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# EPIDEMIOLOGY OF *USTILAGO BULLATA* BERK. ON *BROMUS TECTORUM* L. AND IMPLICATIONS FOR BIOLOGICAL CONTROL

by

Toupta Boguena

A dissertation submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Integrative Biology

Brigham Young University

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BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a dissertation submitted by

Toupta Boguena

This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the dissertation of Toupta Boguena in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirement; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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**ABSTRACT**

EPIDEMIOLOGY OF *USTILAGO BULLATA* BERK. ON *BROMUS TECTORUM* L.
AND IMPLICATIONS FOR BIOLOGICAL CONTROL

Toupta Boguena

Department of Integrative Biology

Doctor of Philosophy

The seedling-infecting pathogen *Ustilago bullata* Berk. is a naturally occurring biological control agent for cheatgrass (*Bromus tectorum* L.). The effects of temperature and nutrients on pathogen teliospore germination behavior and the effects of temperature on host seed germination were examined. The effects of temperature on sporidial proliferation, host infection in a temperature-controlled environment and in a field setting for eight populations were investigated. The infection success of *Ustilago bullata* on *Bromus tectorum* in cultivated fields as a function of seeding date, inoculation method, inoculum density, supplemental watering, and litter was also investigated. Teliospores germinated faster on potato dextrose agar than on water agar. Teliospores germinated slowly at temperatures far from the optimum of 15 and $20^0$C. There were among-population variations in teliospore germination and sporidial proliferation, but differences among populations were much more pronounced at temperatures below $15^0$C. Infection

also decreased and varied far from the optimum with almost no infection at $2.5^{0}$C in a controlled-environment and in the field for the December-planted seeds. Warmer early fall rather than the colder late fall was suitable for successful infection. This agreed with both laboratory and controlled-environment experiments. Intratetrad mating was observed with teliospores at $2.5^{0}$C. Teliospore germination tracked seed germination closely with teliospore germination rate exceeding the host seed germination rate over the range of 10 to $25^{0}$C where both were measured. Below $10^{0}$C, teliospore germination rate fell below host seed germination. This phenomenon was associated with lower infection percentages, suggesting that teliospore germination needed to be ahead of the seed for maximum infection. Inoculum density was positively correlated with infection rate. Litter significantly increased infection, while supplemental watering significantly increased plant establishment. Since teliospores from different populations showed similar germination patterns at temperatures typical of autumn seedbeds in the Intermountain West, it may not be necessary to use locally-adapted pathogen populations in biological control program. A biocontrol program is most likely to be effective under a scenario where autumn precipitation permits emergence of most of the host seed bank as a fall cohort.

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# Chapter 1

## Effect of temperature on propagule germination in the *Ustilago bullata* Berk-*Bromus tectorum* L. pathosystem

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# ABSTRACT

The pathogen *Ustilago bullata* Berk. induces head smut disease of cheatgrass (*Bromus tectorum* L.) in the Intermountain region of the western USA. Despite the threat of epidemic posed by the pathogen, the host often escapes infection. We investigated the effects of temperature and nutrients on *Ustilago bullata* Berk. teliospore germination behavior, and the effects of temperature on *Bromus tectorum* L. seed germination. Co-occurring host and pathogen populations from eight locations were included in the study. Teliospore populations from eight habitats responded differently to temperature. Time to 50% (T50) teliospore germination decreased significantly as temperature increased over the range of 5 to15$^0$C and decreased slightly over the range of 15 to 25$^0$C. Germination differences among teliospore populations were much more pronounced at temperatures below 15$^0$C. Three desert populations had slower teliospore germination than other populations at low temperatures. Average time to 50% germination of *Bromus tectorum* seed decreased from 52 to 16 hours as temperature increased from 10 to 20$^0$C, remained level from 20 to 30$^0$C, and then increased to 31 hours at 35$^0$C. Among-population differences were more pronounced at lower and higher temperatures. Teliospore germination times tracked seed germination times closely over the range of temperature from 10 to 25$^0$C where both were measured. In general, pathogen teliospore germination rate exceeded host seed germination rate at temperatures above 10$^0$C. There was no correlation between optimum temperatures for seed or pathogen germination and habitat attributes. Teliospores not only germinated slowly at lower temperature (2.5$^0$C), but also failed to produce sporidia and instead sent out dikaryotic hyphae. This behavior suggests intratetrad mating at low temperatures. Teliospores also germinated faster on potato

dextrose agar than on water agar. Teliospores were probably responding to higher soluble nutrient availability in potato dextrose agar, a feature that would cue them to germinate quickly on germinating seeds. Our study suggests that teliospores from different populations show similar germination patterns at temperatures typical of autumn seedbeds. It may therefore not be necessary to use locally-adapted pathogen populations as cheatgrass biocontrol agents.

## INTRODUCTION

Plant pathogens must synchronize the activation of their infectious stage with the initiation of the susceptible phase of their hosts. Genetically susceptible hosts may escape infection when environmental requirements do not coincide with conditions for host susceptibility and pathogen infection (Agrios, 1997). For pathogens like *Ustilago bullata* Berk. to infect seedlings, the infectious stage must coincide with seed germination. Even under conditions where pathogen inoculum is not limiting, seeds of the susceptible host, *Bromus tectorum* L., can escape infection (Meyer et al., 2001). Environmental factors such as temperature or moisture in relation to germination requirements of both the host and pathogen could be responsible for inconsistent infection.

The head smut organism (*Ustilago bullata*) is a systemic seedling-infecting fungal pathogen that commonly infects cheatgrass populations and other grasses throughout the world (Falloon, 1979a; Zundel, 1953). Because it reduces cheatgrass populations in the field, it has potential as a biological control organism (Peeper, 1984). Its hosts include *Bromus*, *Festuca*, *Brachypodium*, *Agropyron*, *Hordeum*, *Elymus*, and *Sitanion*. With a life cycle typical of the family Ustilaginaceae (Alexopoulos et al., 1996), it produces dikaryotic teliospores that undergo karyogamy, and then germinate to produce promycelia and then basidiospores (Alexopoulos, 1952 and Bold et al., 1987). During germination, the promycelium normally emerges as a germ tube from a cracked diploid teliospore that has come out of dormancy (Alexopoulos, 1952). The diploid nucleus migrates to the promycelium and undergoes meiosis forming four haploid basidiospores. Basidiospores can either unite as compatible mating types while on the basidium and

produce the infection hypha, or they can proliferate mitotically to produce sporidia. Sporidia of compatible mating types may then fuse to penetrate the host as a dikaryotic hypha (Agrios, 1997). *Ustilago bullata* infects its host soon after emergence of the coleoptile from the seed (Falloon, 1979b). To effectively control cheatgrass, *Ustilago bullata* teliospores would need to germinate faster than the host seeds under field conditions and also would need to germinate readily under high nutrient conditions conducive to sporidial proliferation.

Cheatgrass is a winter annual grass introduced into western North America from Eurasia in the late 19$^{th}$ century (Mack, 1981). Very persistent, it is now the most common plant in the Intermountain region (D'Antonio and Vitousek, 1992). It invades and replaces native perennial vegetation especially after disturbance such as overgrazing and wildfires (Klemmedson and Smith, 1964). It is dominant on 40 million hectares of former shrublands and continues to invade new habitats from desert shrublands to higher elevation communities (Fleming et al., 1942; Billings, 1990; West, 1994). Its dominance is characterized by increased wildfire frequency resulting in loss of native vegetation, and livestock and wildlife habitat (Whisenant, 1990). The seeds of cheatgrass germinate under a wide range of temperature conditions in the field from late summer through early spring (Mack and Pyke, 1983).

Cheatgrass also possesses considerable adaptive genetic variation, both among and within populations, resulting in variation in flowering and seed germination phenology (Rice and Mack 1991). Patterns of among-population variation are clearly of adaptive significance (Beckstead et al. 1996; Meyer et al. 1997). Cheatgrass seeds are dormant at maturity and lose dormancy through dry-after ripening (Meyer and Allen,

1999a). Meyer and Allen (1999a,b) found variation among and within populations with relation to dormancy loss that could be attributed to genetic and ecological controls. Meyer et al. (1997) speculated on the risk of premature summer germination at various sites based on the level of dormancy at harvest time and the rate of dormancy loss during dry storage. *Ustilago bullata* is also known to exhibit among-population variation in teliospore germination response to temperature (Turnbull and Gossen, 2000). But it is not clear whether this variation is adaptively significant.

This study is part of a larger research effort examining environmental and genetic factors that limit field infection percentages in the *Ustilago bullata-Bromus tectorum* pathosystem. Our goal is to determine the feasibility of using *Ustilago bullata* as a biocontrol agent for *Bromus tectorum* in conjunction with ecological restoration of degraded semiarid shrubland communities.

The purpose of this research project is to examine host-pathogen-environment interactions before and during the infection window, including the effect of genetic variation in pathogen ecological tolerance. The research aims at understanding how temperature affects infection, and whether different ecotypes of the pathogen have different ranges of temperature tolerance for the various stages of the infection process. It specifically aims at determining how the pathogen times teliospore germination to coincide with host seed germination, and under what environmental conditions this synchronization fails. We hypothesized that non-dormant pathogen teliospores have temperature requirements for germination that parallel temperature requirements for host seed germination, and that teliospores of pathogen populations from contrasting habitats would show contrasting temperature responses for germination, in a pattern that parallels

ecotypic differentiation in temperature requirements for seed germination in the host. We also investigated the extent to which initiation of teliospore germination is regulated by nutrient availability.

# MATERIALS AND METHODS

## Population selection

*Ustilago bullata* teliospores were collected from a range of habitats in the Intermountain USA (Table 1). Habitats were selected to represent different temperature zones and elevations from warm to cold deserts to the foothills and the mountains.

## Teliospore germination

The effect of temperature on teliospore germination of *Ustilago bullata* was tested by harvesting teliospores at the study sites and assessing them for germination under a range of temperatures. Teliospores were collected from heads of the host placed in sealed paper bags, and later screened to release spores. Collections were stored in sealed vials at room temperature for at least 16 weeks until fully non-dormant. Spores were suspended in 1% sterile Tween 80 and sprayed onto slides coated with potato dextrose agar (PDA) using a vaporizer. The slides were set on U-shaped glass rods laid on wet filter papers inside sterilized glass petri dishes. The petri dishes were in turn placed in growth chambers at 2.5, 5, 10, 15, 20, and $25^{0}$C. The slides were read at different time intervals from 4 hours to 984 hours depending on temperature. There were two slides per read time per temperature per teliospore collection. Ten randomly selected fields of view on each slide were read with a compound microscope at 200-magnification level. The slides were also photographed at different time intervals at two temperatures (2.5 and $25^{0}$C) using a compound microscope with a mounted Canon camera.

**Seed germination**

Cheatgrass seeds were collected from the same locations as the teliospores (Table 1). The four southern populations (Potosi Pass, Hobble Creek, Strawberry Reservoir, and Whiterocks) were from four lines, each from a single maternal parent, that were greenhouse grown and the four northern populations were individual maternal lines from the field. Inflorescences from mature plants were collected at random, stored at room temperature until fully after-ripened (at least 6 months), and then cleaned for the experiment.

Seed germination percentage was assessed by incubating the seeds at the following temperatures: 10, 15, 20, 25, 30, and 35$^0$C. Ten seeds were set on wet filter papers in petri dishes and placed in incubators. The dishes were replicated five times for each temperature and read at 24, 48, 72, and 96 hours. Radicle emergence to 1mm was the germination criterion.

**Teliospore nutrient requirements**

Fully after-ripened (non-dormant) teliospores from the eight study sites were sprayed on slides coated with either water agar (WA) or PDA and placed in incubators at 15 and 25$^0$C. The slides were read after 24 hours of incubation using a compound microscope. There were two slides per temperature per medium per teliospore collection. There were five fields of view read per slide for each read time using a compound microscope at 200-magnification level.

**Data analysis**

We used analysis of variance (ANOVA) to determine germination responses of eight pathogen teliospore populations to temperature. For each of two replications for

each treatment combination, the proportions of spores germinating after a given time interval in each of ten passes across the slide were averaged and treated as a single replicate. These proportions were then expressed in terms of total viable teliospores by dividing by the proportion of viable spores. Germination proportion after 48 hours at 25$^0$C was used as the estimate of maximum viability; this value varied from 0.80 to 0.95 depending on the teliospore population. Because of very slow, erratic, and abnormal germination of teliospores at 2.5$^0$C, germination response at this temperature was excluded from the statistical analysis.

In order to compare results from temperatures that resulted in widely differing teliospore germination time courses, we used time to 50% germination of total viable teliospores (T50) as the response variable. The T50 values were derived by linear interpolation from the germination time courses for each treatment replication and subjected to two-way ANOVA for a completely randomized design with teliospore population and temperature as fixed main effects. Main effects means separations were carried out using the Ryan Einot Gabriel Welsch (REGWQ) procedure (Quinn and Keough (2002). We also carried out means separations within each temperature, using the REGWQ procedure and the error mean square from the overall experiment as the error term. We treated temperature as a class variable in the analysis because: 1) the response to temperature was strongly nonlinear and 2) we were interested in examining differences among populations at specific temperatures. We then graphically examined the relationship between mean germination rate (defined as 1/T50) and temperature for each teliospore population.

The germination data from the 2.5$^0$C regime were analyzed with ANOVA using

germination proportion at 984 hours as the response variable. Data were converted to proportion of viable spores and arcsine square root transformed prior to analysis and REGWQ mean separation.

We also used ANOVA to examine the germination response of eight host seed populations, with population and temperature as fixed main effects. Temperature was again treated as a class variable for the reasons outlined above. T50 of viable seeds was used as the response variable. T50 was derived by linear interpolation on seed germination time courses. Means were separated as described above for the experiment with pathogen teliospores. We also carried out mean separations within each temperature, using the REGWQ procedure and the error mean square from the overall experiment as the error term. We then graphically examined the relationship between mean germination rate (1/T50) and temperature for each of the eight seed populations.

For the experiment examining the effect of teliospore population, nutrient medium, and temperature on teliospore germination, germination percentage (expressed as percentage of total viable spores as explained above) at 24 hours was the response variable. The experiment was analyzed as a completely randomized design with population, temperature, and medium as fixed main effects. Germination percentage (proportion) was arcsine-square root transformed to improve homogeneity of variance prior to analysis. The REGWQ procedure was used to separate treatment means within populations, using the error mean square from the overall experiment as the error term. We used correlation analysis to examine whether teliospore and seed germination responses of the eight populations were correlated and whether teliospore population responses were correlated across temperatures.

# RESULTS

## Teliospore germination response to temperature – mean germination time

An ANOVA of T50 for the eight fully ripened teliospore populations indicated highly significant ($P < 0.0001$) differences among populations, temperatures and their interaction (Table 2). There was nearly a two-fold difference in germination rate between fastest and slowest teliospore collections (Table 3). Time to 50% differences among teliospore populations were significant for temperatures at or below $15^0$C (Table 3). The three desert populations collected from cold to warm climates (Whiterocks, Moses Lake, and Potosi Pass), had significantly slower teliospore germination at 5 and $10^0$C than the other five populations. Whiterocks and Moses Lake teliospores germinated significantly more slowly than Potosi Pass teliospores at $5^0$C (Table 3). Teliospore germination was very slow at $2.5^0$C. The germinating teliospores displayed intratetrad mating by producing dikaryotic hypha directly with no sporidial proliferation (Fig. 1). There were among-population differences in teliospore germination percentage at 984 hours ($F = 50.50$, model df = 7, error df = 151, $P < 0.0001$) (Fig. 2). Teliospore mean germination time at $5^0$C was significantly correlated with mean germination time at $10^0$C ($r=+0.843$, d.f. =6, $P<0.02$), showing that the trend for slower germination of desert pathogen populations was evident at both temperatures. This trend was also somewhat evident in the $2.5^0$C teliospore germination data, where the three desert populations were among the four populations with the lowest germination percentages after 984 hours (Fig. 2). This correlation was not significant overall because a middle elevation population,

Sagehen Hill, had anomalously high germination in the cold and was an outlier in the correlation analysis.

**Cheatgrass seed germination response to temperature – mean germination time**

Time to 50% of eight fully after-ripened cheatgrass seed populations varied significantly ($P < 0.0001$) among populations and temperatures, and the interaction was also significant (Table 4). Overall, mean germination time decreased from 52 to 16 hours, as temperature increased from 10 to 20$^0$C, remained level from 20 to 30$^0$C, and increased to 31 hours at 35$^0$C (Table 5). Overall, seven of eight populations were not significantly different in their mean germination times. Only the Potosi Pass populations showed significantly slower germination than the other populations. It was the slowest to germinate at every temperature except 10$^0$C, and was also the population that failed to reach 50% germination at 35$^0$C.

The differences among populations were least pronounced at temperatures from 20 to 30$^0$C, with very little variation other than the longer times for Potosi Pass. The differences increased as temperature increased or decreased away from the optimum (Table 5). Just as with the teliospores, the three desert populations from cold to warm climates (Whiterocks, Moses Lake, and Potosi Pass), tended to be slower at low temperature than the other five populations (Table 5). There was a significant positive correlation between pathogen population mean teliospore germination time at 5$^0$C and host population seed mean germination time at 10$^0$C ($r=+0.748$, d.f. $=6$, $p<0.05$). The trend for desert populations of both the pathogen and the host to germinate more slowly at lower temperature than those from foothill and mountain populations was the basis for this correlation (Tables 3, 5).

**Comparison of seed and teliospore germination rates**

Overall, teliospore germination rates tracked seed germination rates from 10 to $25^0$C (Fig. 3). At $10^0$C, the two rates were similar, while mean germination time for the pathogen was shorter than for the host from 15 to $25^0$C. Pathogen germination rate increased linearly as a function of temperature from 10 to $25^0$C, and the slope of this increase was much steeper than the slope of the rate increase for seed germination. The difference in rate between host and pathogen increased from zero at $10^0$C to a maximum at $25^0$C. The different host populations showed different temperature optima for germination, where optimum is defined as the lowest temperature at which the maximum germination rate is observed (Fig. 4). Buckskin Canyon had an optimum temperature for germination of $15^0$C, Arrowrock, Hobble Creek, Potosi Pass, and Strawberry of $20^0$C, Sagehen Hill and Whiterocks $25^0$C, and Moses Lake $30^0$C. These temperature optima were not correlated with climate. Most of the pathogen populations tended to show a linear increase in germination rate as a function of temperature from 10 to $25^0$C. Only Arrowrock and Moses Lake showed a definite leveling off in rate above $20^0$C.

Pathogen germination rate exceeded host germination rate at $25^0$C for all population pairs (Fig. 4). At $20^0$C, only the Whiterocks pathogen population had a slower germination rate than its host population. At $10^0$C, all host-pathogen population pairs had closely similar germination rates except Hobble Creek, whose pathogen germination rate exceeded its host germination rate.

**Nutrient availability and teliospore germination**

Teliospore germination percentage after 24 hours differed significantly for populations, temperature, media and their interactions (Table 6). Teliospore germination

was consistently higher on PDA than on WA across both temperatures (Fig. 5). Germination was much higher at $25^0$C than at $15^0$C with incubation on PDA. The responses to temperature on WA depended on population. WA germination was higher at 15 than $25^0$C for four populations, lower at 15 than $25^0$C for one population, and did not vary as a function of temperature for the remaining populations. Thus, when nutrients were not limiting, germination was faster and increased with temperature. When nutrients were limiting, germination was much slower and generally did not increase with temperature.

# DISCUSSION

## Teliospore germination response to temperature

Teliospores of *Ustilago bullata* collected from the eight sites responded differently to incubation temperature (Tables 2 and 3). Turnbull and Gossen (2000) attributed the difference between two pathotypes of *Ustilago bullata* more to the timing of germination than the rate of development. In our study, at the optimum temperature range for germination of *Ustilago bullata* teliospores (20 to 25$^0$C), all populations had similar rapid germination (Table 3). Turnbull and Gossen (2000) also observed the highest percent germination and growth for the two pathotypes of *Ustilago bullata* they studied between 20 and 25$^0$C. Differences in response to temperature became larger among teliospore populations as the temperature grew colder (Table 3). Mean germination time decreased significantly as temperature increased over the range 5 to 15$^0$C. Turnbull and Gossen (2000) found that pathotypes of Ustilago *bullata* were all less tolerant of high and low temperatures during teliospore germination. Similar results were observed with teliospores of *Gymnosporangium fuscum* (Hilbert et. al., 1990) and those of *Tilletia caries* (Bhuiyan and Fox, 1989), where germination dropped as the distance from optimum increased. Teliospores of *Tilletia caries* were found to germinate best at 18 to 20$^0$C with the germination decreasing as the temperature decreased (Bhuiyan and Fox, 1989). Spores of *Cercosporidium personatum,* which causes the late leaf spot disease of groundnut had an optimum temperature range for germination between 15 and 20$^0$C (Reddy and Subbayya, 1987). In Falloon's (1979c) study, the optimum temperature requirement of *Ustilago bullata* in culture was 25$^0$C with

a minimum of 12.5$^0$C (the lowest temperature he measured) and a maximum of 30$^0$C. In the current study we observed that *Ustilago bullata* teliospores can still germinate at temperatures as low as 2.5$^0$C but germination was extremely slow. Whiterocks, Moses Lake, and Potosi Pass, which are the three desert habitats included in the present study had slower teliospore germination than the populations from cooler, wetter climates as the temperature fell below 15$^0$C (Table 3). *Gymnosporangium fuscum* teliospores did not germinate at 5 and 30$^0$C with temperatures between 15 and 20$^0$C being the optima (Hilbert et al., 1990).

