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FULL PAPER

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Synnemapestaloides rhododendri, a new genus and new species of synnematous hyphomycete, causing synnemapestaloides twig blight disease of *Rhododendron brachycarpum* in Japan

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Abstract A hyphomycete with black synnema and appendaged phragmoconidia was collected on *Rhododendron brachycarpum* showing twig blight symptom in Aomori Prefecture, Japan. The twig blight is macroscopically similar to the disease symptoms caused by *Pycnostysanus azaleae*. The fungus, however, has determinate synnemata, (1–)3-level verticillately branched conidiophores, and *Pestalotia*-like conidia. It cannot be accommodated by any existing genus of anamorphic fungi. A new genus *Synnemapestaloides* is established to accommodate the fungus, which is named *S. rhododendri*. The pathogenicity of the fungus on *Rhododendron* leaves and shoots is confirmed by inoculation. Synnemapestaloides twig blight is proposed for the disease of *R. brachycarpum* caused by the fungus.

Key words New genus · *Rhododendron* · *Synnemapestaloides rhododendri* · Twig blight

Introduction

In July 2001, some blighted twigs with synnematous structures were collected on a living tree of *Rhododendron brachycarpum* D. Don at Tsuta spa, Hakkoda mountains, Aomori Pref. The same blighted twigs were also collected at Sukayu spa, Hakkoda mountains, in the same area, in June 2002, and Osorezan, Mutsu, Aomori Pref., in October 2002. The synnematous fungus partly resembled *Pycnostysanus azaleae* (Peck) E.W. Mason (Ellis 1976; Sutton 1973) with black synnema and conidial head, which causes blighted buds and twigs of *Rhododendron* species (Davis 1939; Schmitz 1920; Kaneko et al. 1988). Although *P. azaleae* has aseptate blastconidia, the fungus has *Pestalotia*-like appendaged phragmoconidia (Guba 1961; Nag Raj 1993; Sutton 1969). To the present, no synnematous pathogenic fungi are reported on Ericaceous plants other than *P. azaleae*. In this article, we report this fungus as a new species and genus of anamorphic fungi.

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Materials and methods

Morphological observation

Synnemata on leaves mounted in Tissue-Tek O.C.T. compound (Sakura, Tokyo, Japan) were sectioned using a microtome (HM 400R; Microme, Walldorf, Germany) equipped with a freezing unit (K 400; Microme) and observed under a light microscope. Sectioned fungal structures as well as the fungus spores were mounted for observation in Shear fluid (Kirk et al. 2001), 80% (v/v) ethyl alcohol, 2% (w/v) KOH, or 85% (w/v) lactic acid aqueous solutions with 0.1% cotton blue, if necessary.

For scanning electron microscopy (SEM) observation, synnemata cut from leaves and twigs were fixed with 2.5% glutaraldehyde for 24 h at 4°C and washed with 0.1 M phosphate buffer (pH 6.6). Fixed materials were then dehydrated in an alcohol series, critical point dried, and then sputter-coated with gold and examined using a JSM-5300 scanning electron microscope.

The descriptive terminology used in Seifert (1985) and Seifert and Okada (1990) were basically followed in this article. Colony and fungal colors were determined using the *Methuen Handbook of Colour* (Kornerup and Wanscher 1978).

Cultural characteristics

Growth of the fungus was recorded from potato sucrose agar (PSA; potato 200 g, sucrose 20 g, agar 20 g, distilled

water 1000 ml), oatmeal agar (OA; oat flakes 30 g, agar 15 g, distilled water 1000 ml), 2% malt extract agar (MA; Difco, Detroit, MI, USA), and corn meal agar (CMA; Difco) in 9-cm Petri dishes, incubated at 23° ± 1°C under diffuse sunlight in a laboratory room. To investigate mycelial growth rate, inoculated PSA plates were incubated at constant temperatures of 5°–30°C at intervals of 5°C under dark.

