Effects of Pregermination Environments on the Germinability of Uredospores of Two Wheat Rust Fungi

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ABSTRACT

Newly produced uredospores of *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita* f. sp. *tritici*, the stem rust and leaf rust pathogens of wheat, respectively, were exposed en masse to different environmental treatments for periods up to 48 h and subsequently tested for germinability. Germination was conducted under standard conditions in which nontreated spores routinely germinated at levels greater than 84%. Light, temp, relative humidity, and the duration of these parameters applied to uredospores, all influenced subsequent germination levels, but had only minor effects on germination rate. Germination was markedly reduced or did not occur after spores were exposed to temp above 25 C, especially in dark, water-saturated (hydration) atmospheres. The magnitude of such detrimental effects increased gradually with exposure time. With both fungi, germinability losses due to hydration were reduced or prevented if hydration was conducted in incandescent light. Light at 10,800 to 12,300 lx, that included wavelengths greater than 500 nm, was significantly effective. Germinability was inversely related to rates of spore respiration in pregermination environments.

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Successful infection of host plants by uredospores of the cereal rust fungi is a complex process regulated by a series of morphological and biochemical events. One critical event in the infection process is uredospore germination. Numerous investigations of uredospore germination have shown it to be induced by contact with water, mediated by endogenous energy reserves, and sensitive to a number of chemical and environmental stimulants and inhibitors (1, 2, 4, 5, 6, 9, 10, 11, 12). Environmental factors can act not only during germination (during formation and elongation of the germ tubes) when spores are in contact with water films (2), but also at points earlier in time to influence the germinability of dormant spores, i.e., mature spores released from sporophores, but as yet not in contact with a water film (4, 5, 9, 10, 11).

The present study sought to describe the regulation of germinability by pregermination environments, and thus gain additional knowledge about the ability of dormant uredospores of the cereal rust fungi to tolerate environmental stresses. Specifically, this research was intended to expand an earlier study of Wiese and Daly (11) in which new effects of pregermination environments on uredospore germinability were described. These workers observed, first, that the common practice of hydration (exposing uredospores en masse to water-saturated atmospheres) which normally increases the germinability of poorly germinating spore lots (4, 7, 9, 10, 11, 12, 13, 14), was detrimental to germinability when applied to fresh stem rust uredospores with an inherent high capacity for germination ($> 80\%$). Secondly, they observed that the detrimental effects of hydration in such circumstances were reduced or eliminated if spore hydration occurred in the presence of light.

Since Wiese and Daly (11) used but a single set of conditions to describe these effects (uredospores of *Puccinia graminis* f. sp. *tritici* hydrated for 24 h at 22 C in the dark or in 8,640 lx of incandescent light), the present study sought, first, to describe the limits of light, temp, and time in which these pregermination effects occur, second, to test the response of another common rust pathogen, *Puccinia recondita* f. sp. *tritici*, to such pregermination treatments, and third, to initiate an exploration of these responses in biochemical terms.

MATERIALS AND METHODS.—The wheat stem rust fungus (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) race 56 and the wheat leaf rust fungus (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Johnston & Browder) race 2 were maintained as separate cultures on wheat (*Triticum aestivum* L. 'Little Club'). Growth and inoculation of plants and collection of uredospores was done as previously described (11), except that uredospores applied to wheat seedlings as inoculum were suspended in Mobilsol 100 (8) rather than in talc.

Freshly collected uredospores were subjected to environmental treatments and subsequently tested for germinability under standard conditions. For treatment, spores were divided into 20-mg lots. Such lots were dispersed uniformly in glass planchets (10×25 mm) and placed into one of four $13 \times 13 \times 5$ cm, water-jacketed, Lucite chambers, each equipped with an external incandescent light source and connected to a circulating source of temp-controlled water (11). Spores in the chamber were sealed either in ambient air [relative humidity (RH) in the laboratory was determined to be 20-30% using a sling psychrometer] or in hydration (water-saturated air) compartments as previously described (11). Incident light was varied in intensity by varying the distance of the light from the chambers and in quality by positioning 13×13 cm Kodak Wratten filters (3) in the light path immediately above the spore compartments in each chamber. The apparatus provided independent control over light intensities from 0-32,400 lx and temp from 15-40 C. Temperatures were monitored by placing remote probes in contact with the glass planchets containing the spores. All light measurements were made at spore level using a color-corrected General Electric, Type 213 light meter.

Incubation in the chambers varied from 0-72 h. Beyond 48 h, growth of contaminant organisms was occasionally visible on and/or among hydrated spores. To avoid possible effects of contaminant microorganisms capable of growth under such conditions, all germination data presented herein was obtained following treatments of 48-h duration unless otherwise indicated.

