PHYTOPATHOLOGICAL NOTES

Effect of Inoculation Sequence and Humidity on Infection of Puccinia recondita by the Mycoparasite Darluca filum

D. P. Swendsrud and L. Calpouzos

Former Research Assistant and Professor, respectively, Department of Plant Pathology, University of Minnesota, St. Paul 55101. Present address of second author: Department of Plant Sciences, University of Idaho, Moscow 83843.

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ABSTRACT

The application of pycnidiospores of *Darluca filum* to wheat seedlings 3 days prior to inoculation with uredospores of *Puccinia recondita* resulted in (i) more severe rust infection and (ii) less uredial infection by the mycoparasite than when the two organisms were applied simultaneously, or when the rust fungus was applied first in the sequence.

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The mycoparasite *Darluca filum* (Biv.-Bern. ex Fr.) Cast., the imperfect stage of *Eudarluca caricis* (Fr.) Eriks., occurs in nature closely associated with species of rust fungi (1, 2, 3, 4, 5). Typically, black pycnidia of *D. filum* develop in the rust sorus, and the number of uredospores is reduced. Thus, a widespread infection of rust uredia by *D. filum* in the field may provide some control against epidemics of rust.

This paper reports the effect of *D. filum* on the incidence of wheat leaf rust (*Puccinia recondita* Rob. ex Desm.); firstly, as influenced by the sequence of spore application to the wheat leaves, and secondly, by the use of either 3- or 15-day periods of mist after inoculation.

D. filum was isolated in pure culture from wheat leaf rust, *Puccinia recondita*. The culture medium consisted of potato-dextrose agar (PDA) supplemented with 2.5 g Bacto peptone/liter of PDA. All cultures were grown at 20-25 C, and were exposed to diffuse light during the day. Seven to 12 days after the agar plates were inoculated with spores of *D. filum*, dark-colored pycnidia formed from which oozed gelatinous masses of pycnidiospores. The colonies were flooded with sterile distilled water, and the resulting spore suspensions were poured into beakers and stirred vigorously to break up spore masses. Uredospores of *P. recondita* (race UN-2), collected from infected wheat seedlings growing in the greenhouse, were stored in a desiccator at 4 C until used, at which time they were suspended in sterile distilled water. The concentration of all spore suspensions was adjusted to 50,000 spores/ml of water.

Fifteen-day-old seedlings of Little Club wheat (*Triticum aestivum* L.) were grown in 10-cm clay pots containing autoclaved loam soil. Grains were sown at 15/pot; the pots were kept in a growth chamber set at 21 C (16-hr day) and 16 C (8-hr night). Incandescent and fluorescent lamps provided 3,000 ft-c of illumination.

To test the influence of the sequence of spore application, three inoculation procedures were used: (i) Separate suspensions of uredospores and pycnidiospores were sprayed on wheat seedlings at the same time; (ii) pycnidiospores were sprayed on wheat seedlings 3 days before uredospores were applied; (iii) uredospores were sprayed on wheat seedlings 3 days before pycnidiospores were applied. After each spore application, seedlings were placed in a mist chamber having the same temperature and light regimes as the growth chamber in which the seedlings were grown. Check plants were inoculated with uredospores only.

To test the influence of mist-period duration on infection of rust by *D. filum*, two further treatments were superimposed on the spore applications mentioned earlier: (i) Half the pots of seedlings from each spore application treatment were transferred from the mist chamber 3 days after the last spore application (short duration) to the growth chamber in which the seedlings grew before inoculation; and (ii) the remaining three pots of seedlings were kept in the mist chamber 15 days (long duration) after the last spore application.

The effects of the treatments were measured in two ways: by the total number of rust uredia on wheat, and by the percentage of uredia infected with *D. filum*. Observations were made 15 days after the last inoculation. Three wheat seedlings were chosen at random from each pot and examined. Counts of sori included some where the leaf epidermis was not yet ruptured, but uredospores and pycnidia were clearly visible through the epidermis. The data reported are based on means of four experiments.

Application of pycnidiospores of *D. filum* to leaves 3 days prior to inoculation with uredospores of *P. recondita* resulted in more severe rust than when both organisms were applied either simultaneously or with uredospores 3 days prior to pycnidiospores (average numbers of uredia/seedling were 182, 84, and 61, respectively, when plants were exposed to 3 days of mist, and 163, 86, and 70, respectively, on plants exposed to 15 days of mist). The average rust severity on the check plants which were inoculated only with rust spores ranged from 72 to 96 pustules/seedling.

Prolonging the mist period to 15 days increased the percentage of uredia which contained pycnidia of *D. filum*. Abundant uredial infection occurred when spores of *D. filum* were applied at the same time (76%) or 3 days after rust spores (67%), but few uredia were infected when spores of the mycoparasite were applied first in the sequence (9%). The same trend was shown in seedlings exposed to the 3-day mist period, but levels of uredial infection were much lower; e.g., the most severe uredial infection, which occurred when pycnidiospores were applied 3 days after inoculation with rust spores, was only 26%.

Thus, the inoculation of *D. filum* spores 3 days prior to inoculation with rust spores resulted in (i) more severe rust infection of seedlings; and (ii) less severe uredial infection by the mycoparasite than when the organisms were applied either simultaneously, or rust spores were applied first in the sequence. The low percentage of uredial infection in (ii) suggests that many pycnidiospores of *D. filum* died during the 3-day interval before rust hyphae were available for colonization. It is possible that these dead pycnidiospores served as a source of nutrients for germinating uredospores, and thus partly accounted for increased rust severity when *D. filum* was inoculated first. Also, pycnidiospores on seedling leaves may have altered stomatal physiology in some way, rendering the leaves more susceptible to rust infection.

The longer mist period appeared to have a greater effect on the mycoparasite than on the rust fungus, since only slightly more uredia per seedling developed on the check plants (rust alone) when exposure to mist was prolonged from 3 days to 15.

An earlier paper suggested that an unidentified substance from rust uredospores enhances the viability of *D. filum* spores (6). The present work indicates than an increase of *Darluca*-infected rust pustules is most likely when uredospores reach the infection court either before or with the *Darluca* spores when moist conditions prevail. Therefore, biological control of rust diseases by spraying wheat with *D. filum* spores before rust infection seems unlikely on the basis of this study.

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