*Ustilago bullata* teliospores produced sporidia at optimum and near optimum temperatures while growing on PDA solid medium (Fig. 1). At 2.5$^0$C, practically no sporidia were produced; only dikaryotic hyphae were present (Fig. 1). This suggests intratetrad mating where early fusions of compatible mating types happen among the first products of meiosis in teliospore germination. Fischer (1940) found in his 28 collections of *Ustilago bullata* that five failed to produce sporidia of one of the two mating types, a phenomenon which he later attributed to haplo-lethal deficiency mutations that prevented saprophytic development. Presumably these populations reproduce through intratetrad mating regardless of temperature. Falloon (1979c) found the number of dikaryons formed by *Ustilago bullata* teliospores to be lower at higher super optimal temperatures than at lower optimal temperatures. This suggests that there was more intratetrad mating at high temperatures. Thus, there seems to be a switch from sporidial proliferation to intratetrad mating at temperatures far from the optimum, both at super optimal temperatures (as in Falloon's study) and at sub optimal temperatures (as in our study).

Kaltz and Shykoff (1997) working with the smut fungus, *Ustilago violacea* (which infects flowers), believed that intratetrad mating must be very important in successful infection as it offered quick infection hyphae that could infect rapidly at the crucial moment before the host flowers fell off. However, in our trials, teliospores of *Ustilago bullata* failed to infect the host at temperatures below $5^0$C despite the exclusive intratetrad mating that we observed at $2.5^0$C (dissertation, chapter 2). Intratetrad mating thus appears to represent a disadvantage for *Ustilago bullata*, which is a seedling-infecting fungus.

Hood and Antonovics (2000) demonstrated that patterns of teliospore germination and dikaryon formation were crucial to the ecology and breeding of *Ustilago violacea*, which causes anther-smut disease of species in the Caryophyllaceae. Hood and Antonovics (1998, 2000) also demonstrated that these patterns were greatly altered by the environment. Hood et al. (2000) concluded that favorable conditions such as high nutrient or temperature levels were responsible for the production of promycelia containing anucleate zones. The formation of anucleate zones between cells of the promycelia could likely influence the mating system by decreasing the intratetrad mating and promoting outcrossing (Hood et al., 2000). Despite the adequate level of nutrients present in the PDA media in the current study, intratetrad mating remained the only mating system observed at $2.5^0$C. Temperature, more than nutrient availability seems to be the controlling factor in mediating intratetrad mating in *Ustilago bullata*.

**Seed germination response to temperature**

Germination rate of Bromus tectorum seeds collected from eight population were not significantly different at temperatures from 20 to 300C, with very little variation

other than the longer times for the Potosi Pass population (Table 5). All populations reached 50% germination at or near the optimum temperature of 200C after less than 24 hours of incubation. The rapid germination rate suggests that **seeds were fully after-ripened.** Bromus tectorum seeds are often dormant at maturity and lose dormancy under dry conditions (Meyer et al., 1997). The seeds used in this experiment were stored at room temperature at least 6 months prior to the tests. Seeds from all eight populations had at least 50% germination at all temperatures except 350C. At the optimum temperature range for germination of Bromus tectorum seeds (20 to 300C), there were few differences among populations (Table 5). The overall mean germination time decreased from 52 to 16 hours as temperature increased over the range 10 to 200C, remained level over the range 20 to 300C, and increased to 31 hours at 350C. Some populations differed in germination time at cooler temperatures (Table 5).

**Seed and teliospore germination synchronization**

Non-dormant *Ustilago bullata* teliospores had temperature requirements for germination that paralleled temperature requirements for *Bromus tectorum* seed germination (Fig. 3). Falloon (1979c) observed that *Ustilago bullata* had temperature requirements that paralleled germination and seedling growth requirements of *Bromus catharticus*. At temperatures below an optimum of $25^0$C, the growth of both *Ustilago bullata* and *Bromus catharticus* were affected in the same way as temperature changed (Falloon, 1979c). In the current study, host germination rate increased linearly as a function of temperature over the range of 10 to $20^0$C, and then leveled off. Pathogen germination rate also increased linearly as a function of temperature but over the range of 10 to $25^0$C, and the slope of this increase was much steeper than the slope of the rate of increase for

seed germination (Fig. 3). The two lines meet at $10^0$C. The difference in response to temperature of the two *Ustilago bullata* pathotypes studied earlier may be associated with the adaptation to their hosts (Turnbull and Gossen, 2000).

For successful infection, the pathogen must be equally able to endure the same conditions during growth and infection as the host does during seed germination and growth (Turnbull and Gossen, 2000). When investigating the temperature relations of *Ustilago bullata* and *Bromus catharticus*, Falloon (1979c) found that the host and the pathogen differed in their minimum, optimum, and maximum temperature requirements leaving some windows of opportunity for each of them.

The differences between pathogen populations and the differences between host populations were most clearly interpretable in terms of habitat of origin and most strongly correlated with each other at low temperature. At optimum temperatures differences among populations were small for both host and pathogen. The significance of these differences at low temperatures under field condition is still not clear.

**Teliospore germination response to nutrients**

*Ustilago bullata* teliospores initiated germination faster on potato dextrose agar (PDA) than on water agar (WA) (Table 7). They germinated to higher percentages at $25^0$C than $15^0$C on PDA after 24 hours of incubation (Table 7). The opposite was observed on WA where an average of 8% germination was recorded at $25^0$C and 14% recorded at $15^0$C (Table 7). While studying teliospore germination in four species of *Ustilago*, Ingold (1989) found that *Ustilago aschersoniana* produced sporidia on malt agar but failed or rarely produced sporidia on water agar. On the host surface, the teliospore may start germination as the germinating seed mobilizes its hydrolytic

enzymes that make the stored nutrients available for both the host and the pathogen. Falloon (1979a) found that the *Ustilago bullata* teliospores germinated better on the embryo than on the seed pericarp. This suggests that the presence of nutrient enhances the teliospore germination *in vivo*. Changes in the temperature and nutrient environment, could affect the number of heterokaryons in individual teliospore colonies and the unequal representation of the meiotic products in the sporidial population, consequently affecting the breeding of *Ustilago violacea* itself (Hood and Antonovics, 1998). Hartman et al. (1999) found that the induction and coordination of discrete morphological transitions during sexual development of *Ustilago maydis* were controlled by nutrient status.

## CONCLUSION

Teliospore germination rate exceeds host seed germination rate at temperature greater than $10^0$C while both teliospore germination rate and host seed germination rate equal each other at $10^0$C. Differences among populations of both pathogen and host were greater at lower temperatures. At lower temperatures, teliospores generally germinate slowly but differences between populations of contrasting habitats are greater than at higher temperatures. The fact that both teliospores and seeds of desert habitats tend to germinate more slowly at sub optimum temperatures than those of higher elevation habitats could be of some ecological significance. Teliospores responded to extremely low temperatures by intratetrad mating. The fact that intratetrad mating occurs regardless of nutrient level suggests that temperature could be the main driving force behind this phenomenon. Under optimum and near optimum conditions, however, both temperature and nutrients interact in order to impact teliospore germination as evidenced by our nutrient experiment *in vitro*.

## LITERATURE CITED

Agrios, G. N. 1997. Plant pathology. 4th ed. Academic Press, Inc. San Diego, California.

Alexopoulos, C. J. 1952. Introductory mycology. 2nd ed. John Wiley and Sons, New York, New York.

Alexopoulos, C. J., C. W. Mims, and M. Blackwell. 1996. Phylum Basidiomycota. Ustilaginales. The smut fungi. p 639-657. In: John Wiley and Sons (4th ed.) Introductory mycology, New York, New York.

Beckstead, J., S. E. Meyer and P. S. Allen. 1996. *Bromus tectorum* seed germination: Between-population and between-year variation. Canadian Journal of Botany 74: 875-882.

Bhuiyan, K. A. and R. T. V. Fox. 1989. Effect of temperature on germination and growth of teliospores of *Tilletia caries* Dc. Tul. Bangladesh Journal of Microbiology 6(1): 27-32.

Billings, W. D. 1990. *Bromus tectorum*, a biotic cause of ecosystem impoverishment in the Great Basin p 301-322. In: G. M. Woodell (ed.) The Earth in transition: Pattern and processes of biological impoverishment. Cambridge University Press, Cambridge, UK.

Bold, H. C., C. J. Alexopoulos, and T. Delevoryas. 1987. Division Amastigomycota III: Subdivision Basidiomycotina. p 787-790. In: John Wiley and Sons (5th ed.) Morphology of plants and fungi.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass-fire cycle, and global change. Annual Review of Ecology and Systematics 23: 63-88.

Falloon, R. E. 1979a. Further studies on the effects of infection by *Ustilago bullata* on vegetative growth of *Bromus catharticus*. New Zealand Journal of Agricultural Research 22: 621-626.

Falloon, R. E. 1979b. Seedling and shoot infection of *Bromus catharticus* by *Ustilago bullata.* Transactions of the British Mycological Society 73(1): 49-56.

Falloon, R. E. 1979c. Effect of temperature on the relationship between *Ustilago bullata* and *Bromus catharticus*. Transactions of the British Mycological Society 73(1): 41-47.

Fischer, G. W. 1940. Two cases of haplo-lethal deficiency in *Ustilago bullata* operative against saprophytism. Mycologia XXXII (3): 275-289.

Fleming, C. E., M. A. Shipley, and M. R. Miller. 1942. Bronco grass (*Bromus tectorum*) on Nevada ranges. University of Nevada Experiment Station Bulletin 159, Reno. 21p.

Hartman, H. A., J. Kruger, F. Lottspeich, and R. Kahmann. 1999. Environmental signals controlling sexual development of the corn smut fungus *Ustilago maydis* through the transcriptional regulator Prf1. Plant Cell 11 (7): 1293-1305.

Hilbert, U.; H. Schuepp, and F. J. Schwinn. 1990. Studies on infection biology of *Gymnosporangium fuscum Dc.* Zeitschrift-fuer-pflanzenkrankheiten-und-pflanzenschutz 97(3): 299-305.

Hood, M. E. and J. Antonovics. 1998. Two-celled promycelia and mating-type in *Ustilago* *violacea* (*Microbotryum violaceum*). International Journal of Plant Science 159 (2): 199-205.

Hood, M. E. and J. Antonovics. 2000. Intratedrad mating, heterozygosity, and the maintenance of deleterious alleles in *Microbotryum violaceum* (=*Ustilago violacea*). Heredity 85: 231-241.

Hood, M. E., O. J. Rochaand, and J. Antonovics. 2000. Differences in teliospore germination patterns of *Microbotryum violaceum* from European and North American *Silene* species. Mycological Research 105 (5): 532-536.

Ingold, C. T.1989. Basidium development in some species of *Ustilago*. Mycological Research 93 (4): 405-412.

Kaltz, O. and J. A. Shykoff. 1997. Sporidial mating-type ratios of teliospores from natural populations of the anther smut fungus (*Microbotryum* (= *Ustilago*) *violaceum*. International Journal of Plant Sciences 158 (5): 575-584.

Klemmedson, J. O. and J. G. Smith. 1964. Cheatgrass (*Bromus tectorum* L.) Botanical Review 30: 226-262.

Mack, R. N. 1981. Invasion of *Bromus tectorum* L. into western North America: an ecological chronicle. AgroEcosystems 7: 145-165.

Mack, R. N. and D.A. Pyke.1983. The demography of *Bromus tectorum:* variation in time and space. Journal of Ecology 71: 69-93.

Meyer, S. E. and P. S. Allen. 1999a. Ecological genetics of seed germination regulation in *Bromus tectorum* L. I. Phenotypic variance among and within populations. Oecologia 120: 27-34.

Meyer, S. E. and P. S. Allen. 1999b. Ecological genetics of seed germination regulation in *Bromus tectorum* L. II. Reaction norms in response to a water stress gradient imposed during seed maturation. Oecologia 120: 35-43.

Meyer, S. E., P. S. Allen and J. Beckstead.1997. Seed germination regulation in *Bromus tectorum* L. (Poaceae) and its ecological significance. Oikos 78: 475-485.

Meyer, S. E., D. L. Nelson, and S. Clement. 2001. Evidence for resistance polymorphism in the *Bromus tectorum – Ustilago bullata* pathosystem: implications for biocontrol. Canadian Journal of Plant Pathology 23: 19-27.

Peeper, T. F.1984. Chemical and biological control of downy brome (*Bromus tectorum*). In wheat and alfalfa in North America. Weed Science 32(1): 18-25.

Quinn G. P. and S. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Reddy, K. S. and J. Subbayya.1987. Studies on the growth and sporulation of *Cercosporidium personatum* (Berk. & Curt.) Deighton, the incitant of late leaf spot disease on groundnut. Geobios 14: 30-33.

Rice, K. J. and R. N. Mack.1991. Ecological genetics of *Bromus tectorum*. 1. A hierarchical analysis of phenotypic variation. Oecologia 77: 77-83.

Turnbull, G. D. and B.D. Gossen.2000. Pathotypes of *Ustilago bullata* differ in response to temperature and salinity conditions during spore germination. Canadian Journal of Plant. Pathololology 22: 293-299.

West, N. E. 1994. Effects of fire on salt-desert shrub rangelands p. 313. In: S. B. Monsen and S. G. Kitchen, compilers. Proceedings – Ecology and Management of Annual rangelands. USDA Forest Service General Technical Report INT – GTR – 313, Ogden, UT.

Whisenant, S. G.1990. Changing fire frequencies on Idaho's Snake River Plains: Ecological and management implications P.4-10. In: E. D. McArthur, E. M.

Romney, S. L. Smith and P. T. Tueller, compilers. Proceedings—Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management. USDA Forest Service, Intermountain Research Station, Ogden, Utah.

Zundel, G. L.1953. Ustilaginales of the world. Department of Botany, Pennsylvania State College School of Agriculture Contribution. No. 176. State College, Pennsylvania. 410p.

Table 1. Collection sites for *Ustilago bullata* smut and *Bromus tectorum* seed populations (Adapted from Meyer and Allen (1999a) and data from Western Regional Climate Center (www.wrcc.dri.edu).

| Place | State | Latitude Longitude | Elev. (m) | Vegetation Type | Ann. Precip. (mm) | Jan. Mean temp. ($^{0}$C) | July Mean Temp. ($^{0}$C) |
|---|---|---|---|---|---|---|---|
| Arrowrock | Idaho | N43$^{0}$32.8’ W115$^{0}$47.9’ | 1150 | mountain - ponderosa pine | 596 | -4.4 | 19.1 |
| Buckskin Canyon | Nevada | N41$^{0}$45.5’ W117$^{0}$32.0’ | 2132 | canyon - riparian | 505 | -2.7 | 18.3 |
| Hobble Creek | Utah | N40$^{0}$9.7’ W111$^{0}$30.5’ | 1530 | foothill - sagebrush gambel oak | 400 | -2.1 | 24.8 |
| Moses Lake | Wash. | N47$^{0}$17.5’ W119$^{0}$13.9’ | 390 | cold desert sagebrush | 200 | -3.4 | 21.7 |
| Potosi Pass | Nevada | N35$^{0}$60.0’ W115$^{0}$28.7’ | 1500 | warm desert blackbrush juniper | 250 | 1.7 | 26.5 |
| Sagehen Hill | Oregon | N43$^{0}$32.0’ W119$^{0}$17.2’ | 1400 | high-cold desert - sagebrush steppe | 284 | -3.3 | 19.3 |
| Strawberry Reservoir | Utah | N40$^{0}$14.7’ W111$^{0}$9.1’ | 2400 | mountain meadow | 560 | -7.8 | 16.1 |
| Whiterocks | Utah | N40$^{0}$17.3’ W112$^{0}$49.7’ | 1560 | cold desert shadscale | 180 | -2.3 | 25.8 |

Table 2. Analysis of variance for time to 50% germination of eight fully after-ripened *Ustilago bullata* teliospore populations placed under various temperature regimes (n = 2 for each of 40 treatment combinations).

| Source | Df | Mean square | F-value | P-value |
|---|---|---|---|---|
| Population | 7 | 1850.95 | 248.12 | 0.0001 |
| Temperature | 4 | 69609.67 | 9331.08 | 0.0001 |
| Population x Temperature | 28 | 804.91 | 107.90 | 0.0001 |
| Error | 40 | 7.46 | -- | -- |

Table 3. Average time (hours) to 50% germination of eight fully after-ripened *Ustilago bullata* teliospore collections under each of the five temperatures. Means for temperature and population main effects and for populations within temperatures followed by different letters are significantly different at $P<0.05$.

| Population | Temperature ($^0$C) | | | | | Means |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | |
| | Hours | | | | | |
| Whiterocks | $236^a$ | $53^b$ | $27^a$ | $16^a$ | $11^a$ | $69^a$ |
| Moses Lake | $236^a$ | $76^a$ | $23^a$ | $11^a$ | $11^a$ | $72^a$ |
| Potosi Pass | $185^b$ | $60^b$ | $22^a$ | $10^a$ | $9^a$ | $57^b$ |
| Strawberry Reservoir | $159^c$ | $46^c$ | $18^{ab}$ | $9^a$ | $7^a$ | $48^c$ |
| Sagehen Hill | $144^d$ | $43^c$ | $9^b$ | $10^a$ | $7^a$ | $43^c$ |
| Hobble Creek | $136^d$ | $31^d$ | $13^b$ | $10^a$ | $8^a$ | $40^c$ |
| Buckskin Canyon | $120^e$ | $40^{cd}$ | $19^{ab}$ | $11^a$ | $9^a$ | $40^c$ |
| Arrowrocks | $123^e$ | $37^{cd}$ | $15^b$ | $8^a$ | $7^a$ | $39^c$ |
| Means | $167^a$ | $52^b$ | $18^c$ | $11^c$ | $9^c$ | -- |

Table 4. Analysis of variance for time to 50% germination of eight fully after-ripened *Bromus tectorum* seed populations placed under various temperature regimes.

| Source | df | Mean square | F-value | P-value |
|---|---|---|---|---|
| Population | 7 | 213.37 | 8.98 | 0.0001 |
| Temperature | 5 | 3077.33 | 129.49 | 0.0001 |
| Population x Temperature | 34 | 53.30 | 2.24 | 0.0052 |
| Error | 47 | 23.76 | -- | -- |

Table 5. Average time (hours) to 50% germination for eight fully after-ripened *Bromus tectorum* seed collections under six incubation temperatures. Temperature and population main effects and populations within temperatures followed by different letters are significantly different at $P<0.05$.

| Population | Temperature ($^0$C) | | | | | | Means |
|---|---|---|---|---|---|---|---|
| | 10 | 15 | 20 | 25 | 30 | 35 | |
| | Hours | | | | | | |
| Whiterocks | $64^{x}$ | $19^{y}$ | $14^{x}$ | $13^{x}$ | $13^{y}$ | $30^{xy}$ | $25^{b}$ |
| Moses Lake | $52^{xy}$ | $25^{xy}$ | $18^{x}$ | $17^{x}$ | $13^{y}$ | $23^{y}$ | $25^{b}$ |
| Potosi Pass | $61^{x}$ | $37^{x}$ | $22^{x}$ | $25^{x}$ | $36^{x}$ | -- | $36^{a}$ |
| Strawberry Reservoir | $48^{xyz}$ | $27^{xy}$ | $14^{x}$ | $15^{x}$ | $13^{y}$ | $30^{xy}$ | $24^{b}$ |
| Sagehen Hill | $47^{xyz}$ | $18^{y}$ | $15^{x}$ | $13^{x}$ | $15^{y}$ | $45^{x}$ | $26^{b}$ |
| Hobble Creek | $50^{xy}$ | $19^{y}$ | $14^{x}$ | $18^{x}$ | $16^{y}$ | $35^{xy}$ | $25^{b}$ |
| Buckskin Canyon | $48^{xyz}$ | $16^{y}$ | $16^{x}$ | $16^{x}$ | $15^{y}$ | $29^{xy}$ | $23^{b}$ |
| Arrowrocks | $42^{yz}$ | $17^{y}$ | $13^{x}$ | $13^{x}$ | $15^{y}$ | $26^{xy}$ | $21^{b}$ |
| Means | $52^{a}$ | $22^{c}$ | $16^{d}$ | $16^{d}$ | $17^{d}$ | $31^{b}$ | -- |

