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Phytophthora Diseases of Apple in Korea: II. Occurrence of an Unusual Fruit Rot Caused by *P. cactorum* and *P. cambivora*

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사과의 역병: II. *Phytophthora cactorum*과 *P. cambivora*에 의한 사과 과실역병의 발생

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ABSTRACT : An unusual young fruit rot of apple caused by two species of *Phytophthora* was epidemic from late May to early July of 1996 in Andong, Uisung and Chungwon areas of Korea. The disease spread to over 30 apple orchards in the areas and percent of the infected tree and fruit was ca. 10~90% and 1~15%, respectively. Water soaking lesions or spots on leaves and shoot blight were also developed by the pathogen. Among 39 isolates collected, 25 were identified as *P. cactorum* and the others were as *P. cambivora* on the basis of their distinctive morphological characters. While the former fungus was homothallic, all isolates of the latter were A1 mating types. Koch's postulate was fulfilled. Both fungi showed strong pathogenicity not only to young fruits, leaves and shoots of apple but also to those of pear and peach. Several vegetables tested did not show symptoms even by wound inoculation. An occurrence of young fruit rot of apple caused by *Phytophthora* has not been reported in Korea, especially, *P. cambivora* has not been recorded previously as the causal agent of the disease in the world.

Key words : **apple, young fruit rot, *Phytophthora cactorum, P. cambivora***

Phytophthora fruit rot of apple has been reported worldwide including Korea (2, 5, 10, 15). The disease usually occurs on low hanging fruits at late ripening stage on the tree or in storage (5, 10), and its sporadic outbreaks have emerged as a threat to apple production in many countries, especially in European countries since 1970s (5, 10). Immature fruit rot of pear and apple caused by *P. cactorum* has been known as sprinkler rot in the United States and epidemics of the disease resulted in significant losses in several states of the country from 1989 to 1990 (4, 10). Although the fruit rot of apple caused by the fungus has been also listed in Korea (15), it has not been considered as an important disease in general and no reports on economic losses due to the disease has been found.

Among 11 species of *Phytophthora* known to infect fruit, root or trunk of apple, *P. cactorum* and *P. syringae* are the major pathogens causing the fruit rot on the tree or in storage (2, 5, 10). While the former fungus has been distributed to temperate regions around the world, the latter fungus has limited distribution in cool areas of Europe, North America, New Zealand and Japan (2, 3, 5, 9, 10, 12, 17). *Phytophthora cryptogea*, the causal pathogen of gerbera foot rot (2, 8), also has been reported in Australia and Germany to cause fruit rot of apple in storage (1, 5, 18). However, no other species in the genus has been reported to cause rots on apple fruits.

The rot on young fruits of apple cvs. Fuji and Tsugaru caused by two species of *Phytophthora* was epidemic from late May to early July of 1996 in several apple growing regions of Korea. The fungi also caused

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water soaking lesions or spots on young leaves and blight on shoots in the fields. In this study, we identified the causal agents and examined their pathogenicity to inform the etiological importance of the pathogen and the diseases they caused.

MATERIALS AND METHODS

Isolation of causal pathogens. Rotten young fruits, spotted leaves and blighted shoots of apple were collected from infected fields in Andong, Uisung and Chungwon areas of Korea between late May and early July, 1996. The materials were washed under tap water with a small amount of liquid soap prior to isolation of the causal pathogen. Small pieces of the freshly infected tissues were plated onto both a semi-selective medium for *Phytophthora* and a water agar without surface sterilization. The semi-selective medium (8) consisting of corn meal agar (CMA; Difco, 17 g/L) was supplemented with 100 ppm ampicillin, 50 ppm nystatin and 10 ppm pentachloronitrobenzene (PCNB). After incubation at 24℃ for 2 days, growing mycelial tips of the fungi were cut by a scalpel and transferred to 10% clarified V8 agar. The clarified 10% V8 juice was prepared by mixing 5 g of $CaCO_3$ to 163 m*l* of V8 juice (Campbell, USA) before centrifuge at 7,000 rpm for 20 min. Deionized water and 18 g of agar powder were added to 100 m*l* of supernatant of the juice to make 1000 m*l* of 10% clarified V8 agar (9).

