each population in the field. In the third phase, we inoculated *B. tectorum* lines from different populations with paired monosporidial isolates to determine whether isolates from a single *U. bullata* population would show contrasting patterns of pathogenicity. These investigations were designed to determine whether *B. tectorum* exhibits race-specific resistance polymorphism, either among or within populations.

Materials and methods

General experimental protocol

To represent the possible genetic diversity of *B. tectorum* and *U. bullata* for our study, four *B. tectorum* populations were selected (Table 1). These populations are those used by Meyer and Allen (1999) to study genetic variation in seed germination regulation. Greenhouse-produced seeds belonging to lines from these populations were used in most of the experiments in the present study. We also used greenhouse-produced seeds of lines from a Green River (GR), Utah, *B. tectorum* population (Meyer and Allen 1999; Table 1), because they were highly susceptible in preliminary trials, but no *U. bullata* teliospore collection was obtained from that population. For the four principal populations, a bulk collection of smutted panicles from at least 100 plants was made in the area where host lines had been collected. The exterior of paper bags containing the smut fungus was sprayed with 95% ethanol and double bagged before transporting to avoid cross-contamination. Teliospores were harvested by screening, air dried, and stored in glass vials at room temperature (21:17°C, day:night).

Plant growing medium consisted of volumetric proportions (4:3:2:2:1) of vermiculite, peat moss, sandy loam field soil, No. 20 silica sand, and Agsorb (a calcined montmorillonite clay; Oil-Dri Corp. of America, Chicago, Ill.). The

Table 1. Habitat and location information for five *Bromus tectorum* populations included in this study.

Population	Elevation (m)	Plant community type	Mean annual precipitation (mm)	Mean temperature (°C)		Location	
				January	July	County	State
Potosi Pass	1850	Blackbrush–juniper (warm desert margin)	250	1.7	26.5	Clark	Nevada
Whiterocks	1450	Shadscale (cold desert)	180	–2.3	25.8	Tooele	Utah
Hobble Creek	1800	Sagebrush – gambel oak (foothills)	400	–2.1	24.8	Utah	Utah
Green River	1280	Shadscale (cold desert)	150	–5.2	26.6	Emery	Utah
Strawberry	2400	Subalpine meadow	560	–7.8	16.1	Wasatch	Utah

medium was mixed and moisturized to approximately 25% field capacity and subjected to aerated steam at 103 kPA, 60°C for 60 min to selectively eliminate most pathogenic fungi and retain possible antagonistic actinomycetes and bacteria (Baker et al. 1967). Growing containers were six-cell (25 mm × 25 mm × 140 mm) "rootrainer books" (Spencer–Lemaire Inc. Ltd., Edmonton, Alta.). Books were held in redwood crates impregnated with copper napthanate (Baker 1957).

Inoculation trials were grown out under greenhouse conditions. Fluorescent lamps were used to provide supplemental lighting. *Bromus tectorum* requires vernalization, either as seeds or plants, to flower (Richardson et al. 1986). During the growth period prior to vernalization, day length was gradually reduced from 12 to 10 h and temperature from 19 to 15°C during the day and from 10 to 3°C at night. Four to six weeks at or below 4.4°C with a short day length (8 h) was used for vernalization. After vernalization, day length was gradually increased from 14 to 16 h and temperature from 21 to 30°C during the day and from 5 to 16°C at night.

Following seedling establishment, moisture level was held to a minimum, allowing plants to approach wilting point before watering to soil saturation. Supplemental fertilization was also minimal, consisting of one application of 100 ppm 20:20:20 of N:P:K plus micronutrients (Scotts – Sierra Horticultural Products Co., Marysville, Ohio) to soil saturation immediately after the vernalization treatment. After almost full development of the smut fungus, but prior to any rupturing of sori, the proportion of smutted plants was determined and plants were either harvested or bagged to prevent escape of teliospores.

Proportion of plants smutted was arcsine-transformed to increase homogeneity of variance prior to analysis of variance (ANOVA) appropriate to the experimental design for each experiment. In multiple-factor experiments, where each treatment combination was replicated only once, main effects ANOVA's were conducted using interaction variance as the error term (Zar 1984). Means separations were performed, using a Student–Newman–Keuls means separation test, only for the bulk inoculum trials.