Table 6. Analysis of variance for teliospore germination percentage of eight fully after-ripened teliospore populations grown on Water Agar (WA) vs. Potato Dextrose Agar (PDA) under two temperature regimes.

| Source | df | Mean square | F-value | P-value |
| --- | --- | --- | --- | --- |
| Population | 7 | 0.13 | 8.40 | 0.0001 |
| Temperature | 1 | 4.22 | 275.06 | 0.0001 |
| Population x Temperature | 7 | 0.09 | 6.05 | 0.0001 |
| Medium | 1 | 53.17 | 3465.42 | 0.0001 |
| Population x Medium | 7 | 0.06 | 4.01 | 0.0003 |
| Temperature x Medium | 1 | 8.29 | 540.48 | 0.0001 |
| Pop. x Temp. x Medium | 7 | 0.08 | 5.43 | 0.0001 |
| Error | 29 | 0.02 | -- | -- |

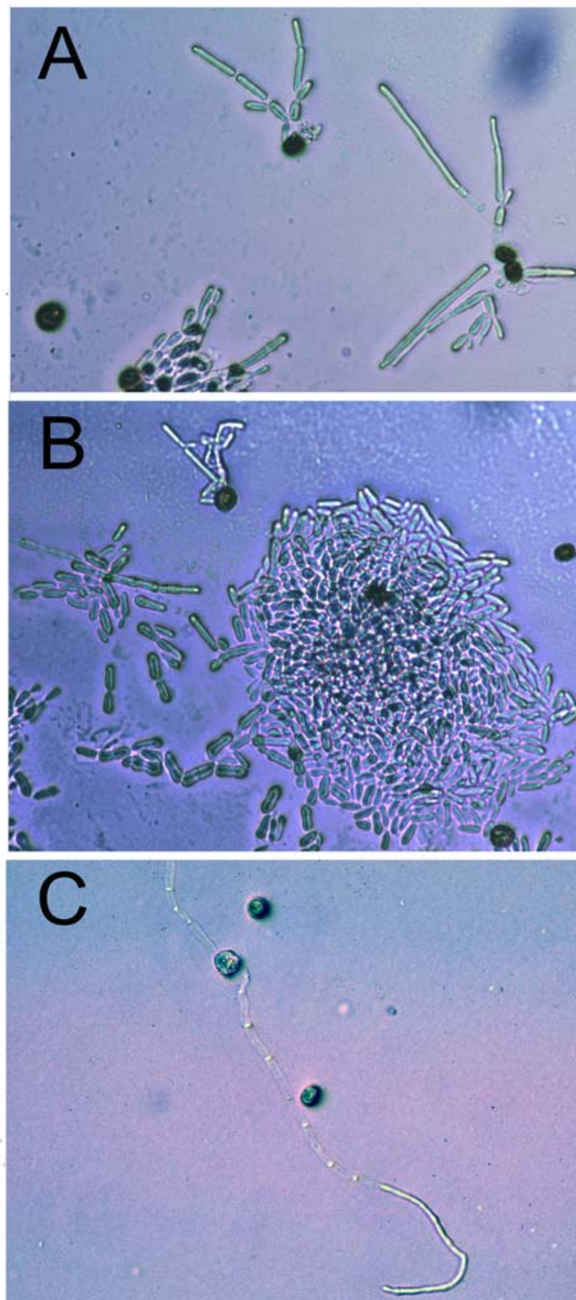


Figure 1. A) Teliospore germination after 24 hrs at optimum temperature (25$^{o}$C). Promycelia have extended from teliospores, meiosis has taken place, sporidial proliferation has been initiated, and a few dikaryons have formed.
B) Teliospore germination after 48 hrs at optimal temperature (25$^{o}$C). Haploid cells of the promycelia of germinated teliospores have produced numerous haploid sporidia via mitosis, C) Teliospore germination after 984 hrs at low temperature (2.5$^{o}$C). Teliospores germinate directly to produce one or two dikaryotic infection hyphae. Meoisis and mating of the primary products of meiosis has taken place within the teliospore wall (intratetrad mating). There is no sporidial proliferation.

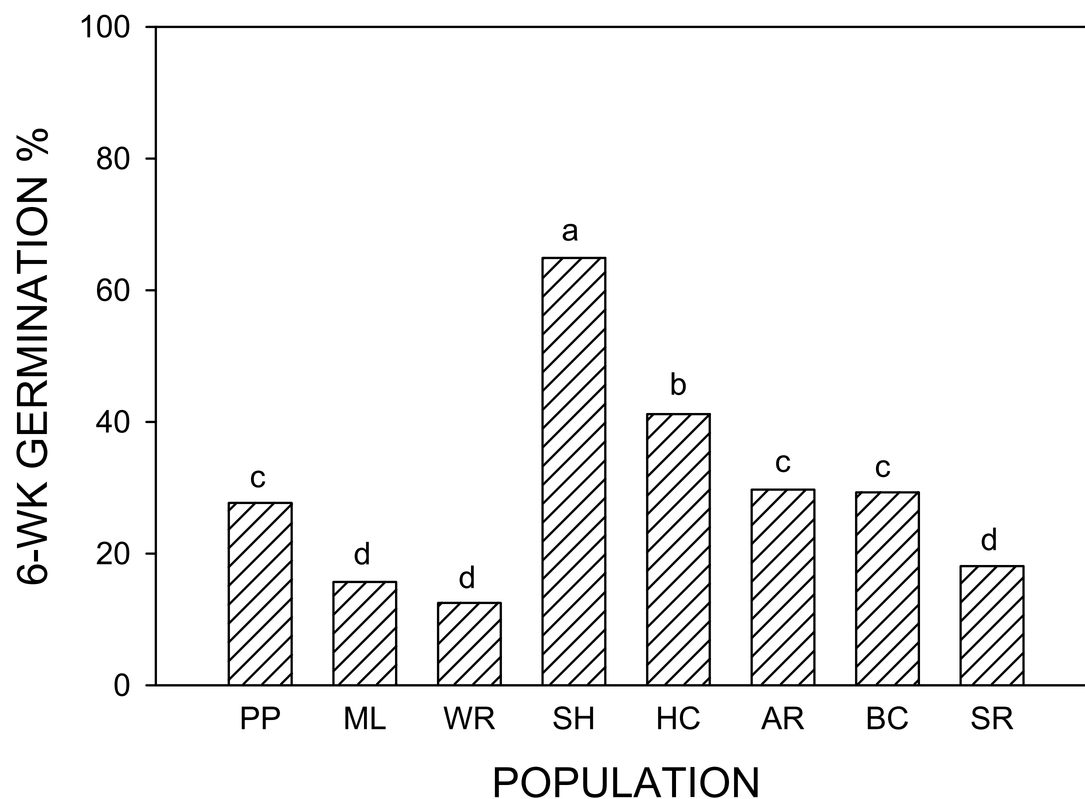


Figure 2. Mean percent teliospore germination of eight *Ustilago bullata* populations after a 6 week (984 hour) incubation period at $2.5^0$C: PP = Potosi Pass, ML = Moses Lake, WR = Whiterocks, SH = Sagehen Hill, HC = Hobble Creek, AR = Arrowrock, BC = Buckskin Canyon, and SR = Strawberry Reservoir. Bars with same letter are not significantly different ($p < 0.05$).

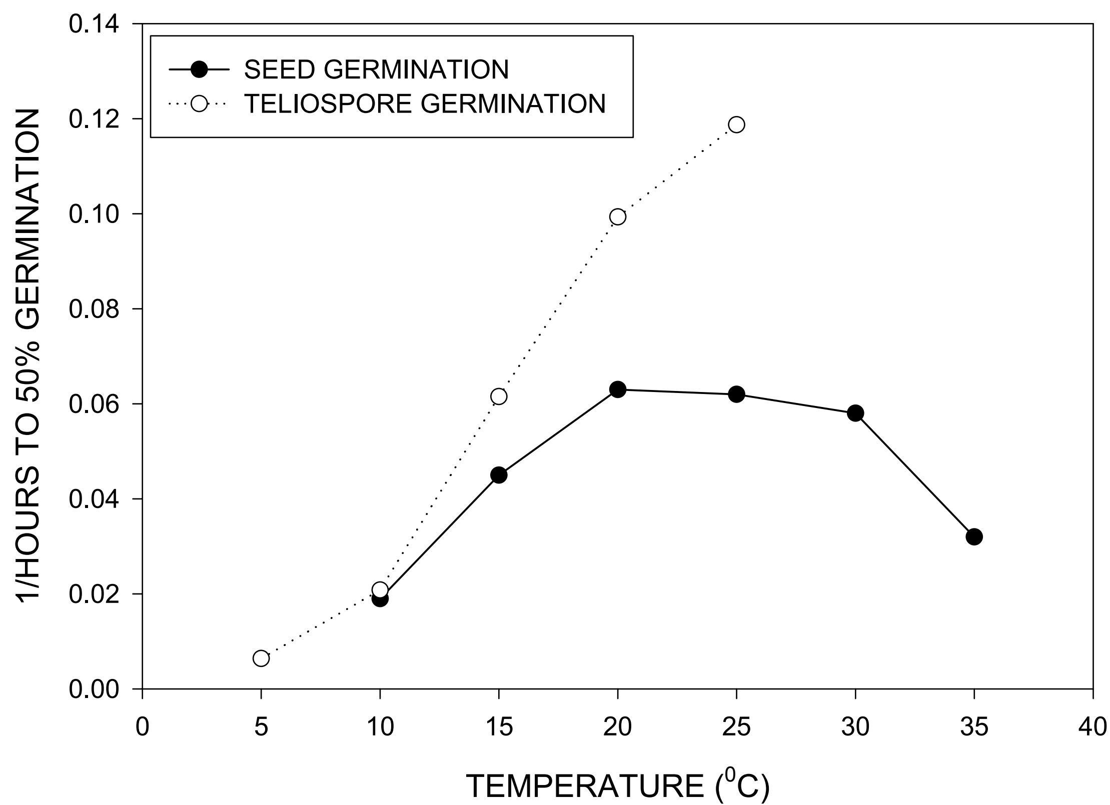


Figure 3. Mean seed germination rates of eight *Bromus tectorum* populations and eight *Ustilago bullata* teliospore populations plotted as a function of temperature.

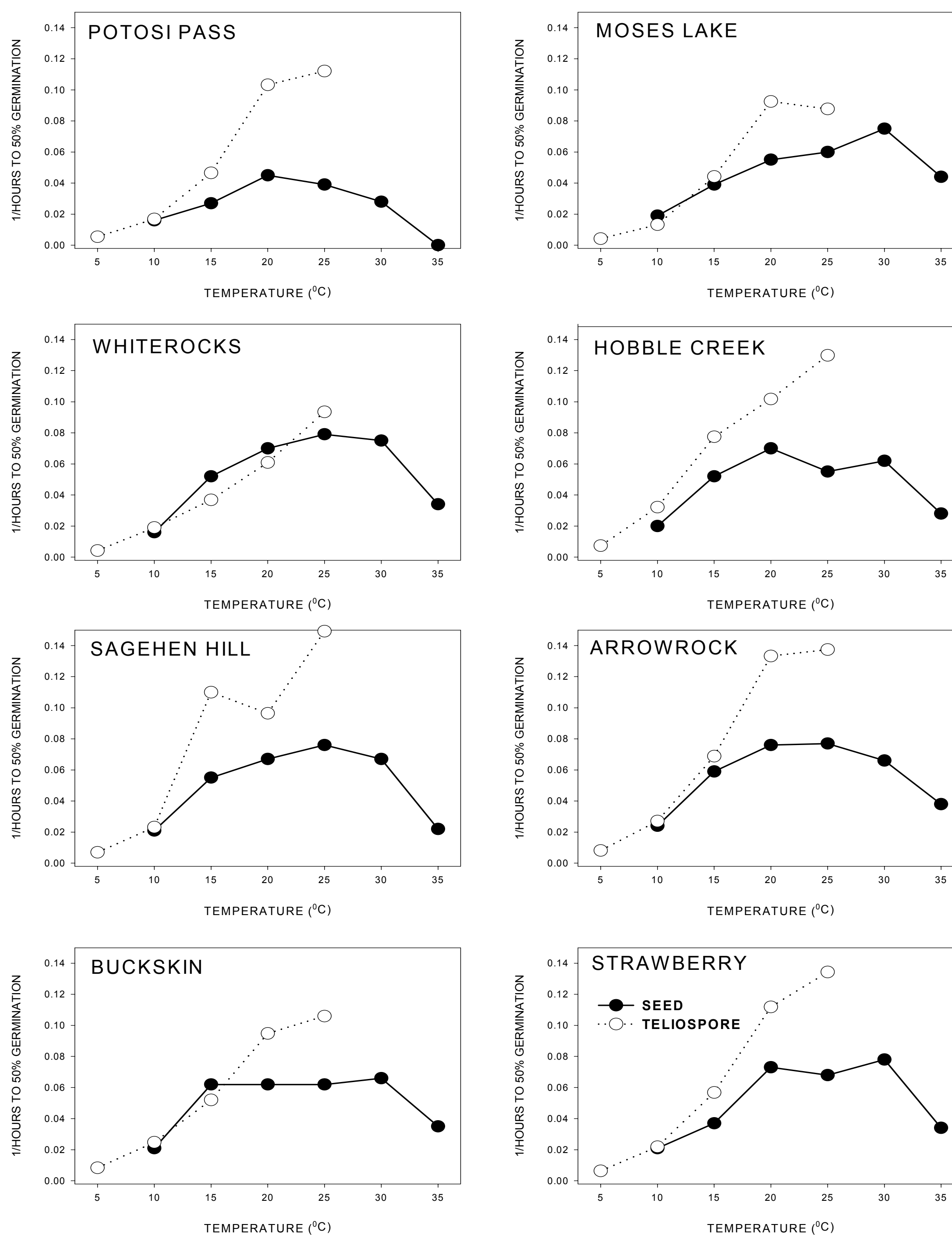


Figure 4. Seed germination rates of eight *Bromus tectorum* populations and eight *Ustilago bullata* teliospore populations plotted as a function of temperature.

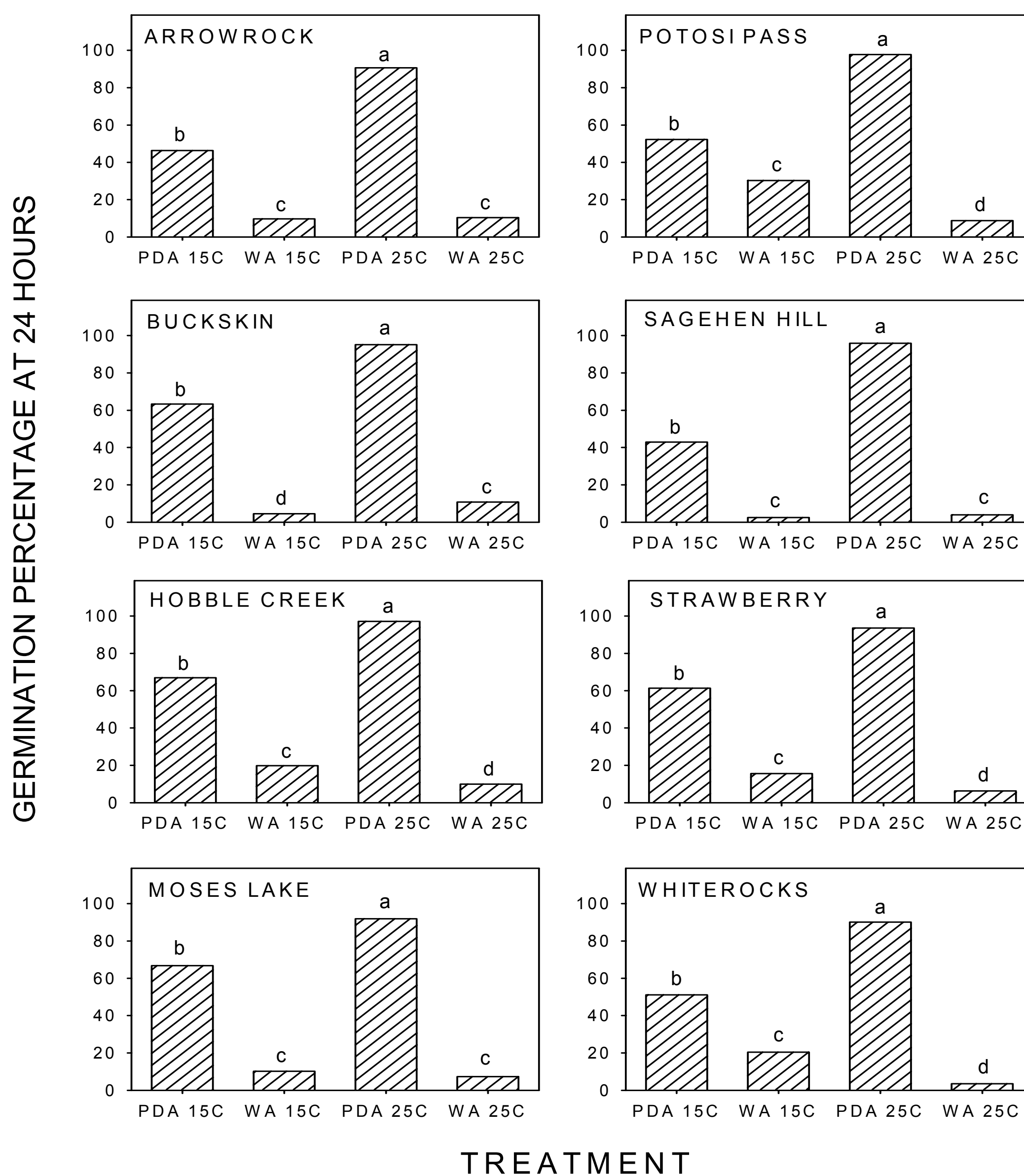


Figure 5. Average percent germination of eight fully after-ripened teliospore populations grown on potato dextrose agar (PDA) and water agar (WA) placed under two temperature regimes after 24 hours incubation period. Bars with the same letter for a population are not significantly different ($p < 0.05$) by REGWQ.

# Chapter 2

## Temperature Relations of the Infection Process in the *Ustilago bullata* Berk-*Bromus tectorum* L. Pathosystem

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**ABSTRACT**

The pathogen, *Ustilago bullata* Berk., naturally infects and causes head smut disease of *Bromus tectorum* L. in the field. But under field conditions, the host escapes infection even under conditions where inoculum and water are not considered limiting. We investigated the effect of temperature on sporidial proliferation and host infection in a temperature-controlled environment for two inoculum densities and with high water availability. We also investigated the effect of inoculum density on infection in the field. High inoculum density produced significantly higher infection than low inoculum density in the controlled environment, except at optimum temperatures for infection (15 to $20^0$C). As distances from optimum temperatures increased, infection decreased and density effect increased. At optimum temperatures for infection, pathogen teliospore germination and sporidial germination were ahead of seed germination suggesting that infection can be achieved regardless of inoculum density. Very little infection was recorded at $2.5^0$C in the controlled environment or in the field for a late planting in December when soil temperatures were minimal. The infection of eight teliospore collections from different geographic locations across the Western United States varied in response to different temperatures both in the field and in the controlled-environment. In the field, the desert populations had lower overall infection percentages than populations collected from montane sites. Sporidia retrieved from the eight teliospore collections differed in growth at different temperatures both within and among populations. Differences in sporidial growth among the collections from different habitats were greatest at 2.5 and $25^0$C but were minimal at the optimum infection temperature of $15^0$C. Our experiments suggest that the efficacy of *Ustilago bullata* as a

biocontrol agent for *Bromus tectorum* will be greatest under climate scenarios associated with autumn host seedling emergence. The failure to obtain infection in later emerging cohorts was seen regardless of pathogen ecotype.

## INTRODUCTION

The development of plant disease epidemics under field conditions depends on three factors: 1) the presence of adequate inoculum in the infection court, 3) the presence of a susceptible host, and 2) environmental conditions favorable for infection. If environmental conditions such as temperature, moisture, and wind are more favorable to the pathogen or increase the vulnerability of the host, the potential amount of the disease becomes greater (Agrios, 1997). To successfully use a plant pathogen as a biocontrol organism, we must understand how environmental conditions interact with inoculum source and density to produce different levels of infection.

*Ustilago bullata* Berk. is a seedling-infecting systemic pathogen that causes head smut disease in a range of grass hosts throughout the world (Zundel, 1953). In the Intermountain West, it is often observed at epiphytotic levels on the winter annual grass weed, cheatgrass (*Bromus tectorum* L.).

