Density of Pythium zingiberum Oospores in Relation to Infection of Chenopodium quinoa Seedlings

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Density of *Pythium zingiberum* Oospores in Relation to Infection of *Chenopodium quinoa* Seedlings

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Abstract

The infectivity tests were repeated and the average results obtained indicated that more than 1 $\times 10^4$ oospores per gram oven dry soil (OPG) caused damping-off of 80% of *Chenopodium quinoa* seedlings. About 20% damping-off occurred with 1×10^3 OPG. With 1×10^2 OPG the average was 6%, and penetrating mycelium and oospores were easily recognized. Neither penetration nor disease incidence was found with 1×10 OPG. Thus, the minimum number of oospores capable of inducing disease was estimated to be $10 - 1 \times 10^3$/g oven dry soil. The fact that *P. zingiberum* needs much more oospores than *P. butleri* to induce disease may be connected with lower germinability of oospores of the former.

Introduction

Pythium zingiberum is the pathogen causing rhizome rots of ginger (*Zingiber officinale* Rosc.) and mioga (*Z. mioga* (Thunb.) Rosc.)[1,2], and this fungus has interesting biological[3] and epidemiological[4] characteristics which differ from those of *P. butleri* causing damping-off of spinach[5].

This study was designed to examine the relationships between the number of oospores of *P. zingiberum* and damping-off of highly susceptible *Chenopodium quinoa* seedlings, following the considerations originally proposed by Mitchell[6], and to compare the results obtained here with those using *P. butleri*[7]. Part of this work has been reported elsewhere[8].

Materials and Methods

Isolate and culture of the fungus used *P. zingiberum* UOP 8001-2 was selected from 28 isolates for producing abundant matured oospores with high virulence and cultured in 10% V-8 juice liquid medium supplemented with 1,000 ppm wheat germ oil (Japan Impex Co., Ltd., Tokyo) at 25 C for 2 weeks. The oospores of this isolate had a relatively uniform germinability (0.4-7.1, usually 1.3-4.2%).

Preparation of idealized soil Two parts of glass beads (GB731, Toshiba Ballotini Co., Ltd., Tokyo) less than 352 mesh (54 μm) were mixed with 8 parts of those (GB708K) of 28-45 mesh (180-604 μm) (w/w) to prepare an idealized soil (Fig. 1). A water characteristic curve (drying) of the idealized soil (pH: about 6.7, see Fig. 3) was measured by tension plate assembly "Dozyo Fuatsu So" (Daiki Rika Co., Ltd., Tokyo)[9] at 25 C.

Induction of oospore germination In *P. zingiberum* this was examined by treating oospore suspensions including a small amount of propagules other than oospores at low

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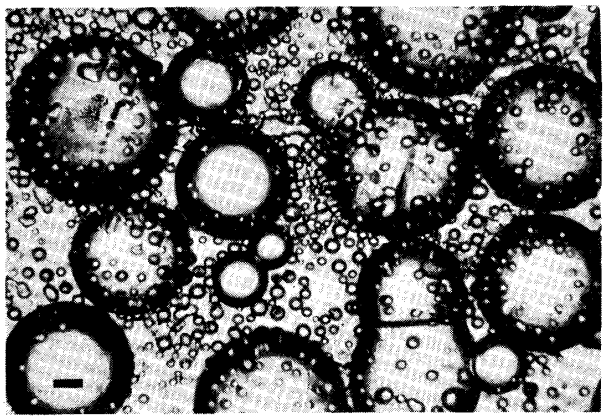

Fig. 1. Idealized soil composing of 2 parts of glass beads which were less than 352 mesh mixed with 8 parts of those of 28-45 mesh (w/w). Bar represents 100 μm.

temperature, heating in a water bath, sonication, use of organic solvents (methanol, ethanol, acetone), and extract of ginger rhizomes. Similarly the induction was determined by placing oospores in the rhizosphere of highly susceptible *C.quinoa* grown in non-amended idealized soil (see Fig. 2).

