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Note

First Report on *Racodium therryanum* Associated with Seed Infection of *Abies koreana* in Korea

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***Racodium therryanum* from Korea is described here for the first time. This fungus is characterized by that it is isolated from conifer seeds and seedlings, including *Abies*, colonies are dark green to dark gray and intricate, and it forms chlamydospores in cultures. This pathogenic fungus was isolated from seeds of *Abies koreana* growing on Mt. Halla, Jeju island. The fungus stopped growth more than at 30°C but grew even at 0°C. This fungus infected seeds of *A. koreana* under the snow during winter season. There is a high probability that this fungus affects the natural regeneration of *A. koreana* on Mt. Halla.**

Keywords : *Abies koreana*, *Racodium therryanum*, seed infection, snow blight

The pathogenic fungus, *Racodium therryanum* causes serious snow blight diseases in conifers in the Tohoku district and Hokkaido, Japan (Sakamoto and Miyamoto, 2005). The disease is associated with significant seedling losses of *Thujopsis dolabrata* var. *hondai*, *Cryptomeria japonica*, *Pinus densiflora*, *Abies sachalinensis*, and *Picea jezoensis* under snow (Sato et al., 1960). *Picea glehnii*, *Picea jezoensis*, and *Larix kaempferi* seeds are attacked by *R. therryanum* under snow in natural stands (Cheng and Igarashi, 1990; Igarashi and Cheng, 1988). The germination rates of *Abies sachalinensis* seeds infected by *R. therryanum* were very low (8-11%) (Hayashi and Endo, 1975). *R. therryanum* is a major factor inhibiting the natural regeneration of *Picea jezoensis* at the seed germination stage (Cheng, 1989). This fungus has high activity at low temperature (0°C) and is distributed at the A_0 layer of soil, where most seed infections occur (Cheng, 1989). *R. therryanum* has been reported only from Japan currently (Sakamoto and Miyamoto, 2005). *R. therryanum* was isolated from *Abies koreana* seeds in Korea for the first time and its morphological features are described in this paper.

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Study site and seeds. This study was conducted in natural *Abies koreana* stands at an elevation of 1700 m on Mt. Halla, Korea. *A. koreana* seeds were collected from Mt. Halla in 2001, and their viability (the percent of germinable seeds) was 72.6%. The seeds were placed in 5 × 5-cm nylon mesh bags, 50 seeds per bag. On 25-26 September 2003, these seed bags, containing dry, unstratified seeds, were placed on the forest floor of an *A. koreana* forest. The seed bags were covered with plastic mesh to prevent rodent predation during the winter. The first snowfall occurred on 12 December 2003, and the soil in the forest was not frozen at that time. The seed bags, remained under the snow, were retrieved on 9-10 April 2004.

The maximum infection rate of *A. koreana* seeds was 88.9% at our study site (Cho, unpublished). No fungi isolated from the seeds were stored in refrigerator. *Racodium therryanum* has been isolated from seeds of *Abies sachalinensis*, which is phylogenetically similar to *A. koreana*, and it infects 100% of *A. sachalinensis* seeds in the field (Hayashi and Endo, 1975). Therefore, fungal infections on *Abies* seeds can result in substantial losses of *A. koreana* seeds.

Isolation of fungus. The seeds, collected from forest floor and stored in refrigerator, were washed in 0.01% Tween 20 (ICN Biochemicals, Germany) for 1 minute. They were surface sterilized in 70% ethanol for 1 minute and in 30% hydrogen peroxide for 2 minutes, washed in distilled water for 1 minute, and then dried on sterile filter paper. They were placed (three seeds per Petri dish) on potato dextrose agar (PDA) and incubated at 5°C for 4 weeks. Discs of fungi were cut from the actively growing colony margins of isolates, transferred on PDA plates and incubated at 20°C. For formation of chlamydospores, fungal colonies were incubated on PDA at room temperature for 4 weeks.

Identificfation of the fungus. The morphological characteristics of the fungus were examined under a compound

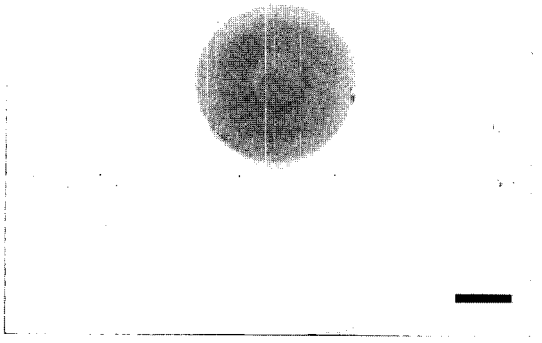

Fig. 1. Colony of *Racodium therryanum* (7-day incubation on PDA at 5°C). Scale bar = 1 cm.

