Production, Storage, Germination, and Infectivity of Uredospores of *Uredo eichhorniae* and *Uromyces pontederiae*

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ABSTRACT

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Uredo eichhorniae and *Uromyces pontederiae* are pathogens of aquatic plant genera in the family Pontederiaceae. Usually less than 20% of freshly harvested uredospores of these fungi germinated on water at suitable temperatures, but more than 70% germinated when also exposed to optimal concentrations of β-ionone, 2-hexanone, 2-heptanone, α-ionone, 5-methyl-2-hexanone, 1-octanol, retinol, retinal, 1-nonanol, or *n*-nonanal (in order of decreasing efficacy). The spores could be stimulated to germinate on water, water agar, or leaf surfaces at temperatures from 10 to 30 C (optimum 20 C). The uredospores lost germinability within 4 wk when stored in ambient air at 20, 5, −5, or −12 C; at 20 C in vacuum; or with anhydrous calcium sulfate, silica gel, or phosphorus pentoxide. At 5 C, relative humidity (RH) of 35, 52, or 61% in storage was more conducive to spore germinability than 0, 82, or 100% RH. Spores stored in liquid nitrogen remained germinable for more than 18 mo and did not require heat shock to activate germination. Consistent host infection occurred and uredia were formed only when newly inoculated plants were incubated with 500 µl of gaseous 2-heptanone, 5-methyl-2-hexanone, or 2-hexanone per milliliter of air in a dew chamber. Spraying leaves with water agar-based inoculum containing 1–100 µl of α-ionone per milliliter was less effective in promoting infection. Uredospores of *U. pontederiae* infected *Pontederia lanceolata* leaves at 15, 20, and 25 C, but uredosori were formed only at 20 and 25 C. Spores germinated but did not form appressoria on the host at 30 C; they failed to germinate at 35 C. No cross-infection occurred with the two rusts on *P. lanceolata* and *Eichhornia crassipes*.

Additional key words: aquatic weeds, biocontrol, chemical stimulants, pickerelweed, water hyacinth.

The potential of *Uredo eichhorniae* Gonz.-Frag. & Cif. (6) as a biological control agent for water hyacinth, *Eichhornia crassipes* (Mart.) Solms (5), and similarities between this fungus and *Uromyces pontederiae* Gerard, a pathogen of pickerelweeds (*Pontederia* spp.) and the anchoring water hyacinth (*E. azurea* (Swartz) Kunth) (4,10,14) prompted us to study these fungi. Unlike *Uromyces pontederiae*, which occurs in the Americas from Canada to Argentina, *Uredo eichhorniae* is found only in South America (4). Attempts to culture these fungi on their hosts showed that the uredospores do not germinate readily on water. Although the literature on the physiology of uredospores is extensive for rusts of terrestrial hosts (2,12,13,19–21,23,24) little is known about rust fungi on aquatic hosts. We sought suitable methods for producing, storing, and germinating uredospores of *Uredo eichhorniae* and *Uromyces pontederiae* and studied host infectivity of the uredospores and host specificity of the pathogens.

MATERIALS AND METHODS

Unless otherwise indicated, experiments included both *Uredo eichhorniae* and *Uromyces pontederiae* and were repeated at least twice with different spore lots. Tests with chemical stimulants were repeated 15–35 times with different spore lots.

Spore production and collection. Uredospores of *Uromyces pontederiae* were initially collected from dried, infected leaves of *P. lanceolata* Nutt. plants and were subsequently obtained from detached, infected leaves. The cut petioles of the detached leaves were immersed in deionized water and kept at 100% relative humidity (RH) with 12 hr of light (1,900 lux) per day. Petioles were incubated at 5, 10, or 20 C; in 10^{-5} and 10^{-7} M kinetin or abscisic acid at 10 or 20 C; or in 60 µl/ml aqueous benzimidazole solution (17) at 10 or 20 C.