Following treatment, the uredospores were immediately atomized in a settling tower onto 7 × 70 mm glass slides layered with 1% Difco Bacto water agar (11). This procedure distributed the spores uniformly on the agar surface in densities averaging 40 spores/mm^2. Slides thus seeded were incubated in the dark at 22 C for varying periods up to 24 h. Normally, incubations were terminated and germination percentages determined after 2, 6, and 24 h in order to monitor germination rate as well as germination percentage. After incubation, the slides were immediately sprayed with lactophenol and cotton blue (11). Germination percentages were calculated from counts of a minimum of 200 spores on each of three slides. Spores with germ tubes of length equal to or greater than the width of the spore were considered germinated.

In all experiments, the germination of treated spores was compared to that of other spores from the same lot that were (i) germinated immediately upon harvest, or (ii) held in a desiccator at 4 C in the dark during the treatment period and germinated simultaneously with treated spores. Such control spores showed germ tubes within 2 h and, germination percentages, which in all experiments exceeded 84%, were maximum after 6-8 h. Any deviation and/or delays in the procedure of spore production, collection, transfer to, or distribution on fresh agar normally were associated with decreased germinability, and thus were carefully avoided. The high germinability, consistent for both stem rust and leaf rust uredospores, was conducive to discerning any environmental treatments that were detrimental to germination.

Spore respiration during treatment was measured using a Gilson Model GRP14 differential respirometer, and standard manometric techniques. Conditions for treatment in the Lucite chambers were simulated in double side-arm respirometer flasks in terms of temp and intensity of incandescent light. Each flask contained 20 mg of newly produced spores dispersed outside the center well which contained 0.1 ml of a 10% aqueous solution of potassium hydroxide on a paper wick. Hydration was accomplished by placing 1 ml of distilled water in each side arm. To induce germination, the spores were floated on 2 ml of distilled water. All treatments were replicated in duplicate flasks.

Measurements of oxygen consumption were made during an 8-h period immediately following a 30-min equilibration. Measurements were discontinued after 8 h because, thereafter, contaminant microbial activity was suspect in contributing to inconsistent respiratory rates, especially among hydrating spores. Our spores were sparsely contaminated with bacteria (6), but consistent respiratory rates within the initial 6-8 h suggested that effects of contaminant microorganisms were not significant during this period.

All germination and respiration data presented are averages from at least three separate experiments conducted with different lots of spores.

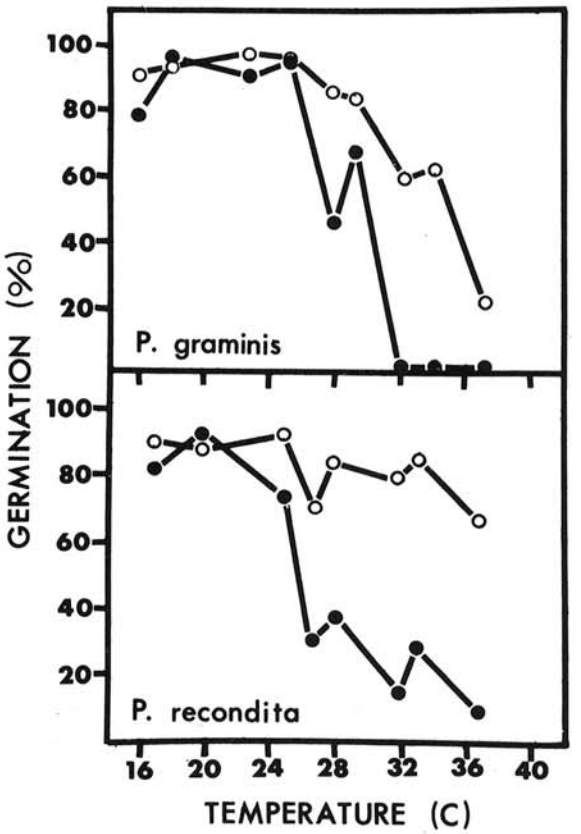

Fig. 1. Germination of newly produced uredospores following 48-h treatment en masse at different temp in the dark in hydrated (- ● -) or nonhydrated (- O -) atmospheres. Differences in germinability between hydrated and nonhydrated spores were significant ($P = 0.05$) at temp between 30 and 36 C.

