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Phytophthora Diseases of Apple in Korea: I. Occurrence of a Destructive Collar Rot Caused by *P. cactorum*

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사과의 역병: I. *Phytophthora cactorum*에 의한 줄기역병의 발생

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ABSTRACT: A destructive collar rot of apple caused by a species of *Phytophthora* has widely occurred in Kyungbuk and less extended in Chungbuk, Chugnam and Chunbuk provinces of Korea. Significantly higher incidence of the disease was observed on cv. Fuji when M26 or M9 was used as dwarfing stocks. Incidence of the disease at several orchards in Uisung, Kunwi, Yesan and Muju ranged from 45 to 80%. Twenty-five isolates of the causal fungus were collected and all isolates were identified as *P. cactorum* on the basis of their cultural and morphological characters. The fungus produced markedly papillate and broadly ovoid deciduous sporangia both on agar and in water, and a short pedicel was attached to each sporangium. Oospores were readily formed on clarified V8 agar by single isolates and all the antheridia were paragynous. The fungus neither grew nor produced oospores under 5 and over 33℃. The destructive collar rot of apple caused by *P. cactorum* has not been reported in Korea previously.

***Key words*: apple, collar rot, *Phytophthora cactorum*, identification.**

A number of *Phytophthora* species have been reported to attack fruit, root, crown and trunk of apple (1, 3, 9). *Phytophthora* diseases of apple in virtually occur at all apple growing regions around the world (1, 3, 7, 9, 14), and are one of the most important soilborne diseases of apple. It has been considered that various *Phytophthora* diseases of apple also occur in Korea and the fruit rot caused by *P. cactorum* is already listed (13). However, researches on the diseases or causal fungi in the genus are rare in Korea. Recently, Lee et al. (8) reported that *Phytophthora* was one of the causal agents of soilborne diseases in apple, however, neither the disease nor the causal fungus was specifically described in their study.

Among the *Phytophthora* species infecting apple, *P. cactorum* and *P. syringae* are the most frequently reported major pathogens causing the diseases in many countries (1, 3, 7, 9). While the former fungus is distributed in temperate regions of the world, the latter fungus has limited distribution in cool areas of Europe, North America, New Zealand and Japan (1, 3, 7, 9). Although *Phytophthora* diseases of apple have distinctive features, they are often confused with other soilborne diseases or unidentified causes such as sour sap, winter injury or wet feet (1, 2, 7). Therefore, isolation of the causal pathogen is essential for diagnosis of the *Phytophthora* diseases (3, 7) since all known species are plant pathogenic and primary causal agents but not secondary microbes (1, 2, 9).

During a survey on apple diseases in 1995 and 1996, we frequently observed a destructive collar rot of apple caused by a species of *Phytophthora*. The disease spread to many apple growing areas and it seemed to be a threat to apple cultivation in Korea. We investigated the disease throughout the country and identified the causal pathogen in order to provide clues to solve the problem.

MATERIALS AND METHODS

Disease survey. A survey on *Phytophthora* diseases

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of apple was conducted in 1995 and 1996 at Andong, Uisung, Kunwi, Chungwon, Chincheon, Yesan and Muju areas located in four provinces. Another survey on the disease throughout the country was performed in cooperation with extension workers at the Rural Development Administration at the respective areas from Oct. 17 to Nov. 20 of 1996. Declining apple trees associated with typical collar rot on basal stem were considered as affected by the disease.

Isolation of the causal pathogen. Direct isolation of the causal fungus was carried out in apple orchards using a water agar and a semi-selective medium for *Phytophthora*. Small pieces (2~3 mm^3) of the newly infected inner tissues of an apple tree were cut by a scalpel after removal of the bark. The pieces were placed on the media without surface disinfection and incubated for 2~3 days at 25℃. Growing mycelial tips were cut and transferred to 10% clarified V8 agar for further studies. The semi-selective medium used in this study consisted of corn meal agar (CMA; Difco, 17 g/L) was supplemented with 100 ppm ampicillin, 50 ppm nystatin and 10 ppm pentachloronitrobenzene (PCNB). The 10% clarified V8 juice was prepared by mixing 5g of $CaCO_3$ to 163 m*l* of the juice (Campbell,