Sporangial production and single zoospore isolation. To examine sporangial production and oospore formation on agar by single isolates, all isolates were cultured on the 10% clarified V8 agar for 7 to 14 days under light and in dark at 20℃ (9). Single zoospore isolate of *Phytophthora* was obtained as follows: 5-day-old culture of the isolate growing on 10% clarified V8 agar was cut by a cork bore (7 mm in diam.) to make agar disks (ca. 3 mm thick). Each two disks of four isolates were transferred to a sterile petri plate and ca. 0.3 m*l* of sterilized water was added to a disk (Fig. 1-A). After incubation at 25℃ for 24-48 hr under light, sporangia formed in water were chilled at 4℃ for 20-30 min and returned to room temperature to release zoospores. About 0.1 m*l* of the zoospore suspension was spread evenly on a water agar and incubated 24 hr at 25℃. Germinated single zoospores on the water agar were selected and transferred to 10% clarified V8 agar for further study. Morphological characters of sporangia were examined under a light microscope (100 or 200x) either directly or after transferred to a slide glass. At least 20 sporangia of an isolate were characterized. Hyphal swellings of the fungus formed in water were also examined concomitantly. Caducity of the sporangia was also determined as described by Jee *et al.* (9).

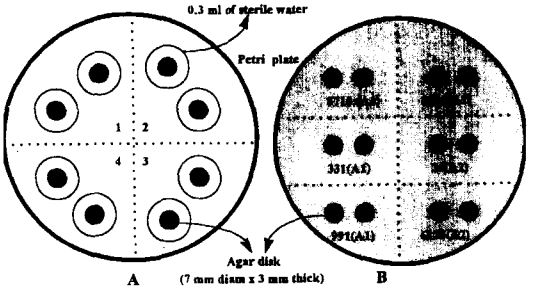

Fig. 1. Agar disk methods for sporangia and oospores production of *Phytophthora*. A; Sporangia were formed after 12~48 h at 20℃ under light. B; Oospores were formed after 7 days at 20℃ in dark. Agar disks of an apple isolate were laid on the bottom and a PC membrane was covered the disks before paired with known mating types of *Phytophthora*: *P. capsici* 8710 and 8812; *P. cryptogea* 331 and 36; *P. parasitica* 991 and 6134.

Oospore formation of heterothallic isolates. To induce sexual reproduction of heterothallic isolates, both A1 and A2 mating types of *P. capsici* 8710 (A1) and 8812 (A2), *P. cryptogea* 331(A1) and 36 (A2) and *P. parasitica* 991 (A1) and 6134 (A2) were used. While the isolates of *P. capsici* and *P. cryptogea* were originated from pepper and gerbera in Korea (8), isolates 991 and 6134 of *P. parasitica* were originally supplied by Dr. G. A. Zentmyer and by Drs. P. J. Ann & W. H. Ko, respectively (7). Five-day-old cultures of the apple isolates and 7-day-old cultures of above six isolates growing on 20% clarified V8 agar were cut by a cork borer (7 mm in diam.). Twelve agar disks of an apple isolate were distributed in a petri plate (Fig. 1-B) and a sterilized polycarbonate membrane (PC MB 90 mm, 0.2 μm, Nucleopore Co., USA) was placed on the top of the agar disks. Each two agar disks of A1 and A2 mating types of the three species were laid upside down on the top of disks of the testee isolates. The PC membrane was used to avoid hyphal contact between oospore inducers and oospore producers. After incubation at 20℃ for 2 wks in dark, the PC membrane was removed along with the disks on the top. At least 20 oospores formed on bottom agar disks of the testee were examined under a light microscope at 100 or

200x.