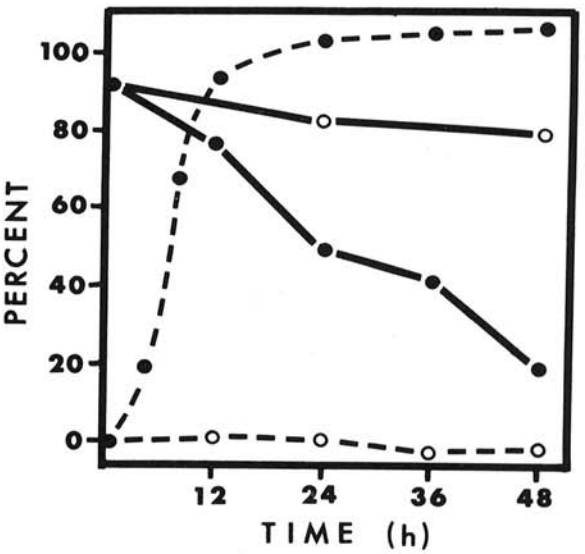

Fig. 2. Change in germinability (____) and weight (- - -) of newly produced leaf rust (*Puccinia recondita* f. sp. *tritici*) uredospores versus treatment time en masse in the dark at 33 C in hydrated (- ● -) or nonhydrated (- O -) atmospheres.

RESULTS.—Uredospore germinability was affected most in spores exposed to warm, dark, water-saturated atmospheres. High temp, high RH, and the duration of exposure to these parameters all adversely affected germinability. Treatment in ambient or in water-saturated air for 48 h was not appreciably detrimental if conducted at 16 to 25 C. However, such treatments became increasingly, and more consistently, detrimental to germination as treatment temp increased from 26 to 40 C, especially in water-saturated air (Fig. 1).

Spores of both rust fungus species were adversely affected by preliminary exposure to warm, humid conditions. Those of *P. graminis,* however, were less tolerant to high temp than those of *P. recondita*. Germination of stem rust uredospores was prevented by 48-h dark-hydration at temp of 33 C and above, while leaf rust spores retained partial germinability after similar treatment at temp up to 38 C. Leaf rust spores also tolerated warm low humidity environments better than stem rust spores, germinating at levels of 65% compared to 22% for stem rust spores following ambient air treatment at 38 C (Fig. 1).

The dark-hydration and/or warm temp treatments affected germination percentages, but had no appreciable effect on germination rate. Treated spores formed germ tubes more slowly during the first 2 h but, like control spores, reached maximum germination levels within 6-8 h.

To determine the onset of reduced germinability in treated spores, germination was measured after 12-h periods of treatment. This technique showed that the detrimental effects of humid atmospheres and/or warm temp were gradual and related to exposure time (Fig. 2). Spores of both rust species showed similar gradual losses in germinability as treatment time increased.

Within the temp range of this study (15-40 C) hydrating spores normally doubled in wt within 24 h, whereas the wt of spores treated in ambient air was not changed appreciably over periods up to 48 h (Fig. 2). Hydrating spores swelled to symmetrical shapes and became visibly lighter (orange-brown) in color compared to nonhydrating spores which retained the red-brown color and indented walls they possessed upon harvest from host plants.

In this study as in the initial one (11), incandescent light at 8,640 lx added to the treatment scheme reduced the detrimental effects of hydration. The greater germinability of light-hydrated spores, however, did not appear to be closely related to light intensity (Table 1). Intensities from 3,200 to 32,400 lx were effective; in some tests, however, spores hydrated in light at these extremes germinated as poorly as dark-hydrated spores. Uredospores of *P. graminis* and *P. recondita* most consistently tolerated hydration in light at 10,800 lx.

TABLE 1. Germination of newly produced rust (*Puccinia* spp.) uredospores following 48-h hydration en masse at 30 C under different intensities of incandescent light

Illumination (lx)	Germination	
	P. graminis (%)	*P. recondita* (%)
0 (Dark)	54	48
3,200	62	65
8,640	71	64
10,800	82*[a]	74*
32,400	70	49

[a]*Denotes germination different (P = 0.050 from that of corresponding dark-hydrated spores.

Using light at intensities as close as possible to that which appeared optimum (10,800 lx, Table 1), another series of experiments was conducted in which light quality was varied. When the shorter wavelengths were progressively eliminated by filters, a positive response in germinability was still realized (Table 2). In this regard wavelengths above and below 510 nm proved effective and unnecessary, respectively, for a positive germinability response.

In an attempt to more fully describe the hydration and light effects on germinability, subsequent spore treatments were conducted in respirometers where oxygen consumption could be monitored. As with preincubation in the Lucite chambers, the light-influenced detrimental effects of hydration on uredospore germinability were in evidence among spores submitted to 48-h treatment in the respirometers. However, oxygen consumption during this same period varied widely even among replicates of the same treatment. Since such inconsistencies appeared to develop during treatment (usually after 12 h) comparisons of respiration among incubating spores were limited to the initial 8-h period. During this period, rates of oxygen consumption for spores of both rust species were directly related to temp and especially to relative humidity, and thus inversely related to subsequent germinability (Table 3). Light that maintained germinability during 12-h and longer hydration periods reduced respiration but not significantly during the initial 8 h.