Environmental factors

Multiple environmental factors

Five environmental factors that could influence infection levels were tested in a factorial design: (1) growth and development under low vs. high temperature, (2) vernalization as imbibed seed vs. young plants, (3) low vs. high fertility, (4) growing medium containing natural soil vs. a soilless mix, and (5) selective heat treatment by aerated steam to retain pathogen-suppressive microorganisms vs. untreated medium. In addition, both Whiterocks (WR) and Hobble Creek (HC) bulk inoculum sources were included in the trial. Twelve *B. tectorum* lines were included, six from WR and six from HC. One replication of six planted cells was used per line per treatment combination. An uninoculated control for each treatment combination was used as a check for smut contamination of seeds. The design was a multiple level split plot with development temperature as the main plot and subsequent factor plots hierarchically arranged within the main plot in the order listed above.

The inoculation method was adapted from the partial-vacuum method (Fisher and Holton 1957). Inoculum density was 0.1 g germinable teliospores per 100 mL water. Seed lots (>95% viability) of each host line were placed in vials and covered by the spore suspension. Vials were then placed in a vacuum desiccator jar that was in turn placed on an orbital shaker. While spores were kept in suspension by the shaker motion, a partial vacuum was drawn down to –172 kPa and released three times. During planting, vials were frequently agitated.

The native-soil-containing medium consisted of volumetric proportions (3:3:2:3:1) of peat moss, vermiculite, sand, sandy loam soil, and Agsorb. The soilless medium was commercial Sunshine No. 3 potting medium. The soil media were either subjected to aerated steam at 50°C for 30 min after moisturizing or not heat-treated.

Young plant vernalization was carried out for 2 weeks in the greenhouse and then 4 weeks in a lathhouse, with temperatures ranging from –6 to 18°C. For the low temperature regime, temperatures were set at 10:5°C, day:night, after vernalization, then ramped to 21:16°C, day:night. Day length was extended from 10 to 15 h. For the high temperature regime, temperatures were set at 21:10°C, day:night, and ramped to 30:18°C, day:night. Day length was extended from 12 to 16 h. The high fertility treatment consisted of an application to soil saturation of a liquid Scotts–Peters (20:20:20 plus micronutrients) solution at 1000 ppm immediately after vernalization and again shortly before plant heading. The low fertility treatment consisted of 100 ppm of the same fertilizer at the same growth states.

Inoculation method and bulk inoculum density

To arrive at an inoculum density threshold for near 100% infection of plants, two inoculum density dilution series

were made: (1) dry spores and (2) spores in water suspension. Two inoculum sources (HC and WR) were used to inoculate three highly susceptible GR host lines. Twelve (dry inoculation) or eight (wet inoculation) seeds from each line were bulked for a total of 36 or 24 seeds per density level.

The dry spore dilution series was made by weighing spores, allowing for a live spore adjustment. The highest density level was 0.005 g live spores in a 4 mL vial with 36 seeds. Five density levels were achieved by reducing the mass of spores per vial by 50% to level 5 at 0.0003 g per vial. A sixth level (0.00015 g) was approximated by doubling the seed number per vial. The water suspension dilution series was made by repeated 50% dilutions beginning with 0.01 g/20 mL water (adjusted for live spores). Using continuous agitation with a magnetic stir bar, this method was used to achieve 12 dilution levels. Twenty-four seeds were immersed in inoculum suspension for each density level. During planting, suspensions were agitated often.

These experiments were planted out in a randomized block design with three replications. For each replicate, the entire dilution procedure was repeated. Culture followed the standard, except plants were reared for 5 weeks prior to a 9-week vernalization treatment.

Vernalization regime variation

In this experiment on the effect of variation in vernalization regime, the goal was to determine the shortest experimental duration (prevernalization growth + vernalization period + postvernalization time to heading) that would permit full *B. tectorum* flowering without disrupting infection and disease development. We used a single, highly susceptible GR host line and a single inoculum source (HC). Thirty-six seeds were used per treatment. Dry live spore inoculum of 0.0025 g per 36 seeds was applied by vibration using a hydraulic sander with a clamp attachment. Plants were reared as described previously. Prevernalization growing times were 2, 4, or 6 weeks after planting. Plants were then vernalized for 4, 6, or 8 weeks. One set of plants was held in greenhouse vernalization having a wide day–night temperature fluctuation (0–18°C), while the other was held in a growth chamber with only a small fluctuation (3.3–4.4°C).

