Teliospore Germination in *Puccinia grindeliae*, a Rust of the Rangeland Weed *Gutierrezia sarothrae*

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ABSTRACT

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Broom snakeweed, *Gutierrezia sarothrae*, is a weedy native shrub of the western rangeland. It is difficult and expensive to control by conventional means. *Puccinia grindeliae* has been identified as a potentially effective biocontrol agent. *P. grindeliae* is microcyclic in the field. Field-collected teliospores were 37.5–55.0 μm long by 17.5–28.8 μm wide, with a sidewall thickness of 2.5–5.0 μm and an apical wall thickness of 5.0–10.25 μm. The pedicel length of detached spores was 62.5–142.5 μm and pedicel width was 3.8–7.5 μm. Teliospore germination was favored by washing with sterile distilled water and was optimal at 15 C and 98.8% relative humidity. Under these conditions, teliospores germinated in 4–8 hr to form basidiospores, which germinated within 1 hr. Basidiospores produced in the laboratory from field teliospores were 12.5–15.0 μm long by 7.5–11.3 μm wide. Urediniospores from young pustules on plants inoculated to confirm Koch's postulates were globose and 15.3–27.5 μm in diameter. Mature teliosori formed on plants 4–8 wk after inoculation. These results suggest that germination and infection of the host generally occurs during the evening and early morning hours in the desert Southwest.

Broom snakeweed (*Gutierrezia sarothrae* (Pursh) Britton & Rusby) is a rangeland shrub native to the western United States, Mexico, and southern Canada (6). Populations of *G. sarothrae* have expanded, in both range and density, in New Mexico and Texas over the past 100 years in response to management practices and climatic factors (5,12).

The ecology of New Mexico rangelands currently favors development of large stands of broom snakeweed. This plant is an aggressive invader of disturbed areas and has displaced many forage grasses in New Mexico rangelands (8,12). A comprehensive rangeland management strategy is required to limit spread of *G. sarothrae*. The strategy should consist of a multifaceted approach including herd management, land husbandry, and weed control. Broom snakeweed is difficult to control by conventional means, such as mechanical and chemical control. Therefore, release of one or more biological control agents which specifically attack *G. sarothrae* is being investigated.

The greatest advantage of biological weed control methods over all other methods, in a rangeland setting, is the potential for natural proliferation of the biocontrol agent (2). Natural reproduction and dissemination of the biocontrol agent reduces the need for repeated applications and hence reduces costs. However, broom snakeweed is polyploid and genetically variable (6), making biological control potentially difficult.

The rust fungus *Puccinia grindeliae* Peck is a common broom snakeweed pathogen (3). However, it is only locally abundant and does not appear to be present on every stand of snakeweed in the region (7). *P. grindeliae* is associated with small areas of high snakeweed mortality and is virulent to *G. sarothrae*. It has a host range of about 24 genera of weedy Asteraceae (3) but is not reported to be a pathogen of any crop species. For these reasons, *P. grindeliae* is a promising biological control agent.

Surveys of the historical range of the pathogen, using herbarium specimens of *G. sarothrae*, showed that *P. grindeliae* has been present in New Mexico since 1906 (7). Approximately 3% of more than 1,000 snakeweed herbarium specimens were infected with it. All rust-positive collections made prior to 1990 were made east of the Rio Grande. A field survey in 1990 and 1991 showed that the rust is sparsely distributed across its range and may be expanding to the west (7).

The objective of this paper is to demonstrate the pathogenicity of *P. grindeliae* to *G. sarothrae* and to report the temperature and relative humidity requirements for teliospore germination of the pathogen.

MATERIALS AND METHODS

Plant and rust collection. Plants of *G. sarothrae* infected with *P. grindeliae* were collected from three locations in New Mexico during the fall and summer of 1990: 1) north of the Las Cruces International Airport exit ramp, 13 km west of Las Cruces, along Interstate 10; 2) 30 km north of Deming, on U.S. Route 180; and 3) 20 km east of San Antonio, along U.S. Route 380. Plant parts bearing teliosori were transported in brown paper bags. Individual teliosori were excised from plants and stored in glass petri dishes at room temperature. Both healthy and rusted whole plants were also removed from the airport site, potted, and maintained in the greenhouse as a source of telia.

