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	作成者: ICHITANI, Takio, KINOSHITA, Tomio
	メールアドレス:
	所属:
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Materials for Pythium Flora of Japan (IV) Pythium graminicola from rhizosphere soil of zoysia green*

Takio Ichitani and Tomio Kinoshita**

Laboratory of Plant Pathology, College of Agriculture and
**Hyogo Women's Junior College
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Abstract

Several isolates of *Pythium graminicola* Subramaniam were obtained from rhizosphere soil of a zoysia green. One of the isolates is described and illustrated.

During a study on Pythium diseases of manilagrass (Zoysia matrella Merr.), several isolates of Pythium graminicola Subramaniam were obtained mainly from rhizosphere soil of a golf green having typical symptoms C¹⁾. The fungus was maintained as described previously²⁾.

Pythium graminicola was originally described from Triticum aestivum L. in India³, occurring very commonly there since. In Japan, also it is encountered extensively^{1,4}.

This paper deals with the description and illustration of the species, following methods described previously⁵⁾.

Pythium graminicola (Plates I-VI)

A detailed description of this fungus is as follows.

Colonies on Bacto-CMA forming very little aerial mycelium and having an arachnoid habit of growth, and on Bacto-PDA producing a rosette (Plate I). Main hyphae up to 8 μ m wide. Appressoria subspherical. Sporangia intercalary, less frequently terminal; inflated with irregular complexes, rarely filamentous. Zoospores formed at 23–25°C; evacuation tubes up to 250 μ m long on CMA; encysted zoospores 10–13 μ m diam.. Oogonia globose, mostly intercalary, sometimes terminal, 20–30 μ m, average 25 μ m diam., smooth. Antheridia 2–6 (–8) per oogonium, originating at various distances from the oogonium, clavate, crook-necked, 1–2 borne on each antheridial stalk, predominantly diclinous, occasionally monoclinous; persistent after fertilization. Oospore plerotic, rarely aplerotic, 17–25 μ m, average 21 μ m diam.; wall up to 1.5 μ m thick; percentage abortion of oospores on CMA*** fairly high.

Cardinal temperatures: minimum $4-10^{\circ}$ C, optimum 34° C, maximum $37-40^{\circ}$ C. Daily mycelial growth on Bacto-CMA at 25° C 30 mm.

Description: based on UOP 380 (=IFO 32330, MAFF 02-35183)

Isolation: UOP 380 (=IFO 32330, MAFF 02-35183), on Bacto-CMA selectively¹⁾

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^{***20} g corn-meal (sold in the market as a chicken feed containing fish meal), 15 g agar, 1,000 ml distilled water.

from rhizosphere soil of zoysia green, Mure-cho, Kita-gun, Kagawa Prefecture, May 7, 1989, by T. Ichitani.

Host range: This isolate was parasitic on aerial parts of creeping bentgrass (Agrostis palustris) seedlings. It was pathogenic on Gibberellin A_3 -treated adult plants of Zoysia matrella, but not on untreated ones⁴). Seedlings of Z. japonica were infected⁴), pathogenicity occurring under low temperature conditions, even at 5° C⁶).

A length of plasmolyzed protoplasm may extend inside a hypha (Plates II-1, III-37). This mycelium-like filament emerges through the hyphal cell wall (Plates II-2, III-38 (arrow), -39 (shorter arrow)). The plasmolyzed protoplasm itself also emerges through the hyphal cell wall (Plates II-3, III-39 (longer arrow), -40). One hypha can penetrate another where they contact (Plates II-4, III-41, -42 (arrows)). A penetrating hypha extends longitudinally inside the host hypha and then emerges. These phenomena can be seen in young (2-3 day) cultures.

Sex organs were difficult to observe and measure when wheat-germ oil (Japan Impex, Tokyo) was added to CMA because their formation, and that of the hyphae, was unusually uneven (Plate IV-45~47). Appressoria-like bodies were also observed under these conditions (Plate IV-48).

Zoospores were easily formed at 25°C. Inflated zoosporangia (Plates II-6, IV-52 (arrow), V-59~62 (arrows)), although filamentous ones (Plates II-7, IV-53 (arrow)) were occasionally observed. Differentiation of zoospores, however, ceased on incubation at 32-35°C (Plate V-59 (arrow)). Zoospores were produced in 90-180 minutes after floating infected grass blades on water and were observed within 4 hr after placing agar discs of 1 month-old CMA culture in water. No zoospores were produced from newly formed hyphal swellings⁷⁾ (Plates II-30,31, VI-81~84).

Abortion of oospores on CMA was about 50%. Aplerotic oospores were sometimes found in young cultures, but numbers of plerotic ones increased with age of the culture. Regardless of wheat-germ oil, sex organ production decreased with subculturings.

Drechsler⁸⁾ and Middleton⁹⁾ examined antheridia as distinguishing factors in the separation of *P.graminicola* from *P.arrhenomanes*. *Pythium graminicola* differs from *P. arrhenomanes* and *P.aristosporum* by having predominantly monoclinous and fewer antheridia, and smaller oogonia, from *P.torulosum* and *P.vanterpoolii* by larger oogonia and more antheridia per oogonium, and from *P.myriotylum* by plerotic oospores and a different incubation temperature for mycelial growth.

