

Pythium irregulareおよびP. aphanidermatumによるカーネーション根腐病(新称)

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著者名	君島,悦夫 小林,慶範 西尾,健
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Root Rot of Carnation Caused by *Pythium irregulare* and *P. aphanidermatum*

Etsuo KIMISHIMA*, Yoshinori KOBAYASHI* and Takeshi NISHIO**

Abstract

Root rots of carnations were found in Tokyo Metropolis and Chiba Prefecture, Japan, in March of 1984 and in July of 1989, respectively. The symptoms were characterized by root rot and discoloration of stems and leaves. The pathogens obtained from Tokyo and Chiba were identified as *Pythium irregulare* Buisman and *P. aphanidermatum* (Edson) Fitzp., respectively, on the basis of their morphological and physiological characteristics. This is the first report of the disease occurrence of carnations caused by *Pythium* spp. in Japan.

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Key words: carnation, root rot, *Pythium irregulare*, *Pythium aphanidermatum*.

INTRODUCTION

In March of 1984 and in July of 1989, root rots were found on carnations, *Dianthus caryophyllus* L., in Tokyo Metropolis and Chiba Pref., Japan, respectively. The symptoms appeared on roots, leaves and stems. *Pythium* spp. were isolated from affected roots and stems as causal fungi. Root rot of carnation caused by *Pythium ultimum* Trow and *P. vexans* de Bary were reported in USA¹⁾. The causal fungi described here were different from those reported in USA. This paper describes the results of etiological studies including the symptoms, serological tests for detection of the causal organisms and their identification. A brief report of this work has been published elsewhere⁷⁾.

MATERIALS AND METHODS

Serological tests. Four kinds of antisera^{6,8)} prepared for *Phytophthora erythroseptica* Pethybridge, *Ph. capsici* Leonian, *Pythium aphanidermatum* (Edson) Fitzp. and *P. ultimum* were tested to detect the pathogen. Two serological techniques were used and they were enzyme-linked immunosorbent assay (ELISA) described by Clark and Adams¹⁾ and dot immunobinding assay (DIBA) by Hibi and Saito²⁾. Antigen samples for ELISA and DIBA were prepared by the following method. Carnation extracts were obtained by grinding and precipitating 0.5 g of the leaves and/or stems of healthy or naturally infected carnation plants in 10-20 ml of phosphate-buffered saline (PBS) -0.05% Tween 20. Their supernatants were used as antigen samples.

Plates for ELISA tests were coated with γ -globulin diluted to 2.5 μ g/ml in carbonate buffer. Conjugate was used at 1/400 dilution. The absorbance was measured with photometer at 405 nm. Antigen samples for DIBA were applied onto the nitrocellulose membrane (NCM) (Millipore) and dried on filter paper. The NCM was saturated by incubation with conjugate, diluted

* Yokohama Plant Protection Station, Naka-ku, Yokohama 231, Japan 横浜植物防疫所

** Plant Protection Division, MAFF, Kasumigaseki, Chiyoda-ku, Tokyo 100, Japan 農林水産省
植物防疫課

at 1/3,000, prepared by coupling γ -globulin with alkaline phosphatase (Sigma). After color development, the reaction was stopped and then the NCM was washed in distilled water and air-dried for visual observation.

Isolation and identification. For isolation, diseased host tissues were washed under running tap water for 1–2 min, air-dried and placed on water-agar (WA) in 9-cm petri dishes. After culturing at 25°C for 1–3 days, *Pythium* isolates grown out of host tissue on WA were obtained by single hyphal tipping method. Representative isolates, DC-2 and CC8901, were used for describing the morphology on WA and/or V-8 juice agar. These isolates were obtained from Tokyo and Chiba sample, respectively.

For identification, the autoclaved rice blades, 1–2 cm in length, were placed in contact with V-8 juice agar where *Pythium* was pre-cultured at 25°C, and incubated at room temperature (18–22°C) for 1–2 days. The infected blades were transferred into sterile distilled water and the morphology of the fungus was observed at room temperature, following Ichitani⁹). The fungi were identified following Waterhouse^{13,14}) and Plaats-Niterink⁹).

Temperature responses of the fungi were studied by determining the mycelial growth rates on V-8 juice agar incubated at various temperatures ranging from 5 to 40°C.

