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Pink Root of Onion Caused by *Pyrenochaeta terrestris* (syn. *Phoma terrestris*)

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Pink root of onion occurred in the fields of the Onion Experimental Station and in the main onion cultivation area in Korea in 1998 and 1999, respectively. The casual fungus of pink root was isolated only from apricot agar. Formation of pycnidia and pycnidiospores of the fungus was highest in alternating cycles of 12 hours near ultraviolet light and 12 hours in dark condition. Its morphological characteristics and pigment formation on water agar were identical with that of *Pyrenochaeta terrestris*. The optimum temperature for the growth of the fungus and disease development was 25-28°C. When onion seeds were inoculated with the spore suspension, incubated in test-tube and sown in potted soil, disease symptoms developed in onion roots 7 and 30 days after inoculation.

Keywords **:** Pathogenicity, pink root of onion, *Pyrenochaeta terrestris*, sporulation.

Onion (*Allium cepa* L.) has been widely cultivated in the southern area of Korea as an important vegetable crop. Pink root of onion is known as one of the most devastating diseases in crops grown in warm climates and occurs worldwide in tropical or subtropical regions (Summer, 1995). Pink root was reported to occur in onions and garlic in Japan (Nishimura, 1986; Yamashita, 1991). However, the occurrence of this disease has never been reported in Korea. The disease was first found in the nursery of the Onion Experimental Station at Changryung, Kyungnam province in 1998 and occurred locally in the growers' field in 1999. The outbreak of the disease may have caused the infection of onion seedlings by the causal fungus in the nursery. Symptoms of the disease were similar to those of *Fusarium* basal rot. However, there was a distinct difference between pink root and symptoms caused by *Fusarium* species. This study was conducted to identify the causal agent of pink root and to confirm its pathogenicity.

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Materials and Methods

Isolation of the causal agent. Diseased roots of onion were washed with tap water for 1 hour and cut into 5 mm length. The diseased root pieces were washed again for 24 hours with running tap water and surface-disinfected with 0.5% NaOCl for 5 minutes. The root pieces were rinsed with sterilized water, cut into 2-3 mm length, and placed on apricot decoction agar (pH 5.0) at 20°C incubator. Mycelial tips of the fungal isolates grown on the medium were cut and transferred to potato dextrose agar (PDA) slants.

Characterization of the causal agent. To initiate pycnidial formation of the isolates, one per ten isolates was cultured on apricot decoction agar for 10 days in alternating cycles of 12 hours near ultraviolet light (NUVL) and 12 hours darkness, 24 hours NUVL, 24 hours fluorescent light, and 24 hours darkness. The morphological characteristics on the medium were examined by light microscope and compared with those of *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson described by Punithalingam and Holliday (1973). Twenty (20) conidia, pycnidia, and setae chosen randomly from each culture were observed and measured under light microscope. In addition, pigmentation of cheesecloth and agar by the fungus was investigated by putting the cheesecloth on water agar, placing diseased root tips on the cheesecloth, and incubating plates at 25°C incubator for 10 days.

Growth tests of the pathogen at different temperature, pH, and growth media regimes. Mycelial growth of the fungus at different temperatures (10°, 15°, 20°, 25°, 28°, 30°, 35°, 40°C), pH (4, 5, 6, 7, 8, 9, 10), and culture media (apricot media, PDA, malt agar, V8 agar, Czapek-Dox agar) was investigated by measuring each colony diameter of the isolates.

Pathogenicity test. Inoculum was obtained from pycnidia of 10-day-old colonies of the pathogen formed on apricot agar. Healthy onion seeds were soaked in spore suspension (10^6 spores/ml) for 24 hours and sown on agar in test tubes (4 cm diameter). Three seeds per one test tube were treated as described above. Half of the onion seeds were surface-disinfected before inoculation. Onion seeds inoculated were incubated at different temperatures (20°, 25°, 28° and 30°C) for 10 days, while color changes of onion roots elongating from the inoculated seeds, seed germination and pigmentation on agar were examined. In addition, pathogenicity test was conducted under potted soil condition as follows: inoculated onion seeds were sown in plastic pots ($10 \times 14 \times 7$

cm), then onion seedlings were grubbed up 30 days after sowing and examined for color change.