Field-collected pickerelweed and water hyacinth plants transplanted in greenhouses were also used as sources of uredospores. *Uredo eichhorniae*-infected water hyacinth plants collected in Argentina were transported to a quarantine greenhouse in Gainesville and acclimatized for 3 days. Uredospores were then collected with a cyclone collector (Instrument Shop, Iowa State University, Ames 50010) and stored. In addition, field-collected *Uredo eichhorniae* spores were flown to Gainesville and tested. In all methods, spores were collected only from fully open uredia that were covered with powdery masses of uredospores (5).

Germination. Attempts were made to germinate uredospores on sterile, deionized water or 1.0% water agar (Bacto agar; Difco Laboratories, Detroit, MI 48232), using 1 mg of uredospores per milliliter. Spores were also hydrated or heat-shocked before being floated on water.

We tested the influence of several concentrations of 2-heptanone, 5-methyl-2-hexanone, 1-nonanol (Eastman Kodak Co., Rochester, NY 14650), α-ionone, β-ionone (Polysciences, Inc., Warrington, PA 18976), 2-hexanone, *n*-nonanal (PolyScience Corp., Niles, IL 60648), and 1-octanol (Fisher Scientific Co., Richmond, VA 23230) on uredospore germination. Because of the chemicals' poor solubility in water, suspensions were shaken vigorously before aliquots were removed. Ten drops of stimulant suspension were added to the annulus of a Conway diffusion vessel (60-mm

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chamber diameter, 10-mm chamber depth, 35-mm central well diameter, and 4-mm annulus and central well depth) (7) in which uredospores were floating on 2 ml of water in the central well. Stimulants were prepared and dispensed under a fume hood to avoid cross-stimulation by the chemicals. The vessels were made airtight by lightly greasing the rims and covering them with the lids, then incubated at 20 C in the dark for 12 hr.

Germination was determined on a loopful of spores placed on a microscope slide with a cover glass. At least 300 spores were assessed for germination per treatment. To compare different stimulatory treatments, the maximum germination potential or "germination index" of each spore sample was determined by a procedure referred to hereafter as the standard procedure. Spores were floated on 2 ml of sterile, deionized water in the central well of a Conway vessel and incubated at 20 C while being exposed to vapors from 50 μl of α-ionone per milliliter of water in the annulus. Control spores were floated on 2 ml of sterile, deionized water in Conway vessels without stimulant (the "baseline" procedure), incubated, and counted as described. Unless specified otherwise, 1 mg of spores was used in germination studies, and the spores were spread evenly in the central wells.

To determine the effects of retinol (vitamin A) and retinal (vitamin A aldehyde) on *Uromyces pontederiae* uredospore germination, aliquots of a stock solution of 25 mg of retinol or retinal (Sigma Chemical Co., St. Louis, MO 63178) in 5 ml of Tween 20 (polyoxyethylene sorbitan monolaurate; Sigma) and 5 ml of absolute ethanol (9) were added to molten 2% water agar to achieve the desired concentrations. Stock solutions and media were stored in the dark and handled under light intensity of 1 lux. Water agar was used for the control. A 1 mg/ml suspension of uredospores in 0.1% Tween 20 was plated, and the plates were incubated at 20 C in darkness for 12 hr before determining spore germination.

The influence of temperature on germination was evaluated on chemically stimulated and nonstimulated uredospores of *Uromyces pontederiae*. The germination procedures were similar to the standard (stimulated) and baseline (nonstimulated) procedures, except the spores were incubated at 5, 10, 15, 20, 25, 30, or 35 C.

To test the effect of light on germination of chemically stimulated spores, two sets of Conway vessels with uredospores of *Uromyces pontederiae* were set up as in the standard procedure including the stimulant, except they were placed under a fluorescent light. The temperature under the light was 25 C. Vessels in one set were covered with frosted glass lids and those in the other with transparent lids. The incident light was 1,960 lux under the frosted glass and 2,476 lux under the transparent lid. Another set of vessels was incubated in darkness, also at 25 C. After 9 hr, germination was determined and the vessels held under light were transferred to darkness for another 9 hr.