Soil pH

The influence of soil pH on infection was tested by two methods, formulation of a growing medium series of increasing pH and use of five native soils with a range of pH values. Growing media were formulated by combining varying proportions of peat moss (pH 4.2), vermiculite (pH 7.5), Agsorb (pH 3.9), silica sand (pH 6.9), sandy loam field soil (pH 6.9), and limestone (pH 8.6). Nine media were prepared, ranging from pH 4.0 to 8.0 in approximately 0.5 pH unit increments. Media were treated for 30 min at 60°C with aerated steam. Final pH was not changed after heat treatment. Field soils were mixed with vermiculite and silica sand (volumetric proportions: 3:2:1) to render them more amenable to container culture. Native soils used, with their amended pH, were: WR (7.9), Rush Valley (8.0), HC (6.1), Point of the Mountain (7.0), and Midway (6.0).

Three host lines (one each from WR, HC, and GR) and two inoculum sources (WR and HC) were included (only HC for the native soils test). A check treatment was included. Twenty-four seeds of each line were dry-inoculated with 0.0025 g live teliospores for each pH treatment.

Planting depth and mulch type

The effects of planting depth and surface mulch type on infection were tested in separate split-plot experiments with depth or mulch treatment as the main plot, using the standard protocol described above. For each test, six host lines (three each from WR and GR) were dry-inoculated with 0.0025 g of live HC inoculum per 36 seeds. Twelve seeds were used per treatment per line. For the planting depth experiment, seeds were planted at depths of 3, 6, and 12 mm. Duff covers with different leachate composition and pH values were tested for their influence on infection. Covers included ground *B. tectorum* duff, peat moss, elm leaf compost, No. 16 silica sand, vermiculite and ground coconut hulls. Duff was autoclaved at 121°C for 30 min. Seeds were placed in shallow depressions and covered with 6–7 mm of surface mulch.

Bulk inoculation trials

For the bulk inoculation trials, we inoculated seeds of six host lines belonging to each of four *B. tectorum* populations with *U. bullata* bulk teliospore collections (Table 1). Each line was inoculated with bulk inoculum from four pathogen populations, namely those cooccurring with each of the four host populations. These tests were made over a 2-year period. Each experiment was with a single pathogen population and consisted of two blocks with 18 plants per host line per block, with line nested within population. In the first set of experiments, HC and WR bulk teliospore were applied as dry inoculum at a density of 0.005 g of live spores per 36 seeds. The cultural regime was standard with the following exceptions. After emergence, plants were reared for 3 weeks, then vernalized in the greenhouse for 12 weeks. Fertilization was 500 ppm Scotts–Peters 20:20:20. In the second set of experiments, Strawberry (ST) and Potosi Pass (PP) bulk inoculum was used. All procedures were the same as the earlier test, except for an 8-week vernalization period and a 0.0025 g inoculum density.

Paired monosporidial isolate inoculation trials

We developed a technique for smut monosporidial culture to produce single genotypes of the smut. Teliospores of HC smut were atomized onto potato dextrose plus antibiotics agar. After sporidial proliferation, sporidia were collected aseptically, dispersed, and diluted in sterile water, then plated on water agar plates. Apparent single sporidia were marked and later, colonies were lifted from the plates and transferred to potato dextrose shaker culture. Monosporidial cultures were then mixed in all combinations and observed for dikaryon formation. Plus and minus mating strains were increased in broth culture to produce monosporidial inoculant. Three pairs of sporidial isolates of opposite mating types were mixed and used at three concentrations prepared by 10× dilutions of an arbitrary cultural high. An evaluation of the midlevel concentration was made using a hemocytometer. The average of ten 1/400 mm^2 was: pair 1 = 65.6, pair 2 = 76.5, and pair 3 = 53.6 sporidia. Two milliliter of the paired sporidial inoculum was added to vials containing 36 seeds. Just prior to planting, vials were vi-

Fig. 1. Infection percentage plotted as a function of log(inoculum density (g/100 mL)) for wet *Ustilago bullata* inoculum applied to *Bromus tectorum* seeds using the partial-vacuum method. The fitted line is a three-parameter Hill function fitted using iterative nonlinear regression.