Results

Disease incidence and symptoms. Pink root of onion was first found in a nursery of the Onion Experimental Station at Changryung in 1998, with the disease incidence reaching up to 10%, and occurred locally in the growers' field in 1999. Symptoms appeared as early drying of leaves and discoloration of roots, at first light pink which turned deeper pink to dark purple in the late stages of the disease (Fig. 1). Infected onion bulbs were smaller than healthy ones.

Isolation and identification. A total of ten isolates of fungi were obtained from infected onion roots on apricot decoction agar with pH adjusted to 5.0. The isolates were confirmed as of the same fungal origin in terms of morphological and cultural characteristics. Pycnidia formation of the present isolates was best in alternating cycles of 12 hours NUVL and 12 hours darkness, followed by 24 hours NUVL and 24 hours fluorescent light radiation (Table 1). Meanwhile the present isolates did not form any pycnidia under 24-hour dark condition. The fungus produced dark brown pycnidia (globose to subglobose, 180-250 μm) with brown setae 70-170 μm long. Setae were produced primarily around an astiole. Pycnidiospores (4.5-5.5 × 1.5-2.0 μm) were hyaline, oblong ovoid, and oozed from

Table 1. Effect of illumination on pycnidial formation of *Pyrenochaeta terrestris* on apricot decoction agar

Radiation[a]	Formation of pycnidia[b]
NUV/Dark	+++
NUV	++
FL	+/–
Dark	–

[a] NUV/darkness, near ultraviolet/darkness (12h/12h); NUV, near ultraviolet (24h); FL, fluorescent light (24h); dark, darkness (24h).
[b] Investigated 10 days after incubation at 25°C. +++ = abundant, ++ = moderate, +/– = little or no formation.

Table 2. Comparison of the characteristics of the present isolate and *Pyrenochaeta terrestris* described in CMI Description of Pathogenic Fungi and Bacteria

Characteristics	*P. terrestris*	Present isolate
Conida		
Color	Hyaline	Hyaline
Shape	Ovoid to allantoid	Ovoid to allantoid
Size	4-7 × 1.5-2.0 μm	4.5-5.5 × 1.5-2.0 μm
Pycnidia		
Color	Dark brown	Dark brown
Shape	Globose	Globose
Size (diameter)	Up to 400 μm	180-250 μm
Setae		
Color	Brown	Brown
Size	60-180 μm	70-170 μm

Fig. 1. Symptoms of onion pink root. (**A**) Early drying of infected leaves; (**B**) Early harvested diseased onion bulbs in the field; (**C**) Typical symptom of onion pink root.