To obtain conidia for inoculation experiment, PSA plates were incubated at 20°C/12 h L:D under a fluorescent lamp (~1500 lux, FLD15; National, Osaka, Japan)

Inoculation experiment

To demonstrate the pathogenicity of the fungus, inoculation experiments were conducted with leaves and shoots of 2-year-old potted plants of *Rhododendron hybridum* Hort. "Pink Dream," which were kept in a growth chamber maintained at 18°–22°C and 12-h photoperiod under a metal halide lamp (~18 000 lux, DR400/T (L); Toshiba, Tokyo, Japan). Before inoculation, leaves were stung by a sterilized needle, and shoots were given wedge-shaped incision by a sterilized scalpel. The wounded parts were either burnt with a soldering iron or not burnt. Conidial suspension (approximately 6.3×10^5 conidia/ml) obtained from PSA culture of the fungus was put into the wounded parts with a small pipette, and the shoots were wrapped with parafilm. Inoculated leaves and shoots were covered with plastic bags to maintaine moisture. After 7 days, parafilm and plastic bags were removed. When inoculated parts began to discolor, about 1 month after inoculation, plants were moved to the field. Sterilized distilled water was used instead of spore suspension for a control experiment.

Results

Description

Synnemapestaloides T. Handa & Y. Harada, anam. gen. nov.

Conidiomata synnematica, definita, atra, ab stromate basali polygonali exorientia; hyphae stipitis parallelae; massae conidii atrae, globosae vel subglobosae, subgelatinosae. Conidiophora aliquoties verticillate ramificantia. Cellulae conidiogenae cylindricae vel subcylindricae, annellatae. Conidia fusiformia, basi truncata, recta, septata, pallide olivacea, apice centro appendiculata, ad basim eccentrice appendiculata.

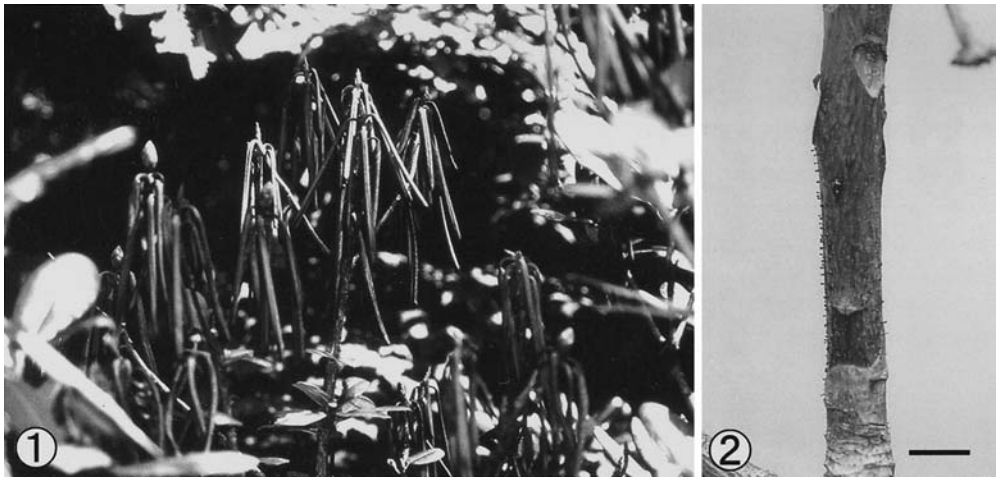
Species typica: *Synnemapestaloides rhododendri* T. Handa & Y. Harada

Etymology: *Synnema* in Greek = synnema, *pestaloides* = *Pestalotia*-like conidia.