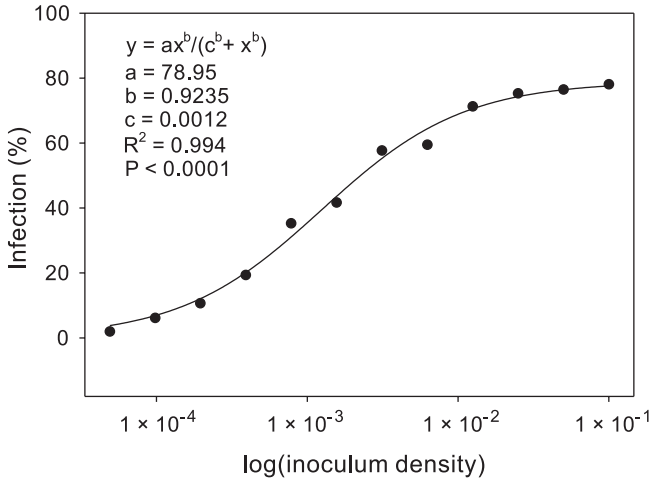
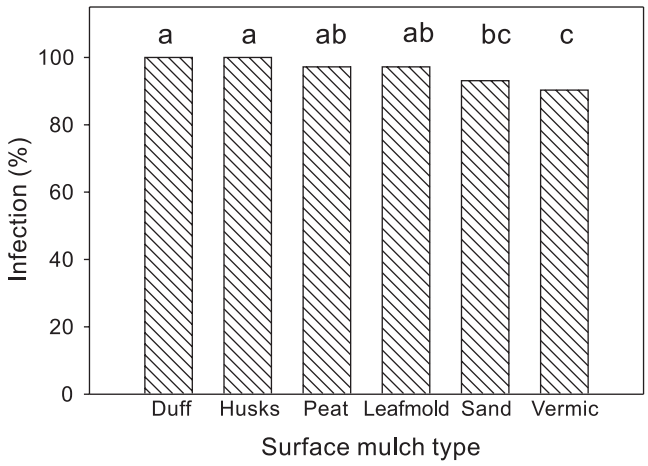


Fig. 2. The effect of surface mulch type on infection percentage following dry inoculation of six *Bromus tectorum* parental lines with Hobble Creek bulk inoculum. Bars headed by different letters are significantly different at the $P < 0.05$ level according to a Student–Newman–Keuls means separation test. Duff, ground *B. tectorum* duff; husks, ground coconut husks; peat, peat moss; leafmold, elm leafmold; vermic, vermiculite.



brated to disperse sporidia. Two replications of 12 seeds were used per treatment in a split-plot design with line as the main plot, paired sporidial isolate as the subplot, and inoculum concentration as the subsubplot. Planting depth was 5 mm. Following planting, the soil surface was sprinkled with water and covered with plastic film to slow drying. The test setup was held in the preparation room for 48 h at 21°C. Plants were then grown out for 3 weeks and vernalized at 4.4°C for 14 weeks.

Results

Environmental factors

The infection process proved to be largely unaffected by many environmental factors over the ranges that we studied. In the split-plot factorial experiment, mean infection percentage for 12 *B. tectorum* lines following inoculation with bulk teliospore inoculum from two sources was not significantly affected by soil mix, soil fertility, soil heat treatment, or temperature during development, though there was a nonsignificant trend for higher infection percentages at the cooler temperature (30 vs. 24%; $F = 2.9$, df = 1, 3, $P < 0.25$). The only significant factor was vernalization regime ($F = 426.8$, df = 1, 3, $P < 0.0001$). Plants vernalized as seeds showed very low infection percentages (3% overall), while plants vernalized after establishment showed much higher infection (55%). There was a marginally significant difference between inoculum sources (HC, 32%, vs. WR, 22%; $F = 11.7$, df = 1 and 2, respectively, $P < 0.10$). The overall difference among lines was highly significant ($F = 7.77$, df = 11 and 20 respectively, $P < 0.0001$), with 2 WR lines (40–42%) significantly higher than the other 10 lines (19–27%). No controls were smutted in this or any test, indicating excellent control of cross-contamination.