Pathogenicity test. A small agar disk (2 mm thick) made by a cork borer (5 mm in diam) from 7-day-old cultures of Pb-2, 6, 9 and 36 grown on 10% clarified V8 agar was inoculated to wounded fruits of apple, pear, peach, plum, orange, lemon and kiwi, and vegetable fruits of eggplant, pepper, potato, cucumber, zucchini, melon, tomato, pumpkin and watermelon. Isolates Pb-2 and 9 were isolated from young fruits showing pale brown rot and dark brown rot, respectively. Whereas, isolates Pb-6 and Pb-36 were obtained from each a blighted shoot and rotten basal stem tissues. Sizes of inoculated fruits of apple and pear were ca. 3 cm in diameter, which were about the same age of the fruits infected in the fields. After incubation at 25℃ for 4 days in dark, degree of the rot developed on the fruits was rated arbitrarily. Young branch cuttings of apple cvs. Fuji and Tsugaru, pear cvs. Niitake and Chojuro and peach cv. Okubo were inoculated by zoospores of Pb-6 and Pb-9. Zoospores prepared by similar procedures as described above were adjusted to ca. 100 zoospores/m*l*. Each 10 m*l* of the suspensions was sprayed evenly to a branch before incubation in a growth chamber at 25℃ for 4 days. Pathogenicity of the isolates was examined arbitrarily based on the degree of symptoms developed on the leaves and shoots. At least two fruits and three branch cuttings of each cultivar were used in this experiment. Each 10 m*l* of sterilized water was sprayed as the same manner as control.

RESULTS

Occurrence and symptoms. The young fruit rot

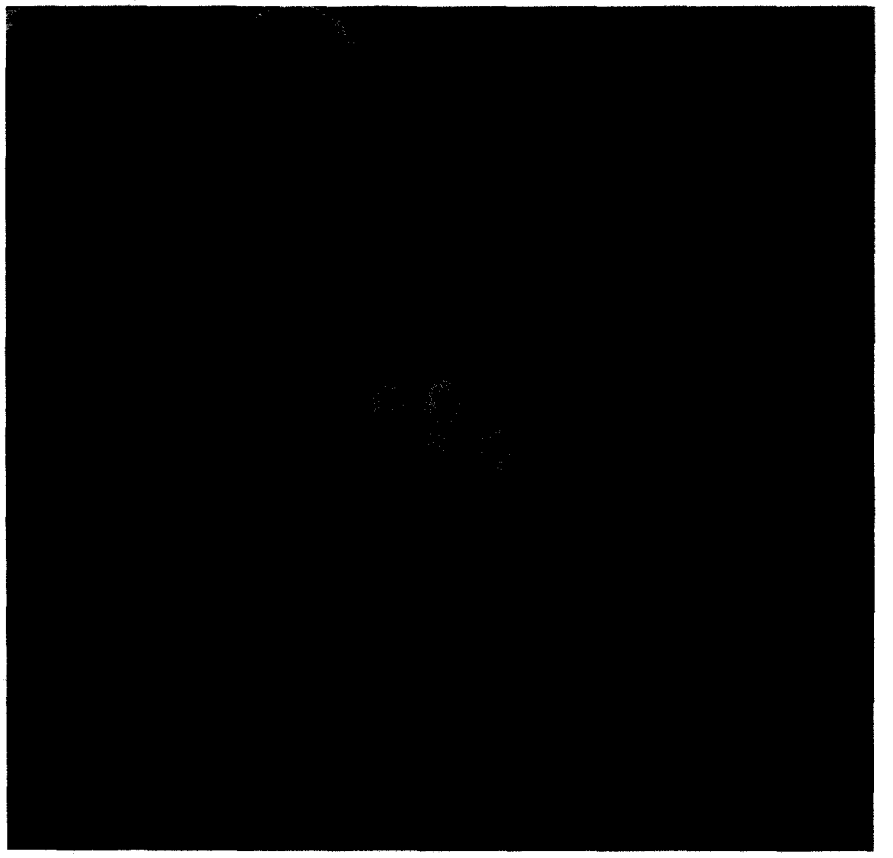

Fig. 2. Symptoms of young fruit rot, leaf spot and shoot blight of apple caused by *Phytophthora*. A; An infected young fruit on the tree. B; Marbled dark brown rot caused by *P. cactorum* (lower row) and pale brown rot caused by another species of *Phytophthora* (upper row). C; Leaf spot caused by *P. cactorum*. D; Shoot blight caused by another species of *Phytophthora*.