Respiration rates during all treatments were minimal compared to that of spores induced to germinate by being in actual contact with water. Spores of *P. recondita,* for example, rarely used oxygen in excess of 2 μl/mg/h during treatment en masse; whereas during germination, rates of oxygen consumption normally exceeded 5 μl/mg/h (Table 3).

DISCUSSION.—As in the original report (11) fresh rust uredospores again responded negatively to hydration treatment. The detrimental hydration effect most consistently occurred at temp above 25 C and was of significance among spores hydrated for 12-h or longer periods. Since hydration for less than 12 h, such as the 8-h treatments in respirometers, did not produce appreciable changes in germinability, the results with our spores may mirror those of Maddison and Manners (5) who, using outdoor treatments, found that germinability declined after an initial lag.

Light significantly prevented losses in germinability due to hydration when applied in the range of 10,800 to 12,300 lx and when it included wavelengths greater than 510 nm (Tables 1 and 2). Since these same wavelengths reduce germination when applied later in time to spores germinating on a water surface (2), light energy above 500 nm apparently acts in different capacities prior to and following germination induction. Similarly, the light reaction expressed in spores must depend on the spore's water status since light has a positive influence on spores

TABLE 2. Germination of newly produced rust (*Puccinia* spp.) uredospores following 48-h hydration en masse at 31 C under different light regimes

Wratten Filter	Transmission (λ)	Transmission (lx)	Germination *P. graminis* (%)	Germination *P. recondita* (%)
Opaque	...	0	29	21
No. 26 (Red)	>585 nm	7,030	31	39
No. 16 (Yellow)	>510 nm	11,000	46*[a]	54*
No. 0 (Clear)	>240 nm	12,300	48*	60*

[a]*Denotes germinability significantly different ($P = 0.05$) from that of corresponding dark-hydrated spores.

TABLE 3. Oxygen consumption by newly produced uredospores of *Puccinia recondita* in different environments

Temp (C)	Light[b]	O_2 consumption (μl O_2/mg spores/h[a]) In ambient air (nonhydrating)	In water-saturated air (hydrating)	On water surface (germinating)[c]
22	Dark	0.3	1.5	5.0
	Light	0.4	1.7	...
33	Dark	0.5	2.1	5.2
	Light	0.6	1.9	...

[a]Differences in respiratory rates between nonhydrating, hydrating, and germinating spores were significant ($P = 0.05$).
[b]Flasks containing spores were dark or in 10,800 lx of incandescent light.
[c]Germination after 8 h was 30-40%.

in water-saturated air (11, and Tables 1 and 2) and a negative (5) or no influence (11) on spores in less humid air.

In our study, hydrating spores accumulated generous amounts of water and showed increased respiration and reduced germinability compared to nonhydrating spores (Fig. 2, Table 3). Germinability, therefore, may depend on conservation of endogenous energy reserves in environments not conducive to germination. Previous anatomical and biochemical comparisons of hydrated and nonhydrated spores demonstrate that substrate maintenance and metabolic activation are requisites for germination (6, 10, 13, 14).

While respiratory rates among dormant spores were inversely related to their germinability, they may be more closely linked to the water status of the spore. Maheshwari and Sussman (6) found a self-inhibitor in uredospores to be more effective at reducing the germination than the respiration of spores in contact with water. Similarly, even though hydrating spores at 33 and 22 C in our study differed markedly in germinability (Fig. 1), respiration differed more significantly between hydrating and nonhydrating spores regardless of temp (Table 3).

Hydration in promoting the germinability of older spores has been shown to maintain their anatomical integrity (7) and to remove or dilute inhibitors (1, 12). In reducing the germinability of newly produced spores through action on inhibitors, hydration would have to promote their production or effectiveness; an unlikely possibility, but also one that has not been investigated. Hydration, in the case of older spores, may solubilize and/or mobilize metabolic machinery and stimulators for germ tube formation that would otherwise be nonfunctional upon sudden contact with water (4). On the other hand, fresh spores hydrated for extended periods (> 12 h) may unnecessarily overuse such machinery and substrates prior to contact with water.

This study further characterized the effects of pregermination light and hydration on the germinability of newly produced uredospores and provided insight for a better understanding of uredospore survival. Since newly produced uredospores of two rust fungi showed the detrimental hydration effect (Fig. 1), the phenomenon may be as general in occurrence as the promotive effect of hydration on the germinability of older spores. In nature, old and new uredospores likely become hydrated under conditions of high RH. Developing night-time dew periods, for example, likely increase the germinability of older spores and then provide the water surface on which both old and new spores germinate. By virtue of being light-sensitive, any ungerminated newly produced spores would be protected from losses in germinability in instances where high moisture conditions persist during daylight hours. Environmental factors that restore or maintain uredospore germinability would bolster infection efficiency, disease development and pathogen survival.

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