Rust Uredospores Increase the Germination of Pycnidiospores of Darluca filum

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ABSTRACT

Uredospores of *Puccinia recondita* significantly enhanced the germination of *Darluca filum* pycnidiospores at temp ranging from 12 to 35 C. *Darluca* spores stored at 7 and 33% relative humidity remained viable longer and germinated better when uredospores were present. A concn of at least 78,000 uredospores/ml was required to promote germination of *D. filum*. Uredospores exerted their positive effect on *D. filum* on either nutrient or water agar media. Uredospores of *P. recondita* and *Uromyces phaseoli* enhanced germination of *D. filum*, whereas spores of nonrust fungi did not. Phytopathology 60:1445-1447.

The fungus *Darluca filum* (Biv.-Bern. ex Fr.) Cast., the imperfect stage of *Eudarluca caricis* (Fr.) Eriks. (6), is found in nature closely associated with rust fungi. Typically, *D. filum* is evident by its black pycnidia in the rust sorus whose production of uredospores is usually impaired or stopped. Because of these unusual ecological characteristics, *D. filum* may become an effective biological control agent of rust diseases, particularly if we understand some of the critical developmental stages of *D. filum* such as spore germination. Although several workers already studied the germination of *D. filum* spores in vitro (2, 3, 7, 10), none measured the germination of *D. filum* in the presence of uredospores. The objective of the present report was to test the effect of uredospores on the germination of *D. filum* spores.

MATERIALS AND METHODS.—The isolate of *D. filum* originated from wheat leaf rust, *Puccinia recondita* Rob. ex Desm. The Darluca isolate was grown on potato-dextrose agar (PDA), supplemented with 2.5 g peptone/liter of PDA, at room temp of 20-25 C (unless otherwise stated), and exposed to diffuse sunlight and artificial light during the day. Seven to 12 days after the agar plates were inoculated, the fungus formed dark-colored pycnidia from which the spores oozed as gelatinous masses. The spores were harvested by flooding the culture dishes with sterile distilled water, and the spore suspension produced was poured into a sterile beaker and stirred aseptically to break up spore clumps. Spore concn were determined by means of a hemacytometer.

Uredospores of *P. recondita* (race UN-2) were stored in a desiccator at room temp and used within 3 days of collection from wheat seedlings. For each experiment, the rust spores and other nonrust spores were diluted and counted as described above for spores of *D. filum*, except that one drop of a wetting agent (Tween 20 [polyoxyethylene sorbitan monolaurate]) was added to about 10 ml of the spore suspension.

In most of the experiments, spore suspensions were applied uniformly with an artist's airbrush across the agar surface of a 10-cm petri dish so that 0.5 ml of suspension was applied/dish. Whenever a mixture of spores was used in an experiment, the mixture was prepared first, then sprayed onto the agar medium. Nine hr later, at room temp, the spores were either observed for germination or stored in a refrigerator at 4 C for not more than 15 hr before being observed. Three randomly selected areas of the dish were examined with the aid of a dissecting microscope, and the numbers of germinated spores/50 spores of each type present were counted.

The effect of temp on germination of *D. filum* was tested by using six temp cabinets, accurate to ± 0.5 C, which were set at 5, 12, 20, 25, 30, and 35 C. Another experiment measured spore germination under several relative humidities which were controlled by preparing saturated solutions of the following salts: $NH_4H_2PO_4$, $NaC_2H_3O_2 \cdot 3H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, $MgCl_2 \cdot 6H_2O$, and $NaOH \cdot H_2O$. At room temp, these solutions in sealed desiccators yielded, respectively, the following relative humidities: 93, 76, 52, 33, and 7%. Suspensions of *D. filum* and rust spores, singly and mixed, were prepared so that the final count of each spore type was 2.5×10^5 spores/ml of water. Three drops of a spore suspension were placed on a slide and dried under a dust-free hood. Pairs of slides having the mixture of dried spores and those having only dried spores of *D. filum* were placed in each desiccator containing a saturated salt solution. At 2-day intervals until the 10th day, a slide with *D. filum* plus rust, and one having only *D. filum*, were taken from each desiccator, sprayed with a fine mist of distilled water, and kept at 100% relative humidity for 7 hr before being examined for percentage germination of spores of *D. filum*.