Storage. Freshly collected uredospores of *Uromyces pontederiae* were stored in vials without evacuation at 20, 5, −5, and −12 C. In another experiment, uredospores were stored at 20 C in vials with separate containers of anhydrous calcium sulfate (Drierite; W. A. Hammond Drierite Co., Xenia, OH 45385), silica gel, or phosphorus pentoxide. A third batch of spores was dried for 3 hr under 20-μm vacuum, sealed, and stored at 20 C. Spores were tested for germinability weekly for 8 wk by the standard procedure, with or without hydration before germination. For hydration, 5 mg of spores was spread evenly in the dry central well of a Conway vessel and exposed for 16 hr to moisture from deionized water in the annulus.

Uredospores of *Uromyces pontederiae* were also stored at different relative humidities determined with a hygrometer. Saturated solutions of the following chemicals at 5 C yielded the percentage RH indicated: LiCl, 35%; $CaCl_2$, 52%; KSCN, 61%; and K_2HPO_4, 82%. Drierite and tap water provided 0 and 100% RH, respectively. Spores were placed in 10-ml beakers inside sealed jars containing the saturated solutions and stored at 5 C. Spores were tested for germination by the standard procedure after 4 and 8 wk of storage.

Finally, uredospores were stored in liquid nitrogen, either untreated or after exposure to a stimulant ("pretreatment"). For pretreatment, 5 mg of spores was spread evenly in the dry central well of Conway vessels and exposed for 20 min to vapors from 10 μl of α-ionone in 2 ml of water in the annulus. Presterilized 2-ml Cryule plastic vials (#985731, Wheaton Products, Inc., Niceville, NJ 08332), which are made for storage in liquid nitrogen, and a Biostat Model 400 cryobiological storage container (Cryenco, Denver, CO 80233) were used. Spores were retrieved weekly from liquid nitrogen and thawed for 30 min at room temperature before germination tests. Unused spores were returned to liquid nitrogen. In one experiment, newly retrieved vials of spores were immersed in 40-C water for 2 min before germination to determine the need for heat activation. Subsequently, uredospores were germinated routinely without heat treatment.

Infection. Undiluted dodecane, 0.5–1.0% aqueous gelatin, or 0.27% water agar was used as a carrier for spray inoculum containing 2 mg of uredospores per milliliter. Talcum powder at 10 parts to 1 part of uredospores was also tested as a diluent. Typically, a mixture containing 2 mg of spores was brushed evenly on a 3-cm^2 adaxial surface area of a dry leaf, and the leaf was lightly misted with sterile, deionized water. The spores were then stimulated by placing enough undiluted 2-heptanone, 5-methyl-2-hexanone, or 2-hexanone in an open petri plate in a dew chamber to give 500 μl of stimulant per milliliter of air.

For host infection studies, unless specified, the plants were incubated immediately after inoculation at 20 C and 100% RH for 12 hr in darkness in the dew chamber, then subjected to a 12-hr (1,900 lux) day/night cycle at 20 C in the dew chamber. In addition, *Uromyces pontederiae* spores suspended (2 mg/ml) in 0.27% water agar containing α-ionone at 1.0, 10, 25, or 100 μl/ml were tested on pickerelweed leaves. Finally, uredospores of *Uromyces pontederiae* pretreated as described with vapors of 20 or 50μl/ml of α-ionone were transferred (2 mg/ml) to 0.27% water agar, mixed, sprayed on pickerelweed leaves, and evaluated as inoculum.

Infection of *P. lanceolata* by uredospores of *Uromyces pontederiae* was studied at 15, 20, 25, 30, and 35 C. At least three leaves per plant and three plants per treatment were inoculated with the spore-talc inoculum, stimulated, and incubated. After 10 hr, segments of inoculated leaves were removed, cleared with chlorine gas (11), and stained with 5 mg of cotton blue and 12 mg of acid fuchsin in 50 ml of molten 2.0% water agar. The stain was sprayed or dropped on leaf segments without dislodging the spores. The percentages of germinated spores and those that formed appressoria were determined. After 5 wk, the plants were transferred to a greenhouse (18–35 C; 3,500 ± 400 lux at midday). Seven attempts were made (25 C at 1,900 lux) to cross-infect water hyacinth and pickerelweed plants, respectively, with uredospores of *Uromyces pontederiae* and *Uredo eichhorniae* (three leaves per plant per host for each attempt).