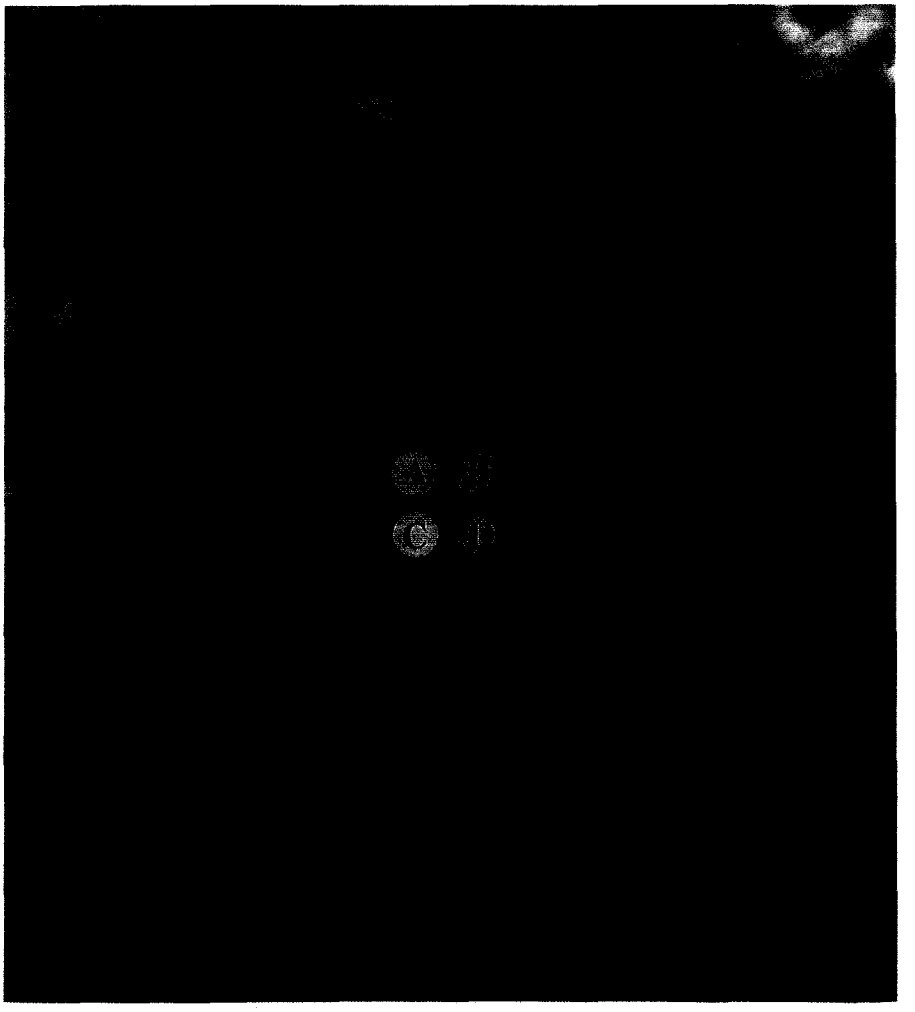

Fig. 1. Symptoms of the collar rot of apple and features of the causal fungus. A: A collar rot shown on the basal stem initiated near the soil-line and progressed upward. B: Decayed inner phloem tissues are clearly distinguished from the healthy tissues. C: Sporangia of the causal fungus; broadly ovoid, deciduous, papillate and close sympodial. D: Oospores of the fungus formed by a single isolate; note that antheridia are paragynous.

USA) prior to centrifuge at 7,000 rpm for 20 min. Deionized water and 18 g of agar powder were added to 100 m*l* of the supernatant of the juice to make 1000 m*l* of 10% clarified V8 agar.

Investigation of mycological characters. All isolates were cultured on the 10% clarified V8 agar for 7 to 14 days under light and in dark at 20℃ to examine sporangial production on agar and oospore formation by single isolates. To investigate the sporangial characters, 7-day-old cultures growing on a 10% clarified V8 agar were cut into small pieces (ca. 10×10 mm). The agar blocks were soaked into water in a petri plate and incubated at 25℃ under light for 24~48 hours. Sporangia formed in water were agitated by a Vortex mixer to examine the caducity. Sporangia and oospores formed on the agar or in water were observed under a microscope either directly or after transferred to a slide glass. At least 20 sporangia and oospores each of an isolate were examined.

