Collar Rot of Apple Caused by *Phytophthora cambivora* (Petri) Buism.

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鈴木孝仁*・星野好博** : *Phytophthora cambivora* (Petri) Buism.

によるリンゴ (矮性台木, MM 106) の疫病

Abstract

Collar and root rot of apple (dwarfing rootstock, MM 106) was detected in Yoichi and Niki, Hokkaido, in 1977. *Phytophthora* sp. was isolated from the diseased trunk under the ground and roots of apple rootstocks. It was identified as *P. cambivora* from the characters of sporangia and sex organs. *P. cambivora* and *P. cactorum* were isolated from soil near by the diseased trees. One hundred fifty isolates of *P. cambivora* proved to belong to the mating type A¹ and 9 isolates to the mating type A². *P. cambivora* was able to induce collar and root rot in apple (MM 106) by the zoospore inoculation test.

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Introduction

Apple dwarfing rootstocks (M and MM series) are spreading for the rationalization of apple cultivation in Japan. Collar rot of size-controlling rootstocks of apple is known to occur universally. *Phytophthora* fruit rot of apple caused by *Phytophthora cactorum* had been observed in apple in Japan unlike collar rot^{7,11)}. The symptoms were noted for the first time in apple rootstock MM 106 in orchards in Yoichi and Niki, Hokkaido in 1977 prompting the isolation, identification and some physiological studies of this microorganism.

Materials and Methods

Phytophthora sp. was isolated from the diseased collar and root of apple rootstock MM 106 (*Malus pumila* cv. Northern SPY × *M. pumila* var. *paradisiaca*) and from soil in orchard located in Yoichi and Niki in 1977. Selective medium was used for isolation of *Phytophthora*⁹⁾. Baiting methods, which were used for isolation from soil, consist of modified Grim's method using small pieces of apple leaves³⁾ and trap method of twig or young fruit of apple trees⁷⁾.

Sporangia were formed in the soil suspension after cultivation on cleared V-8 juice agar (CV-8A)¹²⁾ at 25 C for 10 days. Sex organs were formed in CV-8A when pairing took place with compatibility mating types of P 746 (A¹) and P 635 (A²) isolates from *P. cambivora*, of P 253 (A¹) and P 255 (A²) isolates from *P. palmivora* and of T 131 (A¹) and T 515 (A²) isolates from *P. nicotianae* var. *parasitica*

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(Culture Collection of Univ. Calif.) at 25 C, in the dark for 1 month. For determination of the cardinal temperature and of the fungal growth rate of the isolates, inoculum was transferred to the center of bacto-potato dextrose agar (bacto-PDA) and bacto-corn meal agar (bacto-CMA) and was incubated at temperatures ranging from 3 to 37 C for 11 days. Diameter of colony was measured every day. Layerings of apple tree (MM 106) and nursery plants of Japanese chestnut (*Castanea crenata*), twigs and leaves of apple tree (MM 106), twigs of sweet chestnut (*Castanea sativa*), and fruits of apple (starking delicious) were used in inoculation test. Inocula to apple and chestnut nursery plants were mycelia, crushed infected apple fruit and zoospore suspension. Mycelia and crushed infected apple fruit were mixed with soil near the collar and roots of apple layering in pot. In the zoospore inoculation, plants were steeped in the suspension with aeration for 24 hr, and the plants were planted in pots with soil sterilized by steam. The plants were kept in greenhouse. Plants wounded by 10 burned needles were prepared in every inoculation test.

Results

Symptoms

Diseased plants showed reduced vigor on the whole, and the leaves became smaller than the healthy ones. In early autumn their red coloration became more pronounced, with a dash of purple. They defoliated earlier than the healthy ones. These foliar symptoms were never noticed before the rotting of the rootstock was fairly advanced. Discoloration of cortex and cambium was observed when the bark was stripped off (1 in Plate). Occasionally, however, the presence of such rotting was revealed by a moist appearance of the external bark. When the disease advanced, major changes occurred in the rootstock i.e. browning and softening progressing vertically and laterally, girdled the trunk and extended to the root, including the fibrous roots. As the softening advanced, the remaining bark was easily sloughed off from the root (2 in Plate). In every case, the lesions did not extend beyond the graft union.