RESULTS

Spore production, collection, and germination. Cut infected leaves maintained at 100% RH at 5 or 10 C survived for 3 wk, allowing three spore harvests. In general, leaves maintained at 20 C senesced faster than those at the lower temperatures. Solutions of kinetin and benzimadazole did not delay leaf senescence (at 10 or 20 C) beyond 3 wk. Leaves in these treatments were indistinguishable from those in abscisic acid (10 or 20 C) or deionized water (5 or 10 C). Thus, the detached leaf method was not suitable for culturing *Uromyces pontederiae* because of the poor survival of cut leaves.

Rust-infected pickerelweed plants transplanted in the greenhouse, produced uredospores for up to 2 mo, provided the pustule density on leaves was low. Leaves with more than 15 pustules per square centimeter usually survived less than 3 wk. Uredospores of *Uredo eichhorniae* and *Uromyces pontederiae* from dried leaves did not germinate, whereas 60–90% of *Uromyces pontederiae* spores from cut leaves or from greenhouse-grown plants germinated when exposed to chemical stimulants. But the *Uromyces pontederiae* spores produced in the greenhouse germinated at the highest levels only if they were harvested at 3-day intervals. With 2 days between collections, 40–50% of the spores

germinated, and less than 40% germinated if collected daily.

Stimulation. Uredospores were stimulated to germinate by some of the chemicals at some concentrations, while in nonstimulated controls, only about 5% germinated (Tables 1 and 2, Fig. 1). Except for the varying germination percentages obtained for different uredospore lots, the effects of the stimulants were consistent. In general, the ketones were more stimulatory than alcohols, which in turn were more stimulatory than aldehydes. Among the ketones, the ionones were effective at lower concentrations (20 or 50 μl/ml) than the hexanones or the heptanone (200–500 μl/ml). Seventy and 67% of *Uromyces pontederiae* spores germinated when exposed to 20 μl/ml of β-ionone and 500 μl/ml of 2-hexanone, respectively (Table 1). Only 10–20% of uredospores of *Uredo eichhorniae* germinated, even if the spores were obtained from greenhouse-grown water hyacinth (Fig. 1). Both retinol and retinal stimulated uredospores of *Uromyces pontederiae* (Table 2) but less effectively than the ketones.

Temperature effects on germination. Uredospores of *Uromyces pontederiae* stimulated by vapors of 50 μl/ml α-ionone germinated at 10–30 C; up to 92% germinated at 20 C (Fig. 2). Germination fell from 72% at 30 C to 0% at 35 C. The nonstimulated spores germinated at 15–30 C, with a maximum of 13% at 20 C. The optimal temperature for uredospore germination was 20 C for both fungi.

Light effects on germination. Eighty percent of the spores incubated in darkness germinated, whereas those under light did not. Eighty percent of uredospores held under light and then incubated in darkness germinated. These results suggest that light cancels the influence of the chemical stimulant and reversibly inhibits uredospore germination. Because the baseline or nonstimulated germination percentages of spore lots used in this experiment were below 6%, the effect of light on nonstimulated spores was not studied.

Storage. Freshly collected spores stored at 20, 5, −5, and −12 C declined from 60% germinability at 1 wk to less than 7% at the end of 4 wk; the rate of decrease was slower during the first 2 wk at −12 C. Hydrating spores before floating them on water did not increase

