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Note

Virulence of *Pythium* spp. isolated from pond water

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Pre-emergence damping-off tests indicate that *Pythium* spp. from pond water can be divided into two categories: avirulent *Pythium* (*P. diclinum, P. marsipium, P. middletonii, P. monospermum, P. pleroticum, P. undulatum*) and weakly virulent *Pythium* (*P. catenulatum, P. coloratum, P. dissotocum, P. papillatum, Pythium* 'group F', *Pythium* 'group HS', *Pythium* 'group P', *Pythium* 'group T'). Post-emergence damping-off tests indicate that the pythia tested can also be divided into three categories: avirulent *Pythium* (*P. catenulatum, P. dissotocum, P. marsipium, P. monospermum, P, papillatum, P. pleroticum, P. undulatum, Pythium* 'group F', *Pythium* 'group T'), weakly virulent *Pythium* (*P. coloratum, P. diclinum, P. middletonii, Pythium* 'group P' and moderately virulent *Pythium* (*Pythium* 'group HS').

Key Words——pond water; *Pythium* spp.; virulence.

Diseases caused by *Pythium* spp. are commonly assumed to be the result of soil-borne inoculum, but water-borne inocula may contribute to the disease outbreaks. Water from irrigation ponds is thus one of these sources. Avirulent *P. monospermum* Pringsh. and virulent *P. aphanidermatum* (Edson) Fitzp. have been isolated from pond water in Japan (Takahashi, 1952). Lumsden and Haasis (1964) trapped *P. aphanidermatum, P. carolinianum* Matthews and *P. polytylum* Drechsler with stems and roots of chrysanthemum in shallow vessels of irrigation water in the greenhouse and all these pythia were virulent. *Pythium myriotylum* Drechsler can be carried in pond water for irrigation and this fungus caused root and stem rots of tomato seedlings (Gill, 1970). *Pythium* spp. occurring in irrigation water were capable of causing disease of some commercially grown glasshouse plants under experimental conditions (Pittis and Colhoun, 1984). Recently, Elnaghy et al. (1991) reported that *P. debaryanum* Hesse, *P. graminicola* Subramaniam and *P. ultimum* Trow were found in his study of aquatic zoosporic fungi in freshwater streams for irrigation in upper Egypt.

During a survey of *Pythium* spp. in pond water in Osaka (Abdelzaher et al., 1993), 10 identified species and 4 unidentified species were obtained and used for inoculation experiments using cucumber (*Cucumis sativus* L.) seedlings. Part of the work has been reported elsewhere (Awad et al., 1993).

Isolation and identification *Pythium* spp. were isolated from three ponds (Nakatsu, Tatsumi, Komoda) in the same water system in Sakai, Osaka, Japan, using baits which had been incubated in pond water kept at 25°C and under net house conditions for 5 days (Table 1), and the isolation continued for 18 months. Natural (*Paspalum* leaf blades, internal mandarin rind) and artificial (filter paper disks) baits were used to isolate a wide variety of *Pythium* spp. including cellulolytic and pectinolytic ones. Colonized baits were washed thoroughly in sterile distilled water and excessive water was removed between sterile filter papers. Baits were then plated on VP3 medium selective for *Pythium* (Ali-Shtayeh et al., 1986).

Pythium species free of bacterial contamination were obtained by cutting a small block of agar medium from the distal end of a colony grown in VP3 medium and re-inoculating the block on 2.5% water agar medium in a Petri dish to obtain a colony of about 1 cm diam. Then the whole agar medium in the Petri dish was replaced upside-down with a flamed forceps in the same Petri dish and incubated until the colony reached before the dish wall, during this procedure the mycelia penetrate the agar medium without the contaminating bacteria and reach the top of the agar medium. A thin piece of agar containing a single hyphal tip of the desired fungus was taken from the suface of the margin of the colony on water agar medium under the microscope and transferred to CMA slants for maintaining the fungus and CMA plates supplemented with 500 μg/ml wheat germ oil to check the sexual structure formation. Grass blades were placed in contact with the colonial margin on the same water agar dish for 24 h and then transferred to a sterilized Petri dish (7 cm diam) with 10 ml of sterilized deionized water and incubated at different temperatures to check zoospore (Waterhouse, 1967) and sexual reproductivity. Identification was done with the keys of Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981) and Dick (1990)

and with the reference to the original descriptions. The fungus was maintained as described previously (Ichitani and Kang, 1988).

