SHORT COMMUNICATIONS

Aggregation of *Phytophthora capsici* Zoospores and their Interaction with Zoospores of *P. palmivora*

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INTRODUCTION

Zoospores of *Phytophthora palmivora* Butler aggregated without encysting within 2 min when they were suspended in water (Ko & Chase, 1973). Such zoospore behaviour was temperature-dependent with an optimum temperature of 16 °C. Aggregate formation also depended on both zoospore concentration and depth of suspension. We now report a similar behaviour of zoospores of *Phytophthora capsici* Leonian in water. The interaction between zoospores of *P. palmivora* and *P. capsici* was also investigated.

METHODS

Sporangia of *Phytophthora capsici* (P181, pepper isolate) and *P. palmivora* (18F2P, papaya isolate) were produced by growing the fungus on 5 and 20% (v/v) V-8 juice agar, respectively, under light for 7 days at 24 °C (Miller, 1955). A sporangium suspension was obtained by spraying culture plates with distilled water by means of an atomizer. Zoospores of *P. capsici* and *P. palmivora* were liberated by incubating the sporangium suspension at 24 and 16 °C respectively for 1 h, and separated from sporangia by passing the suspension through a 20 µm screen. Unless otherwise stated, 10 ml of the resulting zoospore suspension in a small Petri dish (60 × 15 mm) was incubated at 16 °C for observations of aggregation, as defined by Ko & Chase (1973). Zoospore concentrations were determined by the microsyringe method of Ko, Chase & Kunimoto (1973). Two drops (1 µl each) of the diluted zoospore suspension were placed on a glass slide and the zoospores were counted under a microscope with a 10 × objective.

RESULTS AND DISCUSSION

Aggregation of Phytophthora capsici zoospores

Zoospores of *Phytophthora capsici* aggregated within 2 min at 16 °C in a way similar to zoospores of *P. palmivora*. First to appear in the suspension were thread-like zoospore masses which soon segregated into umbrella-shaped aggregates, each with a white centre. When the concentrations used were 8.5 and 17.0×10^5 zoospores/ml, the concentrations of aggregate centre and periphery were 32 to 38×10^5 and 12 to 17×10^5 , respectively. The spore concentrations in the centres and peripheries of the aggregates were, respectively, 17 to 70 times and 7 to 24 times higher than that in the non-aggregated area. After aggregation the spore concentration of *P. capsici* in the non-aggregated area ranged from 0.5 to 2.0×10^5 /ml, while that of *P. palmivora* ranged from 5 to 8×10^5 /ml (Ko & Chase, 1973), thus indicating

Table 1. Concentration of zoospores of *Phytophthora capsici* and *P. palmivora* in the same aggregate in a mixed suspension

Aggregate no.	$10^{-5} \times \text{no. zoospores/ml}$					
	Aggregate centre		Aggregate periphery		Non-aggregated area	
	<i>P. capsici</i>	<i>P. palmivora</i>	<i>P. capsici</i>	<i>P. palmivora</i>	<i>P. capsici</i>	<i>P. palmivora</i>
1*	24	29	4.2	3.3	0.3	1.2
2†	24	15	5.3	2.4	0.2	1.2

* The original concentrations of *P. capsici* and *P. palmivora* were 3.0×10^5 and 3.5×10^5 zoospores/ml respectively.

† The original concentrations of both fungi were 2.5×10^5 zoospores/ml.

that *P. capsici* is a stronger aggregate former than *P. palmivora*. Zoospores of two other isolates of *P. capsici* also aggregated under the same conditions.

Zoospore aggregation was compared at 8, 12, 16, 20, 24 and 28 °C. Distinct and large numbers of aggregates were formed at 16 and 20 °C. Aggregates were less distinct and fewer at 12, 24 and 28 °C. Only a few faint aggregates were observed at 8 °C. Apparently, *Phytophthora capsici* is not as sensitive to temperature in aggregate formation as *P. palmivora* (Ko & Chase, 1973). The former also has a wider range of optimum temperature for aggregation than the latter.

Zoospore aggregation was also studied at various spore concentrations. The lowest concentration for zoospore aggregation of *Phytophthora capsici* was between 1 and 2×10^5 /ml, while that of *P. palmivora* was 5 to 10×10^5 /ml (Ko & Chase, 1973). This again indicates that *P. capsici* is a stronger aggregate former than *P. palmivora*. Like *P. palmivora* zoospores, aggregate formation of *P. capsici* zoospores also depended on depth of spore suspension. In 10 ml of suspension (14×10^5 zoospores/ml), zoospores of *P. capsici* aggregated in a small Petri dish (60 × 15 mm) but not in a larger one (100 × 15 mm).

Interaction between *Phytophthora capsici* and *P. palmivora* zoospores

For studying the interaction between *Phytophthora capsici* and *P. palmivora* zoospores, zoospore suspensions of both fungi were adjusted to between 3 and 9×10^5 spores/ml. The treatments consisted of (i) 15 ml *P. capsici* zoospore suspension plus 15 ml distilled water, (ii) 15 ml *P. palmivora* zoospore suspension plus 15 ml distilled water, and (iii) 15 ml *P. capsici* zoospore suspension plus 15 ml *P. palmivora* zoospore suspension. No distinct aggregate was observed on the plates containing either fungus alone. However, in the plate containing zoospores of both species, 5 to 20 distinct aggregates were formed. When higher concentrations were used, more or larger aggregates were formed in plates containing both fungi than in those with either fungus alone. Results indicate that in the presence of both *P. capsici* and *P. palmivora* zoospores, aggregation depended on total zoospore concentration. This also suggests that zoospore aggregation of these two fungi may have been controlled by the same mechanism.

To determine if zoospores of both *Phytophthora capsici* and *P. palmivora* were present in the same aggregate in the mixed suspension, 2 µl of spore suspension were obtained from aggregate centre and aggregate periphery, and 4 µl from the non-aggregated area. The spore suspension was diluted to 10 to 20 zoospores/ml. Diluted spore suspension (0.5 ml) was evenly distributed on a plate of selective medium containing 2% (w/v) agar, 20% (v/v) centrifuged V-8 juice, nystatin (50 mg/l), vancomycin (100 mg/l) and pentachloronitrobenzene

(10 mg/l) (McCain, Holtzmann & Trujillo, 1967). On the second day of incubation at 24°C under light, *P. capsici* formed distinct colonies with macroscopic aerial mycelia while *P. palmivora* formed faint colonies without macroscopic aerial mycelia. On the third day, colonies of *P. palmivora*, but not of *P. capsici*, contained sporangia. Therefore, the number of *P. capsici* and *P. palmivora* colonies on each plate was determined macroscopically on the second day of incubation and microscopically on the third day. In the mixed suspension, centre and periphery of a single aggregate contained zoospores of both *P. capsici* and *P. palmivora* (Table 1). The zoospore concentrations of both fungi in the aggregate centre were about 6 to 10 times higher than the concentrations before aggregation. This further suggests that a single mechanism is controlling zoospore aggregation of these two species of fungi.

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