Influence of Temperature, Hydrogen-ion Concentration and Osmotic Potential on Oospore Germination of Pythium paddicum and P.iwayamai

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Influence of Temperature, Hydrogen-ion Concentration and Osmotic Potential on Oospore Germination of *Pythium paddicum* and *P.iwayamai* *

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Abstract

Two soil-borne fungi, *P. paddicum* and *P. iwayamai,* causing Pythium snow rot of wheat and barley, were compared for the behaviour of oospores under various cultural conditions.

On Bacto-cornmeal agar(CMA) medium, optimum temperatures for oospore germination were approximately 20°C for *P. paddicum* and 10-25°C for *P. iwayamai.* On CMA adjusted to pH values between 4.8 and 8.2 with a MES [2-(N-morpholino)ethanesulfonic acid] buffer, both fungi germinated well. *P. paddicum* reached the best germination at pH 5.5, *P. iwayamai* at 6.5. On CMA amended with a non-ionic mannitol to adjust osmotic potential between -1.3 and -37.2 bars, oospores of *P. paddicum* were able to germinate only above -10.2 bars, but those of *P. iwayamai* germinated above -19.0 bars, with the highest germination rate between -3.4 and -19.0 bars.

The relationship between the results obtained and the ecology of the two fungi is discussed.

Introduction

Pythium iwayamai S. Ito and P. paddicum Hirane are the main causal organisms of Pythium snow rot of wheat and barley in Japan.[1] *P. iwayamai* is dominant in upland fields, whereas *P. paddicum* prevails in ill-drained paddy fields[2]. The different distribution of these pathogens indicates possible differences in their responses to various biological, chemical and physical factors involved in upland- and paddy-field soils. These responses would include mycelial growth, dormancy and germination of oospores. Some work has already been done, concerning tolerance of mycelia to CO_2 [3, 4] and response to pH[5]. But there has been no research on the comparative responses of oospores to various factors in the two different field soils.

This study was designed to compare oospore germination of the two fungi under various cultural conditions, which may explain their difference in distribution. Three main factors, temperature, hydrogen-ion concentration and osmotic potential, were selected.

Materials and Methods

Fungi and isolates studied *P. iwayamai* (UOP381) and *P. paddicum* (UOP382), which can produce abundant oospores, were used in all experiments.

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Media CMA was employed for the investigation of temperature response of oospore germination.

V-8 juice(10%) liquid medium supplemented with 500ppm of wheat germ oil (Japan Impex Co., Ltd., Tokyo) was used for producing oospores. The pH was adjusted to 7.0.

Amended media In the pH experiment, CMA was adjusted to pH values between 4.5 and 8.5 at 0.5 pH unit intervals with MES buffer(50mM)[5]. Double strengths of the buffer salt was dissolved in a fixed volume of distilled water and adjusted to the desired pH with 1N HCl or 1N NaOH, using a Hitachi-Horiba pH-meter(F-7). Double strengths of MES buffer and CMA medium were separately autoclaved at 121°C for 15 minutes, and the buffer was then added to an equal volume of the medium to give a final buffer concentration of 50mM. The final pH was measured and the medium was dispensed into 90-mm diam. shallow Petri dishes (10ml/dish) or Corning disposable multiple well plates with 12 cell wells (0.3ml/well). Since the pH of CMA after mixing was slightly different from the target pH, data are plotted as the actual pH values after mixing.

In the osmotic potential experiment, the osmotic potential of CMA was controlled by adding mannitol, which was found not to be utilized by *Pythium* as a nutrient[6], into CMA. The amount of mannitol added was according to Robinson and Stokes's formula[7]. The osmotic potential was determined with an Advanced osmometer(Advanced Instruments, Inc., Mass.)

Oospore production and germination The fungi were cultured to produce oospores at 20°C for 4 months in 100-ml Erlenmyer flasks containing 12 ml of 10% V-8 juice liquid medium supplemented with 500 ppm of wheat germ oil. Oospore suspensions were then obtained using the method of Kusunoki and Ichitani[8], and the final concentration was adjusted to about 10^5 oospores per milliliter. This suspension was kept at 4°C for 10 days to break oospore dormancy and then incubated on a CMA plate or amended media for 24 hours at fixed temperature in the dark. In pH and osmotic potential experiments, oospore suspensions were further adjusted to the corresponding pH values or osmotic potentials before incubation. Oospore germination was examined microscopically and 200 oospores were chosen at random for calculation of germination rate.