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Explanation of Plates

Plate I Growth habit of *P.graminicola* UOP 380 on Bacto-CMA (left) and Bacto-PDA (right) incubated at 25°C for 2 days (upper), 4 days (intermediate) and 15 days (lower). Plates II-VI Morphology of *P.graminicola* UOP 380.

1-4. 32-44: Mycelia. 45-47: Pieces of mycelial thread. 48: Appressoria-like bodies. 5, 49: Sporangium. 50, 51: Vesicle. 6-8, 52-54: Vesicle, discharge tube and zoosporangium. 9, 10, 55, 56: Discharge tube. 11, 57: Encysted zoospores. 12, 13, 58: Germinating zoospores. 14-16, 59-62: Empty zoosporangium. 17-20, 63-67: Oogonia and monoclinous antheridia. 21, 68: Oogonium and diclinous antheridia. 22, 23, 69, 70: Plerotic oospore. 24-27, 71-74: Oospores with degenerated oospheres. 28, 29, 75-77: Malformed oospores (or more than 2 oospores within 1 oogonium). 78-80: Propagules in leaf tissues of bentgrass (78: hypha extending through cell wall, 79: hyphal swelling, 80: oospore). 30, 31, 81-84: Hyphal swellings.

Bars (10 μ m) on figs. 3, 31, 32, 35, 37 and 63 are applicable to figs. 3–16; 1, 2, 17–31; 32–34, 36, 45–47; 35, 41–44, 49, 52, 55, 57, 58, 60, 78, 81–83; 37–40, 48, 50, 51, 53, 54, 56, 59, 61, 62, 78–80, 84; and 63–77; respectively.

Plate I

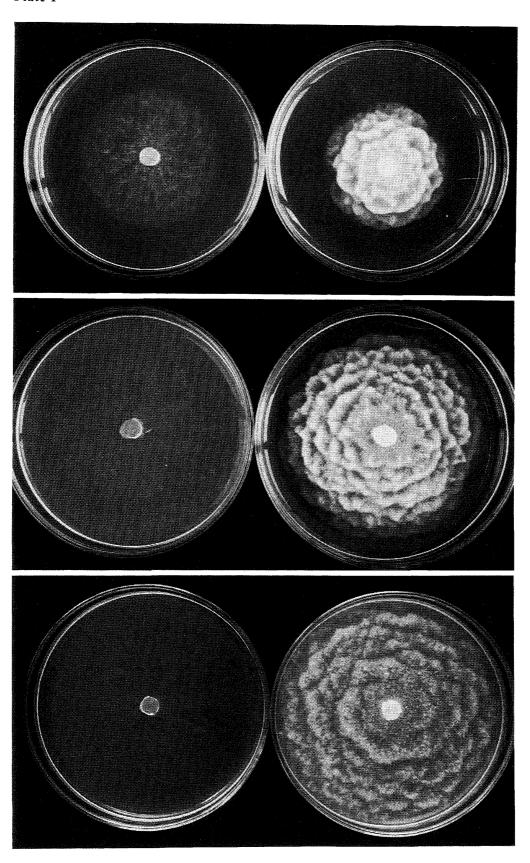


Plate II

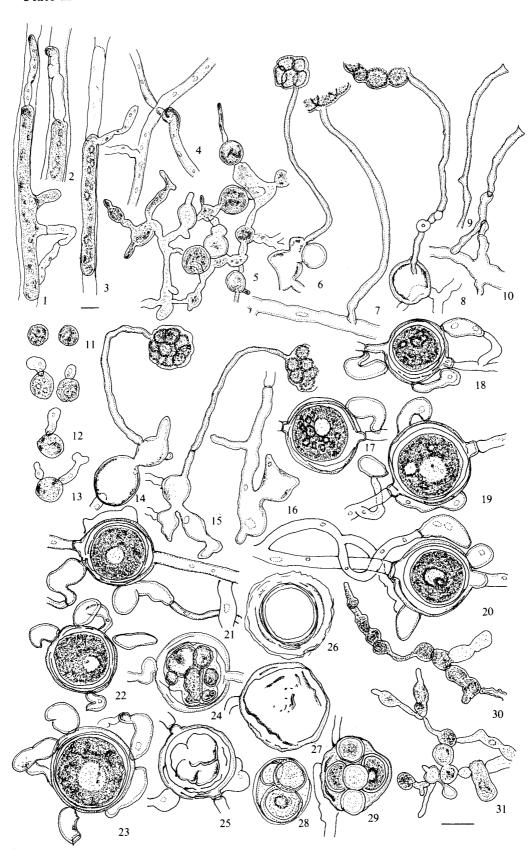
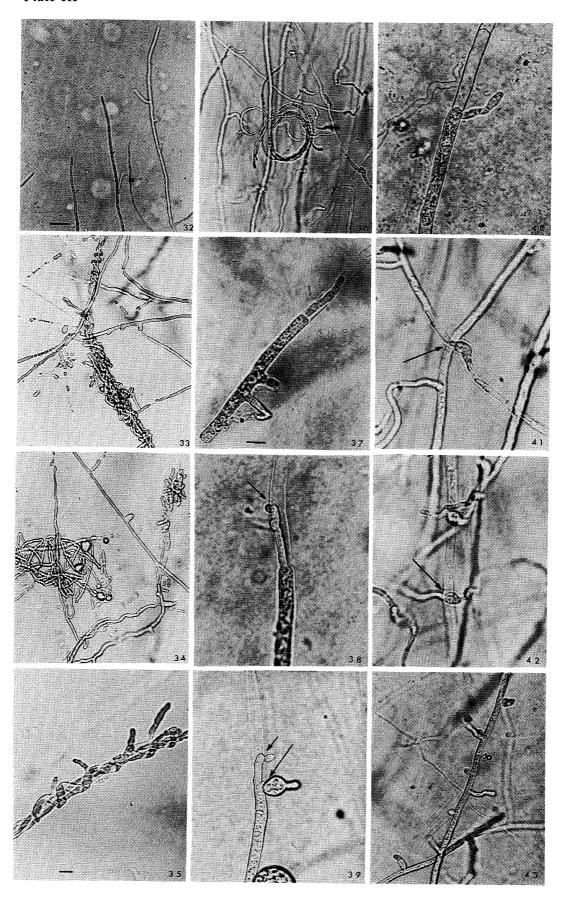