Teliospore dimensions were determined by measuring the length and width of teliospores (exclusive of the pedicel) and length and width of the pedicel. Length and width of basidiospores produced in the laboratory from field collected teliospores were also measured.

Leaching experiments. A series of experiments were undertaken to determine if leaching teliosori induced germination. Excised teliosori from field plants were soaked in sterile distilled water (SDW) for up to 12 hr in nine separate trials. Soaking methods included drip, shake, and stationary soaks at 25 C in the dark. In the constant drip method, an apparatus was assembled with Büchner funnels, in which 10 teliosori were placed on Whatman No. 1 filter paper disks. The drip rate was maintained at 100 drops per minute (6.4 ml/min). In the shake method, 10 teliosori

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were added to 50 ml of SDW in a 250-ml sterile Erlenmeyer flask and placed on a rotary shaker at 100 rpm. For the stationary soak method, 10 teliosori were added to small petri dishes filled with SDW. After 1 hr the lids were removed to allow aeration.

After soaking, teliosori were blotted dry and placed on 18-mm microscope coverslips held horizontally in place with clay plugs. The whole assembly was placed into glass petri dishes with two layers of filter paper wet with 2.5 ml of SWD. Petri dishes were placed in a crisper, which in turn was placed in a dark incubator at 15 C. After 24 hr, coverslips were removed from the humidity chambers, and teliosori removed from the coverslips. Coverslips with displaced teliospores and their basidiospores were stained with lactophenol cotton blue and placed on a microscope slide. Teliospores that had germinated and produced basidiospores were scored for germination.

Temperature. Teliospore germination was studied in the dark at incubation temperatures of 5, 10, 15, 20, 25, 30, and 35 C at 100% relative humidity (RH) in six experiments. For all temperature experiments, 1-qt Mason jars served as humidity chambers. These chambers consisted of two stacked petri dishes separated by 2-cm plastic spacers. A clay plug on top was used to hold four coverslips horizontally in place. This assembly was placed on top of a bent glass triangle placed on a 150-ml glass beaker resting on the bottom of the Mason jar.

Teliosori were soaked in SDW for 1 hr, thinly sectioned (0.3 mm), and placed in small droplets of SDW on the coverslips. The coverslips were gently misted with SDW and put into the humidity chambers. Mason jars were covered with a glass petri dish bottom and sealed with rubber petri dish seals. The beakers inside the humidity chambers were filled with SDW to ensure constant relative humidity (100% RH).

There were four jars placed at each temperature. Temperature was monitored hourly by a datalogger (CR10, Campbell Scientific, Logan, UT), and temperatures remained within ± 1.5 C for all experiments reported here. Preliminary experiments indicated that highest germination rate occurred between 10 and 20 C within 48 hr. Therefore, four experiments were conducted at 5, 10, 15, and 20 C, and jars were sampled at times ranging from 1 to 48 hr.

Basidiospores on coverslips were stained with lactophenol cotton blue and placed on a slide. Basidiospores were counted with a ruler-style ocular micrometer by counting all basidiospores falling within the width of the inner hash marks. Basidiospores were counted on two axes of each teliosorus section, with one count per compass direction. Therefore, four counts were obtained from each teliosorus section.

Relative humidity. The optimum RH for teliospore germination was determined at 15 C in four experiments. RH was controlled by filling beakers in the humidity chambers with various saturated salt solutions: KI (70.9% RH), KCl (85.9% RH), KN (95.4% RH), and $K_2Cr_2O_7$ (98.8% RH) (4). SDW was used as a control (100% RH).

There were four jars per salt solution arranged in a randomized complete block design on one shelf of a 15 ± 1.5 C incubator and incubated in darkness for 24 hr. Coverslips were removed and basidiospores stained and observed under a microscope as described above.

