Short Note  
Hyphal lysis of *Pythium* spp. in a model soil system

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Quantitative studies in *Pythium* ecology in soil are occasionally hindered by the presence of native *Pythium* spp. Recently, Tojo and Ichitan6,8 have identified the threshold of pasteurization required to eliminate *Pythium* spp. in field soil without injuring drastic changes in the physical and chemical properties of soil. To evaluate this pasteurized soil as a model system for ecological studies of *Pythium* spp., propagule behavior of *Pythium* spp. was compared between treated and untreated soils using the nylon fabric technique. Previous studies indicated that the germination patterns of oospores in treated soil in two pathogenic *Pythium* spp. corresponded to those in untreated, natural soil7. In this paper hyphal lysis in *Pythium* spp. was compared between treated and untreated soils.

Soil was collected from a vegetable field, the University Farm, Osaka Prefecture University (Sakai), in spring (April 27), summer (July 30), fall (October 5) in 1993 and winter (February 2) in 1994. Samples were taken from the top 10 cm of the surface soil in 10-15 spots in the field. Soil samples weighing at least 5 kg in total were thoroughly mixed, passed through a 4-mm mesh sieve and stored in a plastic bag at room temperature until use. One kilogram of the sample was treated with water vapor saturated air at 58°C for 20 min as described previously8. The soil consisted of loam with a water content of 10-22% and pH 5.4-5.9 (H2O). At least 10 species of *Pythium* were indigenous to this soil8.

* P. aphanidermatum (UOP 390) and *P. spinosum* (UOP 391), isolated from the above soil at the University Farm in 1992 by the baiting method with spinach seedlings were used for the experiments. They were maintained as described by Ichitan and Kang9.

Hyphal lysis, in which the cellulolytic activity of soil microorganisms was considered to be involved10, was compared to evaluate the suitability of the model soil. The direct assay method of propagules behavior with nylon fabric was adopted to observe the hyphal lysis of *Pythium* spp. in soil9. Mycelia free from oospores were pregrown on Schmittenner's solid medium9 without cholesterol for 2-6 days at 20, 28, 15 and 10°C in the spring, summer, fall and winter experiments, respectively. A fragment of mycelia was introduced into an Erlenmeyer flask (50 ml) containing 7 ml of the same liquid medium and incubated at 20, 28, 15 and 10°C for 48, 24, 72 and 72 hr, in each season, respectively. Mycelia were homogenized in a Waring blender with liquid medium. Fifty µl of the homogenate was then sprinkled within a 7-mm diam. circle, drawn on the surface of a 4.0 cm² piece of nylon fabric (10 µm pore) and incubated in a Petri dish under the same conditions as in liquid culture. Mycelial mats developed with a uniform thickness within each circle after incubation. Fabric pieces containing mycelia were then washed ten times with sterile distilled water and wrapped within the fabric of the same size. The envelopes were buried in soil (25% water content) at a 5 mm depth in Petri dishes and incubated for up to 230 hr at 20, 28, 15 and 10°C in the spring, summer, fall and

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winter experiments, respectively. Total length of hyphae with an intact hyphal wall in ten microscopic fields (×400) was measured. The length was estimated based on the width of a nylon fiber (= 67 μm) (Fig. 1). Relative value of the length was determined by comparing hyphal length measured just before (left) and after (right) burying. All the incubation conditions were examined for both species except for P. aphanidermatum in winter because hyphal growth was not observed at 10°C.

The sequence of hyphal lysis is shown in Fig. 2. The lysis of P. spinosum was completed at 96, 48, 96 and 230 hr in soil in spring, summer, fall and winter, respectively. The lysis of P. aphanidermatum progressed more slowly than that of P. spinosum and was completed at 192, 48 and 192 hr in soil in spring, summer and fall, respectively. These patterns were similar both in treated and untreated soils. The observations suggest that the effect of treatment with water vapor saturated air to eliminate indigenous Pythium spp. may be negligible on the populations of soil microorganisms which were considered to be involved in the hyphal lysis of Pythium spp. The results reported here and a previous study7 suggest that the behavior of Pythium spp. in this pasteurized soil simulates that in naturally infested soil, in part at least for hyphal lysis and oospore germination. The method using pasteurized soil enables to study the behavior of Pythium propagules in soil.

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References

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![Graph showing the relative length of intact hyphae of *Pythium aphanidermatum* and *P. spinosum* in pasteurized (---) and untreated (-----) field soils from spring, 1993, to winter, 1994. Mycelia were buried for up to 230 hr at 20, 28, 15 and 10°C in spring, summer, fall and winter, respectively, and the total length of the hyphae was measured in ten microscopic fields (×400). Relative length was determined by comparing the length of the hyphae just before burying. Standard error in two separate experiments in duplicate is indicated.](image-url)

Fig. 2
to autolysis. *Phytopathology*, 56, 595-602