Isolation

Phytophthora spp. were isolated from 8 specimens of collar, 4 specimens of root rot of apple rootstock, MM 106 and 3 specimens of soil, that were collected from the site of the diseased trees, in Yoichi and Niki apple orchards. Isolation ratio of *Phytophthora* spp. was 0 to 72 % from collar rot, 2.6 to 16.1 % from root rot and 0 to 94.7 % from soil for specimens used in experiments. *Phytophthora* spp. could be divided into 2 types, A 84 and A 230 from the appearance of colonies on PDA, CMA and CV-8A media and from the shape of sporangia. Sixty one and nine isolates were collected from collar and root rot respectively, and all of them belonged to

Table 1. *Phytophthora* spp. isolated from collar and root rot of apple (MM 106) and soil in the vicinity of diseased plants in Yoichi and Niki, Hokkaido

Source	No. of <i>Phytophthora</i> isolated	No. of <i>Phytophthora</i> A 84 type	No. of <i>Phytophthora</i> A 230 type
Collar rot	61	61	0
Root rot	9	9	0
Soil	136	128	8

A 84 type while 128 of 136 isolets from soil into A 84 type and 8 into A 230 type (Table 1).

Identification of isolates

The description of A 84 isolate collected from collar rot is as follows : Hyphae uniform, av. $5.2\ \mu\text{m}$ diam., profuse tough aerial mycelia without coraloid or hyphal swelling as spheroidal inflations. Chlamydospores absent. Sporangiophores proliferating within empty sporangia or simple monochasial sympodium, av. $3.3\ \mu\text{m}$ diam. Sporangia formed in aqueous solution, no papilla, ovoid, broad apex, apical thickening slight, $25-65 \times 17-31\ \mu\text{m}$, av. $46 \times 25\ \mu\text{m}$, L/B ratio 1.8, non-deciduous, exit pore $9.6\ \mu\text{m}$ (3 and 4 in Plate). Sex organs absent in single strain culture, but present when pairing with an opposite strain of *P. cambivora*, *P. nicotianae* var. *parasitica* and *P. palmivora*. Oogonia slightly yellow, spherical, av. $37\ \mu\text{m}$, when pairing with *P. cambivora* mating type A², with protuberance or smooth (5 in Plate). Antheridia amphigynous, bi- and uni-cellular, av. $18\ \mu\text{m}$ width, $28\ \mu\text{m}$ long when pairing with *P. cambivora* mating type A² (6 in Plate). Oospores nearly filling, av. $33\ \mu\text{m}$ when pairing with an opposite strain, *P. cambivora* mating type A². Culture uniform and fluffy.

The surface structure of oogonia of the isolates and its appearance differs depending on pairing of opposite mating type or of compatibility type of other species. When A 84 isolate paired with A 265 isolate the surface of oogonial wall showed a protuberance (18 %) or was smooth (81 %), but when paired with *P. palmivora* oogonia the surface was only smooth (Table 2). Haasis *et al.*⁴⁾ also reported that in the case of intraspecific pairing, protuberant and smooth oogonial wall occurred in varying proportion. Appearance of uni- and bi-cellular antheridia as reported by Ho *et al.*⁵⁾ could be confirmed and the proportion of the two kinds of an-

Table 2. Shape and size of sex organs of *Phytophthora cambivora* A 84 isolate when strain paired with compatibility mating types on cleared V-8 juice agar at 25 C for 1 month