Inoculation test. The pathogenicity of four *Pythium* isolates from carnations listed in Table 2 was tested to seedlings of carnation (cv. Chabot) and dianthus (*Dianthus japonicus* Thunb., cv. Koubai and cv. Hakubai). The infested soil was prepared by mixing 400 ml of autoclaved soil with 10 ml of 3–5 days old agar cultures of each test fungus, usually on V-8 juice agar at 25°C. The seedlings were planted in infested soils and uninfested soils served as controls. The experiments were carried out in a glasshouse (at 15–30°C for *P. irregulare* and at 23–41°C for *P. aphanidermatum*), terminating 35 days after inoculation, and repeated at least twice.

RESULTS

Occurrence and symptoms

In March of 1984, root rots of carnation (cv. Ucon Sim) were found in a glasshouse for nursery stocks at Mizuho, Nishi-tama, Tokyo. The diseased plants were discolored in stems and leaves (Fig. 1-A).

In July of 1989, the similar diseases were found on carnation (cv. Barbara) at Sohse, Chiba Prefecture. The affected roots, stems and leaves were water-soaked, rotted and dark brown (Fig. 1-B). They became soft rotted and brown, and were covered with white cottony mycelia. Many typical aplerotic oospores were observed in leaf tissues.

Serological tests

Extracts of the diseased parts of the carnation cv. Barbara grown in Chiba Prefecture reacted with *Pythium* antibodies by ELISA and DIBA (Table 1). However, they were negative with *Phytophthora* antibody by ELISA or DIBA. The extracts from cv. Ucon Sim were also negative. Healthy plants used as a control gave no positive reaction.

Morphology of two species identified

Pythium irregulare Buisman (Fig. 2, A–C): Main hyphae 5 μ m wide. Sporangia globose, obovate, limoniform, 12.5–32.5 (av. 21.1) μ m in diam., terminal and intercalary, germinated directly by germ tubes or indirectly by zoospores, not proliferated internally. Oogonia globose, terminal and intercalary, 15–20 (av. 17.5) μ m in diam., smooth, occasionally with blunt projections which are various in length and numbers. Antheridia 1–2(3) per oogonium, monoclinal; antheridial cells club shaped, making apical contact with the oogonium. Oospores spherical, aplerotic or occasionally nearly plerotic, 12.5–17.5 (av. 13.7) μ m in diam., with thin wall.

Cardinal temperatures: minimum below 5°C, optimum 20–25°C, maximum 33°C. Daily growth rate on V-8 juice agar at 25°C was 27 mm.

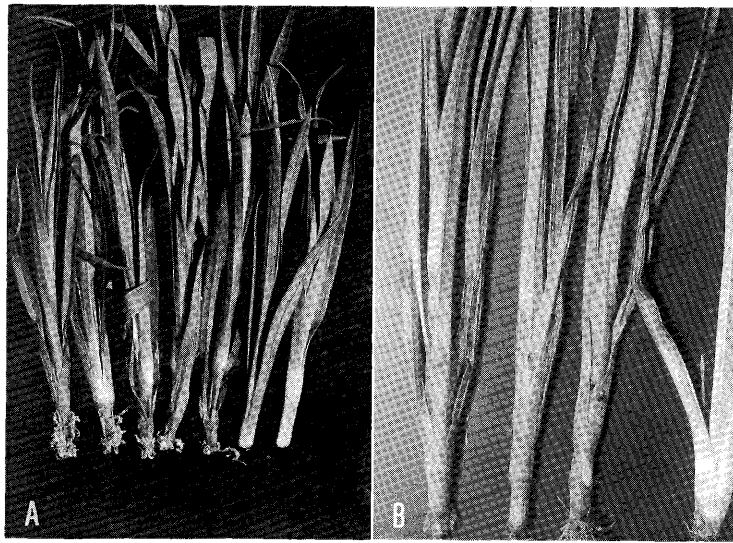


Fig. 1. Diseased carnations naturally infected with *Pythium irregulare* (A) and *P. aphanidermatum* (B).