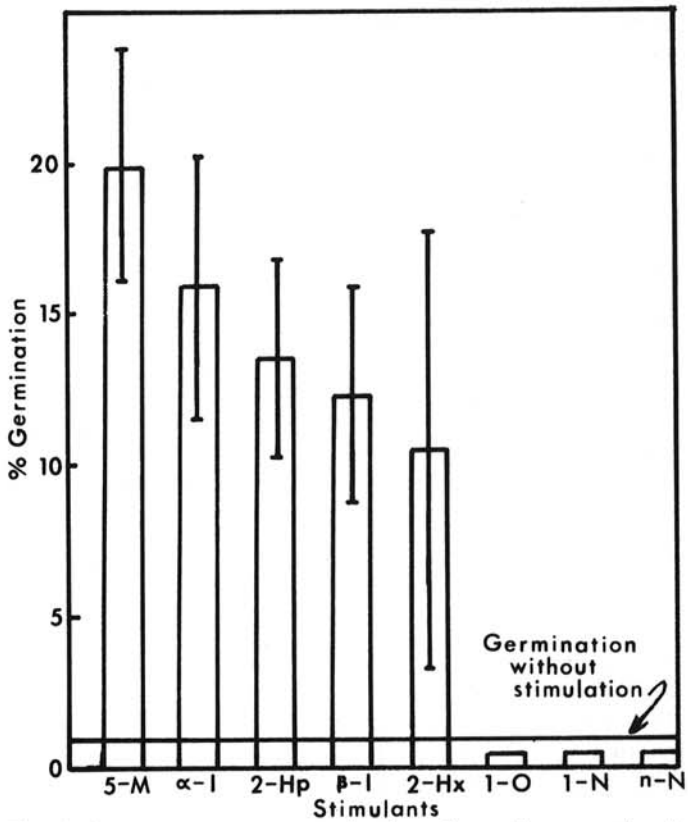

Fig. 1. Average percentage germination of greenhouse-produced uredospores of *Uredo eichhorniae* on water in Conway vessels at the most effective concentrations (μl/ml) of the stimulants 5-methyl-2-hexanone (5-M) (500), α-ionone (α-I) (50), 2-heptanone (2-Hp) (200), β-ionone (β-I) (50), 2-hexanone (2-Hx) (200), 1-octanol (1-O) (100), 1-nonanol (1-N) (10), and *n*-nonanal (n-N) (10). Without stimulation, about 1% of the uredospores germinated. Bars represent standard errors.

TABLE 1. The effects of chemical stimulants on the germination of uredospores of *Uromyces pontederiae* on water in Conway cells[a]

	Concentration in annulus (μl/ml)									
Stimulant	1.25	2.5	5	10	20	50	100	200	500	1,000
β-Ionone	41.8	47.5	66.4	63.6	70.3	58.2	34.1	5.3	2.8	0
	±8.3	±8.0	±6.1	±8.3	±7.1	±7.5	±10.2	±5.1	±2.1	···
2-Hexanone	5.0	9.2	13.3	15.3	13.5	11.1	32.9	49.9	66.6	58.3
	±5.8	±4.4	±11.6	±11.5	±5.5	±8.1	±7.3	±13.3	±5.2	±9.9
2-Heptanone	20.7	19.4	19.1	10.0	17.1	25.6	38.1	55.9	65.7	48.3
	±9.5	±6.6	±8.2	±5.7	±8.5	±7.7	±8.3	±10.1	±6.1	±6.3
α-Ionone	30.4	31.3	37.1	45.5	63.2	63.7	42.9	34.9	19.8	0
	±8.4	±8.6	±8.0	±10.8	±7.6	±5.1	±8.7	±12.7	±8.5	···
5-Methyl-	16.4	15.5	18.1	23.4	27.4	37.5	50.4	54.7	61.4	59.4
2-hexanone	±8.5	±12.2	±6.4	±9.5	±9.7	±8.7	±7.4	±7.4	±5.2	±7.8
1-Octanol	0	37.8	32.3	32.6	38.1	43.8	46.1	32.0	4.3	0
	···	±5.7	±13.2	±9.6	±10.2	±10.8	±9.6	±7.7	±4.2	···
1-Nonanol	19.5	33.0	41.8	42.5	29.7	39.7	9.0	11.0	12.7	0
	±16.6	±18.9	±12.6	±11.4	±10.3	±13.0	±5.7	±11.0	±12.6	···
n-Nonanal	13.5	10.5	12.0	14.3	8.3	9.8	7.5	13.6	8.5	8.3
	±8.6	±10.4	±7.0	±10.9	±4.2	±6.4	±0.8	±9.9	±4.6	±11.8
Control (no stimulant)	5.3 ± 3.5									

[a] Percentage germination (average of at least 300 uredospores per treatment and 35 uredospore samples). Numbers below are standard errors.