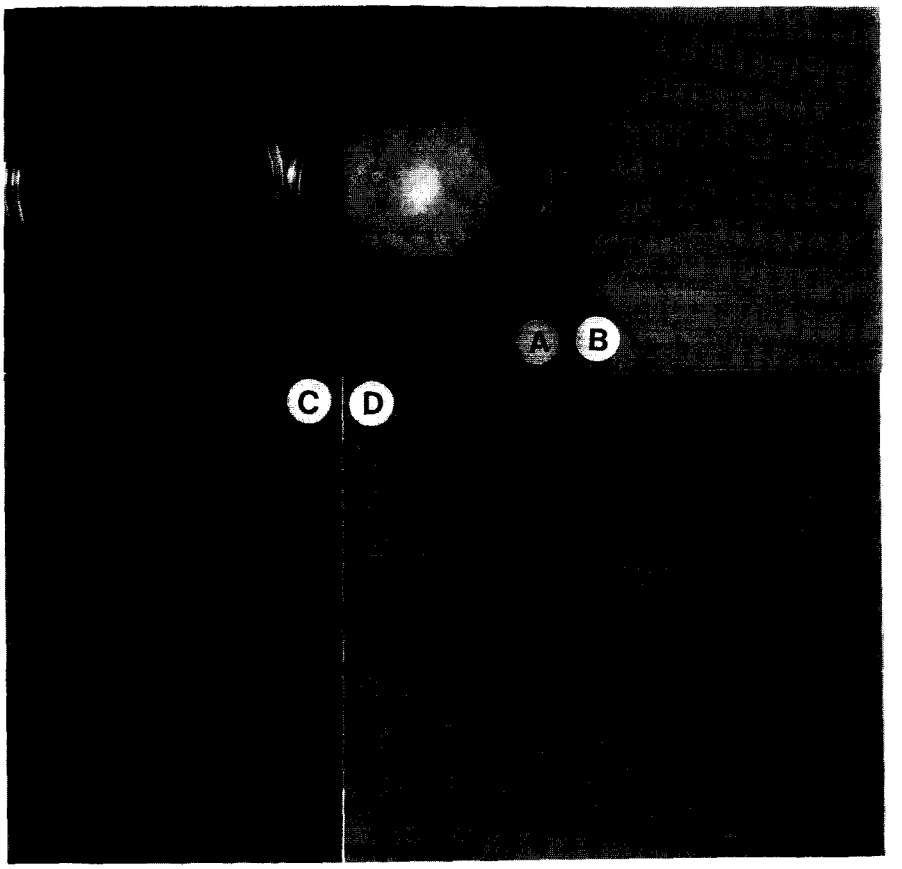

Fig. 3. Morphological characters of the *Phytophthora cambivora* caused pale brown rot on young fruits and shoot blight of apple. A; Colony patterns of *P. cactorum* (left) and *P. cambivora* (right) on 10% clarified V8 agar, B; Internally proliferated sporangiophore, C; Sporangia formed in water (left) and nested in a husk (right), D; Plerotic oospore with elongated 2-celled antheridium. Note that a markedly bullate oogonial wall (bottom right).