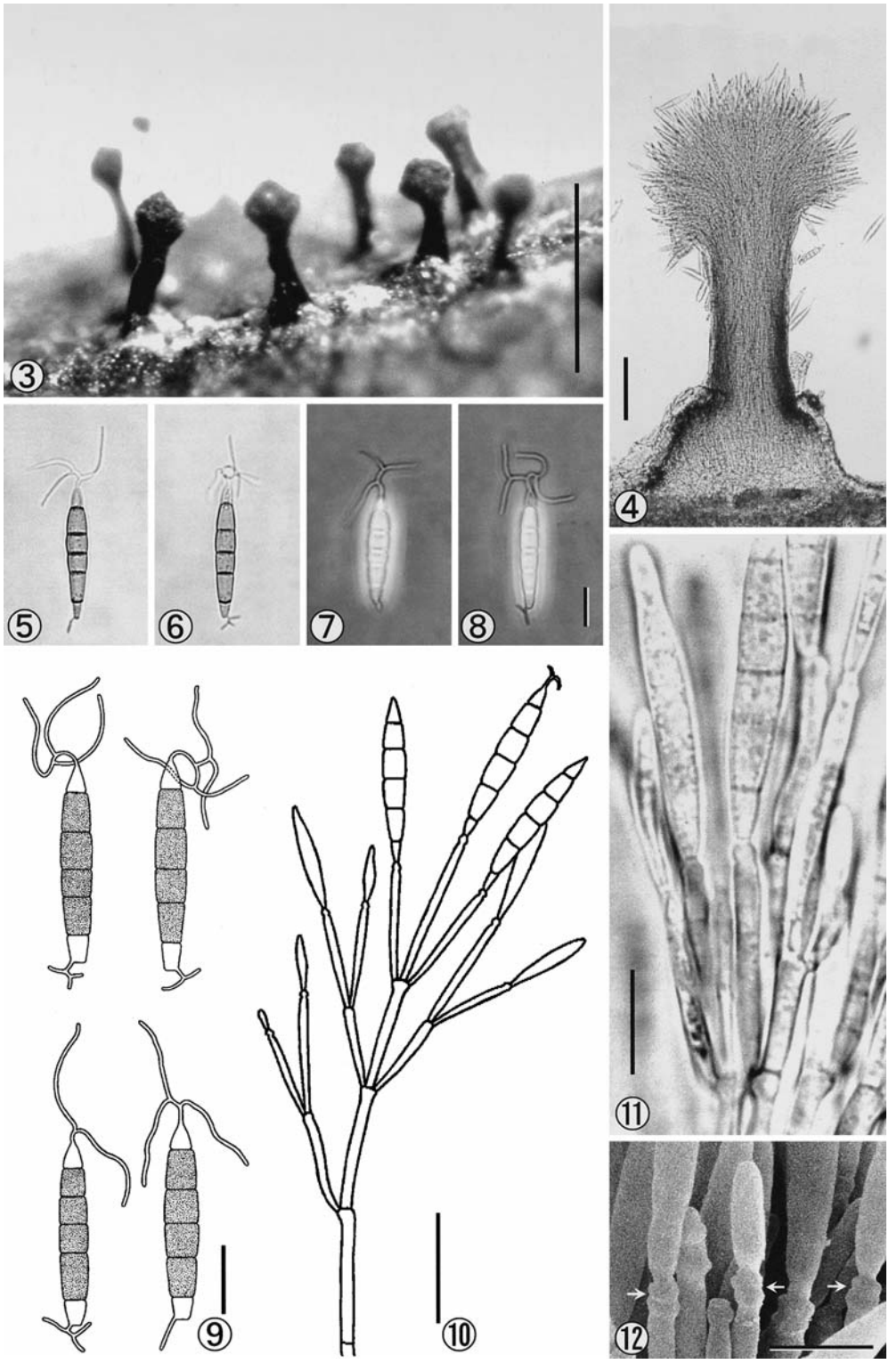
Conidiomata synnematous, determinate, black, not changing color in 2% KOH or 85% lactic acid, arising from a basal stroma composed of textura angularis; hyphae of stipe parallel; conidial mass, black, globose to subglobose, subgelatinous. Conidiophores verticillately branched several times. Conidiogenous cells cylindrical to subcylindrical with annellations. Conidia fusiform with a truncate base, straight, septate, light olivaceous; apical appendage centric; basal appendage excentric.