Virulence test For preparation of inocula, the method of Tojo et al. (1993) was followed in which two inoculum concentrations (2.5 and 75% in pre-emergence damping-off tests and 5 and 25% in post-emergence damping-off tests) were employed to ensure the pathogenicity. Bentgrass seeds (1 and 5 g) were moistened by adding deionized water (5 and 20 ml) each in 300-ml Erlenmeyer flask. After autoclaving at 121°C for 20 min, each flask was inoculated with three disks (7 mm diam) of agar with growing margins of *Pythium* spp. cultured on water agar. The inoculated seeds were held at 25°C for 10 days.

In pre-emergence damping-off tests the inoculum concentration of 2.5% was obtained by mixing thoroughly the 1 g of colonized bentgrass seeds in the Erlenmeyer flask with 50 g of oven-dried (70-80°C for 2 days) clay loam soil using a sterilized mortar and pestle. Two point five grams of this mixture was added to 97.5 g of clay loam soil which had been sterilized by autoclaving at 121°C for 60 min (pH 7.06 after autoclaving) and kept in a plastic bag for 2-3 weeks at room temperature with 25% water content prior to use (CL soil). Seventy-five percent inoculum concentration was also employed, by mixing thoroughly the 5 g of colonized bentgrass seeds in the Erlenmeyer flask with 50 g of oven-dried (70-80°C for 2 days) clay loam soil using a sterilized mortar and pestle, then adding 15 g of this mixture to 85 g of CL soil.

In the post-emergence damping-off tests the inoculum concentration of 5% was obtained by mixing thoroughly the 1 g of colonized bentgrass seeds in the Erlenmeyer flask with 50 g of oven-dried (70-80°C for 2 days) clay loam soil using a sterilized mortar and pestle. Five grams of the mixture was spread around the seedlings in a plastic pot containing 95 g of CL soil. Twenty-five percent concentration was also tested by mixing thoroughly the 5 g of colonized bentgrass seeds in the Erlenmyer flask with 50 g of oven-dried (70-80°C for 2 days) soil using a sterilized mortar and pestle, then spreading 5 g of the mixture around the seedlings in a plastic pot containing 95 g of CL soil.

Soil (100 g) with inocula of each fungal treatment was put into five replicate pots (100 ml capacity and 6 cm diam). For each fungal treatment, eight cucumber seeds which proved to be highly susceptible to damping-off disease by *Pythium* species (Takahashi et al., 1970) were planted in each pot for pre-emergence damping-off test. For post-emergence damping-off test the inocula were added after the emergence. Since inocula are added after emergence in the post-emergence damping-off test, more than 15 g of inoculum soil mixture (25% inoculum density) could not be obtained. On the other hand, high inoculum concentration (75%) could be obtained to ensure the pathogenicity of the species tested in the pre-emergence damping-off test.

The experiments were carried out in a growth cabinet (Nihon Ika Inc., Osaka) at 20°C and 5,000 lux under humid conditions. Healthy and diseased seedlings were counted at regular intervals until the development of 2 true leaves in non-inoculated control. Pre-emergence damping-off was determined as the difference in emergence between healthy control soil and diseased soil. Post-emergence damping-off was determined from the number of diseased plants as a percentage of those