Combination of pairing ^{a)}		No. of sex organs	Percent of oogonia			Percent of antheridia		Oogonia Diam. μm	Antheridia		Oospores Diam. μm
Mating type A ₁	Mating type A ₂		Protuberance %	Echinulate	Smooth	Unicellular %	Bicellular		Length μm	Width	
<i>P. cambivora</i> A 84	<i>P. cambivora</i> A 265	100	19	0	81	6	94	37	28	18	33
" A 84	" P 635	102	11	0	89	67	33	34	20	15	30
" P 746	" A 265	101	91	0	9	37	64	37	22	17	35
" A 84	<i>P. nicotianae</i> var. <i>parasitica</i>	160	1	90	9	74	26	36	22	17	32
" A 84	<i>P. palmivora</i>	100	0	0	100	90	10	26	19	15	21

a) *P. cambivora* A 84 and A 265 ; from apple in Hokkaido. *P. cambivora* P 635 and P 746 ; from Castanea and apricot, respectively, which were supplied from Calif. Culture Collection.

theridia also varied depending on pairing of opposite mating types (Table 2). Size of oogonia, antheridia and oospores was somehow in conformity with pairing of mating A¹ and A² types (Table 2). Growth curve of A 84 isolates at different temperatures 5 days after inoculation on bacto-PDA and bacto-CMA is shown in Fig. 1. Minimum, optimum and maximum temperatures for A 84 isolate growth on bacto-PDA were 3, 22–24 and 32 C, respectively. On bacto-CMA, optimum temperature was 25 C for fungal growth of A 84 isolate, and minimum and maximum temperatures were the same as on bacto-PDA. Growth rate of the isolate on bacto-PDA and on bacto-CMA was 5.0 and 5.8 mm a day at 24 and 25 C, respectively.

Fig. 1. Hyphal growth of *Phytophthora* A 84 isolate at varying temperatures 5 days after transfer on bacto-PDA (○—○) and on bacto-CMA (▲—▲).

From the above mentioned results, the characteristics of A 84 were compared with those of *P. cambivora*¹⁰⁾ as shown in Table 3. *P. cambivora* is characterized by the presence of a protuberance on the wall surface in oogonia and of bi-cellular antheridia. A 84 isolate showed the same characteristics and other experimental results were almost in agreement with the description of *P. cambivora*. A 84 isolate was identified as *P. cambivora* (Petri) Buism. Morphological characters of A 27, A 265 and other A 84 type isolates were the same as those of A 84 isolate. *P. cambivora* A 84 (mating type A¹) was deposited at the Institute of Fermentation Organization (IFO 30471) and at the Commonwealth Mycological Institute (IMI 229178), and *P. cambivora* A 265 isolate (mating type A²) was also deposited at IFO (IFO 30472).

The description of A 230 isolate collected from soil is as follows: Hyphae av. 4.1 μ m diam., without characteristic hyphal swellings. Chlamydospores seldom formed, spherical, 30–36 μ m diam. Sporangiophores produce simple monochasial sympodium, av. 1.8 μ m diam. Sporangia abundant on solid media (PDA, CMA, OMA, CV-8A), subglobose, broadly-ellipsoidal, obpyriform with a conspicuous hemispherical papilla up to 5 μ m, mostly 22–32 $\times$ 17–27 μ m, av. 27 $\times$ 22 μ m, L/B ratio 1.2, deciduous with a pedicel (up to 4 μ m). Sex organs abundant on solid media in single strain culture. Oogonia spherical, 22–37 μ m diam., av. 31 μ m, wall thin, slightly yellow and smooth. Antheridia paragynous, irregularly club-shaped, av. 15 $\times$ 10 μ m, and monoclinal. Oospores nearly filling, spherical, 19–31 μ m, av. 26 μ m. Culture uniform, slightly aerial mycelia.

