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Citrus Brown Rot Caused by *Phytophthora palmivora* and *P.nicotianae*

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Abstract : Grapefruit showing fruit decay, brown rot, were found at the plant quarantine inspection in Yokohama, 1993 and their pathogens were revealed by this study. Three isolates obtained from the diseased fruit caused brown rot on the fruit of grapefruit and other citrus. One isolate was identified as *Phytophthora palmivora* and the others were identified as *P. nicotianae* on the basis of their morphological characteristics and cardinal growth temperatures.
This is the first report on the disease of citrus caused by P. palmivora in Japan, and it is proposed that *P. palmivora* and *P. nicotianae* are new pathogens of citrus brown rot disease.
Key words : Grapefruit, Citrus brown rot, Identification, *Phytophthrora palmivora*, *Phytophthora nicotianae*.

Introduction

Citrus brown rot caused by *Phytophthora citrophthora* (R.E. Sm. & E. H. Sm.) Leonian is a common pre- and post- harvest problem of citrus fruit (Yamamoto, 1981 ; Brown and Eckert, 1989). In our previous work (Kimishima *et al.*, 1993), *P. citrophthora* and *P. syringae* (Kleb.) Kleb. were isolated as causal agents of brown rot of sweet orange fruit imported from California State, USA.

In May of 1993, fruit decay, brown rot, of grapefruit (*Citrus paradisi* Macf.) was found at the plant quarantine inspection, in Yokohama, Japan. They were imported from Florida State, USA. Some diseased fruit were collected and used for this study. This paper describes the results of etiological studies including the symptoms, identification of the causal organisms and their pathogenicity. A brief report of this work has been published elsewhere (Kimishima *et al.*, 1995).

Materials and Methods

Isolation of fungi

For isolation, diseased rind tissues were washed under running tap water for 1 - 2 min, air - dried and placed on a selective medium for *Phytophthora* (Masago *et al.*, 1977). After culturing at 25 ℃ for 2 - 3 days, hyphal tips grown out host tissue on the agar medium were transferred onto water agar (WA) for single hyphal tippings. Representative isolates, UG - 9301, UG - 9302 and UG - 9303, were used for describing the morphology on V - 8 agar (200 mℓ Campbell V-8 juice, 2.5 g calcium carbonate, 15 g agar per liter) and for the pathogenicity test.

Identification

Isolates were identified on the basis of colony morphology, mycelial characteristics, cardinal temperatures and production, morphology and size of sporangia, oogonia and antheridia (Waterhouse, 1963 ; Stamps *et al.*, 1990 ; Hall, 1994 ; Stamps, 1985).

For observation of sporangia, cultures were maintained on V - 8 agar and/or hemp seed saturated in water at 25 ℃ for 4 - 5 days. To study the production and morphology of sex organs, hemp seed agar

(HSA : 100 g hemp seed, 15 g agar per liter) were used. UG - 9301 was paired with each A1 mating type isolate of *P. palmivora* (Butler) Butler * (University of California, Riverside : UCR, Collection P6281) from cocoa and *P. parasitica* Dastur * (= *P. nicotianae* Breda de Haan) (UCR, Collection P1751) from tabacco, paired also with each A2 mating type isolate of *P. palmivora* * (UCR, Collection P6278) from cocoa and *P. parasitica* * (UCR, Collection P3118) form tomato, or grown alone on HSA at 12 ℃ for 3 - 4 weeks. UG - 9302 and UG - 9303 were paired with each A1 mating type isolate of *P.* palmivora (UCR,Collection P6281), *P.parasitica* (UCR, Collection P1751) and *P. capsici* Leonian (IFO 30696) from pumpkin, paired also with each A2 mating type isolate of *P. palmivora* (UCR, Collection P6278), *P. parasitica* (UCR, Collection P3118) and *P. capsici* (IFO 30698) from pumpkin, or grown alone in the same conditions described above.

Temperature responses of the fungi were studied by determing the mycelial growth on V8 agar incubated at various temperatures ranging from 5 to 40 ℃.