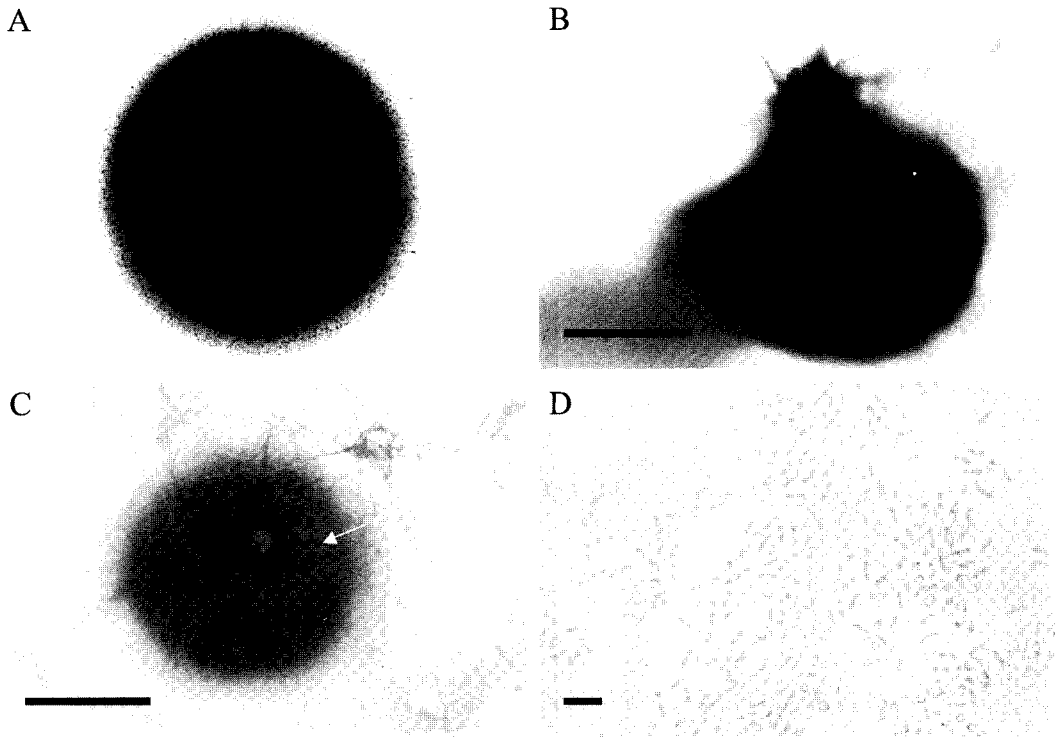

Fig. 2. Pycnidial formation of *Pyrenochaeta terrestris* on potato dextrose agar. (**A**) Pycnidia (arrow) formed on agar medium; (**B**) A pycnidium. Scale bar indicates 100 μm; (**C**) Setae (arrow) on a pycnidium. Scale bar indicates 100 μm; (**D**) Released pycnidiospores. Scale bar indicates 10 μm.

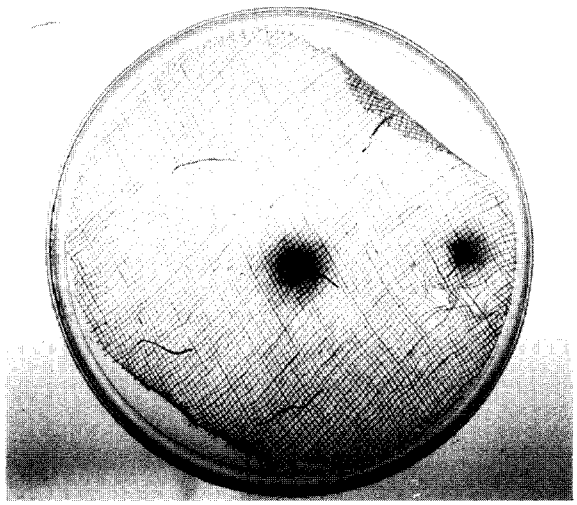

Fig. 3. Pigmentation of cheesecloth and agar by *Pyrenochaeta terrestris*.

rupture or through the ostiole (Table 2 and Fig. 2). The morphological characteristics of the isolates examined by the authors were consistent with those of *Pyrenochaeta terrestris* described by Punithalingam and Holliday (1973). When this fungus was cultured on water agar covered with cheesecloth, characteristic pink pigment was produced as shown in Fig. 3. The present isolates produced typical pigment on cheesecloth and agar, when the diseased root tips were placed on the cheesecloth on top of the water agar and incubating plates at 25°C incubator for 10 days. The present isolate was identified as *P. terrestris* based on the morphological characteristics mentioned above.

Cultural characteristics. The pathogen grew best on apricot agar media, followed by PDA, malt agar, V8 agar, and Czapek-Dox agar (Table 3). It grew well at temperature range of 20-30°C with optimum temperature of 25°C, and on media with broad pH range of 4.0-10.0 (Fig. 4). Optimal pH for mycelial growth of the fungus was 5.0-6.0.

Pathogenicity. When the onion seeds were inoculated with the spore suspension of *P. terrestris* by simple agar-test

Table 3. Mycelial growth of *Pyrenochaeta terrestris* on different agar media

Medium	Colony diameter (mm)[a]
Apricot decoction agar	40.4 a
Potato dextrose agar	37.0 b
Malt agar	36.4 b
V8 juice agar	32.9 c
Czapek-Dox agar	29.8 d

[a] Measured 5 days after incubation at 25°C. Values followed by same letters are not significantly different by DMRT at 0.05% level.