Minimum, optimum and maximum temperatures for growth of A 230 isolate were below 3, 27 and 32 C on bacto-PDA respectively, and below 3, 28 and 32 C on bacto-

Table 3. Comparison of the main characters of *Phytophthora* A 84 isolate collected from collar rot of apple caused by *P. cambivora*

Item	<i>P. cambivora</i> ^{a)}	<i>Phytophthora</i> A 84 isolate ^{b)}
Hyphae : Angular swelling	Absent	Absent
Sporangia : Size	55-65 × 40-45 μm	46 × 25 μm
L/B ratio	1.4	1.8
Vacuole	Without	Without
Apical thickening	Slight	Slight
Oogonia : Surface	Protuberances	Protuberances or smooth
Size	43 μm	37 μm
Antheridia : Cell	Bi-, uni-cellular	Bi-, uni-cellular
Size	25 μm long	28 μm long
Oospores : Size	36 μm	33 μm
Hyphal growth temp. : Min.	About 2 C	Under 3 C
Opt.	22 - 24 C	24 - 25 C
Max.	32 C	32 C

a) ; Waterhouse *et al.*¹⁰⁾b) ; Sporangia formed on apple fruit. Sex organs were formed when strain paired with *Phytophthora* A 265 isolate from collar rot of apple.

CMA respectively. Growth rate of A 230 isolate at 27 C was 3.5 mm a day on bacto-PDA and 5.6 mm on bacto-CMA, respectively. From the above mentioned results, A 230 isolate was identified as *P. cactorum* (Leb. & Cohn) Schroet. *P. cactorum*, A 230 isolate was deposited at IFO (IFO 30474).

Mating types of isolates

Mating types of 159 isolates of *P. cambivora* collected from infected tissues of collar, root rot and soil were determined by pairing with compatibility mating type. One hundred fifty isolates belonged to the mating type A¹ and 9 isolates to the mating type A² (Table 4). The mating type A¹ was dominant in isolates but both types were recovered from all isolates from portions of collar, root and soil, and from the same diseased trees.

Table 4. Mating types of *Phytophthora cambivora* isolated from collar and root rot of apple (MM 106) and soil in Hokkaido

Source	Number of <i>Phytophthora cambivora</i> isolates	
	Mating type A ¹	Mating type A ²
Collar rot	50	6
Root rot	7	2
Soil	93	1
Total	150	9

Pathogenicity to apple and chestnut trees

Following the inoculation test with *P. cambivora* A 84 and A 27 isolates and with *P. cactorum* A 221 and A 230 isolates, appearance of symptoms, on young apple fruits (starking delicious), detached leaves (MM 106) and twigs (MM 106) of apple was only recognized in the case of wounded specimens in all of the inoculations except in the inoculation of unwounded leaves with *P. cactorum* A 230 isolate. When infected apple fruit was used as inoculum, *P. cambivora* A 84 and *P. cactorum* A 230 produced symptoms of collar rot on the trunk and root rot in case of inoculation through wounding and pathogen was recovered from these diseased tissues. In the inoculation without wounding, however, no symptoms appeared in both inocula. Also mycelia inoculation failed to give rise to collar rot in any trees following with and

without wounding treatments. *P. cambivora* A 27 isolate was used for zoospore inoculation and for abundant production of zoospores of this isolate. The results of zoospore inoculation by *P. cambivora* A 27 and *P. cactorum* A 221 and A 230 are shown in Table 5. Symptoms of collar rot appeared in the case of wounding and when pouring method of zoospore suspension of *P. cambivora* A 27 was applied without wounding. A 27 isolate was recovered from infected trunk and roots (Table 5). Symptoms did not appear on either trunk or roots in all experimental plots of *P. cactorum* following zoospore inoculation.

Table 5. Pathogenicity of *Phytophthora cambivora* (A 27 isolate) and of *P. cactorum* (A 221 and A 230 isolates) in apple layerings (MM 106)