Synnemapestaloides rhododendri T. Handa & Y. Harada, sp. nov. Figs. 1–15

Conidiomata synnematica 130–490 μm longa, 17.5–75 μm lata, atra, clavata, cylindro-capitata, raro sessilia et pustuliformia, simplicia, dispersa vel aggregata, definita, ab stromate basali polygonali exorientia; hyphae stipitis parallelae, griseo-aurantiacae; massae conidii 30–150 μm diametro, atrae, globosae vel subglobosae. Conidiophora ramificantia, mono-, bi- vel praecipue triverticillaria; metulae 10–20.8 μm longae, 1.5–2 μm latae. Annellides acrogenae, bi- vel triverticillares, cylindricae vel subcylindricae, rectae, 11.5–31.5 × 1.3–2.5 μm, apice truncatae, noduliforme annulatae, spissitatem periclinalem et collarette non visae. Conidia fusiformia, basi truncata, recta, quinqueseptata, ad septa leviter constricta, exsporis glabris, 25–32.8 μm longa, 4–6 μm lata, apice utrinque appendiculata; quadricellulae medianae 18–23.5 μm longae, pallide brunneae; cellula apicalis conica, hyalina; cellula basilaris obconica, hyalina, basi truncate. Appendix apicalis



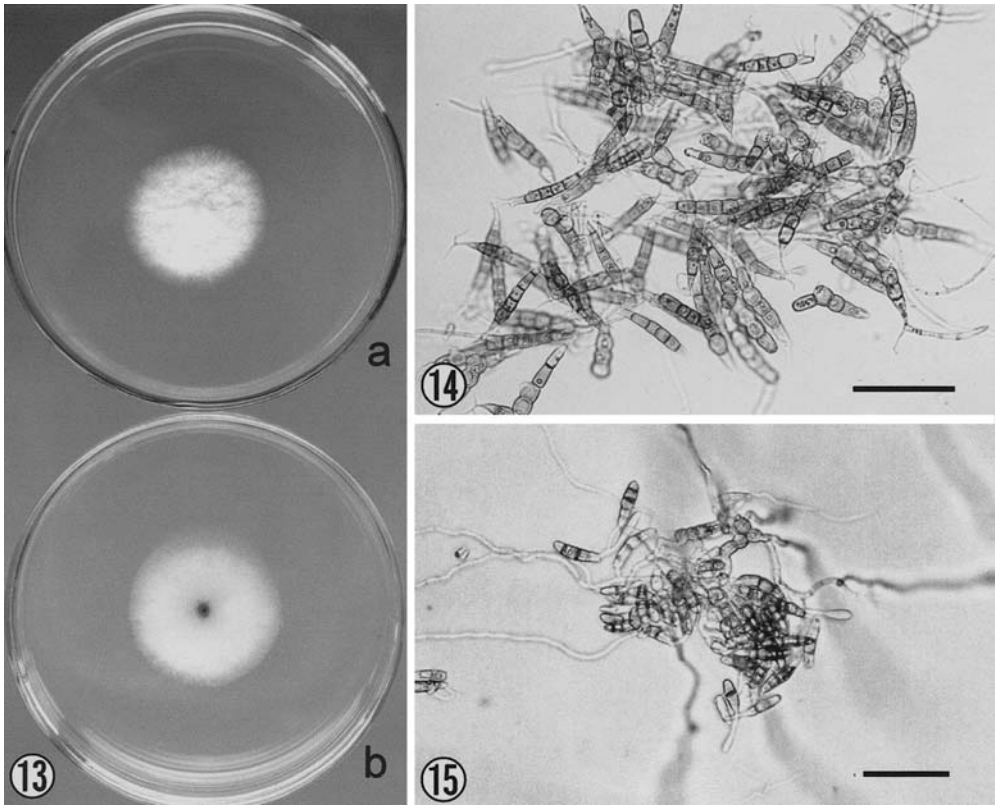
Figs. 1,2. The twig blight of *Rhododendron brachycarpum* caused by *Synnemapestaloides rhododendri*. **1** Infected leaves and twigs. **2** Infected twig (note very tiny synnemata on the surface). *Bar* **2** 5 mm