Inoculation tests

The pathogenicity of each isolate was tested several times to following citrus fruit and/or leaves : Grapefruit, Sweet orange (*Citrus sinensis* Osbeck), Lemon (*C. limon* (L.) Burm. f.), Natsudaidai (*C. natsudaidai* Hayata), Hassaku (*C. hassaku* hort. ex Tanaka), Satsuma mandarin (*C.unshiu* Marc.), Kumquat (*Fortumella sp.*) and Trifoliate orange (*Poncirus trifoliate* (L.) Raf.). The wounded plant surface was prepared by injuring with a cork borer or with a razor. Mycelial mats with an agar culture were placed onto the wounded and/or unwounded parts of each plant. Inoculated plants were maintained under moist condition at 25 ℃ for 4 - 7 days. Uninoculated plants were served as controls.

Results

Symptoms

Symptoms were characterized by brown discoloration of the rind (Fig. 1). The affected area was firm and leathery, and it retained the same degree of firmness as the adjacent healthy rind. Some fruit showed a water soaked area surrounding the diseased part. Diseased fruit had characteristic pungent and rancid ordor.

Fig. 1. Symptoms of brown rot of grapefruit caused by *Phytophthora palmivora* (left) and by *P. nicotianae* (right).

* : Import permit number issued by Ministry of Agriculture, Forestry and Fisheries is 3Y1882.

Identification

UG-9301 (Fig. 2, A - D)

The fungus exhibited slightly radiate pattern on V8 agar. Cardinal growth temperatures were as follows : minimum below 10 ℃, optimum 30 ℃, maximum below 35 ℃. No growth occurred at 35 ℃ (Fig. 3). Sporangiophores were narrow and developing as well defined. Sporangia (av. 39.3 × 24.2 μm, range 25 - 57.5 × 20 - 32.5 μm, L/B ratio = 1.62) were formed readily on V8 agar, papillate, ellipsoid or ovoid, caducous, and had short pedicel (up to 5 μm). Internal proliferation of sporangia was not observed. Chlamydospores (av. 27.6 μm in diam, range 20 - 35 μm in diam) were formed abundantly. Sex organs were not found in single cultures but occasionally produced only when the fungus was paired with A1 mating type of *P.palmivora* (Table 1). Oogonia (av. 35 μm in diam, range 32.5 - 37.5 μm in diam) in dual cultures were spherical with smooth walls. Antheridia (range 15 - 20 × 15 - 30 μm) were amphigynous. Oospores (av. 30.9 μm in diam, range 27.5 - 35 μm in diam) were nearly plerotic and becoming yellowish brown with age.

UG-9302 (Fig. 2,E - H)

The fungus exhibited no pattern on V8 agar. Cardinal growth temperatures were as follows : minimum below 10 ℃, optimum 30 ℃, maximum below 40 ℃. The fungus showed good growth at 35 ℃ (Fig. 3). Sporangia (av. 47.5 × 35.6 μm, range 30 - 82.5 × 20 - 47.5 μm, L/B ratio = 1.33) were produced abundantly on hemp seed saturated in water, papillate (up to 7.5 μm), often with 2 papillae, spherical,ovoid, ellipsoid or obpyriform, occasionally with finger like projections. Chlamydospores (av. 29.4 μm in diam, range 17.5 - 42.5 μm in diam) were formed abundantly on V8 agar, spherical, light brown and delimited from somatic mycelium by a septum. Sex organs were not found in single cultures but abundantly produced when the fungus was paired with A2 mating type isolates of *P.palmivora* (P6278) and *P.capsici* (IFO 30698) (Table 1). Oogonia (av. 31.2 μm in diam, range 27.5 - 35 μm in diam) in dual cultures were spherical with smooth walls. Antheridia (av. 11.4 × 12.1 μm) were amphigynous and cylindrical. Oospores (av. 26.5 μm in diam, range 25 - 30 μm in diam) were nearly plerotic and becoming yellowish brown with age.