of apple first appeared in late May in Andong and spread to Uisung and Chungwon areas until early July of 1996. The disease occurred at more than 30 apple orchards in the areas. The rot was observed not only on low hanging fruits but also high hanging fruits, over 2 m above from the ground (Fig. 2-A). In some heavily infected orchards in Andong area, percentage of infected trees and the fruits was over 90 and 15%, respectively. The fruits were ca. 1-month-old and sizes were ca. 2.5~3.5 cm in diameter. Infected fruits showed either uniformly pale brown (upper row of Fig. 2-B) or marbled dark brown (lower row of Fig. 2-B) with diffusing margins of the rot. The rots were firm and look alike scalded. Wax layers of the fruits showing pale brown rot were readily peeled off when severely infected (upper row of Fig. 2-B). Spots and water soaking lesions on leaves (Fig. 2-C) and blights on shoots (Fig. 2-D) were also observed in the same fields. These symptoms developed only on young leaves and growing shoot tips.

Isolation of the pathogen. Two morphologically distinctive types of *Phytophthora* were constantly isolated from infected fruits, shoots and leaves. Among 39 isolates collected from several areas, 25 isolates were obtained from the fruits showing marbled dark brown rot and leaf spot. The others were isolated from the fruits showing pale brown rot and the blighted shoot. While the former fungus (left plate in Fig. 3-A) was collected from Andong, Uisung and Chungwon areas, the latter fungus (right plate in Fig. 3-B) was collected only from Andong area.

Cultural and morphological characters. All in-

Table 1. Characteristics of asexual and sexual reproduction structures of apple isolates of *Phytophthora* caused pale brown rot on young fruits and shoot blight of apple

Investigated characteristics	Mycological characteristics of Pb-2 and Pb-6
Sporangium	Produced only in water, single, nonpapillate, ovoid to obpyriform, noncaducous, internally proliferated, rounded base, size: 34~80×32~44 (av. 58.8×40.4) μm
Sporangiophore	Single, no branch, thin (<4 μm) and long, internally proliferated
Sexuality	Heterothallic, A1
Oogonium	Spherical, ornamented (bullate), 32~48 (av. 40.2) μm
Oospore	Plerotic, spherical, 33~44 (av. 36.5) μm
Antheridium	All amphigynous, 2 celled, elongated and long: 18~32 μm

[a] Pb-2 and Pb-6 were isolated from a young fruit showing pale brown rot and a blighted shoot, respectively.

vestigated characters of 25 isolates of the former fungus were well matched with *P. cactorum* which was identified as the causal pathogen of the collar rot of apple in our previous work (9). Consequently, characters of the latter fungus were discussed here. The fungus produced fluffy aerial mycelia and fairly uniform hyphae on the media (Table 1). Optimum temperature for mycelial growth of the fungus was 25~28 ℃ and none of the isolates grew over 35℃ or under 8 ℃. Sporangiophores sometimes were formed internally (Fig. 3-B). Nonpapillate sporangia were formed solely at the end of a thin (ca. 4 μm in diam.) and long sporangiophore only in water but not on agar (Fig. 3-C, left). Sporangia which were ovoid to obpyriform sometimes nested in sporangial husks (Fig. 3-C, right), and measured as 34~80×32~44 (av. 58.8×40.4) μm. Hyphal swellings were not formed abundantly even in water, however, a few rounded or irregular swellings were found in some cultures. Chlamydospores were not observed in all isolates.

Sexual reproduction. While the former fungus, *Phytophthora cactorum*, was homothallic, the latter fungus was heterothallic. Because all isolates formed oospores only when mated with A2 types of *P. capsici*, *P. cryptogea* and *P. parasitica*, they were A1 mating types. Spherical oogonia were light yellow to brown and measured 32~48 (av. 40.2) μm. About a half of the oogonial walls were distinctly bullate (Fig. 3-D). Oospores nearly filled oogonia (plerotic) were spherical and measured 33~44 (av. 36.5) μm in diam. All antheridia were amphigynous and either 1-celled or 2-celled. Elongated antheridia were ranged between 18 and 32 μm in length (Fig. 3-D). An isolate Pb-6 produced maximum number of oospores at 20℃ and next 15, 10, 25, and 30℃ in decreasing order when mated with A2 mating type of *P. capsici* 8812 (Table 2). However, the fungus did not form oospores at 5 and 35 ℃.