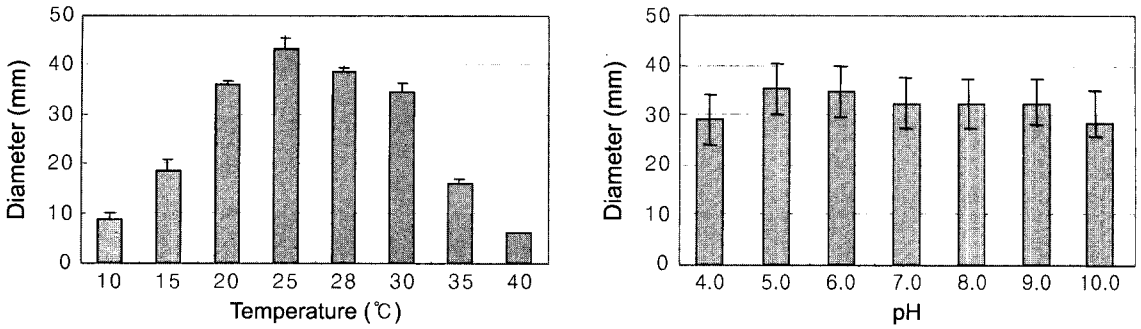

Fig. 4. Mycelial growth of *Pyrenochata terrestris* on potato dextrose agar at different temperatures (left) and pH (right).

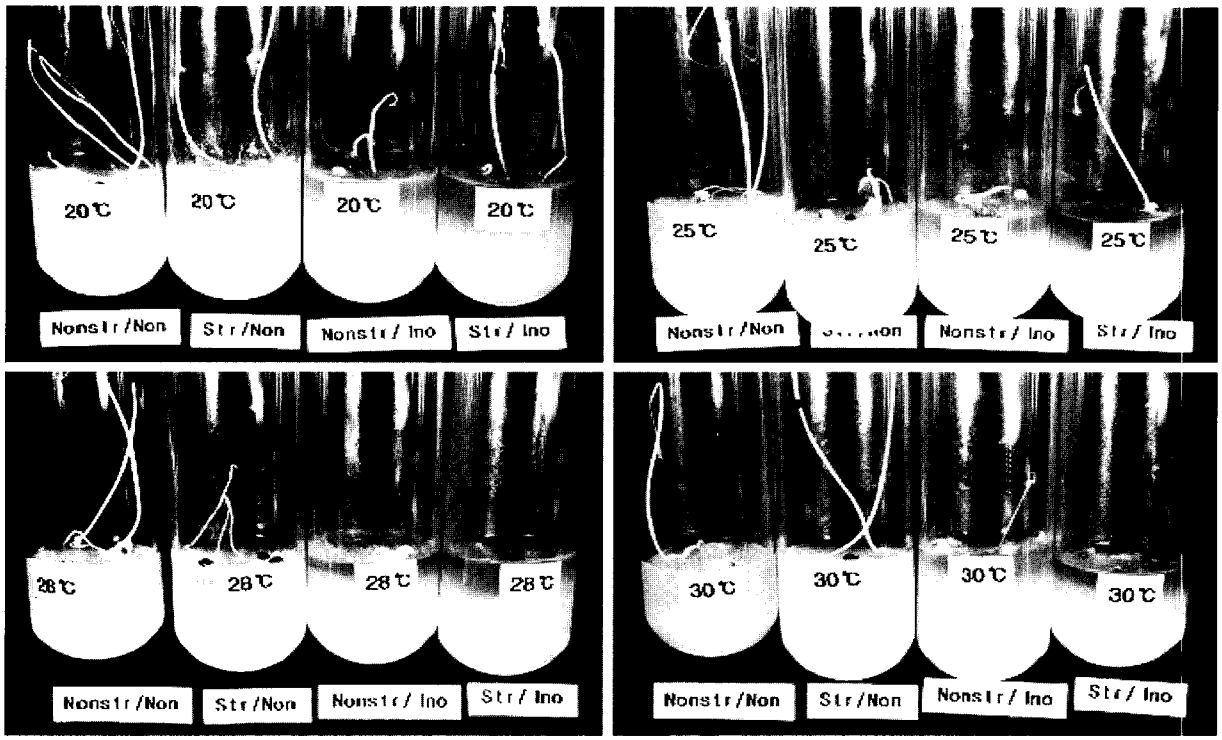

Fig. 5. Pathogenecity of *Pyrenochata terrestris* at different temperatures. Note: Nonstr, Non-sterilization of onion seeds; Non, non-inoculation of *P. terrestris*; Str, Sterilization of onion seeds; Ino, Inoculation of *P. terrestris*.