Preparation of mycelium-free oospores as an inoculum Mycelial mats of *P.zingiberum* were washed twice with sterile distilled water (SDW) and homogenized at 14,000 rpm for 2 min in a Waring blender. The homogenates were filtered through a 200-mesh sieve and oospores including a small amount of other propagules were recovered by using a 635-mesh sieve. After washing the propagules 3 times with SDW, they were resuspended in the same water, followed by pouring into 125 ml Erlenmeyer flask and mixing thoroughly with 100 g of the idealized soil. The propagules other than oospores were killed by drying over calcium chloride in a desiccator at about 8 C for 5 days.

Susceptibility of indicator plants Susceptibility was examined by pouring a suspension of propagules (1.2×10^4 oospores mixed with 5.1×10^4 mycelial fragments/g oven dry soil) of *P.zingiberum* on 15 seedlings and incubating at 30C for 5 days in a growth chamber (Nihon Ika Co., Ltd., Osaka) with 14 hr daily illumination of ca. 1,500 lux.

Direct and indirect germination of oospores in idealized soil Germination of oospores of *P.zingiberum* was examined by using essentially the same method[7] with idealized soil inside a 125 ml Erlenmeyer flask under aseptic conditions (Fig. 2). Oospores were sandwiched

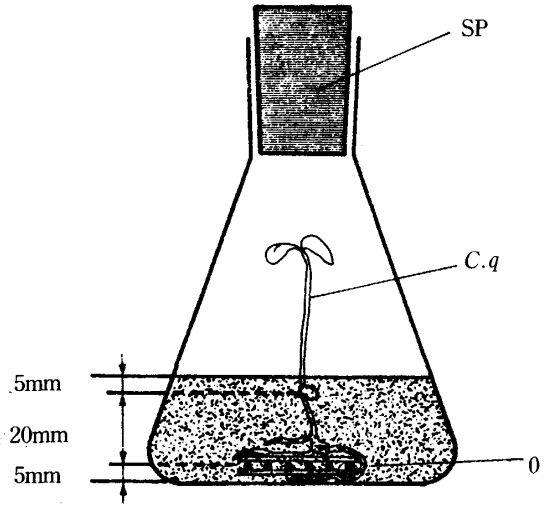

Fig. 2. Germination test of *Pythium zingiberum* oospores (0) in the rhizosphere of a highly susceptible *Chenopodium quinoa* (*C.q.*) seedling grown in non-amended idealized soil at matric potential of –50 mb.
SP: Silicone plug

between two cellulose nitrate filters (8.0 μm pore size, 25mm diam, Sartrius-Membran-filter GmbH, Göttingen). The percentage germination was calculated after removing adhering soil from the filter, followed by clearing with water-saturated phenol and staining the oospores with 0.1% cotton blue in lactophenol at 50-60 C for 1 hr.

Infectivity test One hundred grams of the inoculum of *P. zingiberum* obtained as above was laid uniformly in each 125 ml Erlenmeyer flask. The matric potential of the inoculum was adjusted to –50 mb prior to the infectivity test. One seed of surface-sterilized *C. quinoa* per flask was sown in the idealized soil to prevent secondary infection[6]. The flask, with silicone plug, was incubated at 30 C for 14 days in a growth chamber under 14 hr daily illumination of ca. 1,500 lux. Five flasks were used for each inoculum density.

All the experiments were repeated at least twice.

Results

Water characteristic curve of idealized soil This curve (drying) was similar to those of clay loam and sandy soils (Fig. 3). Using this curve, the matric potential in the idealized soil could be calculated from the water content (wt %).