Cheatgrass has invaded a variety of habitats on semiarid western wildlands of America within the last 100 years (Mack, 1981). It quickly invades rangelands and replaces native vegetation after wildfire or severe overgrazing, and constitutes one of the most important plant invasions in the modern history of North America (D'Antonio and Vitousek, 1992). It now dominates tens of millions of hectares in North America and increases the frequency of wildfires (Whisenant, 1990).

This study is part of a larger research project examining environmental and genetic factors that limit field infection percentages and evaluating the feasibility of using *Ustilago bullata* as a biocontrol organism for *Bromus tectorum* in conjunction with

restoration seedings with native plants. Our goal is to understand how factors other than host resistance and pathogenicity of the pathogen affect infection percentage, i.e., how do susceptible host individuals escape infection? We examined the role of environmental factors, especially temperature, and their interaction with pathogen ecotype and inoculum density in mediating the probability of infection for susceptible host individuals.

The purpose of this study was: 1) to determine how temperature during infection affects disease incidence in highly susceptible host genotypes, 2) to measure differences among pathogen populations from a range of habitats in response to temperature during infection, 3) to examine the interaction of inoculum density with temperature in mediating levels of disease incidence, 4) to estimate the duration of the infection window at different temperatures and examine the ability of the pathogen to time its development to coincide with the infection window, 5) to determine the effect of planting date and therefore temperature on infection success for different pathogen populations in the field.

# MATERIALS AND METHODS

## Pathogen population selection

*Ustilago bullata* teliospores were collected from a range of habitats in the Intermountain USA. (Table 1). Habitats were selected to represent different temperature zones and elevations from warm to cold deserts to the foothills and the mountains.

## Teliospore harvest, cleaning, and storage

Teliospores of *Ustilago bullata* were harvested at the study sites in midsummer from heads of the host and placed in sealed paper bags. Samples were screened to release spores and stored in sealed vials at room temperature for at least 16 weeks until fully non-dormant.

## Greenhouse infection test

The growth chamber experiment testing the effect of pathogen population, temperature, and inoculum density on infection (disease incidence) consisted of two separate experiments run consecutively (i.e., repeated or replicated in time). Each experimental run had a completely randomized design with eight populations, seven temperatures (2.5, 5, 10, 10/20, 15, 20, and $25^0$C), two inoculum densities and two replications for a total of 224 experimental units. Each experimental unit consisted of a group of 18 seeds that were inoculated and planted into individual cells. The two trials were identical except for the use of different Whiterocks host lines (15-3 and 15-7). This change was necessitated by inadequate seed supplies of any one line at the initiation of the experiment. Both host lines were known to be highly susceptible to all eight pathogen populations (Meyer et al., 2001, Meyer and Nelson, unpublished data). Infection proportion was the response variable.

Seeds were collected from two cheatgrass lines, which were descendents of individual genotypes at the Whiterocks, Utah site (Table 1). Inflorescences from mature plants were collected at random, stored at room temperature until fully after-ripened (at least 6 months), and then cleaned for the experiment. Dry inoculum (teliospores) was applied to the seeds following the standard of 3.7 g of teliospores per 1.0 kg of seeds (Falloon and Rolston, 1990; Falloon, 1976). Seeds inoculated with two concentrations of teliospores (high with 3.7g/kg of seeds and low with 0.3g/kg seeds) were planted in trays and allowed to germinate under various temperature regimes in growth chambers in the dark. The seeds were planted in 72 sectioned plastic trays containing soil mixture composed of four parts vermiculite, three parts peat moss, two parts field soil, two parts sand, and one part agsorb; the mixture was steam-pasteurized for 90 minutes at $60^{0}$C.

We kept cheatgrass plants in a controlled-temperature environment until emergence was more or less complete. Plants were considered emerged as soon as the plumule was visible above the tip of coleoptile. After emergence, the plants were transferred to the greenhouse where they were allowed to grow for 3 weeks or until the appearance of the fourth true leaf. At that stage, they were moved to the cold room with fluctuating temperatures of 2.2 to $3^{0}$C for vernalization. After 10 to 12 weeks in the cold room, they were then brought back to the greenhouse for final growth until flowering. Percent infection was determined by counting the number of smutted plants as evidenced by the inflorescence filled with teliospores.

In the analysis of variance for the growth chamber experiment, population, temperature, and inoculum density were treated as fixed main effects. A preliminary examination of the shape of the temperature response showed that the response was not

linear. We chose to treat temperature as a class variable in the analysis rather than make assumptions about the shape of the response curve in a nonlinear regression procedure. Preliminary data analysis also showed that there was no significant difference between the two experimental replications in time. We therefore pooled the block (repetition in time) term and its interactions into the error term, and analyzed the two experiments together as a single completely randomized design with four replications. Infection proportion was arcsine square root transformed to improve homogeneity of variance before analysis.

**Seed germination and coleoptile emergence and elongation**

In order to quantify the time parameters of the period of susceptibility for the host, as well as to make sure that the two Whiterocks host lines used in the growth chamber experimental repetitions did not differ in their infection windows, we performed a germination experiment using seeds of these two lines.

Seed germination percentage, coleoptile emergence and elongation were assessed by incubating cheatgrass seeds at the following temperatures: 2.5, 5, 10, 15, 20, and $25^0$C. Ten seeds were set on wet filter papers in petri dishes with ten replications for each line and placed in incubators. Seeds were scored for germination, coleoptile emergence, and coleoptile elongation to 2 cm at time intervals depending on each of the temperatures under investigation. Linear interpolation was used to estimate time to 50% germination, time to 50% coleoptile emergence, and time to 50% coleoptile elongation to 2 cm for each replicate of each line by temperature combination.

Time to 50% was then used as the response variable in analysis of variance with host line and temperature as fixed main effects. This analysis was performed for each of

the three response variables, and host line main effects and interactions were never significant, regardless of whether temperature was included as a class or continuous variable (analysis not shown). We therefore concluded that there were no differences in parameters of the infection window between the two host lines, and used mean values in our description of the infection window.

**Sporidial proliferation**

To measure differences in response of sporidial cultures to temperature as a function of population, we used a partly nested design, with temperature and population as fixed main effects and sporidial line or isolate as a random effect nested within population. Five isolates or lines were included for each of the eight populations, and experiments were conducted at three temperatures, 2.5, 15, and $25^0$C. Three replications were included for each sporidial isolate by temperature combination (except in a few cases where individual replicates were lost to contamination).

The effect of temperature on sporidial proliferation was determined in Potato Dextrose Broth (PDB) culture in 50 ml flasks in shakers placed in growth chambers set at the above temperatures. A stock suspension was prepared at room temperature for each of the 40 sporidial lines randomly selected to represent the eight populations with five lines per population. Each line came from a single monosporidial culture. The stock solutions were produced according to the protocol in Meyer et al. (2001) and stored in 5 ml tubes at $-80^0$C prior to the experiment. There were six optical density readings of sporidial cultures per temperature per line. Sporidial stock solutions of 100 microliters were pipetted into 50 ml flasks containing 20 ml of PDB. The mixtures were allowed to grow in shaker flasks in growth chambers. The flasks were retrieved periodically, their

contents centrifuged using a Damon IEC HN-S centrifuge for 10 minutes at 2000 RPM, the supernatant discarded, and the pellets resuspended in distilled water. The pellet and the distilled water were thoroughly mixed using a Thermolyne type 37600 mixer and then read through a Bausch and Lomb spectronic 700 set at 600 nm. The final sporidial population was calculated using the standard equation for bacterial exponential growth. Since an optical density (OD) of 0.25 corresponds to 1 x $10^8$ colony forming units (CFU)/ml, sporidial population was determined as: OD/0.25 x ml of distilled water used for re-suspension x $10^8$.

The response variable for analysis of variance was time to $10^{10}$ sporidial cells. This density represents approximately half the maximum density that was achieved under our cultural conditions and is thus a mean proliferation time that is loosely analogous to mean germination time. The sporidial proliferation experiment was not analyzed as a block design because, even though the replicates for each sporidial line by temperature combination were run at different times, a run did not include all the possible combinations due to space constraints. We therefore treated the experiment as completely randomized rather than blocked. Temperature was treated as a class variable because of the strongly nonlinear temperature response. Significance tests were carried out with error terms appropriate to the partly nested experimental design (Quinn and Keough 2002).

**Field infection test**

The experimental design for the field experiment was a randomized block design with ten blocks and with pathogen population, planting date, and inoculum density as fixed main effects, for a total of 320 experimental units. Each experimental unit was

represented by a 0.093 $m^2$ plot. The response variable was infection (disease incidence) proportion.

Field tests were conducted at the Brigham Young University Spanish Fork Farm. The field test was conducted with *Bromus tectorum* bulk seeds from the Whiterocks site (Table 1), artificially inoculated (dusted) with *Ustilago bullata* teliospores from the eight sites. Two levels of inoculum were used to see if disease incidence was positively correlated with inoculum density. Dry inoculum (teliospores) was applied to the seeds following the standard of 3.7 g of teliospores per 1.0 kg of seeds (Falloon and Rolston, 1990; Falloon, 1976). Seeds inoculated with two levels of teliospores (high with 7.4g/kg of seeds and low with 3.7g/kg seeds) were planted at two planting dates, October 5th and December 7th. The two inoculum densities used in this trial were twice as high as the ones in the greenhouse experiment to maximize infection. All the plots were immediately covered with autoclaved dried cheatgrass litter. No irrigation was applied. Percent disease incidence was assessed at the end of the following growing season.

The field experiment was analyzed using analysis of variance for a randomized block design. Main effects and interactions were tested using the appropriate block interaction terms (Quinn and Keough 2002). The variable infection proportion was arcsine square root transformed prior to analysis to improve homogeneity of variance. Differences among population means were tested using the Ryan Einot Gabriel Welsch (REGWQ) procedure with the appropriate error term as the denominator.

# RESULTS

## Growth chamber experiment

Infection of *Bromus tectorum* seeds varied significantly ($P<0.0001$) among teliospore populations, among temperatures, and between inoculum densities (Table 2). The interactions between the main effects were not significant except for the interaction between population and temperature ($P < 0.0360$) (Table 2). The infection levels were generally high, indicating a good experimental protocol (Fig. 1). The optimum temperatures for pathogen infection were 15 and $20^{0}$C, with infection decreasing above and below the optimum. There was little or no infection at $2.5^{0}$C. Higher inoculum density resulted in greater infection proportion than the lower density for all eight teliospore populations used in the experiment. The density effect was more apparent as distance from the optimum increased. The overall infection proportion varied from 0.61 to 0.76. All populations showed fundamentally similar responses to temperature and had similar optima (15-$20^{0}$C). Populations varied in the degree to which infection was reduced at super and sub optimal temperatures, with most of the variation among populations at 5, 10, and $25^{0}$C. Variation among populations was not correlated with habitat attributes. The inoculum density by temperature interaction was not significant but there was a clear trend, which showed little effect of inoculum density at optimum infection temperature but with inoculum density becoming more limiting at temperatures further from the optimum. The exception was at $2.5^{0}$C, where there was little infection regardless of inoculum density.

**Infection window estimate**

*Bromus tectorum* seed germination was faster at higher temperatures than lower temperatures (Fig. 2). Coleoptile elongation times tracked seed germination and coleoptile emergence times at high temperatures but lagged far behind at $2.5^0$C, so that the window of infection was longer at that temperature. Mean rate of seed germination for host lines used in the growth chamber experiment was plotted along with mean rate of teliospore germination for eight pathogen populations, as a function of temperature in order to see the degree to which pathogen teliospore germination was able to track seed germination at different temperatures (Fig. 3). Pathogen and seed germination rates were nearly identical at $10^0$C, and pathogen germination rates exceeded seed germination rates at higher temperatures. At temperatures below $10^0$C, teliospore germination rate clearly lagged behind seed germination rate, and this differential increased as temperature decreased. At a temperature generally above the optimum for infection ($25^0$C), teliospore germination rate exceeded seed germination rate by a considerable margin (teliospores averaged 8 hours to 50% germination, seeds averaged 17 hours).

**Sporidial proliferation experiment**

Sporidial proliferation time to $10^{10}$ cells/ml varied significantly ($P < 0.0001$) among temperatures, populations, and lines within populations (Table 3 and Fig. 4). The interactions between main effects were also highly significant ($P < 0.0001$). The significant interaction of pathogen population and temperature indicates that populations differed in the shape of the response to temperature. Not surprisingly, mean proliferation times were longest at the lowest temperature ($2.5^0$C) and shortest at high temperature ($25^0$C). But sporidial proliferation was not halted at near-freezing temperature, just

slowed down. Differences among populations and also among lines within populations were more evident at low and high temperature and less evident at intermediate temperature (15$^0$C). Populations also differed in the amount of among-line variation. For example, the Arrowrock population showed very little among-line variation at any temperature, whereas the Whiterocks and Hobble Creek populations showed much more among-line variation, especially at the lowest temperature.

Overall, mean sporidial proliferation rate (1/time to $10^{10}$ sporidial cells/ml) increased exponentially as a function of temperature across the range of 2.5 to 25$^0$C (Fig. 5a). That is, the plot of $\log_{10}$ (sporidial proliferation rate) as a function of temperature is linear. Optimum temperature for sporidial proliferation was 25$^0$C.

In general, all populations had similar mean proliferation rates at 15$^0$C (Fig. 5b). The Strawberry population had faster mean proliferation rates at low temperature and shorter mean proliferation rates at high temperature, resulting in a proliferation rate relationship with a relatively shallow slope (Fig. 5b). In contrast, the Potosi Pass population had the slower proliferation rates at low temperature and faster rates at high temperature. The other populations had intermediate slopes. The Whiterocks population had relatively fast mean proliferation rates at both low and high temperature, resulting in a temperature by mean proliferation rate relationship with slope similar to the average slope but with a higher intercept. The slopes represent the rapidity with which sporidial proliferation rate drops as temperature decreases below the optimum.

Population sporidial proliferation rates at 25$^0$C were not correlated with ability to produce high infection at this temperature. For example, both the Strawberry and Potosi Pass pathogen populations had high infection percentages at 25$^0$C, even though they had

low mean proliferation rates at this temperature, and Whiterocks, which had the highest proliferation rate at $25^{0}$C, showed a definite drop in infection percentage at this temperature. Population sporidial proliferation rates at $2.5^{0}$C also showed no relationship with infection percentage.

**Field experiment**

*Bromus tectorum* infection percentage varied significantly for pathogen population ($P<0.0175$) and planting date ($P < 0.0001$), but not for inoculum density (Table 4). There were significant differences among pathogen populations in the ability to infect the highly susceptible Whiterocks host population, but these differences were small (Fig. 6). The differences among populations were more apparent for the early planting date. The warm desert Potosi Pass pathogen population had the lowest infection percentage (74%) and the mountain meadow Strawberry population had the highest (91%). The planting date main effect was much larger. Infection percentages for the early October planting date were in the epidemic range, averaging 84%, while infection percentages for the December planting date averaged less than 2%. The lack of a significant effect of inoculum density was probably because the lower inoculum density was not limiting to infection. We used relatively high inoculum densities in the field experiment because of relatively low infection percentages obtained with lower densities the previous year (unpublished data). All populations responded similarly with respect to planting date and inoculum density, and the null effect of inoculum density was consistent across planting dates.

# DISCUSSION

## Temperature relations of infection

The idea that *Ustilago bullata* has an optimum temperature for infection, with infection percentage decreasing as distance from the optimum temperature increases, was supported by our growth chamber study (Fig. 1). Overall, the optimum temperature for infection was 15 to 20$^0$C, which is a good match with temperatures encountered in the field during autumn precipitation events. At temperatures in this range, all populations showed high infection levels (mean 90%). *Ustilago bullata* caused the highest infection on *Bromus catharticus* at 25$^0$C (Falloon, 1979b). In the present study, infection dropped to an average of 70% at 25$^0$C. Average infection was 82% at 10$^0$C, 65% at 5$^0$C and 5% at 2.5$^0$C. With the optimum temperature for infection being 25$^0$C, the infection of *Ustilago bullata* on *Bromus catharticus* dropped substantially when inoculated seeds were sown and incubated at 32.5$^0$C (Falloon, 1979a). This response of decreasing infection with increasing temperature above optimum agrees with our findings, except that our observed optimum temperature was lower.

Decreasing inoculum density resulted in lower infection percentages overall (mean of 77% at high density vs. 64% at low density), indicating that the lower density in the greenhouse experiment was below the threshold for maximum infection. Bruckart (1999) found a density effect in that two of the three inoculation methods he studied required more inoculum (teliospores) to cause the highest infection of *Puccinia carthami* on safflower (*Carthamus tinctorius* L.). In our study, the effect of inoculum density was less evident at temperatures in the optimum range and generally increased with distance from the optimum.

Differences among populations and their interaction with temperature were significant but generally not large. Mean infection percentage varied from a low of 61% for Arrowrock to a high of 76% for Potosi Pass. Lower mean infection was generally associated with sharper decreases in response to super- and sub optimal temperatures as well as greater reduction in infection at the lower inoculum density.

**Infection window**

The ability of the pathogen to infect at different temperatures was generally interpretable in terms of its ability to synchronize development to coincide with the host infection window. Over the optimum range for infection from 15 to $20^0$C, pathogen teliospore germination and sporidial proliferation were initiated in advance of seed germination, so that high infection levels were achieved regardless of inoculum density and in spite of the rapid post-germination development of the seedling. At sub optimum temperatures of 10 and $5^0$C, pathogen teliospore germination was delayed so that it either coincided with host seed germination or was slightly behind, but slower seedling development coupled with relatively fast sporidial proliferation at these temperatures made considerable infection possible, especially at the higher inoculum density.

At $2.5^0$C, teliospore germination lagged so far behind host germination and seedling development that little or no infection was possible, even at the higher inoculum density. The fact that teliospores germinate directly to produce dikaryons at this temperature means that there is no sporidal proliferation if teliospores are used as the inoculum source (Boguena et al. unpublished data). The sporidia do proliferate well in culture in the cold, however, at rates that match or exceed seed germination and seedling development rates. This means that it may be possible to obtain high infection at low

temperatures by using paired sporidial cultures (i.e., of opposite mating type) as the inoculum rather than teliospores. Sporidial proliferation was slower at lower temperatures just like host seed germination but never stopped even at $2.5^0$C. It was found that teliospores of *Gymnosporangium fuscum* do not germinate at $5^0$C; the basidiospores, on the other hand, stop proliferating only at $0^0$C (Hilbert et al., 1990). In this study, sporidia grew exponentially in relation to temperature. Falloon (1978) found that the growth of *Ustilago bullata* was linear at all stages except for the sporidial proliferation, which was exponential. There was a positive correlation between sporidial growth and temperature (Fig. 5a). The sporidia grew faster at $25^0$C and slower at lower temperatures (Fig. 4). At $15^0$C, there were no difference between pathogen populations from different habitats but more differences were observed at $2.5^0$C and $25^0$C (Fig. 5b).

At $25^0$C, infection dropped, even though rates of teliospore germination and sporidial proliferation were favorable for infection. We hypothesize that some other stage of the infection process, such as sporidial mating, dikaryon formation, dikaryon growth, or host tissue penetration, may have a lower optimum temperature (and thus reduced rate at $25^0$C relative to lower temperatures) than the pathogen development processes we measured, resulting in reduced infection due to the rapid growth of the host seedling beyond the susceptible stage.

Coleoptile emergence appeared to be faster around temperatures considered optimum for the pathogen teliospore germination (Fig. 3). *Bromus tectorum* coleoptile emergence and elongation to 2 cm coincided with *Ustilago bullata* sporidial proliferation, dikaryon formation (Boguena and others unpublished data), and teliospore germination (Fig. 4). At temperatures of $25^0$C and $20^0$C, 50% seed germination happened on average

by 15 and 19 hours respectively, 50% coleoptile emergence by 28 and 32 hours, and 50% coleoptile elongation to 2 cm by 74 and 70 hours (Fig. 3). Since *Ustilago bullata* dikaryons infect cheatgrass seedlings at the coleoptiles, it was understandable that 50% of cheatgrass coleoptiles emerged on average after a 30-hour incubation period and reached 2cm after 70 hours at $20^0$C (Fig. 2) while most dikaryons were observed to be formed by 48 hours under the same temperature. Coleoptiles of *Bromus catharticus* were most susceptible to *Ustilago bullata* when they were less than 10 mm long (Falloon, 1979b) and can probably remain susceptible longer at lower temperatures (Falloon, 1979a). The optimum temperature requirement of coleoptile development of *Bromus catharticus* was 25 to $30^0$C with a minimum of $15^0$C and a maximum of $35^0$C (Falloon, 1979a). At a temperature generally above the optimum for infection ($25^0$C) in our study, teliospore germination rate exceeds seed germination rate by a considerable margin (teliospores average 8 hours to 50% germination, seeds 17 hours). Lower infection levels at $25^0$C are therefore not due to inability of pathogen teliospore germination to track seed germination.

