Foot Rot of Gerbera Caused by *Phytophthora cryptogea* Pethyb. & Laff. in Japan

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Key words: *Phytophthora cryptogea*, gerbera, foot rot.

In February, 1984, destructive foot rots of gerbera (*Gerbera jamesonii* Bolus) were observed in a glasshouse for nursery stocks at Fujisawa, Kanagawa Pref., Japan. A foot rot of gerbera was reported in 1969 in Japan by Morita⁹⁾, who attributed the cause to an unidentified species of *Phytophthora*. This paper reveals the species of causal fungus of the disease. A brief report of this work has been published elsewhere⁴⁾.

Symptoms. The main diagnostic symptoms of the disease consisted of sudden wilting of the leaves accompanied by a conspicuous color change from normal green to dark violet. Affected crown and root tissues were soft, water-soaked, rotted and blackish brown in color. These were similar to the symptoms caused by *Phytophthora* sp. reported previously⁸⁾.

Diagnosis. Enzyme-linked immunosorbent assay²⁾ (ELISA) was applied to diagnose the disease of the symptomatic plants. An antiserum prepared for *Phytophthora erythroseptica* Pethyb. showing genus specific reactivities³⁾ was used. The preparation of antigen samples and the procedures of ELISA were performed as previously described⁹⁾. Extracts of the diseased parts of the gerbera reacted with *Phytophthora* antibody. Healthy parts used as control gave no positive reaction. The serological test using ELISA indicated that this disease might be caused by *Phytophthora*.

Isolation and identification. Affected leaf tissues were washed and cut into pieces. They were placed on a selective medium for *Phytophthora*⁶⁾ and incubated at 25°C for 3 days. After incubation, hyphal tips on the selective medium were transferred onto water agar for single hyphal tipplings. Isolates were cultured on V-8 agar (V-8: 200 ml Campbell V-8 juice, 2.5 g calcium carbonate, 15 g agar per liter). Representative isolate, SG-1 (=IFO 32325), was used for describing the morphology. To study the production of sex organs, the amended V-8¹⁾ (aV-8: 200 ml V-8 juice, 30 mg β -sitosterol, 20 mg tryptophane, 100 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 mg thiamine, and 15 g agar per liter) was used. SG-1 was paired with each A1 mating type isolate of *P. cryptogea* Pethyb. & Laff.** (University of California, Riverside: UCR, Collection P1738) and *P. cinnamomi* Rands** (UCR, Collection P2138 and P3664), paired also with each A2 mating type isolate of *P. cryptogea*** (UCR, Collection P3850) and *P. cinnamomi*** (UCR, Collection P2457 and P2392), or grown alone on aV-8 at 15°C for 2 weeks.

Young hyphae had no septa, but became septate with age. On V-8, the fungus exhibited no characteristic pattern and clusters of hyphal swellings were observed. Cardinal growth temperatures on V-8 were as follows: minimum below 5°C, optimum 25–30°C, and maximum 33°C. Typical sporangia were frequently found on the diseased plant tissues in water (Fig. 1-A and B). Sporangia (av. $44.4 \times 29.3 \mu\text{m}$, range $62.5\text{--}20.0 \mu\text{m} \times 37.5\text{--}12.5 \mu\text{m}$, L/B ratio=1.51) were non-papillate, non-deciduous, ovoid obpiliform or ellipsoidal, and internal proliferation and sympodial elongation occurred. This isolate resembled *Phytophthora* sp. reported by Morita⁹⁾ according to the sporangia and cardinal growth temperatures. Sex organs were not produced in single culture but were produced only when paired with A1 mating type isolates of *P. cryptogea* and *P. cinnamomi*. Oogonia (av. $35.5 \mu\text{m}$, range 25–45 μm) in dual cultures were spherical with smooth walls and becoming yellowish with age. Antheridia (av. 15 μm) were amphigynous, spherical, occasionally oval. Oospore (av. 30.1 μm , range 20–40 μm) nearly filled the oogonium (Fig. 1-C).

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Fig. 1. Morphology of *Phytophthora cryptogea* causing foot rot of gerbera. A: non-papillate sporangia that developed on the China-aster seedling flooded with water ($\times 110$); B: typical sporangium ($\times 330$); C: oogonium with amphigynous antheridium and oospore ($\times 800$).