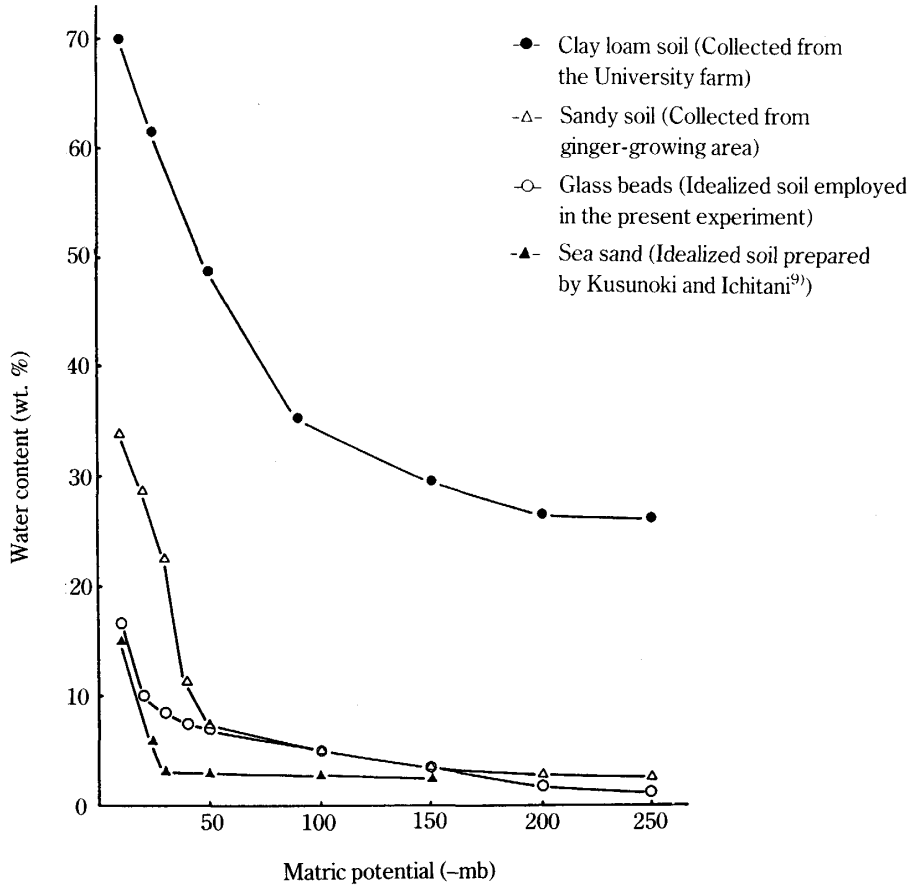

Fig. 3. Water characteristic curve (drying) for different soils (at 25 C).

Induction of oospore germination Germination was not induced by the treatments used in this experiment. There was also no enhancement of oospore germination by the rhizosphere of highly susceptible *C.quinoa* in the idealized soil (Table 1).

Preparation of mycelium-free oospores These were obtained by drying over calcium chloride (Table 2).

Table 1. Germination of *Pythium zingiberum* oospores in the rhizosphere of highly susceptible *Chenopodium quinoa* seedlings grown in non-amended idealized soil[a)]

No. of plants / flask	Germination after 1-14 days' incubation			
	1 day	3 days	7 days	14 days
0	3.5 (–)	3.0 (–)	2.4 (–)	2.6 (–)
1	2.9 (–)	3.8 (–)	4.3 (–)	4.1 (–)
5	3.6 (–)	3.9 (–)	4.2 (–)	4.3 (–)

a) Measured with 1,000 oospores per flask. Numerals indicated average percentage of 3 replications.
(–): No zoospore formation.

Table 2. Killing of the propagules other than oospores by drying

Exp.	Oospores	Propagules other than oospores
Before drying[a)]	Live	Live
After drying[b)]	Live	Killed

a) Living of all propagules was determined by dilution plate[11)] incubated for 1-3 days.
b) Living of oospores was determined by dilution plate[11)] incubated for 7 days and killing of the propagules other than oospores was judged by observing no mycelial growth even after 7 days' culture.