Among-population differences in pathogen teliospore germination rate as a function of temperature (data not shown here) were not well correlated with differences in ability to infect as a function of temperature, suggesting that teliospore germination is not the only phase of pathogen development that affects infection percentages. We consider that the window of infection extends from the time of coleoptile emergence from the seed until the coleoptile is no longer susceptible (estimated at 2 cm length based on Falloon (1979b) and our unpublished data). The duration of this window obviously varies as a function of temperature. Coleoptile emergence and elongation is delayed at

low temperatures relative to seed germination (radicle emergence). This makes it possible for the pathogen to infect at relatively low temperatures (10 and $5^0$C), even though teliospore germination merely matches or lags behind seed germination at these temperatures. At $2.5^0$C, however, teliospore germination is delayed even more than coleoptile elongation, i.e., the coleoptile is generally beyond the susceptible stage even before the teliospores germinate.

**Field Inoculation Trial**

Results of the field trial were in broad agreement with predictions made from the growth chamber experiment. There was little infection in plants that developed from seeds inoculated and planted under cold conditions in early December, while seeds inoculated and planted in early October, while temperatures were still warm, showed infection levels that could be called epidemic (mean of 84%). In a similar study with another smut pathogen, it was observed that sorghum seeds sown in warm soil had a greater disease incidence of covered kernel smutted panicles by *Sporisorium sorghi* than those sown late in the season (Nzioki et. al., 2000).

Precipitation sufficient to support cheatgrass emergence and establishment was recorded soon after planting in early October and good follow-up moisture was received in early November that year (Fig. 7). The winter, in contrast, was much drier than average, and there was little establishment from the late planting and almost no infection (mean <2%). These results are in accordance with our laboratory studies, which suggest that this pathogen will have limited ability to infect at the colder temperatures associated with winter/early spring host seedling emergence.

There were differences among populations in infection percentage in the field experiment, with the warm desert Potosi Pass population giving the lowest infection (74%) and the mountain meadow Strawberry population giving the highest (91%). In general, the desert populations showed lower infection percentages than the foothill and montane populations, possibly due to their slower teliospore germination at cool temperatures (Dissertation, chapter 1).

# CONCLUSION

In general, the differences in temperature response among populations for different stages of pathogen development were minor, not readily interpretable ecologically, and not closely correlated with one another. This suggests that it may not be necessary to match pathogen ecotypes closely to habitat when selecting pathogen populations for use as possible biocontrol agents. On the other hand, this means that the temperature limitations for different stages of the infection process are not likely to be overcome by use of a pathogen population with markedly broader or different temperature relations that are a better match for the host window of infection at limiting temperatures. Our best hope for overcoming the inability of this pathogen to infect at low temperature, as observed in both growth chamber and field experiments, may be to circumvent the problem by skipping the limiting teliospore germination step. The relationship of sporidial proliferation rate to temperature suggests that it may be possible to obtain infection across a broader range of temperatures by using sporidial inoculum in place of teliospore inoculum.

Population sporidial proliferation rates at 2.5$^0$C also showed no relationship with infection percentage. This is not surprising, given that teliospore germination at this temperature is not only dramatically delayed but results in direct production of dikaryotic infection hyphae, with little or no sporidial proliferation (Boguena et al. unpublished data). The fact that sporidia, once produced in culture, are capable of proliferation at this temperature has little relevance under natural conditions but could be important in development of the sporidial stage as a delivery system for biocontrol. We already know how to obtain high infection at near-optimum temperatures using paired sporidial inocula

(Meyer et al. 2001). Experiments are now underway to determine whether this type of inoculation results in high infection percentages at the low temperatures where infection from teliospores is limited. Additional advantages of sporidial inoculum as the vehicle for inoculum delivery are: it can be produced in vitro, it can survive dehydration, and it can retain viability for prolonged periods in the dehydrated state (personal communication with D. L. Nelson).

**LITERATURE CITED**


Agrios, G. N.1997. Plant pathology. 4th ed. Academic Press, Inc. San Diego, California.

Bruckart, W. L. 1999. A simple quantitative procedure for inoculation of safflower with teliospores of the rust fungus, *Puccinia carthami.* Plant Disease 83: 181-185.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass-fire cycle, and global change. Annual Review of Ecology and Systematics 23: 63-88.

Falloon, R. E. 1976. Effect of infection by *Ustilago bullata* on vegetative growth of *Bromus catharticus.* New Zealand Journal of Agricultural Research 19: 249-254.

Falloon, R. E. 1978. Growth of *Ustilago bullata* in culture. Transactions of the British Mycological Society 70 (1): 103-108.

Falloon, R. E. 1979a. Effect of temperature on the relationship between *Ustilago bullata* and *Bromus catharticus*. Transactions of the British Mycological Society 73(1): 41-47.

Falloon, R. E. 1979b. Seedling and shoot infection of *Bromus catharticus* by *Ustilago bullata.* Transactions of the British Mycological Society 73(1): 49-56.

Falloon, R. E. and M. P. Rolston, 1990. Productivity of prairie grass (*Bromus willdenowii* Kunth.) affected by sowing date and the head smut fungus (*Ustilago bullata* Berk). Grass and Forage Science 45: 357-364.

Hilbert, U., H. Schuepp, and F. J. Schwinn. 1990. Study of infection biology of *Gymnosporangium fuscum* Zeitschrift fuer pflanzenkrankheiten und pflanzenschutz 97(3): 299-305.

Mack, R. N. 1981. Invasion of *Bromus tectorum* L. into western North America: an ecological chronicle. AgroEcosystems 7: 145-165.

Meyer, S. E., D. L. Nelson, and S. Clement. 2001. Evidence for resistance polymorphism in the *Bromus tectorum – Ustilago bullata* pathosystem: implications for biocontrol. Canadian Journal of Plant Pathology 23: 19-27.

Nzioki, H. S., L. E. Claflin, and B. A. Ramundo. 2000. Evaluation of screening protocols to determine genetic variability of grain sorghum germplasm to *Sporisorium sorghi* under field and greenhouse conditions. International Journal of pest management 46(2): 91-95.

Quinn G. P. and S. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River Plains: Ecological and management implications P. 4-10. In: E. D. McArthur, E. M. Romney, S. L. Smith and P. T. Tueller, compilers. Proceedings—Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management. USDA Forest Service, Intermountain Research Station, Ogden, Utah.

Zundel, G. L. 1953. Ustilaginales of the world. Department of Botany, Pennsylvania State College School of Agriculture Contribution. No. 176. State College, Pennsylvania. 410p.

Table 1. Collection sites for *Ustilago bullata* smut and *Bromus tectorum* seed populations (Adapted from Meyer and Allen (1999a) and data from Western Regional Climate Center (www.wrcc.dri.edu).

| Place | State | Latitude Longitude | Elev. (m) | Vegetation Type | Ann. Precip. (mm) | Jan. Mean temp. ($^0$C) | July Mean Temp. ($^0$C) |
|---|---|---|---|---|---|---|---|
| Arrowrock | Idaho | N43$^0$32.8' W115$^0$47.9' | 1150 | mountain - ponderosa pine | 596 | -4.4 | 19.1 |
| Buckskin Canyon | Nevada | N41$^0$45.5' W117$^0$32.0' | 2132 | canyon - riparian | 505 | -2.7 | 18.3 |
| Hobble Creek | Utah | N40$^0$9.7' W111$^0$30.5' | 1530 | foothill - sagebrush gambel oak | 400 | -2.1 | 24.8 |
| Moses Lake | Wash. | N47$^0$17.5' W119$^0$13.9' | 390 | cold desert | 200 | -3.4 | 21.7 |
| Potosi Pass | Nevada | N35$^0$60.0' W115$^0$28.7' | 1500 | warm desert blackbrush juniper | 250 | 1.7 | 26.5 |
| Sagehen Hill | Oregon | N43$^0$32.0' W119$^0$17.2' | 1400 | high-cold desert - sagebrush steppe | 284 | -3.3 | 19.3 |
| Strawberry Reservoir | Utah | N40$^0$14.7' W111$^0$9.1' | 2400 | mountain meadow | 560 | -7.8 | 16.1 |
| Whiterocks | Utah | N40$^0$17.3' W112$^0$49.7' | 1560 | cold desert shadscale | 180 | -2.3 | 25.8 |

Table 2. Analysis of variance for infection percentage of eight teliospore populations on *Bromus tectorum* grown under various temperatures in a controlled environment experiment (n = 4 for each treatment combinations).

| Source | Df | Mean square | F-value | P-value |
|---|---|---|---|---|
| Population (P) | 7 | 0.38 | 6.18 | 0.0001 |
| Temperature (T) | 6 | 12.40 | 202.31 | 0.0001 |
| P x T | 42 | 0.09 | 1.47 | 0.0360 |
| Density (D) | 1 | 3.81 | 62.23 | 0.0001 |
| P x D | 7 | 0.06 | 0.97 | ns |
| T x D | 6 | 0.10 | 1.58 | ns |
| P x T x D | 42 | 0.03 | 0.53 | ns |
| Error | 336 | 0.06 | -- | -- |

Table 3. Analysis of variance for rate of sporidial proliferation (time to 100 x $10^8$ sporidia - $ml^{-1}$) of eight smut populations placed under various temperatures using appropriate line (population) and temperature by line (population) interactions as error terms.

| Source | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|
| Population | 7/32 | 132788.63 | 6.83 | 0.0001 |
| Population x Temperature | 14/63 | 164660.86 | 10.95 | 0.0001 |
| Temperature | 2/63 | 18095200.75 | 1202.96 | 0.0001 |
| Line (Population) | 32/216 | 19440.55 | 3.50 | 0.0001 |
| Temp. x Line (Population) | 63/216 | 15042.18 | 2.71 | 0.0001 |

Table 4. General linear models analysis of the arcsine square root transformed data of infection percentage of *Bromus tectorum* sown in the field at two dates after inoculation by eight populations of *Ustilago bullata* at two inoculum densities.

| Source | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|
| Population | 7/63 | 0.10 | 2.67 | 0.0175 |
| Density | 1/9 | 0.07 | 3.31 | ns |
| Date | 1/9 | 69.71 | 2211.56 | 0.0001 |
| Population x Density | 7/62 | 0.03 | 0.58 | ns |
| Date x Population | 7/51 | 0.08 | 1.46 | ns |
| Date x Density | 1/9 | 0.08 | 0.80 | ns |
| Date x Population x Density | 7/25 | 0.11 | 1.51 | ns |

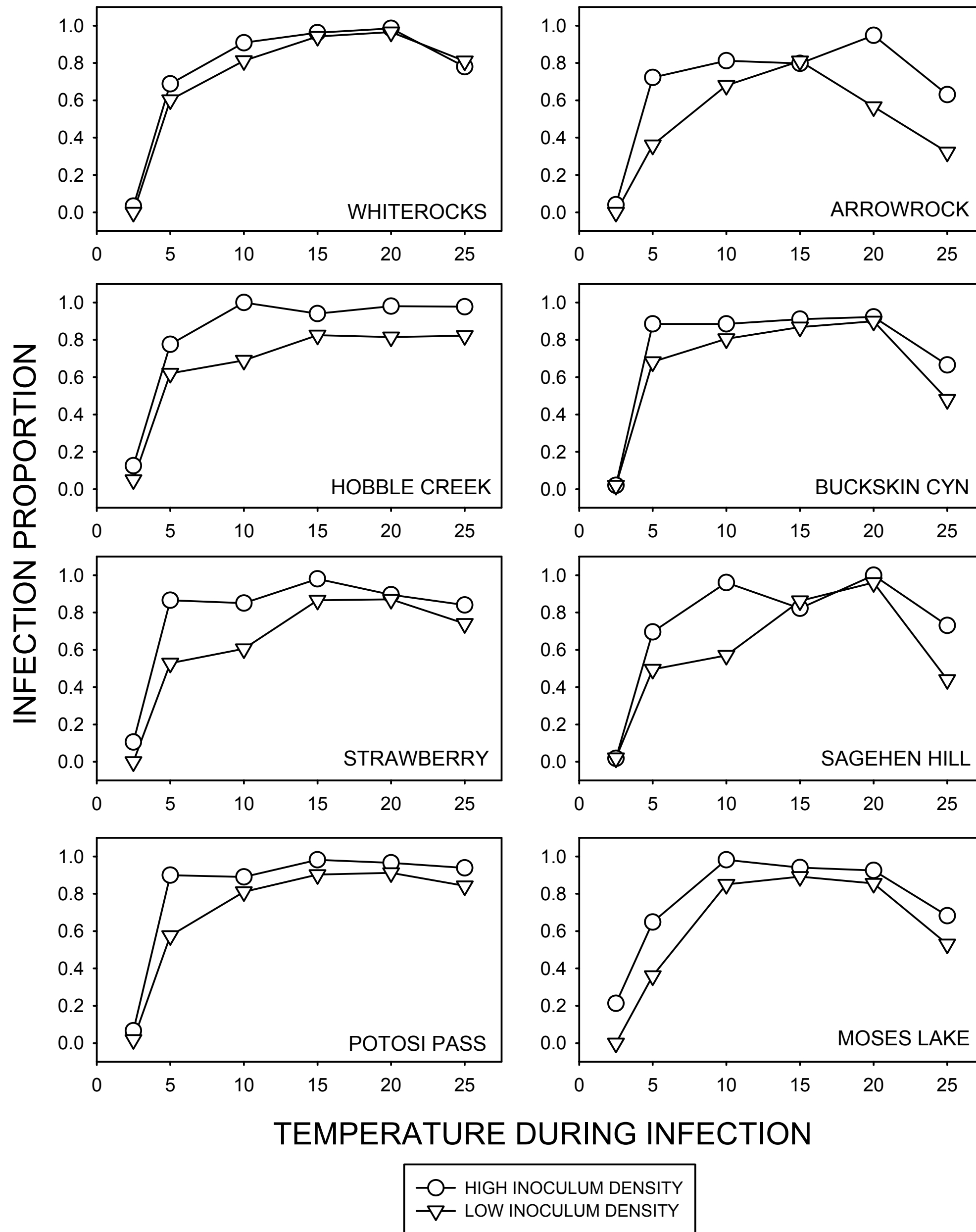


Figure 1. Infection proportion caused by each of eight teliospore populations on *Bromus tectorum* at high (3.7 g teliospores/kg seeds) and low (0.3 g teliospores/kg seeds) teliospore densities under various temperatures as an average of two greenhouse experiments. See table 2 for statistical analysis.

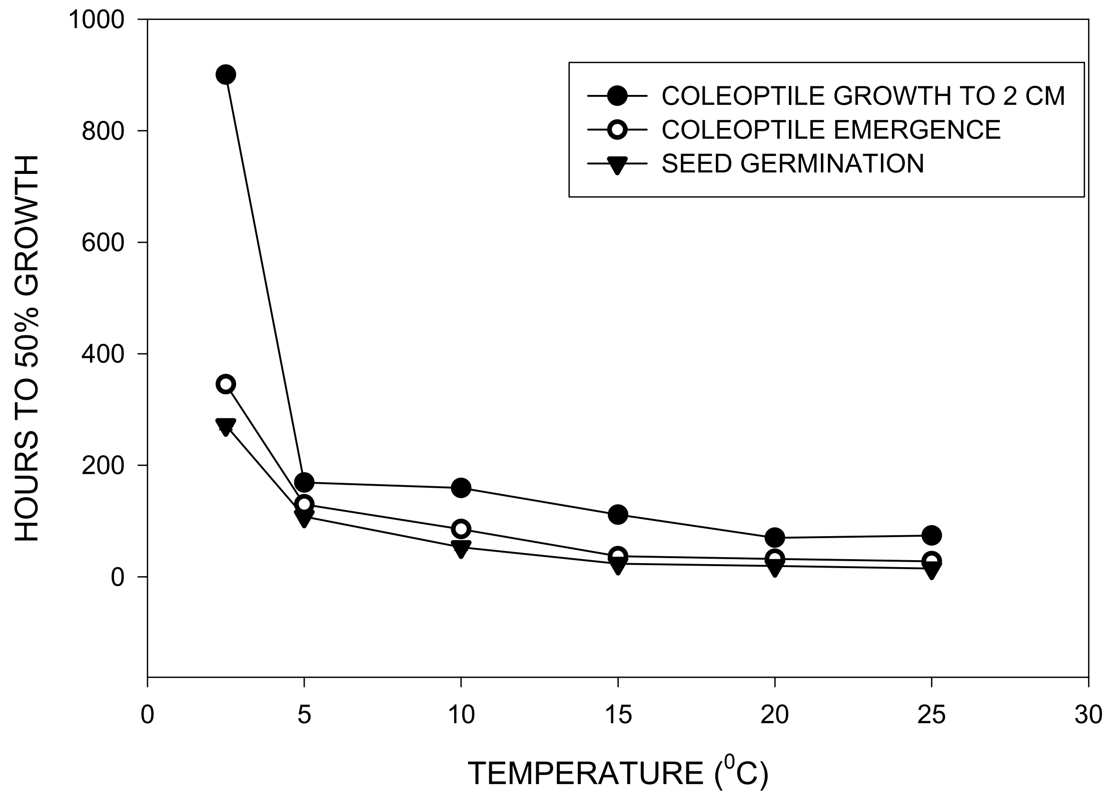


Figure 2. Mean hours to 50% seed germination, coleoptile emergence, and coleoptile elongation to 2 cm for two host lines of a Whiterocks, Utah population of *Bromus tectorum*. See text for statistical analysis.

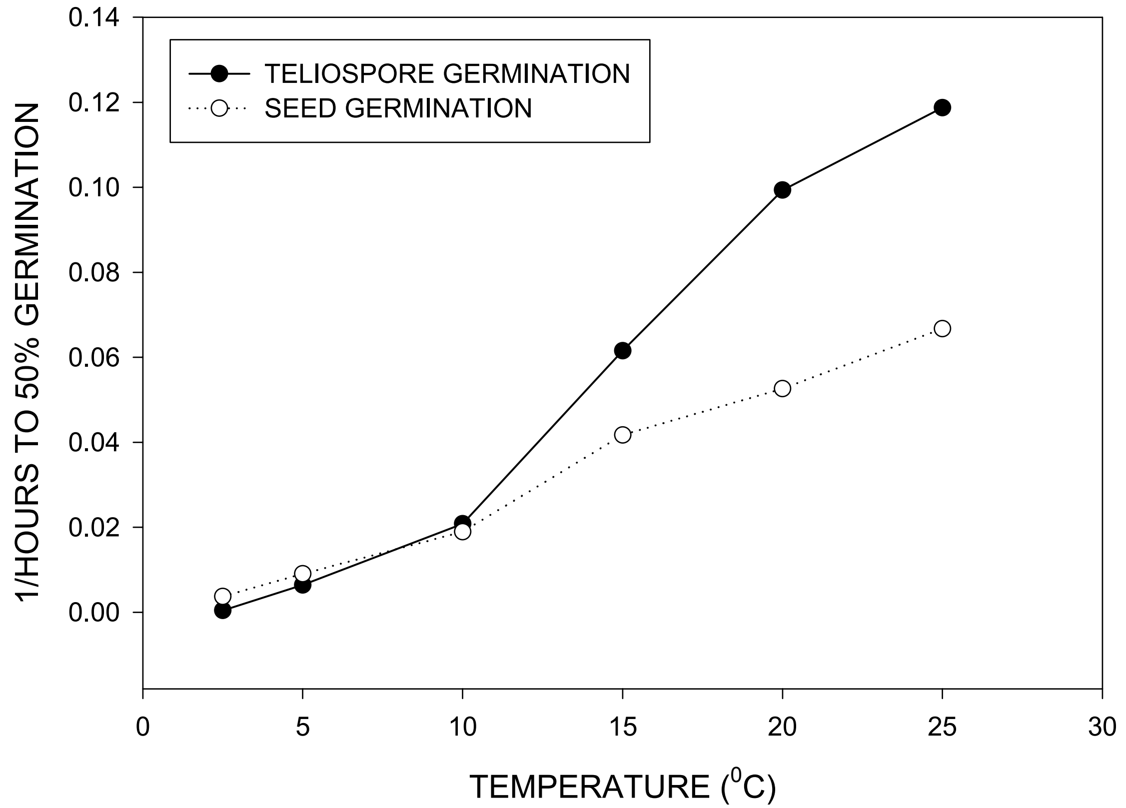


Figure 3. Mean teliospore germination time courses of eight *Ustilago bullata* populations and mean seed germination time courses of two Whiterocks *Bromus tectorum* lines used in a greenhouse experiment.