Koch's postulates. Healthy, well-watered plants, 6 mo to 1 yr old, 10–15 cm high, with ample foliage consisting of 10-cm crowns, were used in these experiments. Plants were placed in 15 × 38 cm plastic bags to which 100 ml of distilled water was added (to maintain high RH). Rusted stems, approximately 8 cm long, were removed in the laboratory from field-collected plants containing numerous teliosori. The rusted stems were soaked in SDW for 3 hr, blotted dry with paper towels, and placed on the canopy of test plants within 15 min.

Plants were misted with distilled water before the plastic bags were sealed over the plants. Plants were incubated at 15 ± 1.5 C for 18 hr in a growth chamber with a 12-hr photoperiod. Plants were removed, and the plastic bags were opened for several hours, allowing for some drying to reduce contaminant fungal populations. Plants were then misted and bags resealed after 3 hr. Plants were returned to the incubator for 5 days and then moved to the greenhouse.

Statistical analyses. Enumeration data, such as counts of germinating teliosori or telia sections, were first analyzed by a chi-square homogeneity test to determine if results from different experiments could be pooled. Pooled enumeration data were analyzed by a chi-square or Fisher's exact test with two-dimensional tables of treatment against count. The counts of germinating telia sections in humidity experiments were analyzed by a one-way analysis of variance (ANOVA). The ANOVA was confirmed with a Kruskal-Wallis test (chi-square approximation) because of the high number of zeros in the data. Continuous variables, such as the number of basidiospores produced by teliosori in the temperature experiments or the dimensions of teliospores and basidiospores, were anaiyzed by a one-way ANOVA by time, where appropriate. Results from the temperature experiments were also analyzed by a repeated measures ANOVA to evaluate the effect of time on production of basidiospores by telia (10,11).

RESULTS AND DISCUSSION

The size of teliospores collected from the field was 37.5–55.0 μm long by 17.5–28.8 μm wide, with a sidewall thickness of 2.5–5.0 μm and an apical wall thickness of 5.0–10.3 μm. The pedicel length of detached teliospores was 62.5–142.5 μm, and the pedicel width was 3.8–7.5 μm. No significant differences were found between the size of teliospores or teliospore pedicels collected at the Las Cruces, Deming, or San Antonio sites. These teliospore dimensions are consistent with Cummins (3) and the original description of *P. grindeliae*, wherein teliospores were reported to be 40.6–50.8 μm long and 20.3–25.4 μm wide (9).

Field teliospores germinated readily in the dark, producing basidiospores that measured 12.5–15.0 μm long and 7.5–11.3 μm wide. Basidiospores were limoniform to reniform, and occasionally obovoid to subglobose (Fig. 1). Basidiospores typically were produced on simple sterigmata. Occasionally, however, branched sterigmata were observed producing five or more basidiospores on each metabasidium.

Urediniospores found in young teliosori, on plants inoculated to confirm

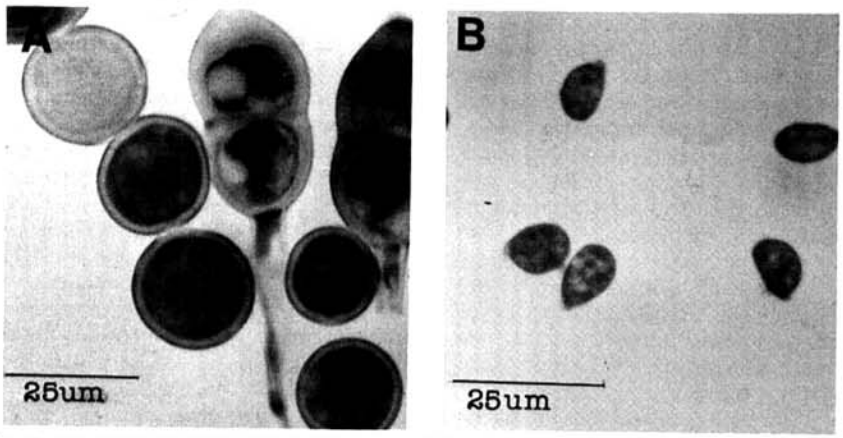

Fig. 1. (A) Urediniospores and a teliospore of *Puccinia grindeliae*. **(B)** Basidiospores of *P. grindeliae*. Bar = 25 μm.