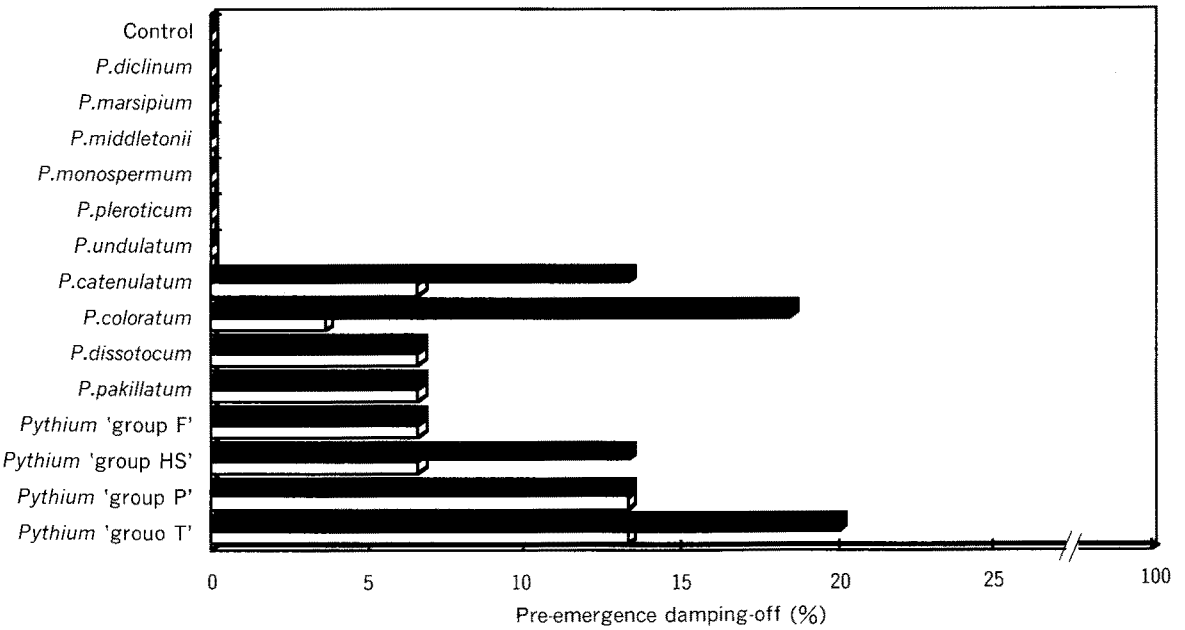

Fig. 1. Pre-emergence damping-off of cucumber seedlings grown in clay loam soil infested with different inoculum densities (□ 2.5%, ■ 75%) of *Pythium* spp.

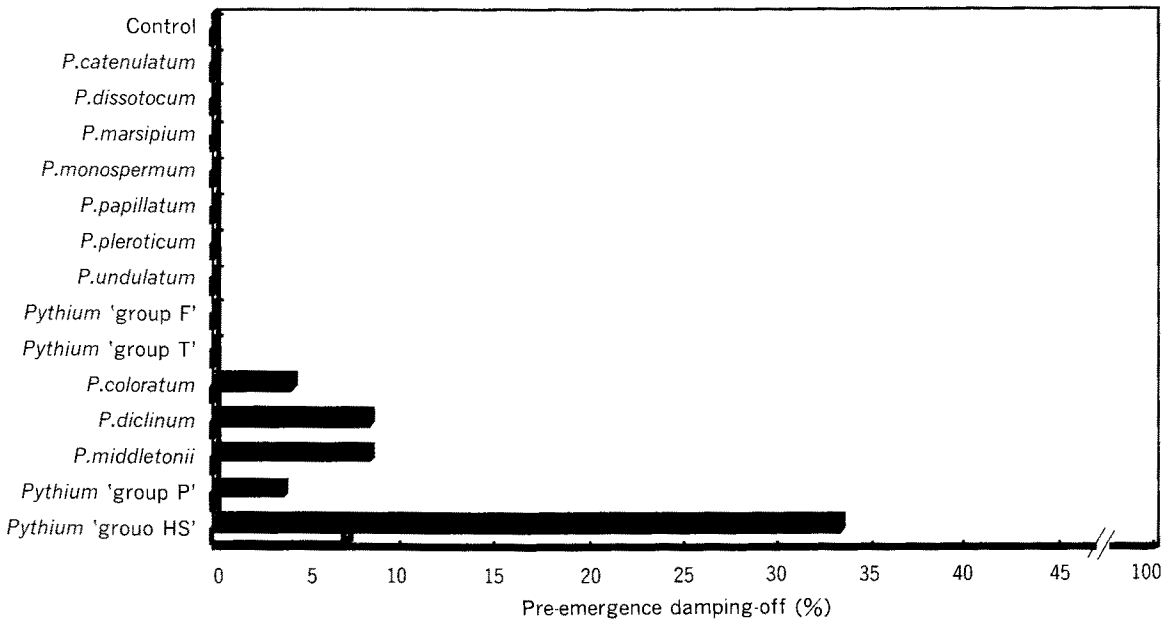

Fig. 2. Post-emergence damping-off of cucumber seedlings grown in clay loam soil infested with different inoculum densities (□ 5%, ■ 25%) of *Pythium* spp.

emerged. The experiments were repeated twice.