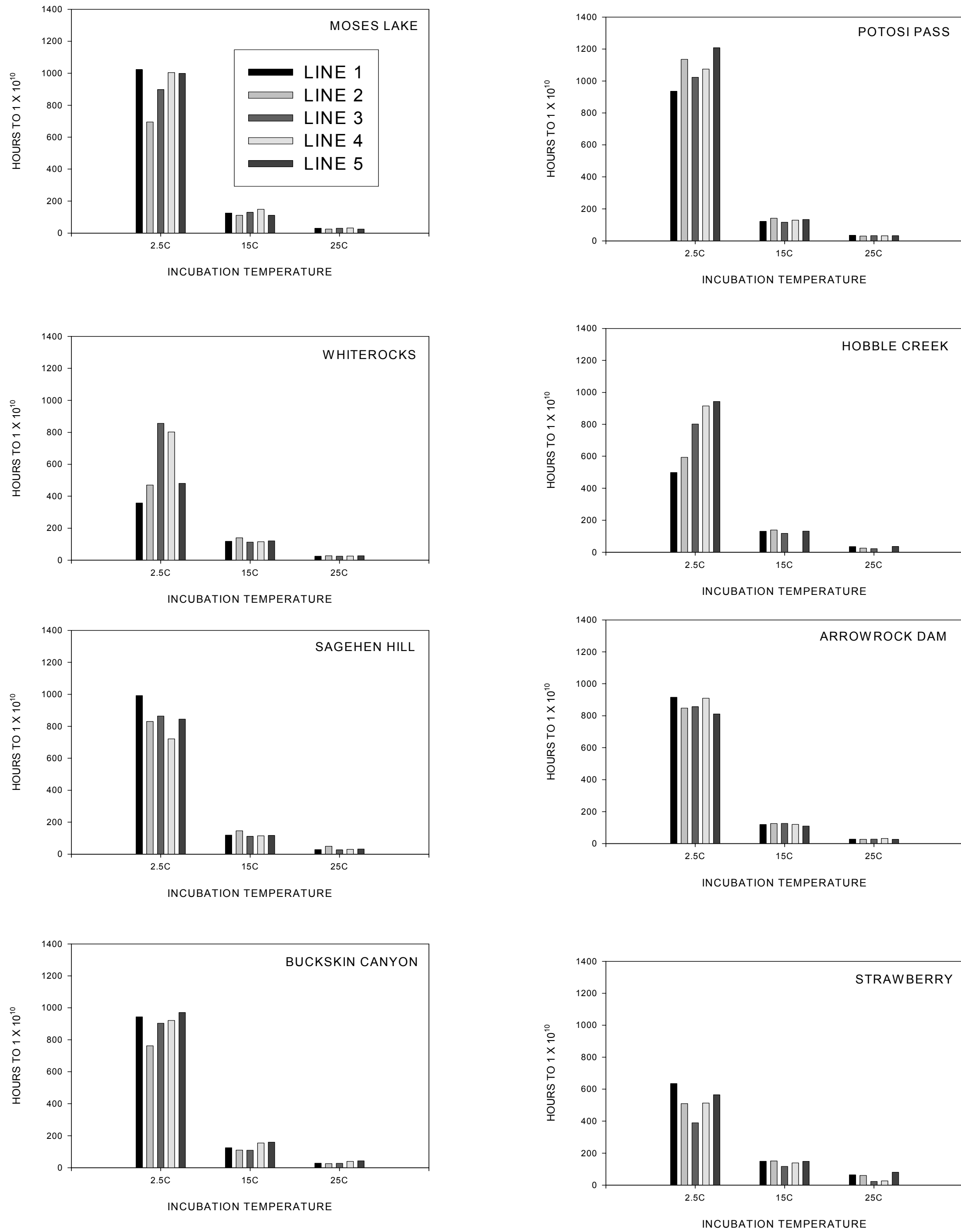


Figure 4. Sporidial proliferation rate (time to$10^{10}$) of five lines of *Ustilago bullata* selected randomly from each of eight smut populations and placed under three different temperatures. See table 3 for statistical analysis.

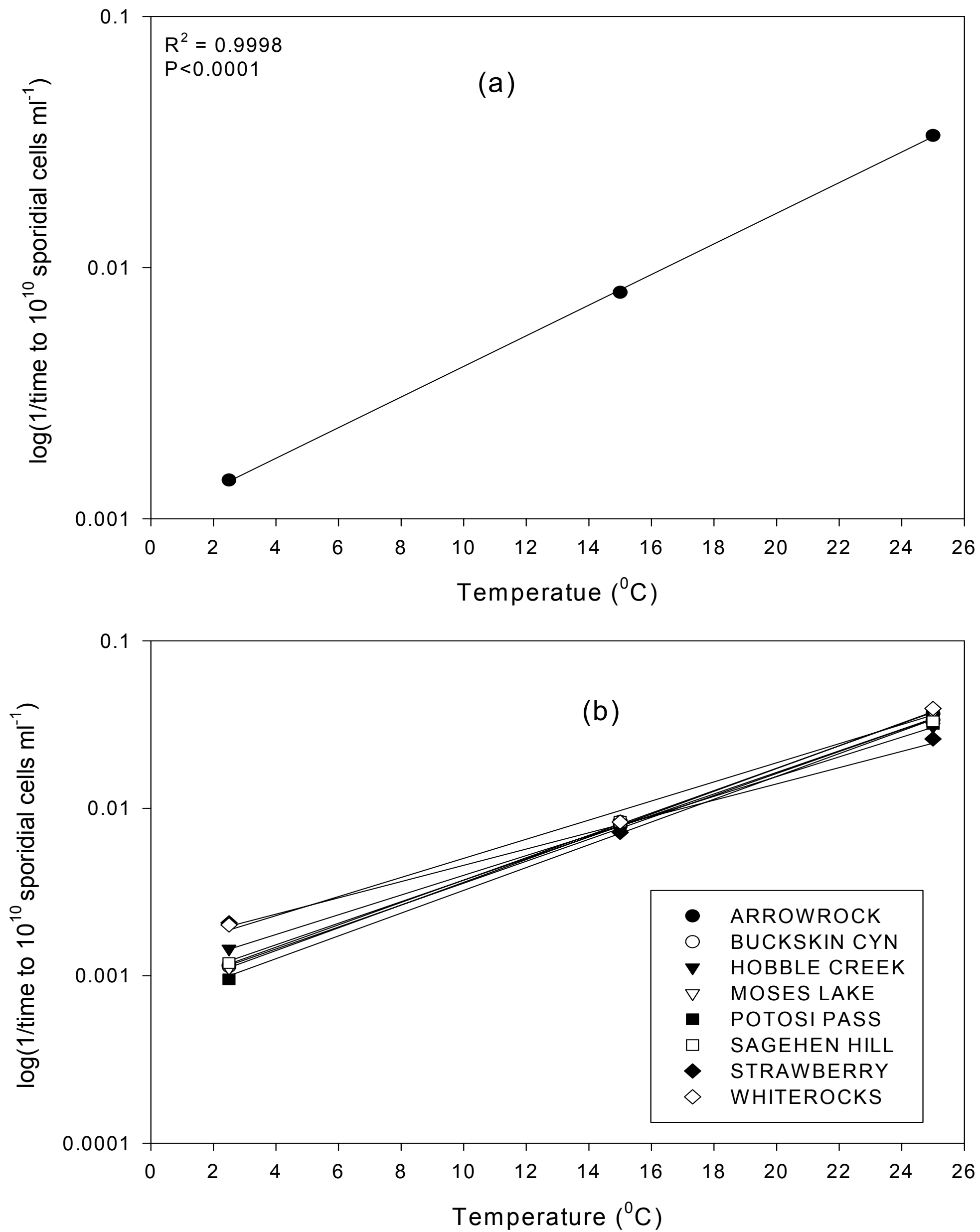


Figure 5. (a) Linear regression for the average of eight populations of *Ustilago bullata:* sporidial proliferation (log (1/time to $10^{10}$ sporidial cells/ml) regressed on temperature; (b) Linear regressions for each of the eight populations of *Ustilago bullata* sporidial proliferation (log (1/time to $10^{10}$ sporidial cells/ml) regressed on temperature.

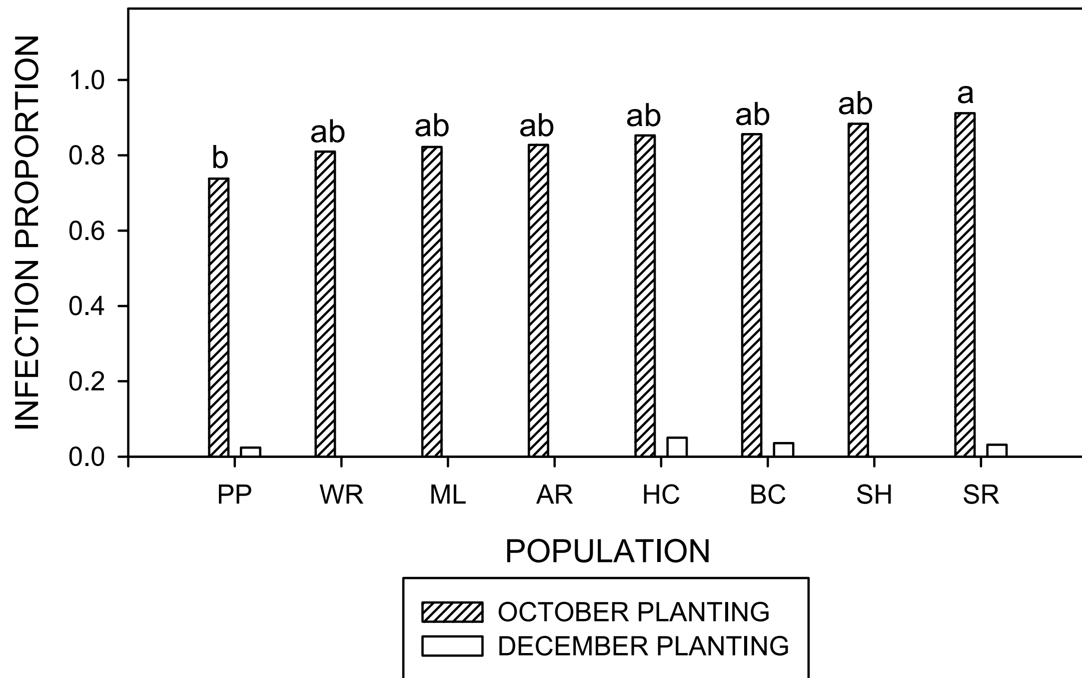


Figure 6. Disease incidence in a field experiment with a Whiterocks bulk seed collection of *Bromus tectorum* inoculated with eight teliospore sources: PP = Potosi Pass, WR = Whiterocks, ML = Moses Lake, AR = Arrowrock, SH = Sagehen Hill, BC = Buckskin Canyon, HC = Hobble Creek, ST = Strawberry Reservoir. Columns headed by the same letter are not significantly different ($p < 0.05$). See table 4 for full statistical analysis.

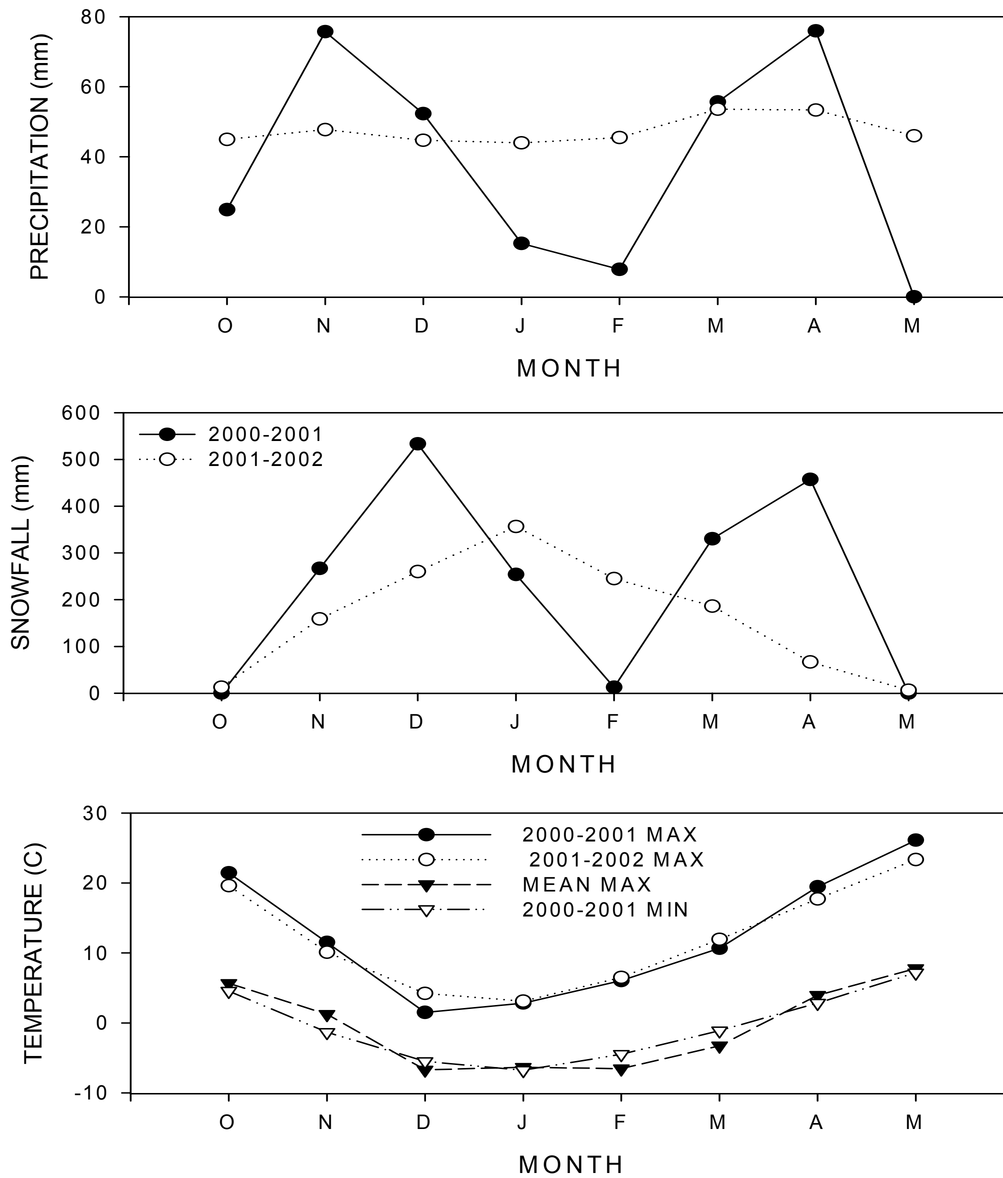


Figure 7. Mean precipitation (mm), snowfall (mm), and temperature ($^{0}$C) from October to May for the years 2000-2001 in Spanish Fork, Utah (Spanish Fork Power Plant NOAA station).

# Chapter 3

## Field infection percentage of *Ustilago bullata* Berk. on *Bromus tectorum* L. as a function of time of planting date, inoculum density, soil moisture, and litter

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## ABSTRACT

The seedling-infecting pathogen *Ustilago bullata* Berk. has potential as a naturally occurring biological control agent for cheatgrass (*Bromus tectorum* L.) in the field. We investigated infection success of *Ustilago bullata* on *Bromus tectorum* in cultivated fields as a function of seeding date, inoculation method, inoculum density, supplemental watering, and litter addition. With two planting dates of early and late fall, the early-emerging cohorts were about 8 to 10 times more likely to be infected than late emerging cohorts. The warmer early-fall rather than the colder late-fall provided an environment suitable for successful infection, a result in accord with laboratory and growth chamber experiments on this pathosystem. The addition of litter also increased infection by modifying the microenvironment around the germinating host seeds. Supplemental watering had little effect on proportion of infected plants, though it greatly increased the number of plants establishing. Applying the inoculum by dusting the seeds prior to planting was more effective than spraying inoculum on the seeded plots, but infection success achieved with the highest level of sprayed inoculum approached that achieved by dusting. Inoculum density was positively correlated with infection success. To efficiently use *Ustilago bullata* as a biocontrol agent for *Bromus tectorum*, teliospores need to be sprayed onto the *in situ* host seed bank at high density (460g/kg of seeds or more) in early autumn. Such a biocontrol program is most likely to be effective under a climate regime where autumn precipitation permits emergence of most of the host seed bank as a fall cohort.

## INTRODUCTION

The systemic seedling-infecting pathogen, *Ustilago bullata* Berk. causes head smut disease in grasses throughout the world (Zundel, 1953). It can significantly reduce cheatgrass (*Bromus tectorum* L.) populations in the field and may have potential as a biocontrol organism (Peeper, 1984). It overwinters in the soil as diploid teliospores (Agrios, 1997). When conditions are suitable for germination, teliospores germinate to produce basidiospores through meiosis (Alexopoulos, 1952; Bold et al., 1987; and Meyer et al., 2001). The basidiospores can either unite as compatible mating types while on the basidium and produce dikaryotic infection hypha or they can divide mitotically producing sporidia that can also later fuse to form dikaryotic hypha to penetrate the host (Agrios, 1997). The infected host shows little or no symptoms during vegetative growth until maturity when teliospores replace seeds at the inflorescence (Fischer and Holton, 1957). In the field, even when *Ustilago bullata* infection reaches an epidemic proportion, some cheatgrass plants are not smutted.

Under field conditions, the ability of a seedling-infecting pathogen like *Ustilago bullata* to infect successfully is partly related to temperature at the time of germination and emergence (Falloon, 1979; Falloon and Rolston, 1990; Thrall and Jarosz, 1994; Gossen and Turnbull, 1995; Nzioki et al., 2000; and Dissertation, chapter 2) and inoculum density and proximity to the host (Pope and McCarter, 1992; Agrios, 1997; Osario and Frederiksen, 1998; Bruckart, 1999; and Meyer et al., 2001). *Ustilago bullata* normally infects seedlings before they emerge from the soil, and then grows internally in the developing plants until it reaches the inflorescence (Agrios, 1997), but under cold temperatures, the infection success decreases (Dissertation, chapter 2). Under harsh

winter conditions, grass plants were found to exhibit reduced disease incidence and severity from the pathogen smut (Gossen and Turnbull, 1995). On the other hand, Falloon (1979) found the infection by *Ustilago bullata* to be reduced when seedlings of *Bromus catharticus* were grown for 2 weeks at 32.5$^0$C. Thus, temperatures much higher or lower than optimum during host seedling emergence are responsible for reduced disease incidence by *Ustilago bullata*.

The inability of *Ustilago bullata* teliospores to successfully infect cheatgrass seedlings at lower temperatures is probably partly due to the higher level of intratetrad matings by teliospores under such temperatures (Chapter 1). We also observed that as distances from optimum temperature for infection increased, infection decreased and density effect increased (Chapter 1). At optimum or near optimum temperatures, teliospores germinated faster than host seeds while the opposite was observed at lower temperatures. This suggested that for successful infection, *Ustilago bullata* teliospores need to germinate faster than the cheatgrass seeds.

The goal of this study was to understand how environmental factors interact with *Ustilago bullata* inoculum density in the field to regulate disease incidence for susceptible *Bromus tectorum* host populations. We hypothesized that infection success under field conditions decreases more as a function of inoculum density under temperature-limiting conditions, i.e., infection success is more limited by low inoculum density when host germination and emergence take place at a season when temperatures are unfavorable for the pathogen. We also proposed that factors that extend the period of high humidity in the infection court, namely litter addition and supplemental watering, would increase the proportion of infected plants. We studied the direct effect of

environmental limitations by sowing pre-inoculated seeds. To examine the feasibility of inoculating the *in situ* seed bank, a scenario more like a biocontrol treatment, we tested a water suspension of teliospores.

# MATERIALS AND METHODS

## First year field test

Field tests were conducted on experimental plots at the Brigham Young University farm east of Spanish Fork, Utah. The field design each year was a randomized block design with 10 replications. In 2000-2001, the experiment included the following variables: two inoculation methods, three inoculum densities, two irrigation treatments, two litter treatments, and two planting dates. Each experimental unit was a 0.093 $m^2$ plot.

## Seed sterilization

We surface-sterilized cheatgrass seeds prior to inoculation to minimize the effect of other microorganisms. Seeds of a bulk collection of the highly susceptible Whiterocks population were cleaned and put on a silver-gray fiberglass screen and dipped in 10% chlorox in a beaker for 3 minutes. The seeds were then rinsed by dipping the screen consecutively into six to seven other beakers filled with distilled water. The screen was then dipped in 95% ethanol for 1 minute, and then rinsed again in distilled water. Seeds were spread and dried overnight under a ventilation hood. About 50 seeds (estimated by weight) were placed into packets or vials for pre-inoculation treatment and eventually planting into each of the 0.093 $m^2$ plots.