TABLE 2. The effects of retinol and retinal on the germination of uredospores of *Uromyces pontederiae* on water agar plates[a]

	Concentration (μg/ml of agar)													
Stimulant	0.025	0.05	0.1	0.25	0.5	1.0	2.5	5	10	25	50	100	150	200
Retinol	3.0	4.1	4.1	1.4	33.0	37.4	31.0	22.0	26.0	32.0	48.0	24.0	0.6	0
	±1.4	±1.3	±1.3	±0.5	±4.2	±5.8	±4.3	±2.4	±3.4	±3.5	±4.3	±1.9	±0.4	···
Retinal	0.6	0.8	0	0	2.6	1.6	2.9	5.2	9.7	4.2	5.7	4.8	0	0
	±0.1	±0.1	···	···	±1.3	±0.5	±0.8	±1.6	±2.5	±1.3	±2.5	±2.1	···	···

[a] Percentage germination (average of at least 300 uredospores per treatment and 15 uredospore samples). Numbers below are standard errors. No germination occurred in the control treatment (water agar alone).

germination. Germinability of uredospores of *Uromyces pontederiae* stored at 20 C with calcium sulfate, silica gel, or phosphorus pentoxide or under vacuum decreased from more than 70% on the day of collection to less than 3% after 4 wk. Again, hydrating spores did not increase germination. These methods, therefore, were not useful for preserving uredospores of the two fungi.

More than 78% of the uredospores of *Uromyces pontederiae* stored at 5 C at 35, 52, or 61% RH germinated after 8 wk; at 52% RH, the optimum for storage, 80–90% germinated. Very low or high RH during storage reduced germinability. At 5 C and 0 RH, the spores were germinable for less than 4 wk. At 82 and 100% RH, 73 and 75% of the spores germinated, respectively, after 4 wk; none germinated after 8 wk.

Uromyces pontederiae uredospores stored in liquid nitrogen germinated 70–80% after 8 wk. Heat treatment after retrieval reduced germination by 52%. Uredospores pretreated with vapors of 50 μl/ml α-ionone and stored for 8 wk in liquid nitrogen required no further chemical stimulation for germination and retained the original level of germinability. Uredospores of both fungi stored in liquid nitrogen have retained germinability and infectivity for more than 18 mo, making this the preferred way to store these spores.