Table 2. Effects of temperature and interspecies matings on oospore production of an apple isolate Pb-6

Temperature (℃)	Pb-6 was paired with					
	P. parasitica		*P. capsici*		*P. cryptogea*	
	991 (A1)	6134 (A2)	8710 (A1)	8812 (A2)	331 (A1)	36 (A2)
5	0[a]	0	0	0	0	0
10	0	7	0	18	0	0
15	0	19	0	31	0	4
20	3	23	0	74	0	7
25	0	11	0	14	0	5
30	0	3	0	7	0	2
35	0	0	0	0	0	0

[a] Number of normal oospores of the apple isolate Pb-6 produced in one ml of 20% clarified V8 juice agar. Values are means of 3 replicates.

Pathogenicity of the fungi. Both fungi showed similarly strong pathogenicity to branch cuttings of the fruit trees tested (Table 3). However, isolates of *P. cactorum* revealed higher pathogenicity to apple cv. Tsu-

Table 3. Pathogenicity of apple isolates of *Phytophthora* to young branch cuttings of apple, pear and peach

Tested plants	Variety	Inoculated isolate[a]			
		P. cactorum (Pb-9)		*P. cambivora* (Pb-6)	
		LWSL[b]	Shoot blight	LWSL	Shoot blight
Apple	Fuji	+++[c]	++	++	+
	Tsugaru	++	+	+	+
Pear	Niitake	+++	++	++	++
	Chojuro	+	+	+	+
Peach	Okubo	+	+	+	+

[a] Pb-9 and Pb-6 were isolated from young apple fruits showing marbled dark brown rot and pale brown rot, respectively.

[b] LWSL: water soaking lesions on leaf.

[c] Severity of the disease: +; weak, ++; moderate, +++; severe.

Table 4. Pathogenicity of the apple isolates Pb-9 and Pb-6 of *Phytophthora* to fruits and vegetable fruits by wound inoculation

Fruit	Inoculated isolate[a]		Vegetable fruit	Inoculated isolate	
	Pb-9	Pb-6		Pb-9	Pb-6
Apple	++[b]	+++	Eggplant	+	-
Pear	+++	+++	Pepper	-	-
Peach	++	++	Potato	++	+
Plum	++	++	Cucumber	-	-
Orange	+	+	Zucchini	-	-
Lemon	-	-	Melon	-	-
Kiwi	-	+	Tomato	+	+
			Pumpkin	+	-
			Watermelon	+	+

[a] Pb-9 and Pb-6 were representative isolates of *P. cactorum* and *P. cambivora*, respectively.

[b] Degree of the rot developed on fruits: -; no, +; weak, ++; moderate and +++; severe, respectively.

garu and pear cv. Niitake than the others. Fuji apple and Niitake pear were the most susceptible and Okubo peach was the least susceptible to both fungi among tested. Young leaves were more vulnerable than shoots of the branch cuttings in general. Both fungi also showed strong pathogenicity to fruits of apple and pear, moderate to peach and plum, and weak to orange, potato and watermelon. While the isolate of *P. cactorum* Pb-9 showed pathogenicity to lemon, eggplant and tomato, the other isolate did not infect those vegetable fruits (Table 4). However, pepper, cucumber, zucchini, pumpkin and orientalmelon were not infected by the isolates even by wound inoculation (Table 4).

DISCUSSION

The fungus caused marbled dark brown rot on young fruits and leaf spots was identified as *Phytophthora cactorum* (2, 9, 13, 17). Since morphological characteristics of the fungus was examined in our previous work (9), the fungus was readily identified as the same species of the causal agent of collar rot of apple. Therefore, it is considered that the fungus is the most important and widely distributed apple pathogen in the genus of *Phytophthora* in Korea as also in many other countries (2, 5, 9, 10).