## Litter collection, sterilization, and application

*Bromus tectorum* straw was collected in the field late in the growing season when almost all the seeds had shed. The straw was chopped, soaked in water, and autoclaved for 30 minutes at $121^0$C. The straw was then spread and dried under the hood for two

days. Twenty grams of sterilized straw were manually spread on the litter-added plots after all the treatments except watering had been applied.

**Inoculation levels and methods**

The dusting inoculation method consisted of by adding a given amount of teliospores to vials containing cheatgrass seeds. The high inoculum density treatments received an equivalence of 3.7g of teliospores/kg of seeds, the medium inoculum density treatment received 1.9g of teliospores/kg of seeds, and the low inoculum density treatment received 0.9g/kg of seeds. The vials were shaken thoroughly to get the teliospores attached to the seeds. The seeds were then spread in the appropriate field plots.

The spray inoculation method was done by adding a given amount of teliospores into 2.5 liters of 1% Tween 80 solution in a pressure sprayer. The high inoculum density treatments received an equivalence of about 230g/kg of seeds or 0.085g/0.09 $m^2$ plot, the medium inoculum density treatment received about 115g/kg of seeds or 0.043g/0.09 $m^2$ plot, and the low inoculum density treatment received about 58g/kg of seeds or 0.021g/0.09 $m^2$ plot. The sprayer was shaken thoroughly before each application and then the solution was evenly spread onto the appropriate plots already planted with seeds.

**Planting date and watering treatment**

The field was planted with *Bromus tectorum* seeds receiving the above treatments at two different planting dates. Seeds were planted both the first week of October and 60 days later during the first week of December. Some plots were irrigated for early emergence using water pillows set above the plots delivering water equivalent to 25.4

mm of precipitation directly to the plots. Only the non-irrigation treatment was included for the December planting date because the ground was saturated at planting time.

Percent disease incidence was assessed at the end of the growing season by harvesting individual plants and determining whether or not their heads were smutted.

**Second year field test**

The second field test was conducted on a different section of the BYU Spanish Fork farm. The experiment consisted of two planting dates, three irrigation treatments, four inoculum densities, and two litter treatments. The inoculum densities were: high dusted (7.4g/kg of seeds), high sprayed (460g/kg of seeds or 0.170g/0.09 $m^2$ plot), medium sprayed (230g/kg of seeds), and low sprayed (58g/kg of seeds). They were applied as in the previous experiment.

**Data analysis**

Data sets for each year were analyzed as two balanced ANOVA designs, one for the first planting date (including the watering main effect) and one for the no water treatments (including planting date as a main effect). Main effects and interactions for each experiment were tested for significance using the appropriate error terms for a randomized block design with fixed main effects (Quinn and Keough, 2002). The response variables were: proportion of infected plants per plot and number of host individuals established per plot (stand density). A General Linear Model (GLM) procedure was used because of missing data cells for infection proportion due to establishment failure in some plots. The Ryan Einot Gabriel Welsch (REGWQ) was used to test main effects means and among sub-set of means when interactions were

significant. The appropriate block interaction term was used as the error term for mean separation.

# RESULTS

## Early planting date

Proportion of cheatgrass plants infected for the 2000-2001 early field planting varied significantly by inoculation method ($P < 0.0001$), litter treatment ($P < 0.0081$), and inoculum density ($P < 0.0037$; Table 1). There were no significant difference between water treatments and no significant interactions (Table 1). Dusting produced more infection than spraying, plots treated with litter produced more infection than those treated with no litter, and infection increased with inoculum density (Fig. 1a).

Infection proportion for the 2001-2002 early field planting varied significantly for litter treatment ($P < 0.0480$), inoculum density ($P < 0.0205$), and the interaction between water and litter ($P < 0.0321$, Table 2). There were no significant differences between water treatments and no other significant interactions. Infection increased with inoculum density, with dusting giving the highest infection percentage (Fig. 1b). Just as in the previous year's experiment, there was more infection with litter than with no litter (Fig. 1b). The greatest litter effect was observed when no water was applied (Fig. 2). Litter may have created a microenvironment that compensated for lack of water.

Cheatgrass stand density (seedlings/plot) for the 2000-2001 early planting experiment varied significantly by water treatment ($P < 0.0001$), litter treatment ($P < 0.0046$), and the interaction between litter and water ($P < 0.0001$) (Table 3). There were no significant differences between inoculation method or density, and no significant interactions. The watered plots yielded more plants than those not watered (Fig. 3a). Litter-treated plots also produced more plants than when no litter was used. The presence of both water and litter gave the highest stand density.

Stand density for the 2001-2002 early planting date varied significantly for water treatment ($P < 0.0001$), litter treatment ($P < 0.0001$), and inoculum density ($P < 0.0331$). The interaction between litter and water was also significant ($P < 0.0001$) (Table 4). There were no other significant interactions. Watering after planting gave the highest stand density, followed by the before-planting water treatment with the no water treatment being the last (Fig. 3b). In general, litter produced more plants than lack of litter. With no water, littered plots yielded three times more plants than non-littered plots (Fig. 3b). With water applied before planting, littered plots produced four times more plants than non-littered plots (Fig. 3b). With water applied after planting, littered plots produced 1.3 times more plants than non-littered plots (Fig. 3b). Dusting, which produced the highest infection, also yielded the lowest stand density suggesting an effect of the pathogen at the emergence stage.

**Unwatered treatment set**

Infection proportion for the unwatered early and late plantings in the 2000-2001 field experiment varied significantly by inoculation method ($P < 0.0001$), date of planting ($P < 0.0001$), inoculum density ($P < 0.0283$), the interaction of inoculation method and planting date ($P < 0.0001$), and the interaction of inoculum density and litter ($P < 0.0412$, Table 5). There were no significant differences between litter treatments and no other significant interactions (Table 5). Dusting produced more infection than spraying in the unwatered treatment set, and infection increased with inoculum density just as in the early planting treatment set. Early planting gave much higher infection percentages than late planting (Fig. 4a). Spraying was relatively less effective in the late planting. Early planting produced 26 times more infection than late planting for dusting and 71 times

more for spraying, accounting for the date by inoculum method interaction. Curiously, litter was more effective at medium inoculum density.

Infection proportion for unwatered plots in the 2001-2002 field planting varied significantly only by planting date ($P < 0.0001$, Table 6). Early planting produced more infection than late planting (Fig. 4a). Even though the inoculum density was not significant it showed a pattern similar to the 2000-2001 experiment with infection increasing with density.

Stand density of cheatgrass for the unwatered plots in the 2000-2001 experiment varied significantly only for planting date ($P < 0.0004$, Table 7). Early planting resulted in greater stand density than late planting (Fig. 4b).

Stand density for the unwatered plots in the 2001-2002 field experiment varied significantly by planting date ($P < 0.0001$), litter treatment ($P < 0.0001$), and the interaction of litter and planting date ($P < 0.0001$, Table 8). There were no significant differences between inoculum densities, and no other significant interactions (Table 8). Early planting produced greater stand density than late planting (Fig. 4b). In general, stand density was greater in 2002 than 2001 (Fig. 4b). By adding litter in the early planting produced three times more plants, while adding litter in the late planting produced 17 times more plants, which explains the significant planting date by litter interaction in 2002.

## DISCUSSION

Under field conditions, the ability of the *Ustilago bullata* pathogen to successfully infect *Bromus tectorum* was related to temperature at the time of seed germination and seedling emergence, and thus to the season of germination and emergence. There was more infection when seeds germinated early in the fall than in the late fall for both field trials. More plants established from the October planting than from the December planting. This result agrees with our earlier finding where little to no infection was recorded when inoculated seeds germinated at $2.5^0$C in the field for a late planting in December (Chapter 2). The field late plantings for both years were done when the December through February temperatures were on average.

Litter addition probably increased infection by conserving moisture and by promoting microbial activities that might have altered the temperature of the soil. The presence of litter provided an environmental condition that was favorable to both the host and the pathogen. More plants emerged when litter was used. On the other hand, watering the plots substantially increased host establishment, but generally did not affect infection success. Unwatered ambient field moisture conditions were enough to sustain maximum infection.

In the 2000-2001 early autumn trial, dusting the seeds before planting resulted in greater infection than that obtained by applying the inoculum in aqueous solution to the plots after planting. In the 2001-2002 trial, infection proportions were much higher overall than the previous year reflecting more favorable environmental conditions for infection, and the spray treatments approached dusting in their effectiveness. We used

higher spray inoculum densities the second year, but even the treatments at comparable densities yielded much more infection in 2001-2002 than in 2000-2001.

Another phenomenon we observed was the density effect on host emergence. Higher inoculum density limited host emergence during the second year. This result is in accord with other reports that smut fungi were also found to influence host seed germination and emergence. The rate of emergence of *Bromus catharticus* can be significantly reduced by *Ustilago bullata* but the final percent emergence may not be affected (Guzman et al., 1996). Falloon (1976) reported that both the rate and the final percent emergence were depressed at high inoculum levels. A field experiment indicated that plants infected by *Ustilago violacea* had higher mortality rate than healthy ones, especially under harsh winter conditions (Thrall and Jarosz, 1994). A similar phenomenon was observed earlier by Falloon (1976) with *Ustilago bullata* on *Bromus catharticus.* He also found that *Ustilago bullata* increased the vulnerability of infected plants to other soil-borne pathogens. In a field trial, Falloon and Hume (1988) observed that all plants infected by *Ustilago bullata* died of a bacterial wilt disease epidemic while none of the healthy ones did. Grass individuals infected with *Ustilago bullata* produced less biomass than non-infected ones partly because fewer tillers were produced by infected plants (Falloon and Hume, 1988). In previous experiments, Falloon (1976) reported decreased numbers of flowering tillers from infected plants in the field but not in the glasshouse.

Conditions for infection for plants emerging from the late planting were generally so poor that low infection proportions made it difficult to detect other treatment effects. Our hypothesis that inoculum density effect would be more evident under such sub-

optimal conditions was not supported. This was also true at winter temperatures in growth chamber experiments where no infection was observed regardless of the inoculum level (Chapter 2). During an investigation of effects of sowing date, soil temperature, fungicide seed treatments, and inoculation of the smut fungus, *Ustilago bullata* on the establishment, seed production, and herbage of *Bromus willdenowii* Kunth, Falloon and Rolston (1990) observed that the smut fungi reduced seedling establishment in all planting dates except late autumn. They also found that infected seeds produced higher herbage in early than late sowings.

The use of *Ustilago bullata* as a biocontrol agent for *Bromus tectorum* can be effective only where most seedlings emerge in response to early autumn rains. The level of inoculum and its proper delivery to the infection court is crucial. Both spraying and dusting can achieve high infection if the right amount of inoculum is used. The presence of litter in the field from previous years can also favor higher infection.

**LITERATURE CITED**

Agrios, G. N. 1997. Plant pathology. 4th ed. Academic Press, Inc. San Diego, California.

Alexopoulos, C. J. 1952. Introductory mycology. 2nd ed. John Wiley and Sons, New York, New York.

Bold, H. C., C. J. Alexopoulos, and T. Delevoryas. 1987. Division Amastigomycota III: Subdivision Basidiomycotina. p. 787-790. In: Morphology of plants and fungi. 5th ed. John Wiley and Sons. New York, New York.

Bruckart, W. L. 1999. A simple quantitative procedure for inoculation of safflower with teliospores of the rust fungus, *Puccinia carthami.* Plant Disease 83: 181-185.

Falloon, R. E. 1976. Effect of infection by *Ustilago bullata* on vegetative growth of *Bromus catharticus.* New Zealand Journal of Agricultural Research 19: 249-254.

Falloon, R. E. 1979. Effect of temperature on the relationship between *Ustilago bullata* and *Bromus catharticus*. Transactions of the British Mycological Society 73(1): 41-47.

Falloon, R. E., and Hume, D. E. 1988. Productivity and persistence of prairie grass (*Bromus willdenowii* Kunth). 1. Effect of the head smut fungus *Ustilago bullata* Berk. Grass Forage Science 43:179-184.

Falloon, R. E. and M. P. Rolston. 1990. Productivity of prairie grass (*Bromus willdenowii* Kunth.) affected by sowing date and the head smut fungus (*Ustilago bullata* Berk). Grass and Forage Science 45: 357-364.

Fischer, G. W. and C. S. Holton. 1957. Biology and control of the smut fungi. Ronald Press, New York.

Gossen, B. D. and G. D. Turnbull. 1995. Head smut (*Ustilago bullata*) of grasses on the Canadian prairies: distribution, impact, and control. Canadian Journal of Plant Pathology 17: 49-56.

Guzman, G. G., Burdon, J.J., and Nicholls, A. O. 1996. Effect of the systemic flower infecting smut *Ustilago bullata* on the growth and competitive ability of the grass *Bromus catharticus*. Journal of Ecology 84: 657-665.

Meyer, S. E., D. L. Nelson, and S. Clement. 2001. Evidence for resistance polymorphism in the *Bromus tectorum – Ustilago bullata* pathosystem: implications for biocontrol. Canadian Journal of Plant Pathology 23: 19-27.

Nzioki, H. S., L. E. Claflin, and B. A. Ramundo. 2000. Evaluation of screening protocols to determine genetic variability of grain sorghum germplasm to *Sporisorium sorghi* under field and greenhouse conditions. International Journal of Pest Management 46(2): 91-95.

Osario, J. A. and R. A. Frederiksen. 1998. Development of an infection assay for *Sporisorium relianum*, the head smut pathogen on sorghum. Plant Disease 82: 1232-1236.

Peeper, T. F. 1984. Chemical and biological control of downy brome (*Bromus tectorum*), in wheat and alfalfa in North America. Weed Science 32(1): 18-25.

Pope, D. D. and S. M. McCarter. 1992. Evaluation of inoculation methods for inducing common smut on corn ears. Phytopathology 82: 950-955.

Quinn, G. P. and S. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Thrall, P. H. and Jarosz, A. M. 1994. Host-pathogen dynamics in experimental populations of *Silene alba* and *Ustilago violacea*. I. Ecological and genetic determinants of disease spread. Journal of Ecology 82: 549-559.

Zundel, G. L. 1953. Ustilaginales of the world. Department of Botany, Pennsylvania State College School of Agriculture Contribution. No. 176. State College, Pennsylvania. 410p.

Table 1. General linear models analysis of infection proportion of *Bromus tectorum* by *Ustilago bullata* for a 2000-2001 early field planting. Treatments were: three inoculum densities, two inoculation techniques, two litter treatments, and two water applications. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Inoculation (I) | 1/9 | 7.07 | 223.85 | 0.0001 |
| | Water (W) | 1/9 | 0.00 | 0.09 | ns |
| | Litter (L) | 1/9 | 0.43 | 11.45 | 0.0081 |
| | Density (D) | 2/18 | 0.62 | 7.75 | 0.0037 |
| | I x W | 1/9 | 0.03 | 0.66 | ns |
| | I x L | 1/9 | 0.07 | 2.49 | ns |
| | L x W | 1/9 | 0.13 | 1.99 | ns |
| | I x D | 2/18 | 0.03 | 0.68 | ns |
| | D x W | 2/18 | 0.01 | 0.17 | ns |
| | D x L | 2/18 | 0.09 | 2.42 | ns |
| | I x L x W | 1/9 | 0.01 | 0.20 | ns |
| | I x D x W | 2/18 | 0.00 | 0.02 | ns |
| | D x L x W | 2/18 | 0.07 | 1.59 | ns |
| | I x D x L | 2/18 | 0.02 | 0.60 | ns |
| | I x D x L x W | 2/18 | 0.03 | 0.46 | ns |

Table 2. General linear models analysis of infection proportion for the 2001-2002 early field planting of *Ustilago bullata* teliospores on *Bromus tectorum*. Treatments were: four inoculum densities, two inoculation techniques, two litter treatments, and three water applications. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Water (W) | 2/18 | 0.04 | 0.39 | ns |
| | Litter (L) | 1/9 | 0.58 | 5.23 | 0.0480 |
| | Density (D) | 3/27 | 0.37 | 3.85 | 0.0205 |
| | W x L | 2/18 | 0.36 | 4.19 | 0.0321 |
| | W x D | 6/54 | 0.17 | 1.74 | ns |
| | W x L x D | 6/43 | 0.10 | 0.98 | ns |

Table 3. General linear models analysis of *Bromus tectorum* stand density for the 2000-2001 early field planting. Treatments were: two *Ustilago bullata* inoculation techniques, two litter treatments, and two water applications. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Inoculation (I) | 1/9 | 0.20 | 3.19 | ns |
| | Water (W) | 1/9 | 28.59 | 70.79 | 0.0001 |
| | Litter (L) | 1/9 | 1.78 | 13.97 | 0.0046 |
| | Density (D) | 2/18 | 0.17 | 0.58 | ns |
| | I x W | 1/9 | 0.81 | 3.84 | ns |
| | I x L | 1/9 | 0.09 | 0.21 | ns |
| | L x W | 1/9 | 5.70 | 81.73 | 0.0001 |
| | I x D | 2/18 | 0.05 | 0.21 | ns |
| | D x W | 2/18 | 0.06 | 0.24 | ns |
| | D x L | 2/18 | 0.09 | 0.82 | ns |
| | I x L x W | 1/9 | 0.15 | 1.06 | ns |
| | I x D x W | 2/18 | 0.80 | 2.87 | ns |
| | D x L x W | 2/18 | 0.05 | 0.19 | ns |
| | I x D x L | 2/18 | 0.38 | 1.63 | ns |
| | I x D x L x W | 2/18 | 0.02 | 0.10 | ns |

Table 4. General linear models analysis of *Bromus tectorum* stand density for the 2001-2002 early field planting of four inoculum densities of *Ustilago bullata* teliospores, receiving two inoculation techniques, two litter treatments, and two water applications. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Water (W) | 2/18 | 32.11 | 32.43 | 0.0001 |
| | Litter (L) | 1/9 | 83.75 | 44.52 | 0.0001 |
| | Density (D) | 3/27 | 2.57 | 3.37 | 0.0331 |
| | W x L | 2/18 | 16.93 | 18.15 | 0.0001 |
| | W x D | 6/54 | 0.37 | 0.33 | ns |
| | W x L x D | 6/54 | 1.20 | 0.93 | ns |

Table 5. General linear models analysis of infection proportion of *Bromus tectorum* by *Ustilago bullata* for a 2000-2001 unwatered field experiment. Treatments were: three inoculum densities, two inoculation techniques, two litter treatments, and two planting dates. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | Df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Inoculation (I) | 1/9 | 2.43 | 85.65 | 0.0001 |
| | Date (DA) | 1/9 | 19.34 | 215.98 | 0.0001 |
| | Litter (L) | 1/9 | 0.01 | 0.30 | ns |
| | Density (D) | 2/18 | 0.24 | 4.38 | 0.0283 |
| | I x DA | 1/9 | 1.62 | 58.60 | 0.0001 |
| | I x L | 1/9 | 0.03 | 3.42 | ns |
| | L x DA | 1/9 | 0.04 | 0.92 | ns |
| | I x D | 2/18 | 0.03 | 0.85 | ns |
| | D x DA | 2/18 | 0.09 | 2.52 | ns |
| | D x L | 2/18 | 0.11 | 3.83 | 0.0412 |
| | I x L x DA | 1/9 | 0.00 | 0.04 | ns |
| | I x D x DA | 2/18 | 0.02 | 0.41 | ns |
| | D x L x DA | 2/18 | 0.05 | 1.65 | ns |
| | I x D x L | 2/18 | 0.01 | 0.32 | ns |
| | I x D x L x DA | 2/18 | 0.00 | 0.14 | ns |

Table 6. General linear models analysis of infection proportion of *Bromus tectorum* by *Ustilago bullata* for a 2001-2002 field experiment unwatered treatment set. Treatments were: four inoculum densities, two inoculation techniques, two litter treatments, and two planting dates. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Date (DA) | 1/9 | 9.17 | 90.99 | 0.0001 |
| | Litter (L) | 1/9 | 0.20 | 1.72 | ns |
| | Density (D) | 3/27 | 0.12 | 0.67 | ns |
| | DA x L | 1/9 | 0.19 | 1.17 | ns |
| | L x D | 3/23 | 0.00 | 0.01 | ns |
| | DA x D | 3/27 | 0.10 | 2.22 | ns |