RESULTS.—*Germination of D. filum among uredospores.*—1) *Temperature.*—The suspension contained 6×10^5 *D. filum* spores/ml of water and a similar concn of leaf rust uredospores. The results of three replicated experiments (Table 1) clearly show a significant increase in percentage germination by spores of *D. filum* when mixed with uredospores and incubated at 12, 20, 25, 30, and 35 C, but not at 5 C, which presumably was too low to allow any germination.

2) *Relative humidity.*—Storage under low relative humidities of 7 and 33% favored longevity of *D. filum* spores, whereas spores stored at higher relative humidities did not germinate (Table 2). The presence of uredospores significantly enhanced germination of stored spores of *D. filum*. Furthermore, the longevity of the spores of *D. filum* increased noticeably at 7 and

TABLE 1. Germination of *Darluca filum* spores alone and in the presence of uredospores of *Puccinia recondita* at different temp

Temp	Germination of spores[a]	
	D. filum alone	*D. filum* with rust[b]
C	%	%
5	0	0
8	8	18**
15	28	47**
20	56	87**
25	50	84**
30	22	76**
35	0	6*

[a] Data shown are avg from three experiments. Concentration of each spore type was 600×10^3 spores/ml of water.

[b] Asterisks indicate significant differences (** = 1%) (* = 5%) between the two treatments at that one temp.

33% relative humidity when uredospores were present.

3) *Spore concn.*—In this experiment, the effect of spore concn was examined in three ways; i.e., both spore types were in a 1:1 ratio over a range of concn; rust spore concn remained constant while spore concn of *D. filum* varied and vice versa. Spore concn were prepared by using a dilution factor of approximately one-half. The results from three replicate experiments show several interesting relationships (Table 3). A significant increase in germination of *D. filum* occurred when: (i) a 1:1 ratio was used and the concn of each spore type was 78,000/ml or greater; and (ii) the spore concn of *D. filum* was constant at 78,000/ml and the uredospores were of equal or higher concn. No significant increase occurred in spore germination of *D. filum* when the uredospore concn remained constant at 78,000 and *D. filum* concn varied (except where both spore types were at 78,000 spores/ml). These results show that a min concn of uredospores is needed to significantly increase germination of *D. filum* spores.

TABLE 2. Effect of relative humidity on germination of *Darluca filum* spores alone and in the presence of uredospores of *Puccinia recondita*

Relative humidity	Days in storage	Germination of spores[a]	
		D. filum alone	*D. filum* with rust[b]
%	*no.*	%	%
7	2	51	87**
	4	32	83**
	6	9	66**
	8	0	11*
	10	0	0
33	2	0	64**
	4	0	24**
	6-10	0	0
52	2-10	0	0
76	2-10	0	0
93	2-10	0	0

[a] Data shown are avg from three experiments. Concentration of each spore type was 250×10^3 spores/ml of water.

[b] Asterisks indicate significant differences (** = 1%; * = 5%) between the two treatments at that one storage and relative humidity regime.

TABLE 3. Germination of *Darluca filum* spores when mixed in various concn with *Puccinia recondita* uredospores

D. filum spore concn (10^3/ml)	Germination of *D. filum* spores[a]							
	Uredospore concn (10^3/ml)							
	1,250	625	313	156	78	39	20	0[c]
	%	%	%	%	%	%	%	%
1,250	44*[b]				23			22
625		42*			26			19
313			42*		21			18
156				36*	23			16
78	44*	34*	39*	34*	29*	15	17	13
39					23	29		21
20					21		28	21

[a] Data are avg of three experiments, except for the 78,000 × 78,000 concn mixture where the value is an average of nine experiments.

[b] Asterisks indicate significant difference (at 5% level) between that figure and its corresponding check in the last column on the right.

[c] No significant differences between any values in this column.