RESULTS

Symptoms of the collar rot. The collar rot shown on the basal stem of apple trees initiated at the grafting site of dwarfing rootstocks near the soil line and progressed upward (Fig. 1-A). Rotting lesions of severely infected trees were readily recognized by their dark brown to black color appeared on the outer layers of bark. The rot nearly girdled the basal stem when heavily infected (Fig. 1-A). Inner phloem tissues showing reddish brown or brown discoloration were also decayed and margins of the lesion were clearly distinguished from the healthy tissues (Fig. 1-B). However, the symptoms on recently infected trees were merely visible only when the bark was removed. Generally foliar symptoms were not readily diagnostic, although leaves of some infected trees turned yellow and defoliated earlier than those of healthy trees.

Disease survey. Collar rot of apple was observed in four provinces and 15 areas located at the central part of South Korea: Kimcheon, Andong, Kumi, Youngju, Youngcheon, Kunwi, Uisung, Chungsong, Yecheon, Bonghwa in Kyungbuk; Chincheon and Chungwon in Chungbuk; Yesan and Youngdong in Chungnam; Muju in Chunbuk (Fig. 2). However, the disease has not been observed in other provinces; Kyunggi, Kangwon, Kyungnam, Cheonnam and Cheju. Incidence of the collar rot reached as high as 80% at a few fields in Uisung, and several fields in Uisung,

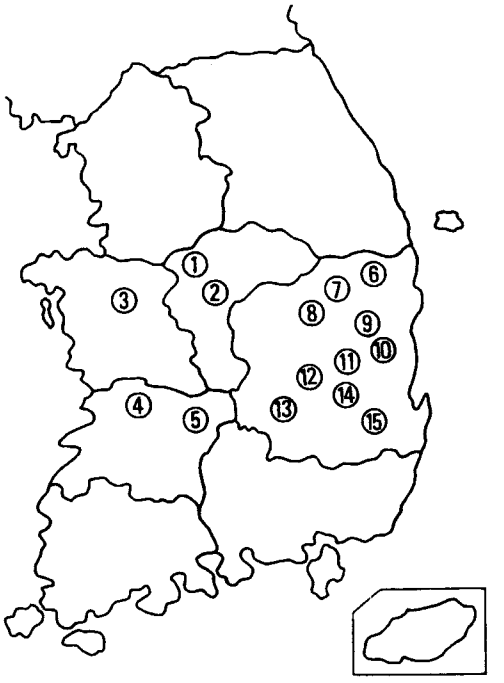

Fig. 2. Distribution of the collar rot of apple caused *Phytophthora* sp. in Korea. Circles indicate where the disease has observed: ① Chincheon, ② Chungwon, ③ Yesan, ④ Iksan, ⑤ Muju, ⑥ Bonghwa, ⑦ Youngju, ⑧ Andong, ⑨ Yecheon, ⑩ Chungsong, ⑪ Uisung, ⑫ Kumi, ⑬ Kimcheon, ⑭ Kunwi, ⑮ Youngcheon.

Kunwi, Yesan and Muju showed over 45% infection (Table 1). Majority of the infected trees were 10 to 20 years old cv. Fuji, however, cv. Tsugaru was also infected. *Phytophthora* fruit rot of apple was insignificant in the fields. It was observed rarely or quite commonly in some orchards at most apple growing areas, although the collar rot was not found in the fields (Table 1).

Identification of the causal pathogen. Morphological characters of *Phytophthora* isolates collected from various areas are summarized in Table 2. All isolates grew well on 10% clarified V8, potato dextrose agar (PDA) and corn meal agar (CMA). The isolates grew maximally around 25~27℃ and did not grow under 5 and over 33℃. While hyphae of the fungus were uneven and irregular, growth pattern was rather uniform and no aerial mycelia were produced on 10% clarified V8 agar and CMA. Sporangia were readily formed on agar and more abundantly in water (Fig. 1-C). Oospores were also formed abundantly by single isolates (Fig. 1-D). Sporangia easily fallen off from

Table 1. A survey on *Phytophthora* collar rot and fruit rot of apple cvs. Fuji and Tsugaru at major growing areas in Korea[a]

Field location		No. of fields investigated	Incidence of collar rot(%)	Severity of fruit rot[b]	Age of the trees(year)
Province	County				
Kyungbuk	Andong	15	0~17	++	7~18
	Uisung	9	10~80	++	6~15
	Kunwi	2	40~70	+	8~12
	Yecheon	5	0~25	+	11~24
Chungbuk	Chungwon	3	0~10	+	10~20
	Chincheon	2	2~10	++	7~20
Chungnam	Yesan	2	3~50	+	8~13
Chunnam	Muju	1	45	++	7~25

[a] The survey was conducted from Sep. 1995 to Nov. 1996.
[b] Fruits either on the tree or the ground (fallen): +; observed scarcely, ++; observed quite commonly.