Table 7. General linear models analysis of *Bromus tectorum* stand density for a 2000-2001 unwatered field experiment. Treatments were: two inoculation techniques, two litter treatments, and two planting dates. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Inoculation (I) | 1/9 | 0.98 | 4.60 | ns |
| | Date (DA) | 1/9 | 13.02 | 28.90 | 0.0004 |
| | Litter (L) | 1/9 | 0.01 | 0.02 | ns |
| | Density (D) | 2/18 | 0.43 | 0.97 | ns |
| | I x DA | 1/9 | 0.29 | 1.56 | ns |
| | I x L | 1/9 | 0.05 | 0.10 | ns |
| | L x DA | 1/9 | 0.92 | 2.44 | ns |
| | I x D | 2/18 | 0.88 | 1.01 | ns |
| | D x DA | 2/18 | 0.32 | 0.81 | ns |
| | D x L | 2/18 | 0.10 | 0.27 | ns |
| | I x L x DA | 1/9 | 0.09 | 0.22 | ns |
| | I x D x DA | 2/18 | 0.04 | 0.09 | ns |
| | D x L x DA | 2/18 | 0.41 | 0.86 | ns |
| | I x D x L | 2/18 | 0.06 | 0.11 | ns |
| | I x D x L x DA | 2/18 | 0.38 | 0.91 | ns |

Table 8. General linear models analysis of *Bromus tectorum* stand density for a 2001-2002 unwatered field experiment. Treatments were: two inoculation techniques, two litter treatments, and two planting dates. Arcsine square root transformed data was used with appropriate block interactions as an error term.

| Source | | Df/df | Mean square | F-value | P-value |
|---|---|---|---|---|---|
| Main Effects | Date (DA) | 1,9 | 152.97 | 143.85 | 0.0001 |
| | Litter (L) | 1,9 | 278.13 | 217.11 | 0.0001 |
| | Density (D) | 3,27 | 0.15 | 0.14 | ns |
| | DA x L | 1,9 | 43.31 | 47.33 | 0.0001 |
| | L x D | 3,27 | 0.08 | 0.08 | ns |
| | DA x D | 3,27 | 1.73 | 1.97 | ns |
| | DA x L x D | 3,27 | 1.19 | 1.45 | ns |

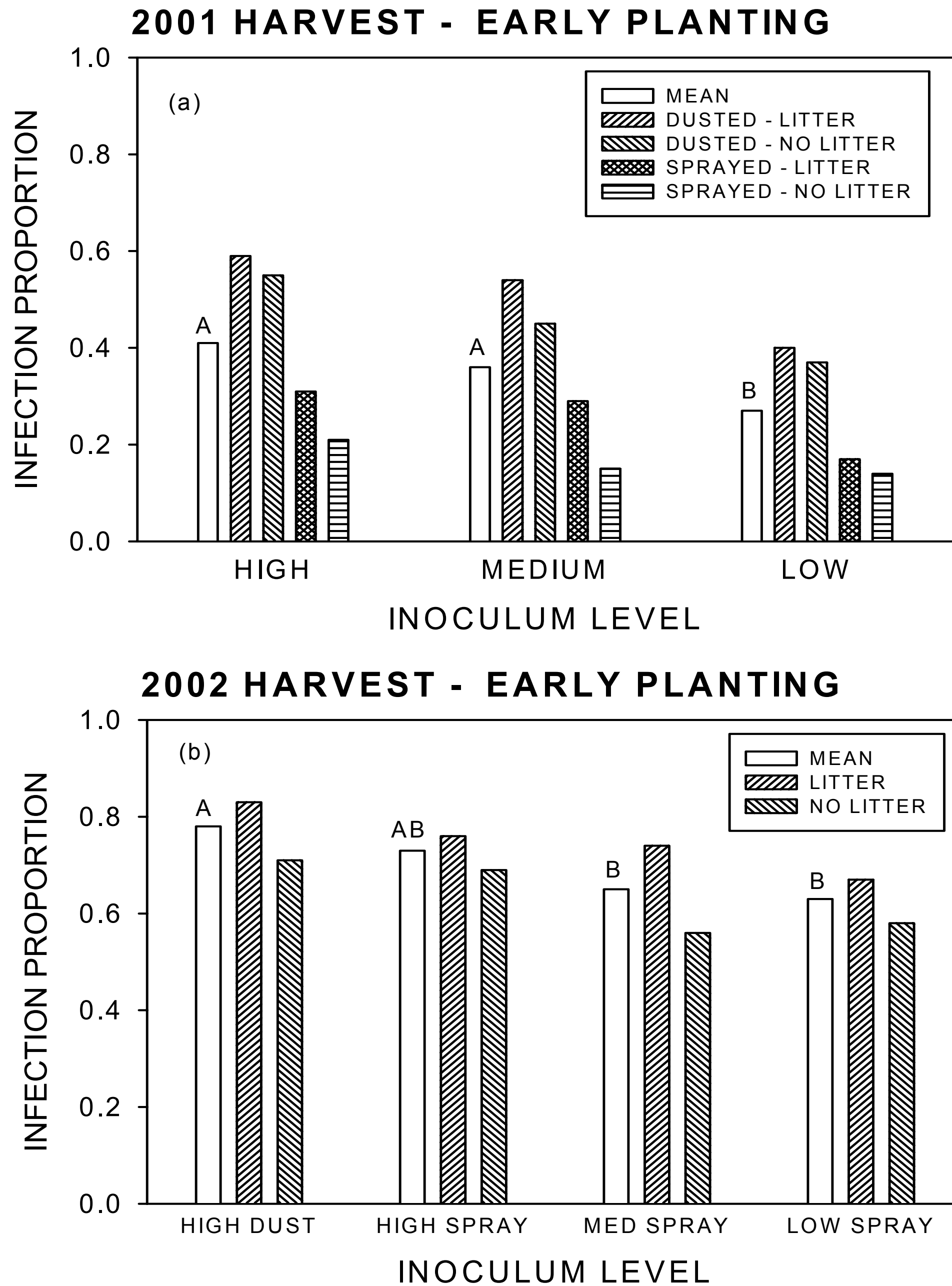


Figure 1. Proportion of *Bromus tectorum* plants infected by *Ustilago bullata* from early fall field plantings in a) 2001 and b) 2002 in response to litter addition and inoculum density and method of application. Columns headed by the same letter are not significantly different ($p < 0.05$). See tables 1 and 2 for full statistical analysis.

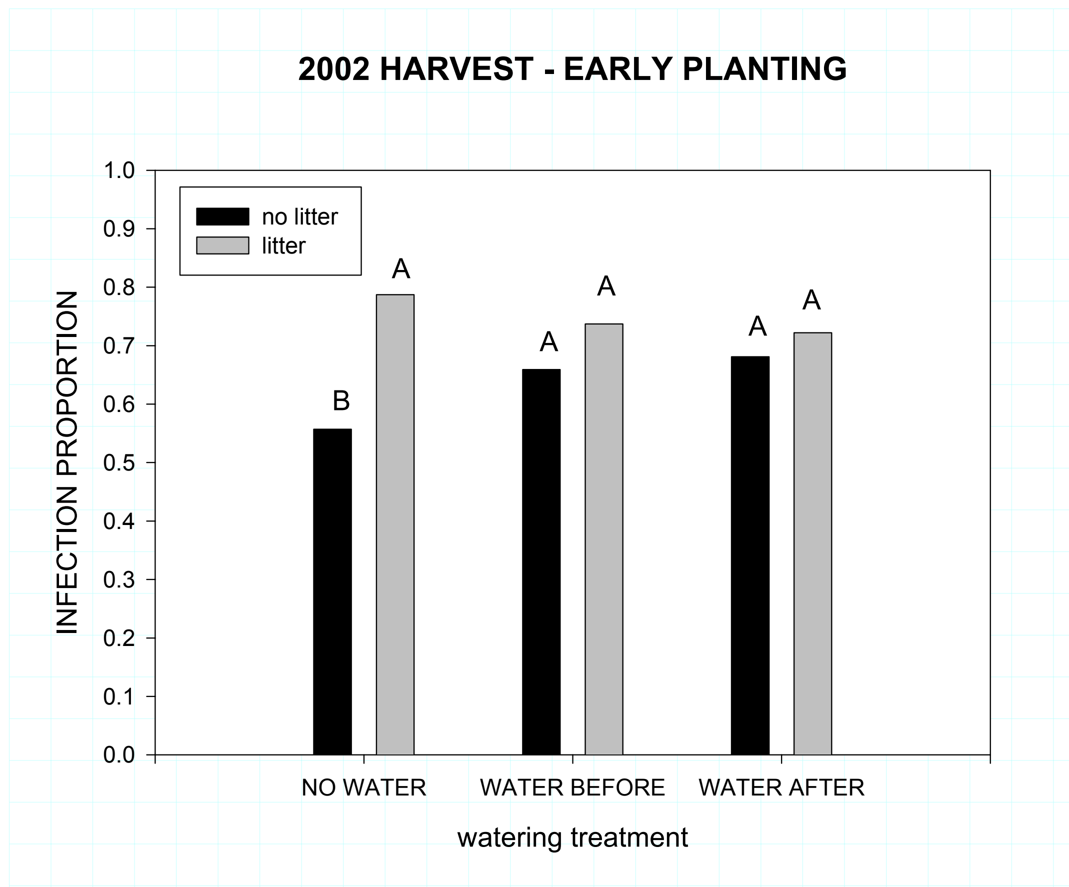


Figure 2. Infection proportion of *Bromus tectorum* by *Ustilago bullata* in a 2001-2002 field test of early fall planting in response to litter and watering treatments. See table 2 for full statistical analysis. Columns headed by the same letter are not significantly different ($p < 0.05$).

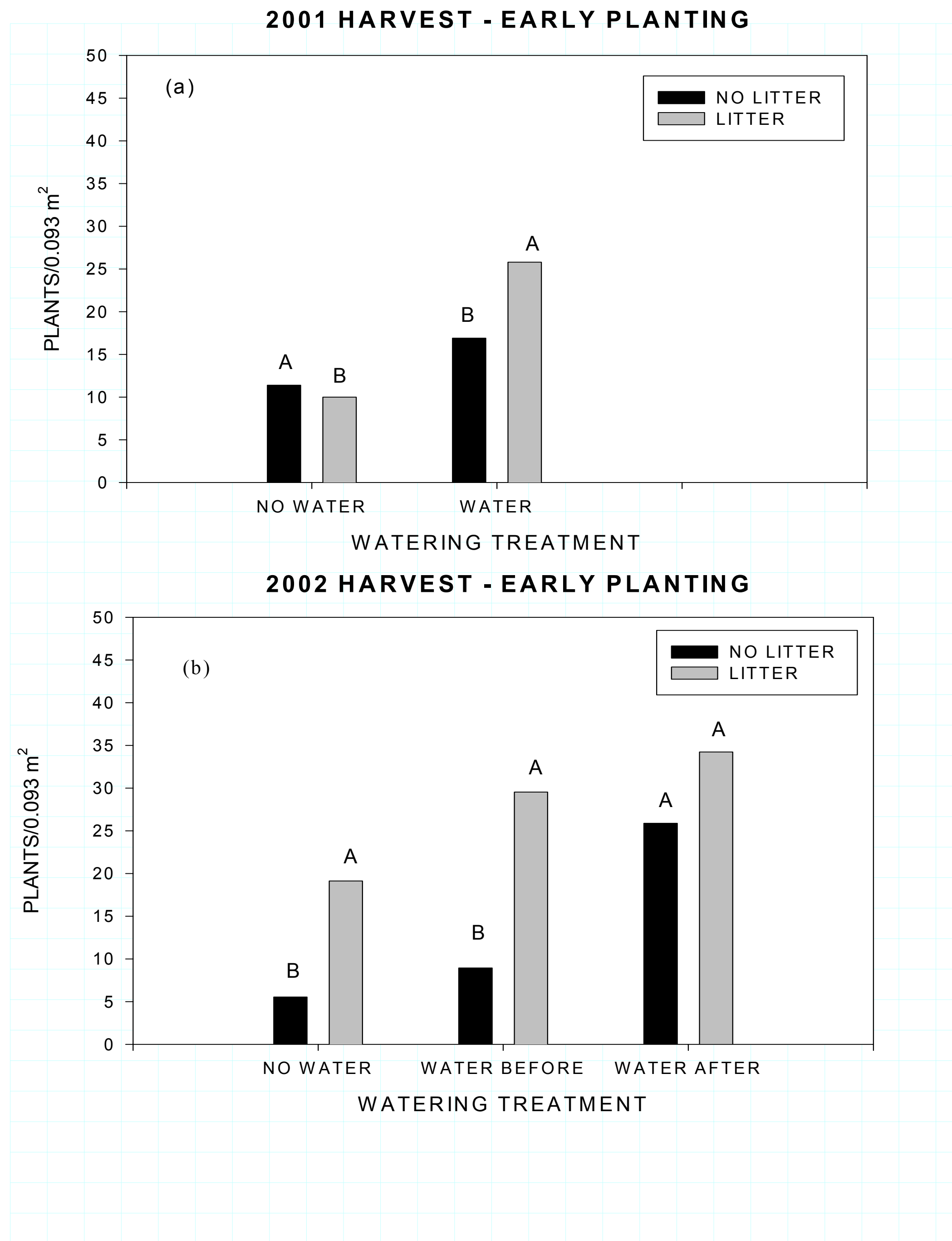


Figure 3. Stand density of *Bromus tectorum* inoculated with *Ustilago bullata* in an early fall field planting in relation to litter additions and watering treatment for a) 2000-2001 and b) 2001-2002. See tables 3 and 4 for full statistical analysis. Columns headed by the same letter are not significantly different ($p < 0.05$).

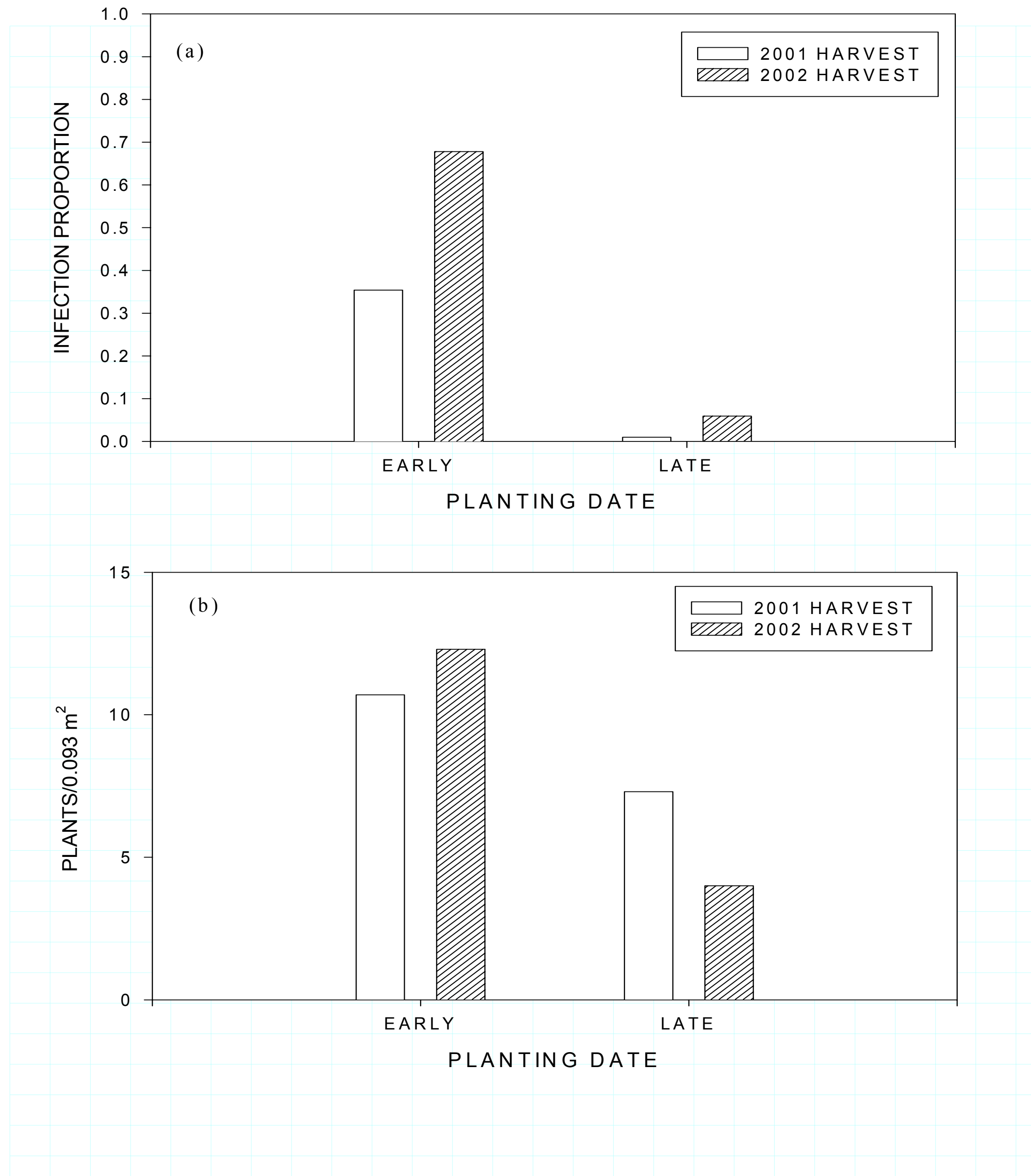


Figure 4. Infection proportion (a) and *Bromus tectorum* density (b) for *Bromus tectorum* inoculated with *Ustilago bullata* in relation to early and late fall planting dates for 2 years. See tables 5, 6, 7, and 8 for full statistical analysis.

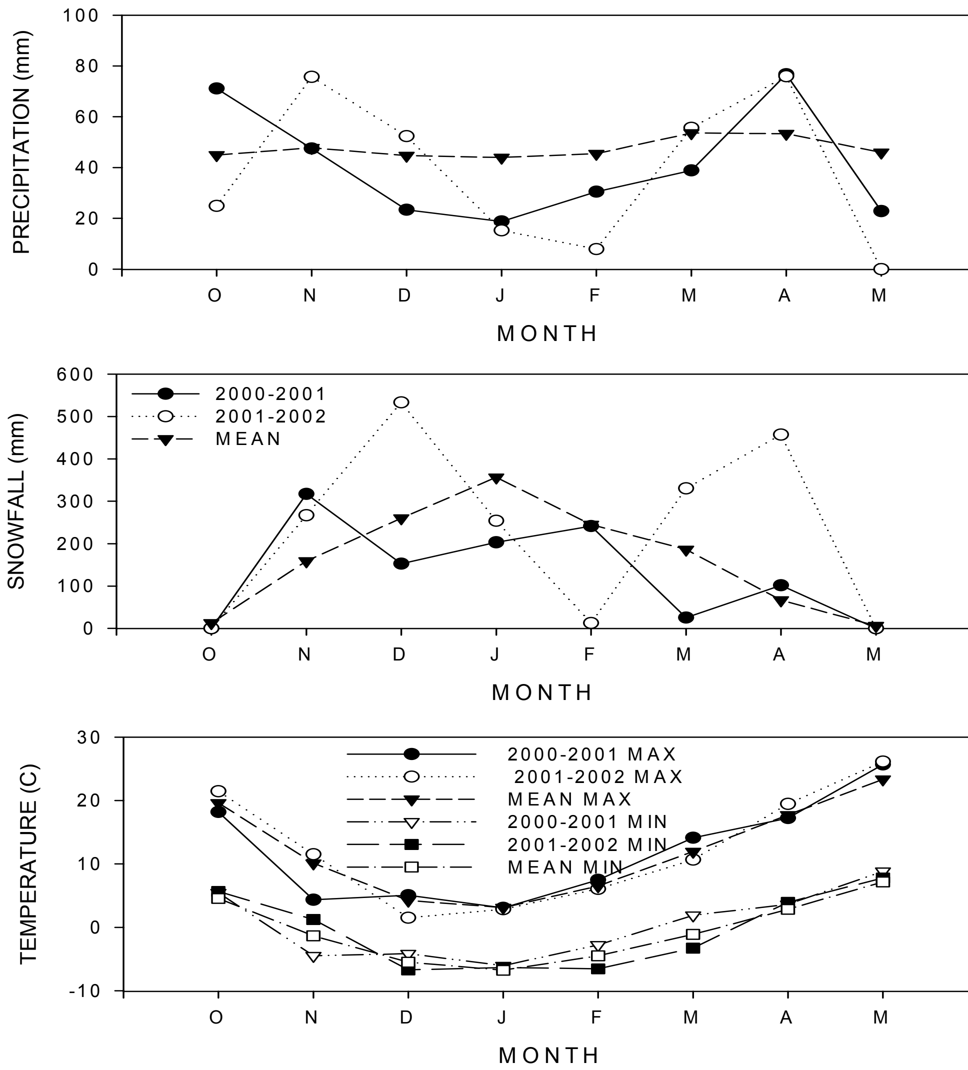


Figure 5. Mean precipitation (mm), snowfall (mm), and temperature ($^{0}$C) from October to May for the growing seasons 2000-2001 and 2001-2002 (Spanish Fork Power Plant NOAA station)