tube method as shown in Fig. 5 at different temperatures, three types of pathogenic characteristics were shown as follows: no germination and seeds were covered with white mycelium, pink root, and pink pigmentation on the agar. At 28°C, all inoculated onion seeds were infected by *P. terrestris* whether they were surface-disinfected or not, while all seeds did not germinate and the colors of agar plates changed into deep pink. At 25° and 30°C, one of the three inoculated onion seeds germinated, but all onion plants died or had pink root. At 20°C, although one to three onion seeds germinated, most of them also died or had pink root. At a temperature range of 20-30°C, all onion seeds inoculated with spore suspension of *P. terrestris* showed typical symptoms of pink root through simple agar-test tube method. Like in the test tube method, the pathogen also caused typical pink root symptom 30 days after sowing on inoculated onion seeds in plastic pots (data not shown).

Discussion

Onion pink root was reported to be caused by *Fusarium malli* by Taubenhaus and Johnson in 1917. Later, Sideris (1924) reported two new varieties of *Fusarium* which were associated with the disease. Hansen (1926) first reported that pink root of onion was caused by *Phoma* sp. based on morphological characteristics. He also confirmed that

typical "pink root" symptom followed by shriveling of invaded parts, were readily produced in roots of onion seedling grown in sterilized soil inoculated with *Phoma* sp.. Gorenz et al. (1948) isolated the casual agents causing pink root of onion and found the pycnidia to be setose in all cases. On the basis of the setose character of the pycnidia, they transferred the species to the genus *Pyrenochaeta* in 1948. In this paper, attempts were made to isolate the causal fungus by culturing surface-sterilized affected parts on water agar and PDA. *Fusarium* species were isolated from all cultured parts, while no isolates of *P. terrestris* were found. Through the method described by Morita (1995), *P. terrestris* was isolated as the causal fungus on apricot medium. The epidermal layer and the vascular elements of onion roots infected by *P. terrestris* may be easily separated as all the intervening tissues have been disintegrated. When roots are in this condition, they are easily invaded by other members of soil flora like bacteria and many species of *Fusarium* (Hansen, 1929). Hence, many saprophytic and pathogenic *Fusarium* species coexist with the casual agent on the diseased roots caused by *P. terrestris*. In addition, because they generally grow faster than *P. terrestris*, it is very difficult to isolate *P. terrestris* from the lesions of pinked roots. This study showed that proper culture medium with pH adjusted to 5.0-6.0 and alternating radiation of 12 hours near ultraviolet light and 12 hours darkness to isolate *P. terrestris* were needed. On pH-adjusted apricot agar, the present isolate grew well and formed abundant pycnidia and pycnidiospores. This confirmed the pathogenicity of *P. terrestris* by agar-test tube method. When the present isolate was inoculated on onion seeds at 20°, 25°, 28°, and 30°C, it caused the most severe symptoms at 28°C. Also, pigmentation on agar produced by the isolate varied depending on growth temperature. This results were consistent with that reported by Biles et al. (1992).

Pink root is a serious disease of onion bulbs. Pathogenesis of onion pink root was described as follows: the development of the characteristic pink discoloration of the root tissue followed by loss of tugor; disintegration of root cortical tissues; intensification of pigmentation; and ultimately complete impairment of root function resulting to root death. According to Ahmed et al. (1974), *P. terrestris* is present in moist soil, builds up rapidly with successive onion crops, and remains destructive for several years even when there are no more onions grown in the soil. Acreage of fields infested with *P. terrestris* seems to increase more and more in Korea. Recently, pink root in onion cultivation fields has been evident in Korea. Therefore, it is now necessary to establish strategies for controlling onion pink root in the country.

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