Plate III



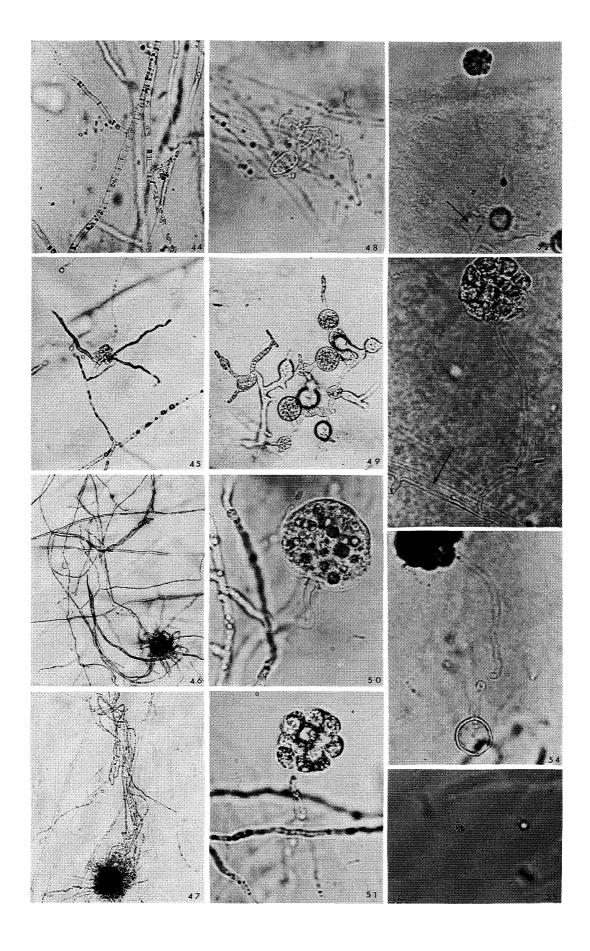


Plate V

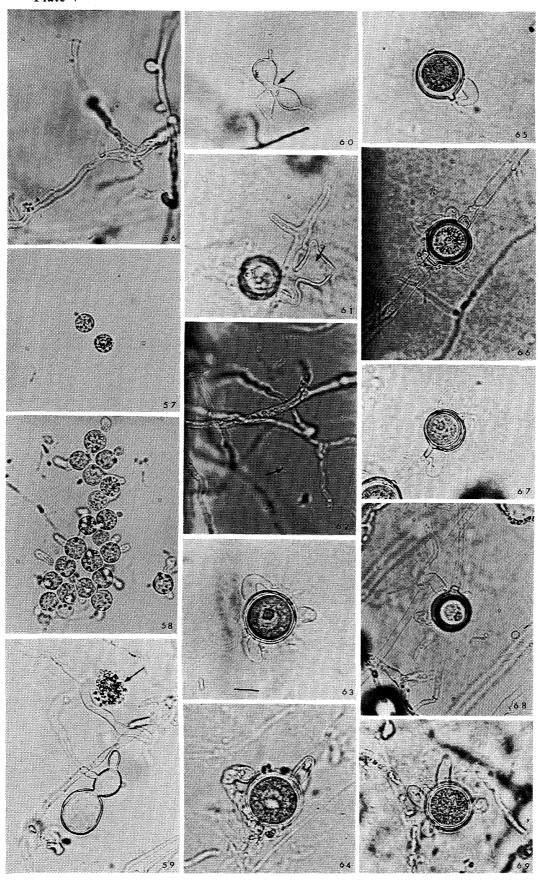


Plate VI