Table 2. Characteristics of asexual and sexual reproduction structures of the apple isolates of *Phytophthora* causing the collar rot of apple

Investigated	Morphological characters of reproduction structures
Sporangium	Produced on agar and in water, papillate*, caducous*, broadly ovoid*, attached a short pedicel* (ca. 4 μm), >1 papilla, laterally attached, 32~56×24~34 (av. 40.6×28.3) μm
Sporangiophore	Aerial on agar, simple close and unichasial sympodia*
Sexuality	Homothallic* (abundantly produced by single isolates)
Oogonium	Spherical, smooth, 20~32 (av. 24) μm
Oospore	Plerotic or aplerotic, 18~28 (av. 24) μm
Antheridium	Paragynous*, 1-celled, spherical or irregular club shape
Chlamydospore	Rare, spherical, 32~40 (av. 35) μm
Others	Hyphae uneven and irregular
	No aerial mycelia
	Uniform cultural pattern
	Temperatures: opt. 25~27℃, min. 8℃, max. 33℃

*Distinguishing characters of the fungus from other species in the genus of *Phytophthora*.

sporangiophores attached a short pedicel (ca. 4 μm) to each. Sporangia, measured 32~52×24~34 (av. 40.6×28.3) μm, were broadly ovoid, markedly papillate, scarcely 2 apexed and laterally attached. Sporangiophores branched close sympodially or unichasially. Antheridia were 1-celled, spherical to irregular club shaped and predominantly paragynous. Spherical and smooth oosporangial walls were measured 20~32 (av. 29) μm. Oospores were either plerotic or aplerotic and measured 18~28 (av. 24) μm. Spherical chlamydospores were not abundant, however, it was formed on the agar and measured 32~40 (av. 35) μm.

DISCUSSION

The morphological and cultural characters of the causal fungus of the collar rot of apple agreed well with *Phytophthora cactorum* described by different workers, Erwin and Ribeiro (1), Ho (5), Ho et al. (6), Stamps et al. (12) and Waterhouse and Waterston (14). Since the fungus produced conspicuously papillate sporangia and predominantly paragynous antheridia, it belongs to *Phytophthora* group I (12). *Phytophthora cactorum* can be readily distinguished from other species in the group by the caducity of broadly ovoid sporangia with short pedicels and abundant oospores formed by single isolates with paragynous antheridia (1, 12). The fungus has been known to attack more than 200 plant species in 150 genera of 60 plant families (1, 2, 9). However, as per the previous report (10), it has been recorded only to cause the root rot of ginseng and the fruit rot of apple in Korea.

The term 'collar rot' has been often confused with crown rot or root rot of apple. But, the disease ob-

served by the authors was obviously the collar rot because the disease was initiated at the dwarfing stock (or interstock) grafted on the lower trunk at or above the soil line and progressed upward (3, 7). The most commonly using dwarfing stocks in Korea M26 and M 9 were recently reported as highly susceptible to *P. cactorum* and *P. cambivora* in Japan (10, 11). Therefore, it is considered that the susceptible stocks need to be replaced with resistant varieties to prevent the *Phytophthora* diseases on apple.

The collar rot of apple has occurred at 15 counties in 4 provinces located at the central part of South Korea. However, the disease has not been found yet in other 5 provinces in southern and northern parts of the country (Fig. 2). Although the collar rot caused by *P. cactorum* has not been recorded previously, it is probable that the disease has long been existed rather than introduced recently because the apple has been cultivated commercially for about a hundred years and the fungus was recorded several decades ago in Korea (13). It is also considered that the fungus is distributed more widely than investigated since the *Phytophthora* rot on fallen fruits in the ground was observed at a number of orchards although the collar rot was not yet found in the fields.