Statistical analysis All experiments had 4-5 replicates, and were repeated at least

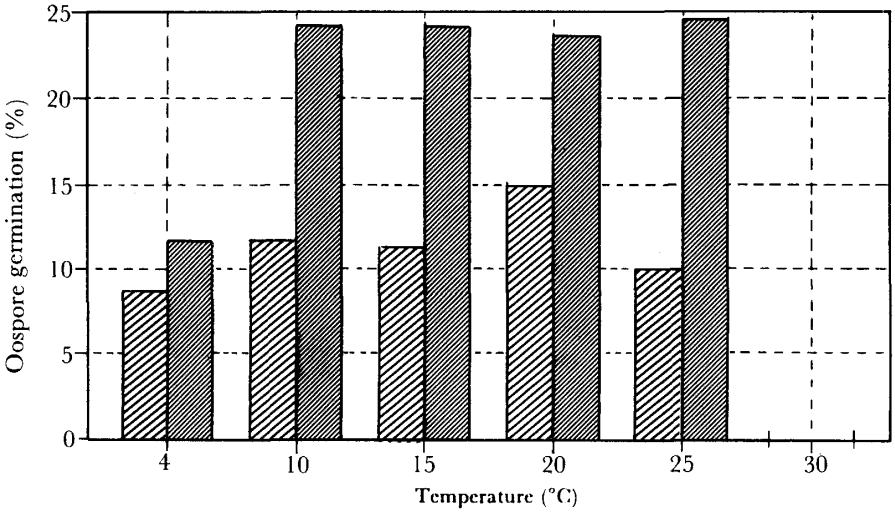

Fig. 1 Effect of temperature on oospore germination of *Pythium iwayamai* (▨) and *P. paddicum* (▨).
Data represent the mean of 5 replicates.

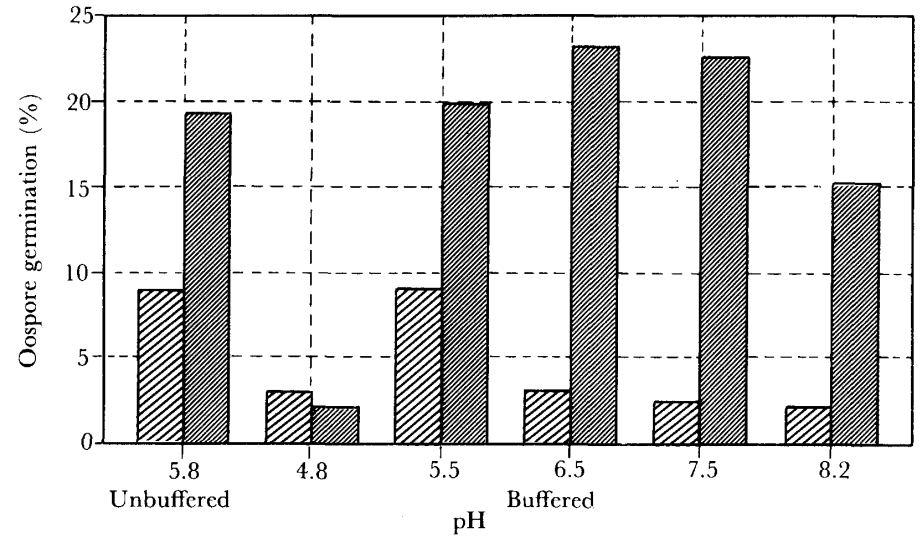

Fig. 2 Effect of hydrogen-ion concentration on oospore germination of *Pythium iwayamai* (▨) and *P. paddicum*(▨) at 20°C.
Data represent the mean of 4 replicates.