Inoculum	Isolate	Inoculation method	Treatment in layering	Appearance of symptoms ^{e)}		Reisolation of pathogen	
				Collar rot	Root rot	Collar rot	Root rot
<i>Phytophthora cambivora</i>	A 27	Steeped in zoospore suspension ^{a)}	Wounded ^{d)}	+	±	+	+
do.	do.	do.	Unwounded	±	±	—	—
do.	do.	Pouring zoospore suspension ^{b)}	Unwounded	+	±	+	+
Control	—	Steeped in water ^{c)}	Wounded	—	±	—	—
do.	—	do.	Unwounded	—	±	—	—
<i>P. cactorum</i>	A 221	Steeped in zoospore suspension	Wounded	—	±	—	—
do.	do.	do.	Unwounded	±	±	—	—
do.	A 230	do.	Wounded	—	±	—	—
do.	do.	do.	Unwounded	—	±	—	—
Control	—	Steeped in water	Wounded	—	±	—	—
do.	—	do.	Unwounded	—	±	—	—

a) : Layerings were steeped in the zoospore suspension for 24 hr.

b) : Zoospore suspension was poured into the soil.

c) : Layerings were steeped in water for 24 hr.

d) : Wounded by 10 burned sewing needles.

e) : +; Appearance of symptoms of collar or root rot.

±; Color change brown to light brown.

—; No symptoms appeared.

Observation 60 days after inoculation.

Inoculation test of *P. cambivora* A 27 to nursery plants of Japanese chestnut was carried out by applying zoospore pouring method into the soil in wounded and unwounded plants. Symptoms of collar rot appeared on the wounded trunk of trees and inoculum was recovered from the diseased trunk and roots. In the plot of plants inoculated without wounding foliage wilt was observed in 2 out of 3 chestnut plants, and inoculum was recovered from the trunk. In the inoculation test of *P. cambivora* A 27 to the detached twigs of sweet chestnut (*Castanea sativa*), symptoms appeared both in wounded and unwounded twigs by agar disk method, and a high percentage of inoculum was reisolated from the diseased tissues.

Conclusion

In the present study, only *P. cambivora* was isolated from infected tissues of collar and root rot of apple (MM 106) rootstock. Both *P. cambivora* and *P. cactorum*, however, were isolated from soil around the diseased apple trees. Although *P. cactorum* has been reported to be the main pathogen of collar rot of apple rootstock by most authors¹⁾, in the present study this species could not be isolated from the diseased plants. *P. cambivora* is known as one of the pathogen of 'ink disease, of chestnut and basal canker of Norway maple¹⁰⁾. In addition, many host plants have been reported²⁾. Recently Mircetich *et al.*⁹⁾ described the same disease of apple caused by *P. cambivora*. More recently, Julis *et al.*⁶⁾ reported that *P. cambivora* as well as *P. cactorum* could be isolated from M and MM rootstocks sampled from various areas in northern America. This is the first report demonstrating that *P. cambivora* is associated with collar and root rot of apple in Japan. The writers propose that the disease be designated as Ekibyō in the same Japanese name with fruit rot caused by *P. cactorum*.

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和 文 摘 要

Phytophthora cambivora (Petri) Buism. によるリンゴ
(矮性台木、MM 106) の疫病

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1977年北海道余市地方のリンゴ矮性台木 (MM 106) に collar rot 症状の発生が認められ、罹病樹とその附近の土壌から *Phytophthora cambivora* が多数分離された。分離菌株のうち150菌株は mating type A¹ に属し、9菌株は mating type A² であった。本菌はリンゴの幼木に病原性を示し、本病の病原菌と考えられた。本病を *P. cactorum* による病名と同じ疫病と呼称したい。

Explanation of plate

- 1 and 2. Symptoms of collar and root rot of dwarfing rootstock (MM 106) of apple caused by *Phytophthora cambivora*. 1. Diseased tree in orchard. 2. Upper part of layering is apple cv. starking delicious, and lower part is rootstock (MM 106).
- 3 and 4. Sporangia of *P. cambivora* isolated from collar rot of apple. 3. Sporangium produced in proliferating sporangiophore. 4. Sporangium produced in monochasial sympodium.
- 5 and 6. Oogonia with bullate protuberances and uni- and bi-cellular antheridia of *P. cambivora* when strain paired with an opposite strain. 5. Oogonium with protuberances. 6. Bi-cellular antheridium.

The scale represents 10 μ m in photographs 3, 4, 5 and 6.