Pre-emergence damping-off tests indicate that the 14 species employed in this experiment can be divided into two categories: avirulent *Pythium* (*P. diclinum* Tokunaga, *P. marsipium* Drechsler, *P. middletonii* Sparrow, *P. monospermum* Pringsh., *P. pleroticum* T. Ito, *P. undulatum* H. E. Pertersen) with 0% damping-off and weakly virulent *Pythium* (*P. catenulatum* Matthews, *P. coloratum* Vaartaja, *P. dissotocum* Drechsler, *P. papillatum* Matthews, *Pythium* 'group F', *Pythium* 'group HS', *Pythium* 'group P', *Pythium* 'group T') with less than 20% damping-off (Fig. 1).

Post-emergence damping-off tests indicate that the pythia tested can also be divided into three categories: avirulent *Pythium* (*P. catenulatum, P. dissotocum, P. marsipium, P. monospermum, P. papillatum, P. pleroticum, P. undulatum, Pythium* 'group F', *Pythium* 'group T') with 0% damping-off, weakly virulent *Pythium* (*P. coloratum, P. diclinum, P. middletonii, Pythium* 'group P') was less than 10% damping-off and moderately virulent *Pythium* (*Pythium* 'group HS') with around 30% damping-off at 25% inoculum density (Fig. 2).

This study established that a number of pythiaceous fungi occurred in pond water over a period of 18 months,

Table 1. *Pythium* spp. used, and physical and chemical data of pond water.

Fungi (Isolate no.)	Date of isolation	Place (incubation temp., °C) and bait used for isolation	Pond [temp., pH, transparency, salinity] (°C) (ml) (ms/ml)
P. catenulatum (94)	Nov. '92	Incubator (25), mandarin	Komoda [14.0, 7.1, 17.0, 0.31]
P. coloratum (187)	Jan. '93	Net house (1-8), *Paspalum*	Nakatsu [5.5, 8.7, 24.5, 0.35]
P. diclinum (114)	Dec. '92	Incubator (25), mandarin	Nakatsu [10.0, 8.1, 32.0, 0.35]
P. dissotocum (250)	Mar. '93	Net house (3-13), *Paspalum*	Nakatsu [9.0, 7.9, 17.5, 0.34]
P. marsipium (29)	Oct. '92	Net house (14-26), mandarin	Komada [21.0, 6.3, N.D., 0.32]
P. middletonii (14)	Oct. '92	Incubator (25), *Paspalum*	Komada [21.0, 6.3, N.D., 0.32]
P. monospermum (237)	Mar. '93	Incubator (25), *Paspalum*	Nakatsu [7.0, 7.9, 26.5, 0.36]
P. papillatum (85)	Nov. '92	Net house (7-18), filter paper	Komada [14.0, 7.1, 17.0, 0.31]
P. pleroticum (125)	Dec. '92	Net house (3-12), filter paper	Tatsumi [N.D., N.D., N.D., N.D.]
P. undulatum (306)	May '93	Net house (10-28), *Paspalum*	Komada [26.5, 10.5, 25.5, 0.24]
Pythium 'group F' (120)	Dec. '92	Net house (3-12), *Paspalum*	Tatsumi [N.D., N.D., N.D., N.D.]
Pythium 'group HS' (163)	Feb. '93	Incubator (25), *Paspalum*	Nakatsu [5.5, 8.7, 24.5, 0.35]
Pythium 'group P' (271)	Mar. '93	Net house (3-13), mandarin	Nakatsu [9.0, 7.9, 17.5, 0.34]
Pythium 'group T' (75)	Nov. '92	Net house (7-18), mandarin	Komada [14.0, 7.1, 17.0, 0.31]

N.D.: Not determined.

revealing the widespread occurrence of pythiaceous fungi in water. Our findings indiate that the aquatic *Pythium* species tested seem to behave as saprophytic fungi, which were adapted to utilize dead materials in the form of leaf litter or plant and animal debris. However, the risks of pathogenicity is also present. Strongly virulent *P. spinosum* and *P. ultimum* were isolated from the bottom soil of Tatsumi pond while there was no water (Awad et al., 1993), but they were not isolated from the pond water itself during our two years of study (Awad et al., 1993). Strong water currents, however, may carry infected soil particles and the pond water could thus provide a source of *Pythium* damping-off organisms. The pythia tested, which were isolated from the pond water, were weakly or even non-pathogenic, especially at lower inoculum density. The high inoculum density may, however, provide a suitable condition for disease occurrence.