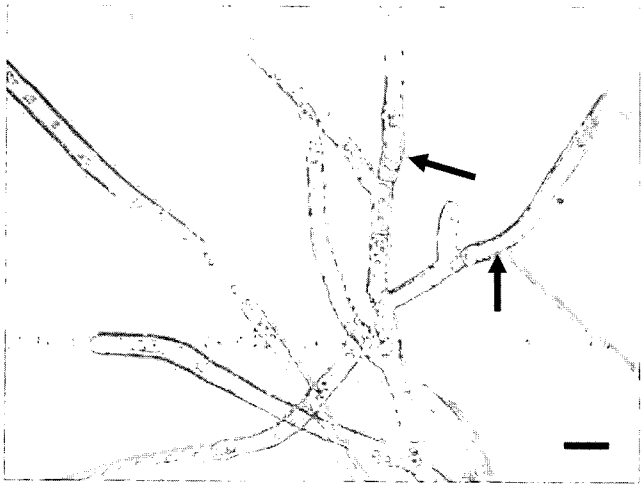

Fig. 2. Hyphae of *Racodium therryanum* (4-weeks incubation on PDA at 5°C) arrows indicates articulate septacum. Scale bar = 10 μm.

microscope (BH2-UCD, Olympus, Japan), and the color, shape, and size of the hyphae and chlamydospores were recorded. The size was determined from 30 and 50 measurements of hyphae and chlamydospores, respectively. The identification and description were based on Sato et al. (1960). In order to investigate the temperature range of growth, fungal colonies were incubated on PDA at 0, 5, 10, 15, 20, 25, 30, and 35°C for 7 days with three replications each. The colony diameter was examined after 7 days.

Based on the morphological and cultural characteristics, the fungus was identified as *Racodium therryanum* Thuemen.

Colonies were reaching 2.2 cm diam. in 7 days at 20°C in the dark on PDA (Fig. 1). Hyphae were dark gray to dark brown, with granules, articulate-septated, 11.2-78.2 μm long, 3.4-6.2 μm wide (Fig. 2). Chlamydospores were sphaeroid, oval, dark-brown, catenulate, with granules, 8.1-10.5 × 6.5-8.6 μm (average 9.3 × 7.6 μm) (Table 1, Fig. 3).

The temperature range of growth was 0-25°C, with an optimum of 20°C and a maximum of 25°C (Fig. 4). *R. therryanum* start to grow vigorously at 10°C and was suppressed at 30 and 35°C.

The following features characterize *R. therryanum.* 1) It is isolated from conifer seeds and seedlings, including *Abies*, 2) Colonies are dark green to dark gray and intricate, and 3) It forms chlamydospores in order cultures (Saccardo, 1899; Sakamoto and Miyamoto, 2005; Sato et al., 1960).

According to Saccardo (1899), the hyphae are 6-7 μm wide with articulate septa. Sato et al. (1960) described the hyphae as 21-87 × 2.2-5.4 μm with articulate septa. Sakamoto and Miyamoto (2005) reported that hyphae are 3.0-7.9 μm in diameter with septa. In our study, the hyphae

Table 1. Comparison of morphological characteristics of *Racodium therryanum*

	Present	Saccardo (1899)	Sato (1960)	Sakamoto and Miyamoto (2005)
Colony				
Color	dark	dark gray	dark gray	dark green to dark gray
Hyphae				
Color	dark gray to dark brown	dark gray	dark gray	–
Size; length (μm)	11.2-78.2 ± 3.4-6.2	6-7	21-87 ± 2.2-5.4	3.0-7.9
Granule	present	–	present	present
Septum	present	present	present	present
Chlamydospore				
Figure	sphaeroid, ovoid	–	sphaeroid, ovoid	–
Color	dark brown, dark black	–	dark-brown, dull black	–
Size (μm)	8.1-10.5 ± 6.5-8.6	–	12-20 ± 6-12	12.9 ± 9.6
Granule	present	–	present	–