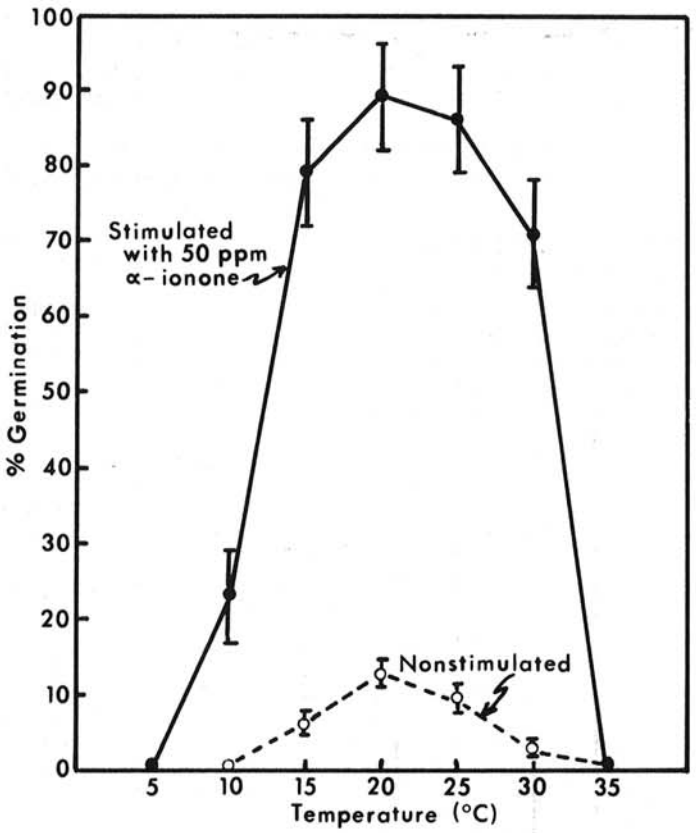

Fig. 2. Influence of temperature on percentage germination of uredospores of *Uromyces pontederiae* on water in Conway vessels. Bars represent standard errors.

Inoculation. The inoculation technique using the spore-talc mixture and stimulation with vapors of 2-heptanone, 5-methyl-2-hexanone, or 2-hexanone yielded up to 80% germination of *Uromyces pontederiae* uredospores on leaf surfaces and typically 10 ± 3 pustules per square centimeter. This technique was also effective with *Uredo eichhorniae* on water hyacinth.

Water agar (0.27%) was an acceptable inoculum carrier. Spores were evenly suspended in this nontoxic, semisolid gel, and spore deposition on leaves was good. The medium was a source of moisture, and runoff in heavy sprays was minimal. By incorporating the ketones, the uredospores could be stimulated to germinate in this medium, although at a level below the germination index.

Dodecane and gelatin solutions were not suitable carriers for inoculum. Dodecane was phytotoxic. Gelatin solutions were nontoxic to the plants and the spores but did not give an even spore distribution. Only 1% of the uredospores of *Uromyces pontederiae* pretreated with 20 or 50 μl/ml α-ionone germinated on pickerelweed leaves, while the germination index for the spore lots was 80%.

Infection. The optimal temperatures for growth of *P. lanceolata* and infection by uredospores of *Uromyces pontederiae* were 20 and 25 C (Table 3). At these temperatures, germination was close to the germination indexes of the spore samples (Table 3). At 15, 30, and 35 C, 51, 45, and 0% of the spores germinated, respectively. At 15, 20, and 25 C, 4, 8, and 7%, respectively, of the germinated spores formed appressoria over stomata. No appressoria were formed at 30 C.

Small brown spots developed on inoculated plants in the 15, 20, and 25 C treatments in 3 days, and uredosori erupted on day 16 and 17, respectively, at the two higher temperatures. Uredospores from these sori germinated at 85–90%. After being transferred to a greenhouse, plants from the 15 C treatment developed uredosori within 1 wk. On the other hand, pickerelweed plants from the 30 and 35 C treatments did not develop uredosori in the greenhouse.

At 25 C, 70% of *Uromyces pontederiae* uredospores germinated on water hyacinth leaves, but only 1% of them formed appressoria on this nonhost. No uredia were formed on water hyacinth even after prolonged incubation. In cross-inoculation trials involving *Uromyces pontederiae*, *Uredo eichhorniae*, and the two hosts, the rusts developed only on their respective hosts. These fungi, therefore, are considered host-selective to *P. lanceolata* and *E. crassipes*, respectively.

DISCUSSION

The ability to germinate uredospores in vitro was essential to the study of *Uredo eichhorniae* and *Uromyces pontederiae*. However, these spores were fastidious in their requirements, and unless chemically stimulated, less than 20% (maximum) germinated. But when exposed to a suitable stimulant at optimal concentration, up to 90% of the spores germinated.