Figs. 3–12. *Synnemapestaloides rhododendri* on *R. brachycarpum*. **3** Synnemata on blighted leaf. **4** Longitudinal section of a synnema. **5–9** Conidia (**7**, **8** phase contrast). **10** Conidiophores with conidiogenous cells bearing conidia. **11** Conidiogenous cells. **12** Apex of conidiogenous cells with annellations at the tip (*arrows*), SEM. *Bars* **3** 500 μm; **4** 50 μm; **5–9, 11** 10 μm; **10** 20 μm; **12** 5 μm

singularis, centralis, filiformis, non ramificans vel dichotome ramosa, diverse tortuosa, usque ad 22 μm longa; appendix basilaris singularis, non ramificans vel saepe irregulariter ramificans, eccentrica, usque ad 5.8 μm longa.

Synnemata produced on blighted buds, leaves, and twigs of *Rhododendron brachycarpum* (Figs. 1–3), 130–490 μm long, 17.5–75 μm wide, black, clavate, cylindrical-capitate, rarely sessile and pustulate, unbranched, scattered to gregarious, determinate, arising from a basal stroma composed of textura angularis (Fig. 4), not changing color in 2% KOH or 85% lactic acid; hyphae of stipe parallel, greyish-orange (5B5); conidial mass 30–150 μm diameter, black, globose to subglobose, subgelatinous. Conidiophores branching once or twice monochasial or biverticillate to mainly



Figs. 13–15. Cultural characteristics of *S. rhododendri*. **13** Colonies of a single conidium culture MAFF239201 on potato sucrose agar (PSA) after 7 d at 20°C under dark: surface (*a*) and reverse (*b*). **14** Conidia with insufficiently developed appendages on PSA. **15** Germination of morphologically immature conidia. *Bars* **14, 15** 50 μm

triverticillate (Fig. 10), metulae 10–20.8 × 1.5–2 μm. Anellides in terminal whorls of 2–3, cylindrical to subcylindrical with a truncate apex, hyaline, straight, 11.5–31.5 × 1.3–2.5 μm, with nodular annellations (Figs. 11, 12); periclinal thickening and collarettes not visible. Conidia fusiform with a truncate base, straight, 5-septate, slightly constricted at the septa, smooth walled, 25–32.8 × 4–6 μm, bearing appendages at both ends (Figs. 5–9); four median cells 18–23.5 μm long, champagne (4B4) to light blonde (4C3); apical cell conic, hyaline; basal cell obconic with a truncate base, hyaline; apical appendage single, filiform, unbranched or dichotomously branched, variously curved, centric, up to 22 μm long; basal appendage single, unbranched or often irregularly branched, excentric, up to 5.8 μm.

Colonies on PSA growing at 5°–30°C, optimum at 20°C, velvety (Fig. 13), white (1A1) to pale yellow (1A3); reverse white (5D6) to oak-brown (5D6). Colonies attained a diameter of 39–40 mm on PSA, 17–18 mm on OA, 17–18 mm on MA, and 17.5–19 mm on CMA after 7 days. Conidial formation began on PSA after 10 days; conidia with insufficient appendages were produced, but they could germinate when seeded on agar media (Figs. 14, 15).

Teleomorph unknown.

Habitat: Blighted buds, leaves, and twigs of *Rhododendron brachycarpum* D. Don.

