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Measurement of Matric Potential and Oospore Germination of *Pythium butleri* in Sea Sand

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Abstract

Tension plate assembly was used for measuring a matric potential in sea sand as a soil sample. Indirect germination of oospores was found in sea sand deficient in nutrition under saturated conditions (0 mb), but not when the matric potential reached −100 mb. Percentage of direct germination was relatively low at 0 mb, compared with that between −10 and −100 mb at which the values showed little difference. While the percentage of direct germination was low in non-amended sea sand, enhancement was found in some degree when carbohydrates, except glucose, were added, and there was a considerable increase with addition of amino acids. Although indirect germination was sometimes observed in non-amended sea sand, no such a germination was found when carbohydrates or amino acids were added.

Introduction

The relationships between soil water potential and incidence of *Phytophthora* diseases have already been studied[1, 2]. The effect of the water potential on zoospore formation from zoosporangia of *Pythium* spp. causing snow rot of wheat has also been examined[3]. There are, however, no experiments on the water potential affecting zoospore formation from oospores in *Pythium*.

This study was designed to measure a matric potential and to examine the nutritional factors influencing direct and indirect germination of oospores of *P. butleri* in sea sand as a soil sample deficient in nutrition. Part of this work has already been reported elsewhere[4].

Materials and Methods

Measurement of matric potential by tension plate assembly As shown in Fig.1, tension plate (porous ceramic) assembly "Dozyo Fuatsu So" (Daiki Rika Co., Ltd., Tokyo) was used for equilibrating the soil sample with a known matric potential value. This assembly is applicable in the range of 0–1 bar. One hundred grams of sea sand (20–35 mesh, Wako Chemical Co., Ltd., Osaka) was placed in a vessel of the assembly and saturated with distilled water. Adjusting the h value to 10–15 cm, excess water was removed through a micropipette. The equilibrium of the matric potential was reached when no more water came out of the pipette. As the h value increased, water again came out and the amount of water was then weighed. When no more water came out even if the h value was increased, the final water content of the sea sand was obtained on a dry weight

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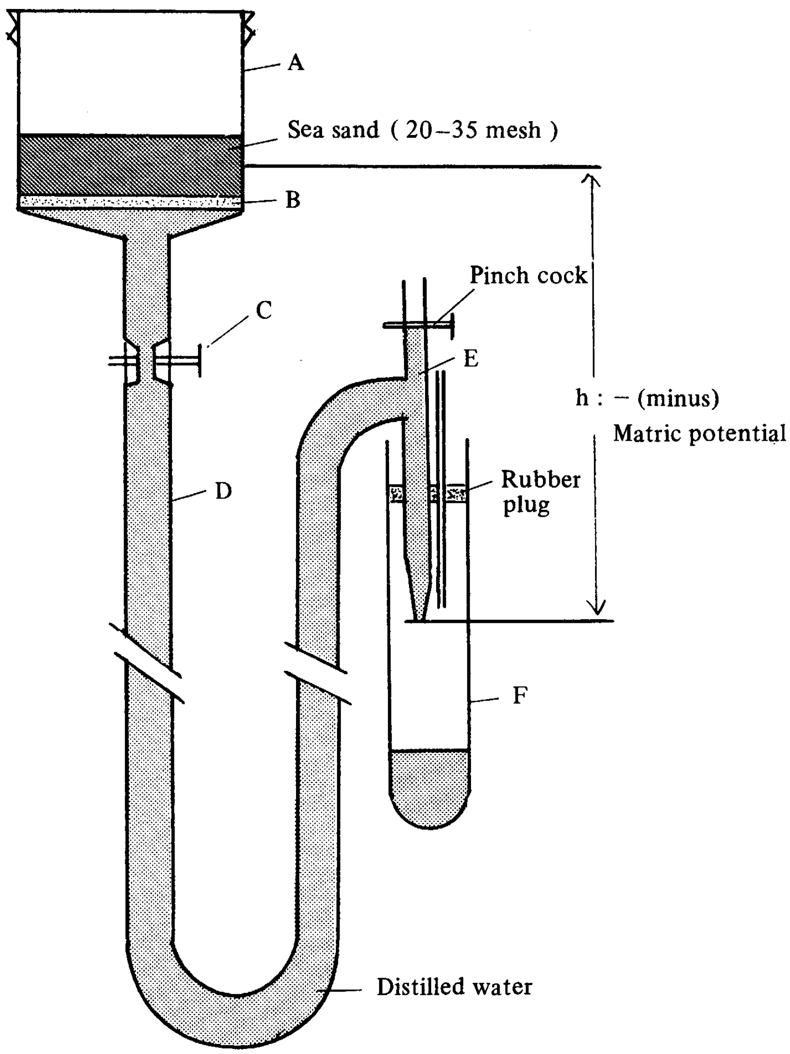

Fig. 1 Tension plate assembly "Dozyo Fuatsu So"
A : Vessel (70 mm diam., 70 mm high)
B : Porous ceramic plate
C : Stop cock
D : Plastic tube (6 mm inside diam., 2000 mm long)
E : Micropipette (ca. 0.3 mm inside diam. of nozzle)
F : Test tube (15 mm diam., 150 mm high)

basis (90°C, 2 days). By plotting relative water content (%) against the h value (which is equivalent to matric potential, –mb), the water characteristic curve (drying) for sea sand can be obtained (see Fig.2). Measurements were repeated 3 times.