Since the isolation of the causal fungus is essential for diagnosis of *Phytophthora* diseases (1, 2, 7), the direct isolation method of the causal fungus used in this study greatly facilitated to identify the disease. Detection frequency of the pathogen using the selective medium from freshly infected tissues in the fields without sterilization was much higher than using a water agar or the selective medium with sterilization in the lab (data not shown).

In conclusion, the collar rot of apple is becoming a threat to safe cultivation of apple and the causal pathogen *P. cactorum* has widely spread to many apple growing areas in Korea. Therefore, more detailed researches on the disease, the causal pathogen and control measures remain to be investigated for safe cultivation of apple in Korea.

요 약

사과 줄기 밑둥을 썩히는 역병(collar rot)이 경북의 사과 재배지역에 널리 발생되고 있으며, 충북, 충남, 전북 등의 일부 지역에서도 발생이 확인되었다. 이 역병은 주로 M26이나 M9를 왜성대목으로 사용한 후지 품종에 심하게 발생되었는데, 발병율은 포장에 따라 큰 차이를 보이지만 의성, 군위, 예산, 무주 등지의 일부 포장에서는 이병주율이 45~80% 정도로 아주 높았다. 수집된 25 균주의 균학적 특성을 조사 결과 모두 *Phytophthora cactorum*으로 동정되었는데, 이들은 배지에서나 물 속에서 유두돌기가 뚜렷하며, 계란형의 쉽게 이탈되는 다량의 유주자낭을 형성하였고 짧은 자루(약 4 μm)를 부착하고 있었다. 자웅동주 균으로 10% clarified V8 배지에서 다량의 난포자를 형성하였는데 모든 장정기는 측착(paragynous) 하였다. 모든 공시 균주는 5℃ 이하와 33℃ 이상에서는 자라지 못하였으며 난포자도 형성되지 않았다. *Phytophthora cactorum*에 의한 사과 줄기역병은 국내에서 기록된 바가 없어 본 병해를 최초로 보고하는 바이다.

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REFERENCES

1. Erwin, D. C. and Riberio, O. K. 1996. *Phytophthora disease worldwide*. APS press, St. Paul, Minnesota, USA. 562 pp.
2. Erwin, D. C., Bratnicki-Garcia, S. and Tsao, P. H. 1983. *Phytophthora: its biology, taxonomy, ecology, and pathology*. APS press, St. Paul, Minnesota, USA. 392 pp.
3. Harris, D. C. 1991. The *Phytophthora* diseases of apple. *J. of Horticultural Science* 66(5) : 513-544.
4. Harris, D. C. 1987. Orchard trials on the control of apple collar rot. *J. of Horticultural Science* 62 : 441-447.
5. Ho, H. H. 1981. Synoptic keys to the species of *Phytophthora*. *Mycologia* 73 : 705-714.
6. Ho, H. H., Ann, P. J. and Chang, H. S. 1995. The genus *Phytophthora* in Taiwan. Institute of Botany, *Academia Sinica Monograph Series* No. 15, 86 pp.
7. Jones, A. L. and Aldwinckle, H. S. 1990. *Compendium of apple and pear disease*. APS press, St. Paul, Minnesota, USA. 100 pp.

8. Lee, Sang-Bum, Chung, Bong-Koo, Jang, Han-Ik, Kim Kee-Hong and Choi Young-Moon. 1995. Incidence of soil-borne diseases in apple orchards in Korea. *Korean J. Plant Pathol.* 11(2) : 132-138.
9. Ribeiro, O. K. 1978. *A source book of the genus Phytophthora.* J. Cramer, Lehre, Germany. 420 pp.
10. Soejima Junichi. 1996. Perspective and present status on the development of dwarfing rootstocks of apple. *Kongesnonoyaku* 40(7) : 26-29.
11. Soejima Junichi. 1996. Characteristics of the new dwarfing rootstocks of apple, JM1, JM7 and JM8. *Kongesnonoyaku* 40(12) : 24-27.
12. Stamps, D. J., Waterhouse, G. M., Newhook, F. J. and Hall, G. S. 1990. Revised tabular key to the species of *Phytophthora. Mycological Papers* No. 162.
13. The Korean Society of Plant Protection. 1986. *A list of plant diseases, insect pests, and weeds in Korea.* 2nd ed. 52 pp.
14. Waterhouse, G. M. and Waterston, J. M. 1966. *Phytophthora cactorum. CMI Descriptions of Pathogenic Fungi and Bacteria.* No. 111.

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