Table 1. Mean percent germination of teliosori of *Puccinia grindeliae* after soaking by three methods[x]

Method	Soaking time (min)			
	0	60	120	180
Drip	0 a[y]	56 b[z]	75 b	75 b
Stationary	0 a	75 b	81 b	94 b
Shake	0 a	63 b	100 b	94 b

[x] Means are based on the pooled results from two experiments with a total of 16 teliosori per each soaking time and soaking method combination.

[y] Means in the same row with different letters are statistically different at the 5% level, using contingency table analysis with Fisher's exact probability test.

[z] Percentages were calculated from raw counts of germinated teliosori after contingency analysis of counts.

Koch's postulates, were globose and measured 15.3–27.5 μm in diameter (Fig. 1). These spores were not abundant and could not be induced to germinate. Urediniospores were orange-tan, and the outer wall was rough but not echinulate.

Longer periods of soaking were more effective at promoting germination of teliosori and basidiospore production, but there was no significant difference between 60, 120, and 180 min of soaking. Germination percentage reached 100% between 3 and 12 hr of soaking in all treatments (data not presented). All soaking times promoted significantly greater germination than the treatment with no soaking (Table 1). There were no differences between soaking methods at promoting germination (Table 1), although the drip method tended to be least effective ($X^2_1 = 2.7$). The stationary method was the simplest, gave a high germination percentage after 3 hr (Table 1), and was used for all other experiments. Soaking probably acted in two ways to promote germination (1), by removing autoinhibitory compounds from teliosori, which are common in rusts, and by hydrating spores before germination.

The optimum temperature for teliospore germination was 15 C (Fig. 2) at 12, 24, and 48 hr. All experiments gave similar results, and results for 6, 12, 24, and 48 hr are reported in Figure 2. According to a repeated measures ANOVA, there were significant time and temperature effects, and a significant time-by-temperature interaction ($P = 0.05$). This indicates that more teliospores continued to germinate with time at 15 C but not at 5, 10, or 20 C (Fig. 2).

The optimal RH for teliospore germination was 95–100% (Fig. 3). Germination was more erratic at lower RH, resulting in many zeros in the data and making analysis of the number of germinating telia sections difficult. Hence, data were analyzed by traditional ANOVA and the nonparametric Kruskal-Wallis test. Both analyses gave similar results. The optimum RH was above 95%. At lower RH little germination occurred regardless of the experiment duration. These results are consistent with the hypothesis that teliospores are susceptible to dehydration below 95% RH and cannot maintain the water imbibed during soaking long enough to initiate germination at low to moderate RH.