Germination test As described elsewhere[5)], a membrane filter (8 μm in pore size, Karl Zeiss Co., Ltd., Germany) to which about 1×10^4 oospores were previously adhered was inserted into sea sand in the vessel, and the filter and sand were saturated with distilled water. After obtaining the desired matric potential by adjusting h (usually the value can be obtained within 1–2 hr), a stop cock directly below the vessel was closed. The vessel was covered by cellophane film with a few tiny needle holes, to prevent evaporation and the oospores in the vessel were incubated at 30°C for 24 hr. The percentage germination was calculated after removing adhered sand from the filter, followed by clearing it with phenol and staining the oospores with 0.2 % rose bengal at 45°C for 1 min. All tests were repeated at least twice.

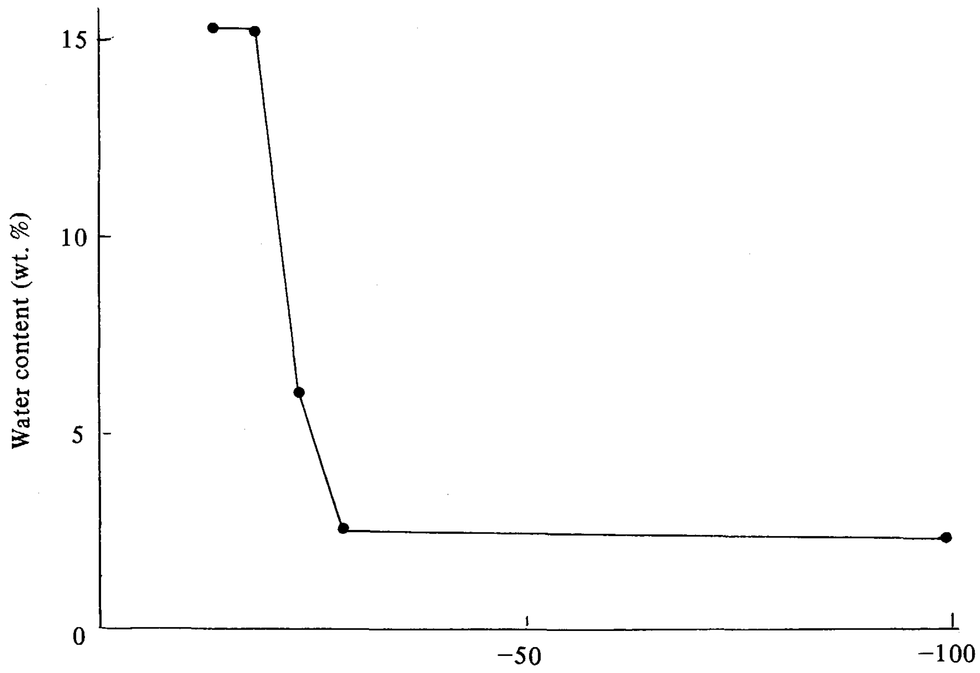

Fig. 2 Water characteristic curve (drying) for the sea sand (at 25°C).

Results

Water characteristic curve (drying) for sea sand From this curve obtained at 25°C (Fig.2), the value of matric potential between −100 and −20 mb can easily be calculated using the relative water content.

Effect of matric potential on oospore germination in sea sand As shown in Table 1, indirect germination of oospores was found in sea sand deficient in nutrition under saturated conditions (0 mb), but not when the matric potential reached −100 mb. Percentage of direct germination was relatively low at 0 mb, compared with that between −10 and −100 mb at which the values showed little difference.

Table 1. Effect of matric potential on direct and indirect germination of *Pythium butleri* oospores in sea sand.[1)]

Matric potential (mb)	Germination	
	Direct (%)	Indirect
0	1.2	+
− 10	7.4	+
− 50	5.3	−, +
− 100	6.2	−

1) Incubated at 30°C for 24 hr in the dark.
Indirect germination: + presence, − absence.

Effect of carbohydrates and amino acids on oospore germination in sea sand Adjusting the matric water potential in sea sand to –20 mb, different carbohydrates or amino acids were compared for oospore germination (Tables 2, 3). While the percentage of direct germination was low in non-amended sea sand, enhancement was found in some degree when carbohydrates, except glucose, were added, and there was a considerable increase with addition of amino acids. Although indirect germination was sometimes observed in non-amended sea sand, no such a germination was found when carbohydrates or amino acids were added.