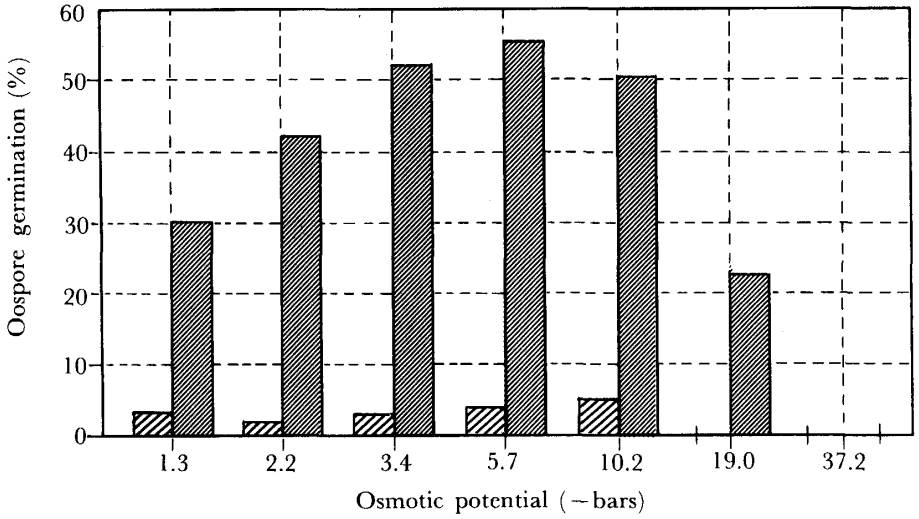

Fig. 3 Effect of osmotic potential on oospore germination of *Pythium iwayamai*(▨) and *P. paddicum*(▨) at 20°C.
Data represent the mean of 4 replicates.

once. Only data from individual experiments are presented. Significant trends were analyzed using Duncan's multiple-range test.

Results

Influence of temperature on oospore germination The germination rates of oospores of the two fungi were measured at 4, 10, 15, 20, 25, and 30°C. The results obtained are indicated in Fig. 1.

Oospores of both fungi were able to germinate between 4 and 25°C, but not at 30°C. Both species may belong to low-temperature favouring fungi.

Influence of hydrogen-ion concentration on oospore germination The pH of unbuffered CMA was 5.8. Both fungi were able to germinate over the whole pH range designed in the experiment at 20°C. Optimum pH for oospore germination was considered as 5.5 for *P. paddicum* and about 6.5 for *P. iwayamai*(Fig. 2).

Influence of osmotic potential on oospore germination The osmotic potential of CMA was controlled to -1.3, -2.2, -3.4, -5.7, -10.2, -19.0, and -37.2 bars by adding mannitol at the ratio of 0.00 (unamended CMA), 0.04, 0.08, 0.16, 0.32, 0.64, and 1.28 mol/kg, respectively. The response of oospore germination to osmotic potential at 20°C is shown in Fig. 3.

The two fungi were quite different in their response. At -37.2 bars, neither of them germinated. At -19.0 bars, *P. paddicum* did not germinate, whereas *P. iwayamai* was still able to germinate although at a considerably reduced rate. For *P. paddicum,* variation of oospore germination among the osmotic potentials between -1.3 and -10.2 bars was not significant ($P<0.05$) whereas for *P. iwayamai* the highest germination rates corresponded to osmotic potentials of -3.4, -5.7, and -10.2 bars. Osmotic potentials above -3.4 bars or below -10.2 bars inhibited oospore germination of *P. iwayamai.*

Discussion

The two species studied here have a distinctly different distribution in upland fields and ill-drained paddy fields although they cause the same Pythium snow rot of wheat and barley. Because of the conspicuous differences in two soils[2], the responses of the fungi to the biological, chemical and physical conditions in the natural environment were assumed to be different(Ichitani, unpublished data). Takamatsu *et al.*[3], and Ichitani and Takamatsu[4] studied the mycelial tolerance of the two fungi to CO_2, and found that *P. paddicum,* which has a paddy field distribution, is more tolerant of CO_2 than *P. iwayamai,* which is dominant in upland fields. On the other hand, Inoue and Ichitani[5], comparing the pH response, showed that the optimal pH values for growth of the two fungi differed, but were consistent with the soil pH in which each existed. These findings suggest some kind of relationship between the behaviour of the two fungi and their environmental distribution.

A similar correlation could be derived from the point of view of oospore germination. Optimum temperature ranges for germination in both fungi were similar, but the optimum pH for *P. iwayamai* was about 1 unit higher (pH 6.5) than that (pH 5.5) for *P. paddicum;* and *P. iwayamai* was more tolerant of lower osmotic potential (-19.0 bars) than *P. paddicum* which ceased to germinate at this level.

The upland field which *P. iwayamai* inhabits is chemically neutral and dry, with greater decomposition of fertilizers and accumulation of inorganic ions, i.e. a lower osmotic potential to which *P. iwayamai* is able to adapt. The ill-drained paddy field where *P. paddicum* is prevalent is, however, acidic and wet, with a relatively high osmotic potential, which may be suitable for *P. paddicum*. Thus, the differences in distribution of the two pathogens may partly be explained by the differences in response to pH and osmotic potential. Further studies are needed on matric potential. The data presented here may, however, help to analyze the ecological mechanisms of these fungi.

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