When *D. filum* spores were alone, there were no significant differences in germination throughout the range of spore concn. Rust spore germination was also observed; the presence of *D. filum* had no noticeable effect on rust germination at any of the spore concn tested.

4) *Nutrient effects.*—The germination experiments described so far were done on water agar. The increased germination of *D. filum* in the presence of rust spores could be due to nutrients from uredospores. If so, then nutrient agar should reduce or nullify the influence of the rust spores. PDA was compared with water agar using a spore concn of 4.5×10^5/ml for each species. *Darluca filum* alone on water agar or PDA had, in two experiments, an average germination of 42% and 46%, respectively (not significant at the 5% level). When uredospores were present, *D. filum* had an average germination of 81% and 84%, respectively (not significant at the 5% level). The difference between germination of *D. filum* spores alone and with uredospores was highly significant, showing that the presence of uredospores was responsible for the large increase in germination. On the other hand, the presence of nutrients had no significant effect, suggesting that nutritional factors may not be responsible for the increased germination.

Germination of D. filum among spores from other fungus species.—Since the presence of uredospores of *P. recondita* stimulated the germination of spores of *D. filum,* we wanted to know whether the stimulation is specific to *P. recondita* and uredospores of other rusts or whether the effect is nonspecific and can be caused by nonrust fungi.

Spores of *D. filum* were germinated in the presence of spores ("companion spores") of each of eight species of fungi (Table 4). The concn of spores from each species was 2.5×10^5 spores/ml. The results are from two experiments. Germination of *D. filum* was significantly greater with *P. recondita* than with *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth., which

TABLE 4. Germination of *Darluca filum* spores in the presence of spores from several fungus species[a]

Species of companion spores	Germination		
	D. filum plus companion fungus		Companion fungus alone
	D. filum	Companion fungus	
	%	%	%
Puccinia recondita	82a	27	24
Uromyces phaseoli	64b	47	50
Ustilago avenae (Pers.) Rostr.	52c	45	38
Penicillium sp.	50c	48	43
Aspergillus sp.	54c	0	0
Septoria nodorum Berk.	54c	3	5
Mucor sp.	52c	90	92
Helminthosporium sp.	52c	53	51

[a] Data shown are average of two experiments. Concentration of each spore type was 250×10^3 spores/ml of water.

[b] Figures followed by different letters are significantly different (5%) from each other according to Duncan's multiple range test.

in turn was significantly higher than the check, *D. filum* alone. In the presence of the nonrust companion spores, germination of *D. filum* was not significantly increased. Darluca spores did not markedly affect germination of any of the species of companion spores.

DISCUSSION.—The results indicate that the parasitic relationship of *D. filum* on its rust host may not be due primarily to nutritional factors. *Darluca filum* grows and sporulates readily on many types of nutrient agars (2, 4, 8, 11, 12); thus, it might be expected that *D. filum* would be found growing saprophytically in nature; however, it has only been reported growing in close association with other fungi, almost exclusively with rusts (5, 6, 7, 9, 12), which we suggest is due primarily to improved survival of *D. filum* spores and only secondarily due to nutrition. At least two mechanisms are probably involved, enhanced germination and enhanced longevity. Other mechanisms may also exist.

The enhanced germination and longevity of *D. filum* is probably due to a chemical compound(s) from the rust uredospores. It is unlikely that the compound is a common nutritional factor, since PDA did not reduce the stimulating effect of uredospores on *D. filum*. The experiment with different species of companion spores indicated that the compound is either absent or limiting in spores of fungi other than rusts. The identity of the compound remains unknown, but it may be one of the germination stimulators known to exist in uredospores (1).

The present evidence suggests that improved infection of rust sori in the field would occur if *D. filum* is applied either together with uredospores or when rust spores are already abundant on the crop. But this situation poses a dilemma, since it is undesirable either to disseminate viable uredospores with *D. filum* spores or to wait until abundant rust sori develop before applying control measures. An alternative for avoiding this dilemma may be possible if the chemical stimulant can be isolated and spores of *D. filum* coated with it prior to dissemination by man.

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