For teliospore inoculum applied in water suspension using the partial-vacuum method, the lowest inoculum level produced 1% infection, and infection percentage increased approximately exponentially with inoculum density over the first six densities (Fig. 1). It then levelled off at approximately 75%, regardless of further inoculum density increase. In contrast, inoculum applied as dry teliospores gave essentially full infection (mean 94%) at all six density levels.

In the experiment examining the effects of variation in vernalization regime, infection was not sensitive to the details of the regime, as long as vernalization took place after establishment. Regardless of plant age at initiation of vernalization, length of vernalization, or vernalization regime, mean infection percentage was 99%.

Soil pH had no significant effect on infection percentage over a remarkably broad range of pH. In the experiment utilizing five native soils with a pH range from 6 to 8.0, infection averaged 100%. In the experiment that utilized soilless media with pH from 4.2 to 8.6, infection averaged 93%, with no sign of a pH-associated trend.

Planting at depths of 3, 6, or 12 mm in the standard soil mix had no effect on infection percentage, which averaged 98%. When seeds were placed on the surface of the mix, then covered with different types of mulch, mulch type had a small but significant effect on infection percentage (Fig. 2). Intact organic mulches (cheatgrass litter and coconut husks) gave 100% infection, while inorganic mulches (vermiculite and sand) gave somewhat lower infection.

Bulk inoculum trials

There were major among-population differences in infection percentage in response to each of four *U. bullata* bulk inoculum sources (Table 2). WC and HC host lines were generally highly susceptible to both HC and WR bulk inocula, while ST lines were generally much less susceptible and PP lines were completely resistant. WR and ST lines were generally highly susceptible to ST bulk inoculum, while HC lines were less susceptible and PP lines were again completely resistant. PP lines were completely and uniformly susceptible to PP bulk inoculum. WR lines were

generally also highly susceptible to PP bulk inoculum, while ST and HC lines were much less susceptible. *Bromus tectorum* populations were generally most susceptible to locally collected bulk inoculum.

Paired monosporidial isolate inoculation trials

When seeds from nine lines representing three populations were inoculated with paired monosporidial isolates from the HC bulk inoculum, isolate pairs 1 and 3 gave essentially full infection on all nine lines (Table 3). Isolate pair 2 gave full infection on the three WR lines and on two of the three GR lines. The remaining two GR lines and all three HC lines were resistant to this isolate pair.

There was a small effect of inoculum concentration in the paired monosporidial line test (Table 3). Infection percentage was 84% for high, 80% for medium, and 66% for low concentration, averaged across all lines and isolates (F = 60.7, df = 2, 81, $P < 0.0001$). At the highest concentration, infection percentages for susceptible lines averaged 100%.

There were a total of three infected plants out of a possible 288 in two of the four lines that were resistant to isolate pair 2. These low levels of infection in otherwise resistant lines could mean that resistance is not absolute in these lines.

Discussion

Environmental factors

We obtained high levels of infection in susceptible *B. tectorum* lines over a wide range of environmental conditions. The two variables that emerged as most important in this success were inoculation method and vernalization regime. In contrast to the results of Fischer and Holton (1957), we obtained much higher infection levels using dry inoculation than with the traditional partial-vacuum inoculation method. This may be due to the anatomy of *B. tectorum* florets. The lemma surface is roughened with small appressed hairs, which cause dry teliospores to cling tightly, while the palea is thin and membranous. This is quite different from many of the perennial grasses in Fischer's experiments, which have smooth florets with much thicker, shell-like lemmas and paleas that may act as effective barriers. For those grasses, the sudden release of vacuum may result in a drawing in of teliospores under these barrier structures. In our case, using wet inoculation simply meant that most of the teliospores were left behind in suspension, while most of the dry teliospores were transported with the florets.