We have found that the ponds surveyed lay below such pollution sources as sanitation from the adjacent residential area, rubbish materials and factory by-products (Abdelzaher et al., unpublished data; see also Table 1). Pollution at a limited level plays an important role in optimizing the factors for sporulation and establishement of pythiaceous fungi, in providing the organic matter necessary for muliplication (Harvey, 1952; Manoharachary and Rao, 1978). Leaf litter, paper, cellulolytic and related polysaccharides provide a good atmosphere for establishement of *Pythium* spp. in aquatic ecosystems. Water currents may carry the colonized rubbish materials toward the crops irrigated and act as a disease source. Under unfavorable conditions *Pythium* species survive in the form of oospores or asexual propagules, which may accumulate in suitable rubbish materials (Webster, 1991). These colonized materials represent a very dangerous source of infection to the plants irrigated by such water.

Literature cited

Abdelzaher, H. M. A., Ichitani, T. and Elnaghy, M. A. 1993. Effect of temperature, hydrogen-ion concentration and osmotic potential on oospore germination of five aquatic *Pythium* spp. Trans. Nishi-Nippon Div. (Mycol. Soc. Japan). p. 3.

Al-Shtayeh, M. S., Lim-Ho, C. L. and Dick, M. W. 1986. An improved method and medium for quantitative estimates of population of *Pythium* spp. from soil. Trans. Br. Mycol. Soc. **86**: 39-47.

Awad, H. M., Ichitani, T. and Elnaghy, M. A. 1993. Pythiaceous fungi from pond water in Osaka and their virulence to cucumber (Suyo) seedlings. Ann. Phytopath. Soc. Japan **59**: 749-750.

Dick, M. W. 1990. "Keys to *Pythium*," College of Estate Management, Reading. 64 p.

Elnaghy, M. A., Hassan, S. K. M., Fadl-Allah, E. M. and Abdelzaher, H. M. A. 1991. Seasonal fluctuations of some aquatic zoosporic fungi in water streams in upper Egypt. Sci. Bull. Minia Univ. **4**: 119-130.

Gill, D. L. 1970. Pathogenic *Pythium* from irrigation ponds. Plant Dis. Reptr. **54**: 1077-1079.

Harvey, J. V. 1952. Relationship of aquatic fungi to water pollution. Sewage and Industrial Wastes **24**: 1159-1164.

Ichitani, T. and Kang, H. T. 1988. Materials for *Pythium* flora of Japan (I) Plant-pathogenic *Pythium irregulare*. Bull. Univ. Osaka Pref. Ser. B **40**: 19-26.

Lumsden, R. D. and Haasis, F. A. 1964. *Pythium* root and stem diseases of Chrysanthemum in North Carolina. North Carolina Agr. Exp. Sta. Tech. Bull. **158**: 1-27.

Manoharachary, C. and Rao, P. R. 1978. Distribution and seasonal variation of some *Pythium* sp., in some soils, pond water and mud soils of Hyderabad district. Mysore J. Agric. Sci. **12**: 280-284.

Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of the genus *Pythium*. Mem. Torrey Bot. Club **20**: 1-171.

Pittis, J. E. and Colhoun, J. 1984. Isolation and identification of pythiaceous fungi from irrigation water and their pathogenicity to Antirrhinum, Tomato and *Chamaecyparis lawsoniana*. Phytopath. Z. **110**: 301-318.

Plaats-Niterinnk, A. J. van der 1981. Monograph of the genus *Pythium*. Stud. Mycol. **21**: 1-244.

Takahashi, M. 1952. A comparative study of the pathogenicity of two species of aquatic *Pythium, P. aphanidermatum* and *P. monospermum*. Ann. Phytopath. Soc. Japan **16**: 19-22 (in Japanese).

Takahashi, M., Ichitani, T., Akaji, K. and Kawase, Y. 1970. Ecologic and taxonomic studies on *Pythium* as pathogenic soil fungi. VIII. Differences in pathogenicity of several species of *Pythium*. Bull. Univ. Osaka Pref. Ser. B **22**: 87-93.

Tojo, M., Fujita, Y., Awad, H. M. and Ichitani, T. 1993. Preparation of *Pythium* inocula using bentgrass seeds for glasshouse studies. Proc. Kansai Pl. Prot. Soc. **35**: 1-5 (in Japanese).

Waterhouse, G. M. 1967. Key to *Pythium* Pringsheim. Mycol. Pap. **109**: 1-15.

Webster, J. 1991. "Introduction to Fungi, 2nd ed.," Cambridge University Press, Cambridge. 169p.