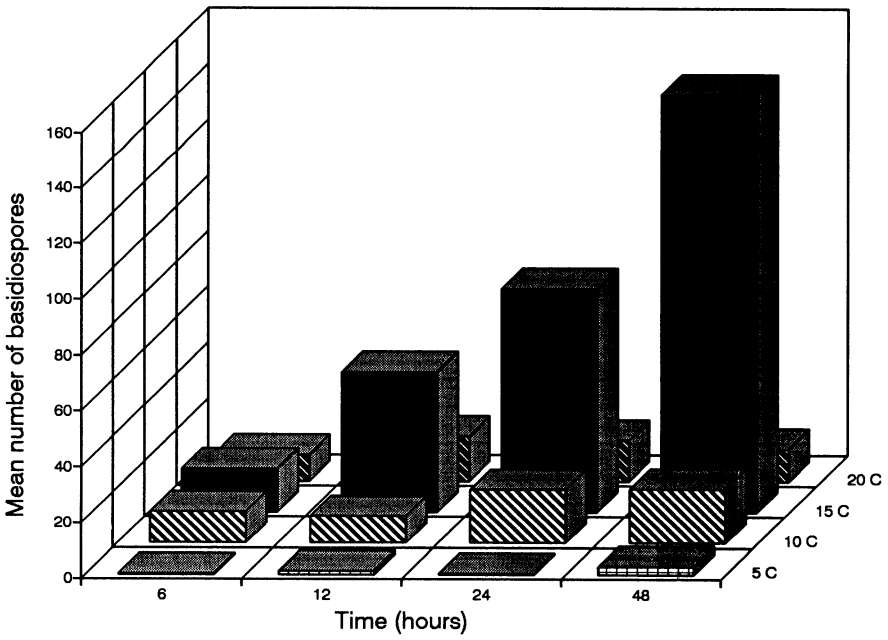

Fig. 2. Mean number of basidiospores produced by teliospores on a 0.3-mm transverse section of field teliosori, incubated in humidity chambers at four temperatures for 6, 12, 24, and 48 hr. The means are based on four sample counts per section. There were five sections per replicate and four replicates (see Materials and Methods).

Inoculation experiments performed to confirm Koch's postulates led to the formation of teliosori in 4–6 wk after inoculation with basidiospores. No aeciospores or spermatia were found, despite careful observation, but urediniospores were present in several young teliosori on these plants. However, urediniospores produced on inoculated plants could not be induced to germinate during this study. The role of urediniospores in the life cycle of this rust is not known, and they are not usually found on field plants. However, a small number of urediniospores in several pustules of *P. grindeliae* were collected from one plant in the field in September 1992. If urediniospores can be induced to germinate, they may provide a valuable method for field inoculation of broom snakeweed.

The fungus *P. grindeliae* is well adapted to the high desert climate of New Mexico, where cool, moist conditions most commonly occur at night and in the early morning after rainfall during the spring and fall. The fungus can produce basidiospores at 15 C and 95–100% RH within 6–12 hr, and telia are able to produce further basidiospores for at least 48 hr under these conditions. These results suggest that germination and infection occur in the field overnight

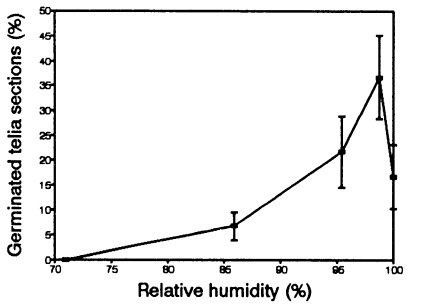

Fig. 3. Mean percent of 0.3-mm sections of field teliosori that germinated at four levels of relative humidity at 15 C after 24 hr (error bars represent ± one standard error).

after evening thundershowers. Rain, soaking the teliosori, may remove autoinhibitory compounds and hydrate teliospores as well as provide cool ambient temperatures and high RH. Germination could then ensue during early morning hours, before the high temperatures, low RH, and high solar radiation of the daylight hours occur. It is common in the high deserts of the Southwest to have summer evening temperatures as low as 10–15 C after day temperatures have exceeded 35 C. Telia apparently can resume germination during subsequent nights, provided conditions remain suitable.

Germination of basidiospores occurs quickly in the laboratory. We hypothesize that germination of basidiospores and infection of the host in the field is also rapid, occurring in one to five consecutive nights. Biological control of *G. sarothrae* with *P. grindeliae* will be difficult, given the high degree of genetic diversity in the host (6) and the microcyclic nature of the pathogen. The

only spore stage currently available for use in field inoculation trials is teliospores, and these cannot be easily separated from host material thereby limiting application options. Furthermore, teliospore germination is erratic, depending on the collection time from the field and conditions and length of storage in the laboratory. Teliospores stored at room temperature lost viability after 1–2 mo in the laboratory.

Successful biological control of *G. sarothrae* by *P. grindeliae* will depend upon the discovery of a spore stage, such as the urediniospores found in this study, that can be easily produced for field inoculation trials. A better understanding of the population genetics of the plant and the natural level of resistance will also be required to fully evaluate the potential efficacy of *P. grindeliae* as a biocontrol agent of *G. sarothrae*.

ACKNOWLEDGMENT

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