The failure to obtain infection when vernalization was carried out on seeds rather than plants is more problematic. *Bromus tectorum* is a facultative winter annual, which means that seed germination and establishment can take place any time from the first effective rains of early autumn though early spring (Mack and Pyke 1983). Mack and Pyke (1984) reported lower percentages of *U. bullata* infected plants in later-emerging cohorts in an eastern Washington field study, but levels in their populations were low overall, around 10%. Hulbert (1955) reported a similar result in row studies with a number of weedy bromes, i.e., that percentage of smutted plants was reduced in cohorts emerging from seeds planted later in the autumn. He attributed this result to inoculum depletion, but it could easily have been due to the effect we observed in our study. Falloon (1979) showed that, for a race of *U. bullata* on *Bromus willdenowii* Kunth, optimum temperature for teliospore germination was relatively high, over 20C. This is also true for *B. tectorum* seed germination (Christensen et al. 1996; Meyer et al. 1997). But *B. tectorum* has a low minimum for germination (0°C), so that seeds can germinate under winter conditions, albeit slowly. *Ustilago bullata* may have a higher minimum temperature, making winter infection unlikely. This may represent an obstacle to effective use of *U. bullata* as a biocontrol organism for *B. tectorum*.

The minor effect of surface mulch type that we observed was probably due to differences in degree of sporidial proliferation. The organic mulches may have provided a nutritional substrate for saprophytic proliferation or they may have improved moisture relations in the proliferation zone relative to inorganic mulches. This effect could be more important under inoculum-limited conditions likely to be found in the field.

Bulk inoculation trials

We obtained clear evidence for among-population resistance polymorphism in this pathosystem, in that PP lines were completely resistant to all but locally collected bulk inoculum, while lines of the other three populations showed some level of susceptibility to all four bulk inoculum sources.

We also obtained evidence for some within-population resistance polymorphism in the bulk inoculation trials, though this evidence was somewhat indirect. It is indicated by among-line differences in infection percentage within populations and bulk inoculum sources. We interpret low infection percentages as evidence for susceptibility to one or more pathogen races that were at low frequency in the bulk inoculum, while high infection percentages would reflect susceptibility to more abundant races. There was no evidence for among-line variation in resistance for the PP population, where all lines showed identical responses of either no infection or 100% infection (Table 2). This was also true for the HC population, where there were no significant differences among lines in response to any of the bulk inoculum sources. For the WR population, significant among-line differences were evident only in response to the ST and PP bulk inoculum sources. For the ST population, significant among-line differences were seen in response to all but ST bulk inoculum. ST lines 6 and 7 showed high infection percentages regardless of bulk inoculum source, while lines 3 and 4 showed low percentages for all but ST bulk inoculum.

The results of the bulk inoculum trials also provided a confirmation of the genetic distinctness of the Mojave Desert PP *B. tectorum* population. *Bromus madritensis* L. (formerly *B. rubens* L., red brome) is the abundant introduced annual brome in North American warm deserts, which have only very recently been invaded by *B. tectorum* (Beatley 1966). Mojave Desert populations may represent a unique and possibly recent introduction from a different part of the Old World range. The PP population has genetically determined seed germination regulation patterns uniquely suited to the warm desert (Meyer et al. 1997; Meyer and Allen 1999). Our recent studies have also shown that its vernalization requirement is essentially nil, also an adaptation to warmer winters (Meyer 1999). This population is also

Table 2. Percentage of smutted plants following bulk inoculation with each of four *Ustilago bullata* bulk teliospore collections for six lines belonging to each of four *Bromus tectorum* populations that were the source of bulk teliospore collection.

		Source of bulk inoculum				
Population	Line	Hobble Creek	Whiterocks	Strawberry	Potosi Pass	Mean
Hobble Creek	1	96	94	42	55	72
	2	90	90	68	72	80
	3	88	85	72	64	78
	4	94	74	83	67	80
	5	91	83	61	47	71
	6	100	81	66	67	79
	Mean	93 x	85 x	66 z	62 z	76
Whiterocks	1	90	62	64 c	97 a	78
	3	97	97	100 a	100 a	98
	4	97	96	84 bc	40 b	79
	7	100	94	89 bc	94 a	94
	8	94	97	97 ab	92 a	95
	10	93	85	75 c	97 a	88
	Mean	95 x	89 x	85 x	87 y	89
Strawberry	1	30 b	77 a	60	23 b	48
	3	38 b	48 b	80	13 b	45
	4	36 b	44 b	82	20 b	45
	5	64 b	80 a	85	97 a	81
	6	97 a	79 a	80	97 a	88
	7	100 a	88 a	86	88 a	91
	Mean	61 y	69 y	79 y	56 z	66
Potosi Pass	1	0	0	0	100	25
	5	0	0	0	100	25
	6	0	0	0	100	25
	7	0	0	0	100	25
	9	0	0	0	100	25
	10	0	0	0	100	25
	Mean	0 z	0 z	0 w	100 x	25

Note: Within a column (bulk inoculum source), population means or parental line means within a population that are followed by different letters are significantly different ($p < 0.05$ level) according to a Student–Newman–Keuls means separation test.