Among the stimulants tested, the ketones were more effective than 1-nonanol, *n*-nonanal, 1-octanol, retinol, or retinal. Of the

TABLE 3. Effect of temperature on germination, appressorium formation, and infection of *Pontederia lanceolata* by *Uromyces pontederiae* uredospores

Temperature (C)	Germination on leaf[a] (%)	Germination index[b] (%)	Appressoria over stomata[a] (%)	Infection rating[c]	Uredosorus appearance (days)	Plant growth[d]
15	51	81	4	+	···	−
20	72	84	8	+	16	++
25	71	82	7	+	17	++
30	45	80	0	−	···	++
35	0	85	0	−	···	+

[a] A total of 300 uredospores per treatment were counted using 5 × 5 mm leaf samples. Percentage of appressoria is based on germinated spores. Spores were stimulated on leaf surface with 500 μl of 2-heptanone per milliliter of air volume.
[b] Determined at 20 C.
[c] − No symptoms, + brown spots that did or did not develop into sori at these temperatures.
[d] Determined visually: − no growth, + slow growth, ++ rapid growth.

ketones, the ionones were stimulatory over a narrower concentration range unlike the heptanone or the hexanones. French et al (8) described the stimulatory effect of the ketones, nonanol, nonanal, and octanol on uredospore germination. Stimulation of uredospore germination by retinol (vitamin A) and retinal (vitamin A aldehyde) has not been previously reported. Retinol and retinal are structurally related to β-ionone, from which they can be synthesized (22). Retinol can also be a degradation product of β-carotene (16), and both retinol and retinal are involved in sporangiophore initiation (differentiation) of *Phycomyces blakesleeanus* (9).

Previously, we reported that some of the compounds effectively stimulated germination in the range of 200–1,000 μl/ml (14,15). These values, based on fewer spore samples, were incorrect. For example, using more spore samples, the α- and β-ionones were found to be effective at 50 and 20 μl/ml, respectively, rather than at 500 and 200 μl/ml as reported (15). Nonetheless, the usefulness of the stimulants in this study is evident. Without chemical stimulation, neither in vitro germination nor host infection was possible.

Uredospores of *Uromyces pontederiae* and *Uredo eichhorniae* are produced in a habitat in which abundant moisture and free water are invariably present (5). Frequently, infected leaves become submerged before the uredospores reach maturity (R. Charudattan, *unpublished*). Therefore, the survival of uredospores of these fungi in nature is limited. A selective advantage would result if the uredospores failed to germinate solely upon contact with free water. In view of their dependence on a stimulus other than water for germination, we suggest that these uredospores survive in the aquatic habitat by resisting precocious, wasteful germination that might otherwise result from contact with water. Although we did not test the possibility, the apparent self-inhibition may be caused by endogenous substances common in uredospores (1,3). Usually, self-inhibition can be overcome by washing the spores, heat shock, or chemical stimulation (2). In nature, the germination of uredospores of *Uredo eichhorniae* and *Uromyces pontederiae* and host infection may be regulated by endogenous or exogenous stimuli (2,18).

In summary, to culture these rusts in the greenhouse, the infected hosts must be maintained at a low vegetative growth rate at which each infected leaf will survive for at least 4 wk to allow time for the uredia to mature (about 3 wk). A rapid growth rate, at which the infected leaves senesce or are submerged in water in less than 4 wk because of the growth of newer leaves, would result in fewer spores (R. Charudattan, *unpublished*). Three days between spore collections are necessary to enable the uredospores to mature. Uredospores of both fungi are best preserved at −196 C in liquid nitrogen. For host inoculation, the method using a spore-talc mixture followed by chemical stimulation is preferred. Although the hexanones and the heptanone were used routinely for inoculation experiments, the ionones are likely to be acceptable substitutes.

Spores of *Uromyces pontederiae* pretreated with α-ionone germinated in Conway vessels but not on the leaf surface; this may have been caused by the different stimulant concentrations used in these experiments. The relatively poor germination obtained with *Uredo eichhorniae* (Fig. 1) appears to be the result of the quality of greenhouse-produced uredospores; such poor quality was not a problem with *Uromyces pontederiae*.

Despite their similarities in uredospore morphology and physiology (4,5,14), *Uredo eichhorniae* and *Uromyces pontederiae* are pathogenically specific to their respective hosts. Therefore, *Uredo eichhorniae* may be a safe biocontrol agent for water hyacinth. The methods described in this paper should facilitate further research on *Uredo eichhorniae*.

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