UG-9303 (Fig. 2, I - L)

The fungus showed rosette colony pattern on V8 agar. Cardinal growth temperatures were as follows : minimum below 10 ℃, optimum 30 ℃, maximum below 40 ℃. The fungus showed good growth at 35 ℃ (Fig. 3). Sporangia (av. 54.7 × 44.7 μm, range 40 - 75 × 37.5 - 55 μm, L/B ratio = 1.22) were produced abundantly on hemp seed saturated in water, papillate (up to 7.5 μm), sometime with 2 papillae, spherical, ovoid, ellipsoid. Chlamydospores (av. 28 μm in diam, range 17.5 - 37.5 μm in diam) were abundant on V8 agar, spherical, light brown.Sex organs were not found in single cultures but abundantly produced when the fungus was paired with A1 mating type isolates of *P.palmivora* (P6281) and *P. capsici* (IFO 30696) and when with both mating type of *P.parasitica* (P1751 : A1; P3118:A2) (Table 1). Oogonia (av. 27 μm in diam, range 22.5 - 30 μm in diam) in dual cultures were spherical with smooth walls. Antheridia (av. 13.5 × 13.5 μm) were amphigynous and cylindrical. Oospores (av. 24.2 μm in diam, range 20 - 27.5 μm in diam) were nearly plerotic and becoming yellowish brown with age.

Inoculation tests

Results of inoculation tests are shown in Table 2. UG-9301, UG-9302 and UG-9303 caused brown rot on the fruit of grapefruit, sweet orange, lemon and hassaku. The decay first observed as a water-soaked discoloration of the rind, and then the affected area becoming light brown. Both UG - 9301 and UG - 9303 caused dark green lesions on the leaves of lemon, sweet orange, natsudaidai, satsuma mandarin, kumquat and trifoliate orange. UG-9302 formed ones on the leaves of kumquat and