Susceptibility of two species of Chenopodium *C.quinoa* was more susceptible than *C.amaranticolor* and all seedlings of the former were damped-off within 5 days.

Infectivity test Factors affecting direct germination of oospores of *P.zingiberum* were first determined in the idealized soil. Direct germination (2.4-4.3%) occurred when oospores were incubated at 30 C in the rhizosphere of *C.quinoa* with matric potential of –50 mb, but no indirect germination was found under these conditions (see Table 1).

The infectivity test was then repeated 4 times and the similar results were obtained. One of the results is shown in Table 3. More than 1×10^4 oospores per gram oven dry soil (OPG) caused damping-off of *C.quinoa* seedlings within 10 days of incubation at 30 C. A low disease incidence was observed with 1×10^3 OPG. Neither penetration nor disease was found with 1×10^2 OPG even after 14 days.

Table 3. Inoculation of *Pythium zingiberum* oospores at different inoculum densities on *Chenopodium quinoa* seedlings grown in a non-amended glass bead medium

Inoculum density (OPG)	No. seeded	No. emerged[a)]	No. of damped-off seedlings after incubation[b)]	
			6 days	10 days
1	5	4	0	0
1×10	5	4	0	0
1×10^2	5	4	0	0
1×10^3	5	4	0	1
1×10^4	5	5	2	4
Uninoculated	5	5	0	0

[a)] All unemerged plants were rooted, without fungal penetration.

[b)] All seedlings damped-off were penetrated.

Discussion

The water characteristic curve of the idealized soil in the present experiment was similar to that of clay loam soil collected from the University farm and also to that of sandy soil from ginger-growing area in Wakayama prefecture where *P.zingiberum* was isolated as the pathogen causing rhizome rot of ginger. This indicates that the idealized soil is suitable for the experiment on inoculum potential of oospores of *P.zingiberum.*

Since there was no induction of oospore germination by different treatments, including root exudates of the suscept, the oospores were considered to have innate dormancy. Thus, the second consideration that the *Pythium* tested should require stimulation by host produced nutrients for germination of oospores[6)] might not be satisfied in the present experiment. This second consideration should be evaluated after examining procedures for breaking dormancy.

Propagules other than oospores of *P.zingiberum* are all killed by drying and oospores seem to be normal morphologically. Thus the oospores can be used for inoculum in the present experiment. However, the percentage of oospore germination was always reduced after drying (unpublished data). Further detailed studies are needed concerning these problems.

Spinach is known to be highly susceptible to *P.zingiberum*[10)] and susceptibility was then compared to that of the seedlings of two *Chenopodium* species. *C.quinoa* was the most susceptible and disease incidence could easily be evaluated as the percentage of plants infected rather than by symptoms or disease index[6)].

The infectivity tests were repeated with conditions determined by the above-mentioned several preliminary experiments and the average results obtained indicated that more than 1×10^4 OPG caused damping-off of 80% of *C.quinoa* seedlings. About 20% damping-off caused with 1×10^3 OPG. With 1×10^2 OPG the average was 6% and penetrating mycelium and oospores were easily recognized. Neither penetration nor disease incidence was found with 1×10 OPG. Thus, the minimum number of oospores capable of inducing disease was estimated to be $10\text{-}10^3$/g oven dry soil. *P.butleri,* however, requires only 3-30 OPG[7)]: The fact that *P.zingiberum* needs much more oospores than *P.butleri* to induce disease may be connected with lower germinability of oospores of the former. Assuming that innate dormancy

in oospores of *P.zingiberum* may break to some extent in the rhizosphere during ginger-growing season in the next year, the minimum number of oospores capable of inducing disease will be less than the above values.

The idealized soil and *Pythium* spp. which differ from each other in biological and epidemiological characteristics should be used for further comparison of environmental factors affecting the relationships between density of oospores and disease incidence.

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