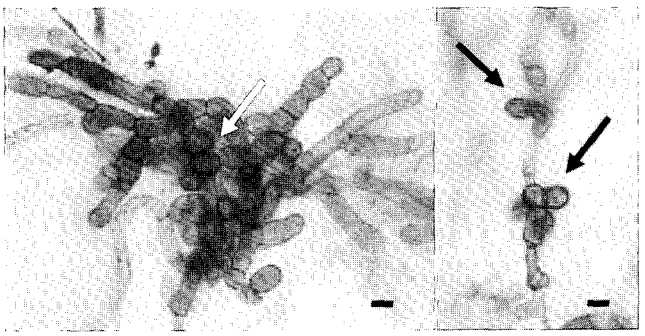

Fig. 3. Chlamydospores of *Racodium therryanum* (4-week incubation on PDA at room temperature) arrows indicates articulate septacum. Scale bar = 10 μm.

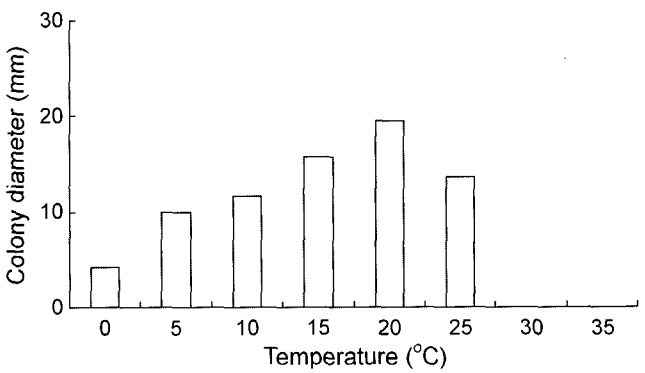

Fig. 4. Colony growth of *Racodium therryanum* at different temperatures on PDA after 7 days.

were 11.2-78.2 μm long and 3.4-6.2 μm wide with articulate septa. Sato et al. (1960) described the sizes of chlamydospores as 12-20 × 6-12 μm., while Sakamoto and Miyamoto (2005) reported that they were 9.0-15.4 × 7.7-11.8 μm, averaging 12.9 × 9.6 μm. In our study, the chlamydospores were 8.1-10.5 × 6.5-8.6 μm (average 9.3 × 7.6 μm). The morphological characteristics we observed did not differ significantly from previous descriptions.

Cheng (1989) reported that chlamydospores of *R. therryanum* formed on glucose filter paper (GFP) at 15 and 20°C after 28 days of, while we found that chlamydospores formed on PDA at room temperature after 4 weeks.

According to Sato et al. (1960), the optimal growth temperature of *R. therryanum* is between 15 and 20°C on PDA. Cheng (1989) obtained the same result on potato sucrose agar (PSA) and our results concurred with these earlier studies.

Sato et al. (1960) showed that the *R. therryanum* grows at very low temperatures (–4°C) on PSA. Endo and Hayashi (1973) examined the mycelial growth of *R. therryanum* on frozen and unfrozen PSA at –3°C to –4°C and reported that the fungus grew on frozen PSA. Cheng (1989) found that *R. therryanum* grows at –5°C to 0°C. He suggested that *R. therryanum* is active and can infect seeds under the snow during winter season. We also found that the fungus can grow at 0°C (Fig. 4), which suggests that *A. koreana* seeds under the snow are vulnerable to attacks by this fungus on Mt. Halla during winter.

Our results agree with previous descriptions of *R. therryanum*. This is the first report on *R. therryanum* in Korea. There is a high probability that this fungus affects the natural regeneration of *A. koreana* on Mt. Halla. The pathogenicity (seed decay by artificial infection test) of *R. therryanum* isolated from Mt. Halla is currently under investigation. The germination rate of *A. koreana* seeds on this fungus culture was 0% 100 days later at 0°C (Cho, unpublished). Although *R. therryanum* is considerable pathogenic fungus, the report on this fungus has been only from Japan and Korea at this time. Furthermore, there is a report that the name *R. therryanum* is scientifically doubtful (Sakamoto and Miyamoto, 2005). A more extensive investigation is needed.

The specimen (KKU-Rt-04, April, 2004, Jeju) examined was preserved at the Herbarium of Konkuk University.

Acknowledgement

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