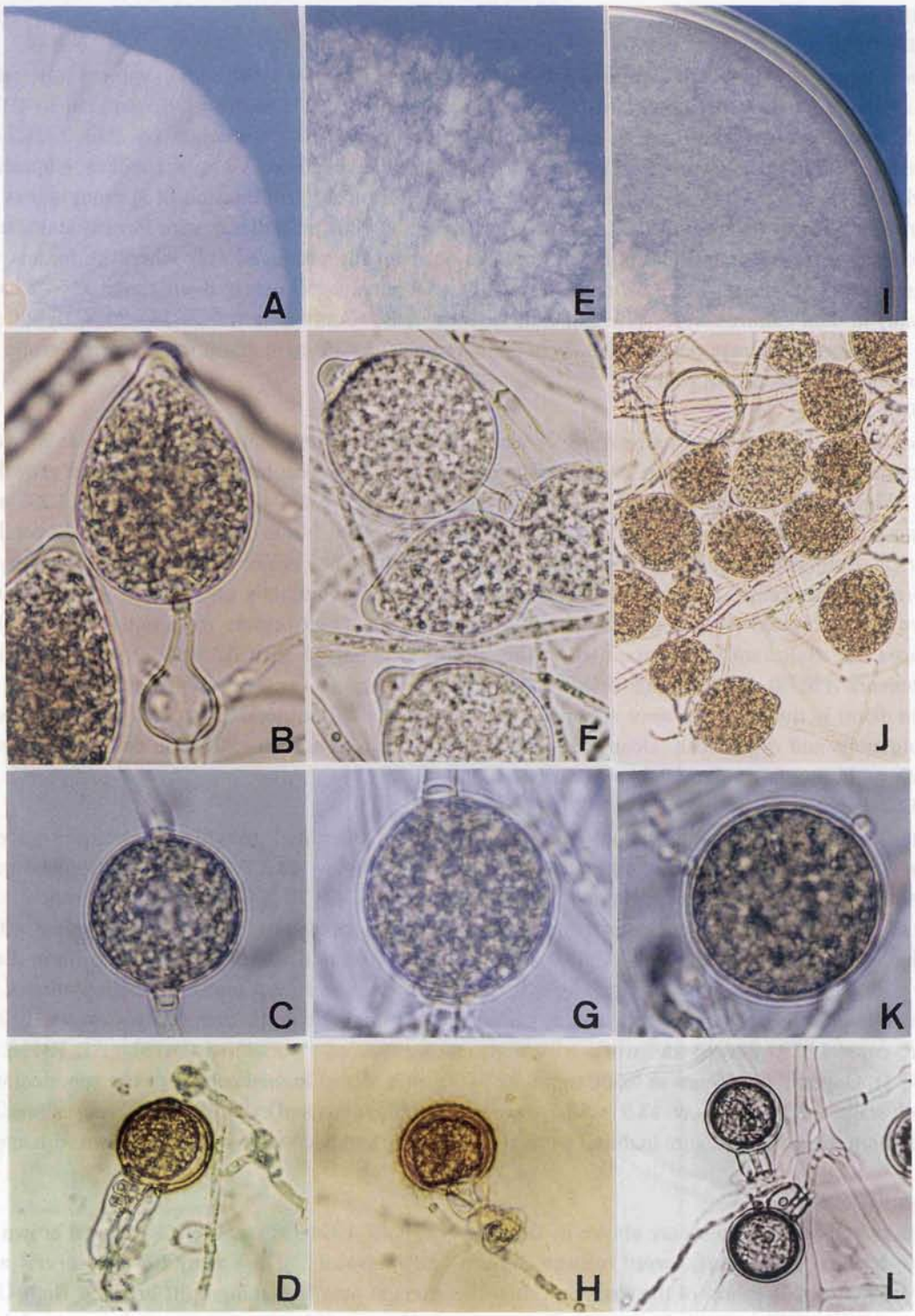

Fig. 2. Morphology of *P.palmivora* (UG - 9301 : A - D) and *P.nicotianae* (UG - 9302 : E - H ; UG - 9303 : I - L).
A : Colony morphology on V8 agar. B : Sporangiophore and papillate sporangium (× 800).
C : Chlamydospore (× 800). D : Oogonium with amphigynous antheridium and oospore (× 400).
E : Colony morphology on V8 agar. F : Papillate sporangia. (× 800). G : Chlamydospore (× 800).
H : Oogonium with amphigynous antheridium and oospore (× 400). I : Colony morphology on V8 agar. J : Papillate sporangia (× 400). K : Chlamydospore (× 800). L : Oogonia with amphigynous antheridia and oospores (× 400).

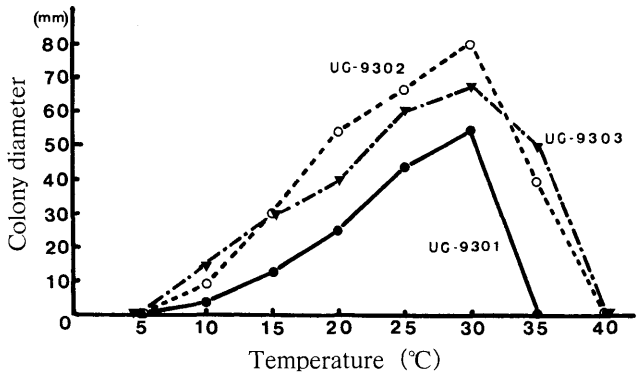

Fig. 3. Colony diameter of *Phytophthora palmivora* (UG - 9301) and *P.nicotianae* (UG - 9302 and UG - 9303) grown at different temperatures on V8 agar for 5 days.

Table 1. Production of oospores of three *Phytophthora* isolates (UG-9301, UG-9302 and UG-9303) with various isolates of *Phytophthora* spp [a]

Isolate		Mating type	Oospore production rating [b]		
			UG-9301	UG-9302	UG-9303
P.palmivora	P6281	A 1	+	−	++
	P6278	A 2	−	++	−
P.parasitica	P1751	A1	−	−	++
(=*P.nicotianae*)	P3118	A2	−	−	++
P.capsici	IFO30696	A1	NT [c]	−	++
	IFO30698	A2	NT	+	−

a) Grown on hemp seed agar at 12 ℃ for 20 - 30 days.
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).
c) Not tested.