Table 1. Effect of temperature on oospore production of *P. cryptogea* (SG-1) in the amended V-8 agar^{a)}

Temperature ($^{\circ}\text{C}$)	Oospore production rating ^{b)}		
	<i>P. cryptogea</i>	<i>P. cinnamomi</i>	
	P 1738	P 2138	P 3664
10	++	++	+
15	++	++	+
20	++	++	+
25	—	—	—
30	—	—	—

a) SG-1 was paired with A1 mating type isolates of *P. cryptogea* and *P. cinnamomi*.

b) The rating (—: 0, +: 1-10, and ++: more than 10) was based on the mean number of oospores observed in microscope field (about 2.5 mm^2).

Table 2. Production of oospores of *P. cryptogea* (SG-1) in various media after 2 weeks at 15°C ^{a)}

Media ^{b)}	Oospore production rating ^{c)}		
	<i>P. cryptogea</i>	<i>P. cinnamomi</i>	
	P 1738	P 2138	P 3664
HSA	++	++	+
CMA	++	+	+
V-8	++	+	+
aV-8	++	++	+
PSA	—	—	—

a) See foot note a) in Table 1.

b) HSA, CMA, V-8, aV-8 and PSA=hemp seed agar, corn meal agar, V-8 agar, amended V-8 agar and potato sucrose agar, respectively, described in text.

c) See foot note b) in Table 1.

From the above results, the isolate was identified as *Phytophthora cryptogea* Pethyb. & Laff.^{10,11)} which has been known as a foot rot fungus of gerbera in USA¹²⁾ and Europe⁹⁾, and was isolated from an alfalfa-field soil, in Hokkaido, Japan⁷⁾.

Effects of temperature and culture media on oospore formation. SG-1 was paired with *P. cryptogea* (P1738) and *P. cinnamomi* (P2138 and P3664) on aV-8 in the petri dishes and then placed in the incubators of $10\text{--}30^{\circ}\text{C}$ at 5°C intervals to determine the effect of temperature. To determine the effect of medium, dual cultures were incubated at 15°C on the following media: V-8, aV-8, corn meal agar (CMA: 20 g corn meal, 15 g agar per liter), hemp seed agar (HSA: 100 g hemp seed, 15 g agar per liter),

Fig. 2. Diseased gerbera grown in the artificially infested soil with *P. cryptogea* (left) and healthy gerbera grown in the uninfested soil (right).

potato sucrose agar (PSA: 200 g potato, 20 g sucrose, 15 g agar per liter). After 2 weeks the bottom of the petri dishes was examined under the microscope and oospores were counted.

Abundant oospores were produced at 15 and 20°C, but no oospore was found at higher temperatures than 25°C (Table 1), though the optimum temperature for vegetative growth was 25–30°C. SG-1 produced oospores in V-8, aV-8, CMA and HSA, but no oospore was observed in PSA (Table 2).

Pathogenicity. The pathogenicity of SG-1 was tested several times to the following seedlings by the method described elsewhere⁹⁾: gerbera, China-aster (*Callistephus chinensis* Nees), stock (*Matthiola incana* R. Br.), godetia (*Godetia grandiflora* Lindl.), Chinese cabbage (*Brassica campestris* L.), corn poppy (*Papaver rhoeas* L.), snapdragon (*Antirrhinum majus* L.), sweet pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), pumpkin (*Cucurbita maxima* Duch.) and cucumber (*Cucumis sativus* L.). SG-1 was highly pathogenic to gerbera (Fig. 2), China-aster and stock (100–76% killed), moderately pathogenic to Chinese cabbage, corn poppy and snapdragon (51–27% killed), weakly pathogenic to sweet pepper and tomato (7–2% killed) and non-pathogenic to pumpkin and cucumber. The fungus was consistently reisolated from the diseased plants.

The present paper reveals that *P. cryptogea* is the causal agent of foot rot of gerbera reported by Morita⁸⁾ in Japan.

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和 文 摘 要

君島悦夫・後藤正昭：わが国における *Phytophthora cryptogea* Pethyb. & Laff. によるガーベラ根腐病について

1984年2月、神奈川県藤沢市内のガーベラ栽培温室内で、根腐病 (*Phytophthora* sp.) とみられる被害が発生した。被害株は *P. erythroseptica* 抗血清を用いた ELISA 試験で顕著な反応を示した。分離菌は土壌接種でガーベラをはじめ多くの植物に病原性を示した。本菌は *P. cryptogea* 及び *P. cinnamomi* の A1 菌株との対峙培養により有性器官を生じた。形態的特徴から分離菌を *P. cryptogea* Pethyb. & Laff. と同定した。本試験によりガーベラ根腐病の病原菌が明らかになった。

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