Table 1. Detection of *Pythium* and/or *Phytophthora* in diseased tissues of carnation cultivars Ucon Sim and Barbara by serological methods

Antisera	Serological method	cv. Ucon Sim		cv. Barbara	
		Infected	Healthy	Infected	Healthy
<i>Phytophthora</i>					
<i>Ph. erythroseptica</i>	ELISA	0.04 a)	0.04	*b)	*
<i>Ph. capsici</i>	ELISA	*	*	0.1	0.1
	DIBA	*	*	—c)	—
<i>Pythium</i>					
<i>P. aphanidermatum</i>	ELISA	*	*	0.5	0.0
	DIBA	*	*	+	—
<i>P. ultimum</i>	ELISA	*	*	0.6	0.0
	DIBA	*	*	+	—

a) ELISA value (A_{405}).

b) Not tested.

c) —: negative response, +: positive response.

Pythium aphanidermatum (Edson) Fitzp. (Fig. 2, D–F): Main hyphae up to 7.5 μm wide. Sporangia consisting of terminal complexes of swollen hyphal branches of various length and up to 20 μm wide, germinated by extension of long exit tube and vesicle formation and zoospore discharge. Vesicles 20–65 (av. 40.4) μm in diam. Oogonia terminal, globose, smooth, 25–32.5 (av. 28.2) μm in diam., with straight oogonial stalks. Antheridia typically intercalary, usually declinuous, occasionally monoclinal, 7.5–20 μm long and 7.5–15 μm wide, commonly 1 per oogonium. Oospores aplerotic, spherical, smooth, 20–27.5 (av. 22.5) μm in diam.

Cardinal temperatures: minimum 10°C, optimum 30–35°C, maximum above 40°C. Daily growth rate on V-8 juice agar at 30°C was beyond 30 mm.

Pathogenicity

Typical data of the inoculation tests are shown in Table 2. Carnation and dianthus seedlings were damped-off in the infested soil within one month after inoculation. Two isolates,