Table 2. Effect of carbohydrates on direct and indirect germination of *Pythium butleri* oospores in sea sand.[1)]

Carbohydrate	Swollen oospores (%)	Germination	
		Direct (%)	Indirect
Glucose	4.4	6.4	–
Fructose	16.4	24.6	–
Sucrose	5.0	13.0	–
Starch	14.8	22.2	–
None added	10.6	6.4	+, –

1) Maintained at the equivalent of –20 mb after adding each carbohydrate to 0.1% and incubated at 30°C for 24 hr. in the dark. Swollen oospores are those neither extending germ tubes nor forming zoospores for 24 hr.
Indirect germination: + presence, – absence.

Table 3 Effect of amino acids on direct and indirect germination of *Pythium butleri* in sea sand.[1)]

Amino acid	Swollen oospores (%)	Germination	
		Direct (%)	Indirect
Glycine	4.6	81.5	–
Serine	9.6	76.9	–
L-asparagine	2.5	88.6	–
Phenylalanine	21.0	52.9	–
None added	5.0	3.3	+, –

1) Maintained at the equivalent of –20 mb after adding each amino acid to 0.1 % and incubated at 30°C for 24 hr. in the dark. Swollen oospores are those neither extending germ tubes nor forming zoospores for 24 hr.
Indirect germination: + presence, – absence.

By addition of carbohydrates or amino acids, the osmotic water potential of the sea sand is lowered drastically. For example, 0.1 % of glucose produce the osmotic potential of about –24 bar. As shown in Tables 2 and 3, this low osmotic potential does not affect direct germination of oospores. On the other hand, indirect germination is prevented completely by addition of the compounds.

Discussion

The limiting matric potential was about −100 mb for zoospore formation from oospores of *P. butleri* in sea sand deficient in nutrition and this value agreed well with those for zoospore formation from zoosporangia in other members of Pythiaceae[6, 7]. Thus, the result may indicate that the oospores of this fungus play a role in the release of zoospores, especially in very wet soils.

Since the lower limit for the tension plate assembly used was −100 mb, the effect of matric potential on direct germination of oospores could not be examined by the present method. Moreover, a minute change in matric potential can not be applied accurately because serious errors will be involved in the region of low matric potential as shown in Fig.2. Thus, a new experimental system should be set up, using finer sand than sea sand to apply the low matric potential by means of either centrifugation[8] or pressure membrane[9].

A few oospores of *P.butleri* germinate by germ tubes in sea sand of −20 mb which is deficient in nutrients and a considerable increase in direct germination, without zoospore formation, is found in the presence of amino acids. This may indicate that oospores in the rhizosphere can penetrate into the host tissue by germ tubes, but not by zoospores, at 25–35°C[5] over the entire range of matric potential (0 to −100 mb); and also by zoospores at 25–30°C at matric potentials higher than −50 mb outside the rhizosphere. This assumption is partially supported by SEM observations that there are only directly germinated oospores, and no zoospores, on the surface of cucumber roots in sea sand[5], and such roots have been known to produce serine[10].

Further studies are needed to be done using different types of natural soils.

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References

1) STERNE, R. E., ZENTMYER, G. A. and KAUFMANN, M. R. (1977). The effect of matric and osmotic potential of soil on *Phytophthora* root disease of *Persea indica*. *Phytopathology* 67, 1491-1494.
2) STERNE, R. E., ZENTMYER, G. A. and KAUFMANN, M. R. (1977). The influence of matric potential, soil texture, and soil amendment on root disease caused by *Phytophthora cinnamomi*. *Phytopathology* 67, 1495-1500.
3) LIPPS, P. E. (1980). The influence of temperature and water potential on asexual reproduction by *Pythium* spp. associated with snow rot of wheat. *Phytopathology*, 70, 794-797.
4) KUSUNOKI, M. and ICHITANI, T. (1983). Germination of *Pythium butleri* oospores in both nutrient solution and in sea sand. *Ann. Phytopath. Soc. Japan*, 49,

99-100 (Abstr. in Japanese).
5) KUSUNOKI, M. and ICHITANI, T. (1984). *Ann. Phytopath. Soc. Japan* (*In press*).
6) PFENDER, W. F., HINE, R. B. and STANGHELLINI, M. E. (1977). Production of sporangia and release of zoospores by *Phytophthora megasperma* in soil. *Phytopathology*, 67, 657-663.
7) DUNIWAY, J. M. (1979). Water relations of water molds. *Ann. Rev. Phytopathol.*, 17, 431-460.
8) RUSSELL, M. B. and RICHARDS, L. A. (1938). The determination of soil moisture energy relations by centrifugation. *Soil Sci. Soc. Amer. Proc.*, 3, 65-69.
9) KUNZE, R. J. and KIRKHAM, D. (1962). Simplified accounting membrane impedance in capillary conductivity determinations. *Soil Sci. Soc. Amer. Proc.*, 26, 421-426.
10) SUZUKI, K. and TAKAHASHI, M. (1978). Chemotactic substances to *Pythium aphanidermatum* zoospores from cucumber root exudates. *Ann. Phytopath. Soc. Japan*, 44, 241-246 (In Japanese).