Table 3. Infection percentages for nine *Bromus tectorum* lines belonging to three populations, after inoculation with three sets of paired monosporidial isolates (PMSIs) from Hobble Creek *Ustilago bullata* bulk inoculum.

		Lines of *B. tectorum*								
		Whiterocks			Hobble Creek			Green River		
Isolate	Level	3	4	8	1	2	3	2	3	5
PMSI 1	High	100	96	100	100	100	100	100	100	100
	Medium	100	92	100	96	96	100	100	88	96
	Low	90	54	75	82	96	74	92	92	62
	Mean	97	81	92	93	97	92	97	93	86
PMSI 2	High	100	92	100	0	0	0	0	96	100
	Medium	96	79	100	8	0	0	4	100	80
	Low	66	62	79	0	0	0	0	71	50
	Mean	88	78	93	3	0	0	1	89	76
PMSI 3	High	100	100	100	100	100	100	100	100	92
	Medium	96	83	100	79	90	100	96	96	188
	Low	71	91	92	79	78	84	88	92	62
	Mean	89	91	97	86	89	94	94	96	80

Note: The PMSIs were applied in water suspension at three concentrations levels.

unique in our studies in having virtually no within-population variation for any trait, suggesting that it is made up of a single line. These results have recently been confirmed through the use of molecular microsatellite markers (A. Ramakrishnan, personal communication). The PP population had unique alleles at three of five loci, and all 10 in-

dividuals had identical marker fingerprints. This could explain their unique and uniform response to bulk inoculation.

Variation in response to bulk inoculation for the WR, HC, and ST populations was also concordant with microsatellite marker fingerprints for these populations, which shared most alleles (A. Ramakrishnan, personal communication). Each population appears to be composed of a small number of lines, some of which are common to two or three of the populations. Sometimes, members of different lines showed consistently contrasting responses to bulk inoculation, as in the ST population, while other times members of different lines had very similar responses, as in the HC population.

Paired monosporidial isolate inoculation trials

Results from the infection trials with pathogen isolates demonstrate within-population resistance polymorphism in two ways. First, the GR parental lines showed contrasting patterns of resistance to the isolates, with two lines susceptible to all three isolates and one line susceptible to only two (Table 2). Second, the three HC lines, all of which were highly susceptible to locally collected bulk inoculum, were resistant to one of the isolate pairs. This strongly suggests that there are at least two pathogenicity races in the bulk inoculum, the one to which these lines are resistant in this experiment, and the one to which they were highly susceptible in the bulk inoculations. The fact that this isolate came from HC bulk inoculum shows that there is at least one inbred line in the HC population that is susceptible to the pathogen race it represents.

The trials with pathogen isolates also support the hypothesis that variation in infection percentage among lines in response to bulk inoculation may be due to susceptibility to pathogen races present at different frequencies in the bulk inoculum. We demonstrated that the HC *U. bullata* population contains at least two pathogen races, and that its corresponding *B. tectorum* population must contain at least two resistance phenotypes. This makes it at least theoretically possible for frequency-dependent selection to operate.

Conclusion

Our results to date suggest that it may be possible to use *U. bullata* for biocontrol of *B. tectorum*, but it will be necessary to acquire more basic knowledge about the pathosystem before this idea can be tested in the field. Our goals now are: (1) to develop a pathogenicity-resistance matrix (Burdon 1994) that describes the races of *U. bullata* that are pathogenic on different subsets of all the known *B. tectorum* lines in these four populations, (2) to examine the relative abundance of different *B. tectorum* lines in the smutted and unsmutted subsets of field populations across years using microsatellite marker fingerprints, to test the hypothesis of frequency-dependent selection, and (3) to critically examine environmental conditions necessary for *U. bullata* teliospore germination, sporidial proliferation, and infection, under laboratory, greenhouse, and field conditions.

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