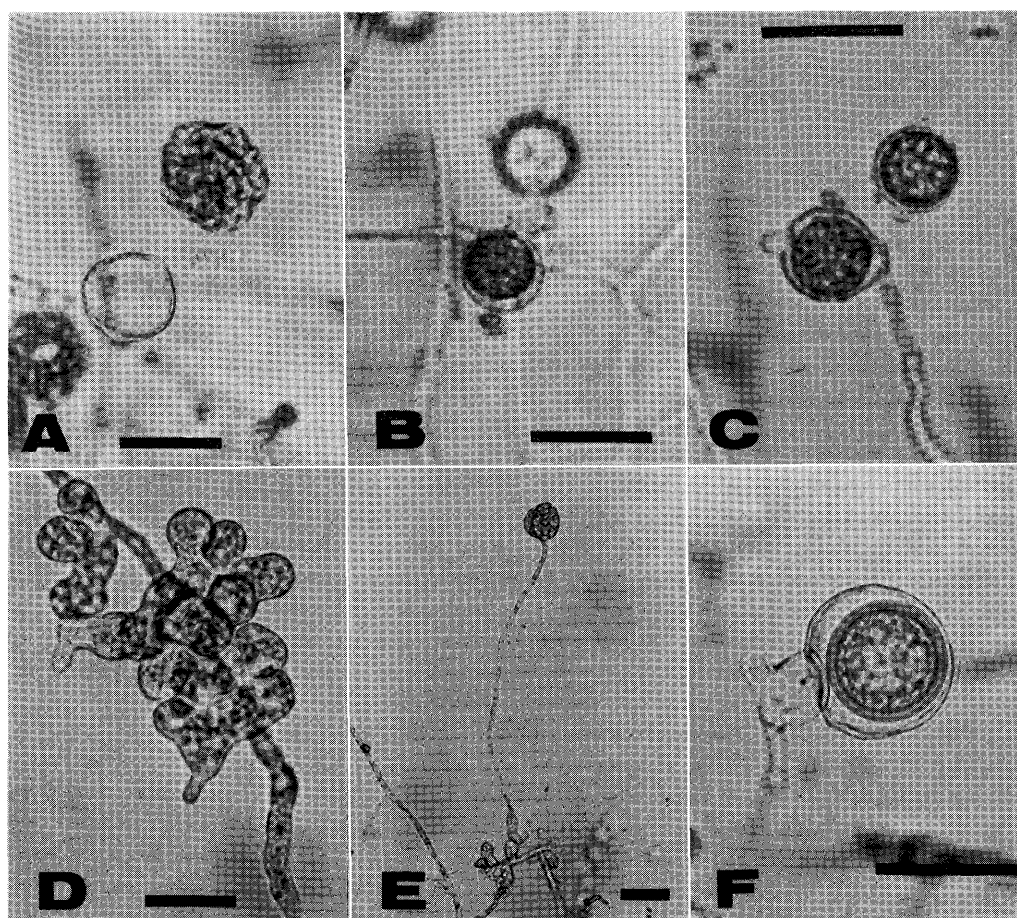


Fig. 2. Morphology of *Pythium irregulare* (A-C) and *P. aphanidermatum* (D-F). A: Zoospore discharge from sporangium. B: Oogonium with monoclinal antheridium and aplerotic oospore. C: Echinulate oogonia with immature and mature oospore. D: Lobate sporangium. E: Sporangium with long extension of an exit tube and a vesicle. F: Oogonium with an antheridium and an aplerotic oospore. Bars: 25 μ m.

Table 2. Pathogenicity of four *Pythium* isolates to seedlings of carnation (cv. Chabot) and dianthus (cv. Koubai and cv. Hakubai) one month after artificial inoculation in a glass-house^{a)}

Fungus	Carnation	Dianthus
<i>P. irregulare</i> DC-1	5/6 ^{b)}	3/6
<i>P. irregulare</i> DC-2	5/5	4/6
<i>P. irregulare</i> DC-3	4/6	0/6
<i>P. aphanidermatum</i> CC8901	9/17	8/21
Control	0/5	0/6

a) Planted in the autoclaved soil artificially infested with agar culture, and incubated at 15–30°C for *P. irregulare* and at 23–41°C for *P. aphanidermatum*.

b) No. of seedlings killed/no. of seedlings tested.

DC-2 and CC8901, caused root rots, water-soaked lesions on stems, and damping-off of both plants tested. Their initial symptoms were observed on the 5th day after inoculation. The inoculated fungi were consistently reisolated from the diseased plants, but not from uninoculated healthy plants.

DISCUSSION

Root rots in carnations have been found in Tokyo and Chiba, recently. The serological tests using ELISA and DIBA indicated that the disease might be caused by *Pythium*, but not by *Phytophthora*.

Two *Pythium* species were isolated from diseased carnations. The Tokyo isolate (DC-2) did not form sporangia on agar media, but occasionally formed them on rice blades saturated with water at room temperature. Sporangia were germinated by extending exit tubes and forming vesicles followed by zoospore discharge. The shapes of sporangia, oogonia and antheridia of the isolate have a close resemblance to those of *Pythium irregulare* and *Pythium paroecandrum* Drechsler. According to Plaats-Niterink⁹⁾, *P. paroecandrum* forms smooth and mostly intercalary oogonia, and has occasionally intercalary or hypogynous antheridia. The isolate, therefore, is different from *P. paroecandrum* because of formation of oogonia with blunt projections and no intercalary antheridia. The Tokyo isolate (DC-2) was identified as *P. irregulare* Buisman.

Pythium irregulare has been recorded from several parts of the world and pathogenic to many plants, especially to seedlings⁹⁾. In Japan, this fungus has been reported as root rot or damping-off fungus of edible burdock⁹⁾, onion¹⁰⁾, and tulip⁴⁾.

On the other hand, *P. aphanidermatum* is a typical plant parasite of warm regions and widely distributed all over the world⁹⁾. It can cause root rot and damping-off, stalk and rhizome rots, soft rot, fruit rot or cottony blight of various plants. Distribution of *P. aphanidermatum* and its significance in Japan were discussed by Watanabe¹²⁾.

Pythium ultimum and *P. vexans* were reported as causal fungi of carnation root rot disease in USA¹¹⁾, but no report on *Pythium* disease of carnation has been available in Japan. This is the first report on the disease of carnation caused by two *Pythium* species in Japan. Therefore, carnation root rot, "Negusarebyo" in Japanese, is proposed for this disease.

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和 文 摘 要

君島悦夫・小林慶範・西尾 健：*Pythium irregulare* および *P. aphanidermatum* によるカーネーション根腐病（新称）

東京都および千葉県において、それぞれ 1984 年および 1989 年にカーネーションの苗に根腐れを伴う病害が発生した。被害株の茎葉は褐変・腐敗し、根も腐敗症状を呈していた。形態的特徴から東京都下からの分離菌を *Pythium irregulare* Buisman, 千葉県下からの分離菌を *Pythium aphanidermatum* (Edson) Fitzp. と同定した。両菌ともカーネーションに病原性を示した。病名として根腐病を提唱した。