Mycological characters of the other 14 isolates causing pale brown rot on young fruits and shoot blight of apple were agreed well with *Phytophthora cambivora* described by Erwin & Ribeiro (2), Ho (6), Stamps et al. (13) and Waterhouse and Waterson (16). Since the fungus produced nonpapillate and internally proliferated sporangia and amphigynous antheridia, it belongs to *Phytophthora* group VI (13). Among species in the group *P. cambivora* is morphologically similar to *P. cinnamomi* (2, 6). However, the former differs in that it did not produce coralloid-type mycelia and abundant big chlamydospores. In addition, conspicuously ornamented (bullate) oogonium produced by the fungus was clearly different from that of *P. cinnamomi* (2, 6, 13). Based on these characters, the fungus was readily identified as *P. cambivora*.

Sporangia of the fungus were readily formed in water within 12 to 48 h by the agar disk method used in this experiment. Since sporangial characters of each two replicates of four isolates could be examined in a petri plate, the technique was economical and time saving. The agar disk method used for sexual reproduction in heterothallic isolates was also very convenient because mating types and oosporangial characters of the testee isolate could be examined by both A1 and A2 mating types of the three species of *Phytophthora* in a petri plate at one time. Since the PC membrane prevented hyphal contact between the testee isolate and the mating type cultures, oospore formation of the apple isolate by selfing was stimulated chemically. Such oospore inducing chemicals produced by A1 and A2 isolates were proved as sex hormones and designated by Ko (11) as $\alpha 1$ and $\alpha 2$, respectively. Sex hormone $\alpha 1$ produced by A1 type only stimulate oospore reproduction of A2 but not A1 isolates, and *vice versa*.

Phytophthora cambivora is not only well known as the causal pathogen of the ink disease of chestnut worldwide, but also causes root or trunk rot of many fruit trees, namely; apple, maple, avocado, walnut, cherry, almond, apricot, peach, elm, and plum (2, 10, 12, 14, 16). However, fruit rot of apple either on the tree or in storage, especially on young fruits, caused by the fungus has not been reported. It is considered that heavy rainfall during the rainy season, which started earlier than previous years in early June of 1996 in the areas, was encountered with high inoculum potentials built-up in the orchards to cause an outbreak of the disease. Since the fungus showed strong pathogenicity to young fruits, leaves, and shoots of pear as well as apple in the pathogenicity test, sporadic outbreak of the disease possibly could occur in the fields on pears when environmental conditions are favorable. On the

other hand, it is not likely that the fungus cause diseases on vegetables since most vegetable fruits tested were not infected even by wound inoculation.

요 약

1996년 5월 하순부터 7월 초순사이 경북 안동, 의성, 충북 청원 지방의 30개 이상의 사과 과수원에서 어린 과일에 역병이 대발생하였는데, 발병된 과수원에서는 이병주율과 이병과율이 각각 10~90%와 1~15% 정도였다. 이병된 어린 과일은 연한 갈색 혹은 진한 갈색으로 썩고 단단하였으며 병반은 건전부위로 경계가 불명확하게 확산되었다. 어린 잎이나 신초 역시 역병균에 감염되면 반점이나 수침상 혹은 마름 증상 등을 심하게 나타내었는데 이들로부터 총 39개 역병 균주를 분리하였다. 이들 중 *Phytophthora cactorum*으로 동정된 25개 균주는 모든 발생지역에서 분리되었으나 *P. cambivora*로 동정된 나머지 14개 균주는 안동지역에서만 수집되었다. *P. cambivora* 균주의 유성생식형은 모두 A1으로 조사되었으며 이들의 균학적 특성은 본문에 자세히 기록하였다. 본 실험에서 Koch의 가설이 증명되었으며, 두 균 모두 사과뿐 아니라 배와 복숭아의 어린 가지와 과일에 강한 병원성을 보였고 여러 채소과일에는 병원성이 없거나 아주 미약하였다. 사과 어린 과일 역병의 대 발생은 국내에 기록된 바가 없으며, 특히 *P. cambivora*에 의한 사과 과일역병의 발생은 세계적으로도 보고된 바 없다.

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