Table 2. Pathogenicity of three *Phytophthora* isolates (UG-9301, UG-9302 and UG-9303) to citrus fruits and/or leaves by artificial inoculation

Plant	Part inoculated [a]	Pathogenicity [b]		
		UG-9301	UG-9302	UG-9303
Grapefruit (*Citrus paradisi* Macf.)	F	+	+	+
Lemon	F	+	+	+
(*C.limon* (L.) Burm.f.)	L	+	−	+
Sweet orange	F	+	+	+
(*C.sinensis* (L.) Osbeck)	L	+	+/-	+
Natsudaidai	L	+	+/-	+
(*C.natsudaidai* Hayata)				
Hassaku	F	+	+	+
(*C.hassaku hort.* ex Tanaka)				
Satsuma mandarin	L	+	−	+
(*C.unshiu* Marc.)				
Kumquat	L	+	+	+
(*Fortunella* sp.)				
Trifoliate orange	L	+	+	+
(*Poncirus trifoliate* (L.) Raf.)				

a): Inoculated wounded part of fruit (F) and/or leaves (L) with mycelial disk.
b): Lesions were formed (+) or not (-).

trifoliate orange, and occasionally on sweet orange and natsudaidai. All isolates caused no lesions on the unwounded leaves tested. No symptoms were shown on the control fruit and leaves. The fungi were consistently reisolated from diseased plants.

Discussion

Grapefruit showing fruit decay were found at the plant quarantine inspection and their pathogens were revealed. The symptoms of the diseased fruit were similar to those caused by *Phytophthora citrophthora* and *P. syringae* (Kimishima *et al.*, 1993).

From the results described above, the isolate, UG - 9301, was identified as *Phytophthora palmivora* (Butler) Butler (Stamps, 1985) which has been known as a pathogen of black pod and canker of cocoa. According to stamps (1985), 138 species of economic, ornamental and hedge plants were known as host of this fungus. *P.palmivora* has been reported from citrus in many humid tropical areas (Klotz, 1978) and has been associated with a blight of grapefruit nursery seedlings in Puerto Rico (USDA, 1960). Zitko *et al.* (1991) reported that a *Phytophthora* sp. recovered from soil in a citrus orchard, Florida, USA was characterized and designated as *P. palmivora.* It was the first report of *P. palmivora* pathogenic to citrus in the United States. Grapefruit samples used in this study were from Florida State, USA. No report on *P.palmivora* pathogenic to citrus has been available in Japan. This is the first report on the disease of citrus caused by *P. palmivora* in Japan.

On the other hand, both UG - 9302 and UG - 9303 were identified as *Phytophthora nicotianae* Hall (Hall, 1994) on the basis of their morphological characteristics and cardinal temperatures. The fungus is known as a destructive plant pathogen with a worldwide distribution, particularly common in the tropics and sub - tropics and also has extensive host range (Hall, 1994).*P. parasitica* (=*P. nicotianae*) is widespread in citrus orchards in Florida (Zitko *et al.*, 1991). In Japan, *P. nicotianae* var. *parasitica* (Dastur) Waterhouse and *P.citrophthora* are known as pahtogens of Phytophthora rot of citrus seedlings (The Phytopathological Society of Japan, 1984). According to Hall (1993), *P. parasitica* and *P.nicotianae* var. *parasitica* are included in *P.nicotianae* as synonymous fungi.

The work described here reveals that *P. palmivora* and *P. nicotianae* are new pathogens of citrus brown rot disease.

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和文摘要

Phytophthora palmivora 及び *P. nicotianae* による カンキツ褐色腐敗病

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アメリカ合衆国産グレープフルーツの輸入検査時に褐色腐敗症状を呈する果実を発見したため、病原菌の分離·同定を行なった。試験の結果、分離菌は形態的特徴及び成育温度から *Phytophthora palmivora* 及び *P. nicotianae* と同定された。分離菌はグレープフルーツ果実に原寄主と同様の病徴を再現し、再分離された。カンキツ褐色腐敗病の新たな病原菌として P. *palmivora* と *P. nicotianae* の追加を提唱した。