Holotype: Japan, Honshu, Tsuta spa, Hakkoda mountains, Aomori Pref., on blighted buds, leaves, and twigs of *R. brachycarpum*, July 21, 2001, leg. Y. Harada (HHUF26527), deposited in the Herbarium of Hirosaki University. Ex-holotype culture (MAFF239201) is kept in the culture collection of the National Institute of Agrobiological Science, Tsukuba (MAFF Genebank).

Etymology: *rhododendri*, from the generic name of the host plant.

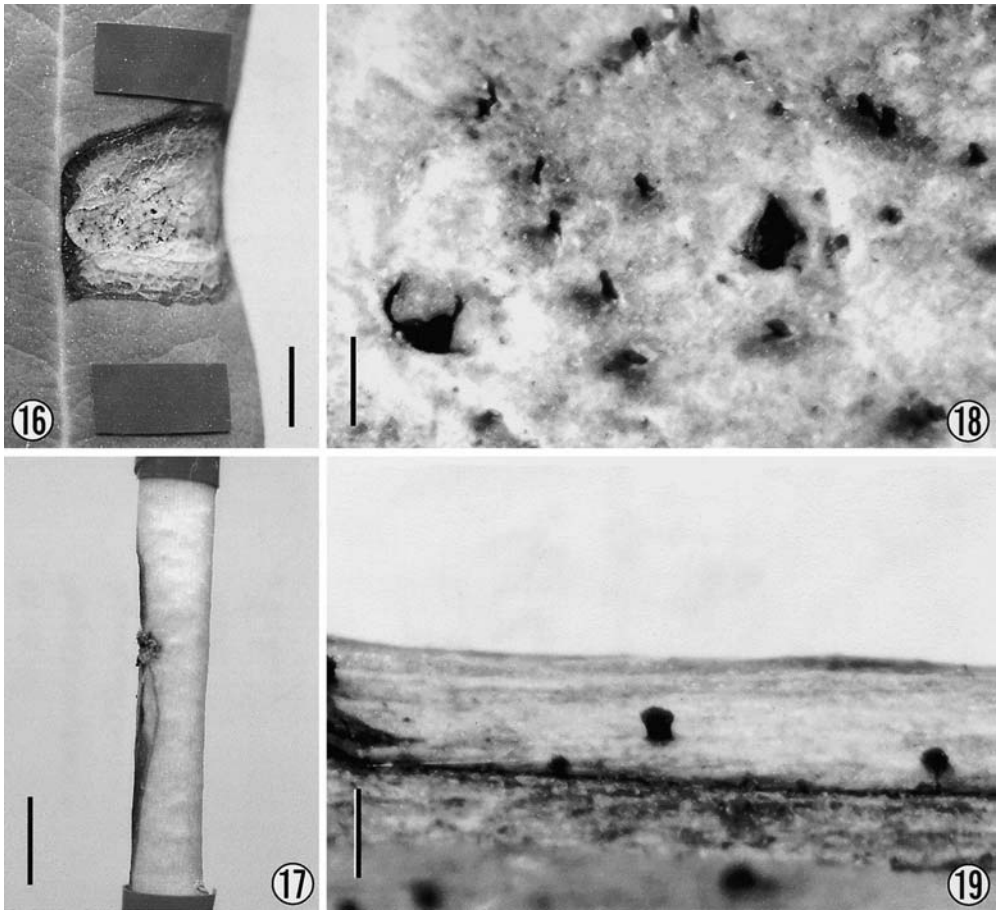
Other specimens examined: On blighted buds, leaves, and twigs of *R. brachycarpum*, Tsuta spa, Hakkoda mountains, Aomori Pref., July 21, 2001, leg. Y. Harada (HHUF26528–26533); Sukayu spa, Hakkoda mountains, Aomori Pref., June 9, 2002, leg. Y. Harada (HHUF27741–27742); Mt. Osorezan, Mutsu, Aomori Pref., Oct. 27, 2002, leg. T. Handa and Y. Harada (HHUF27743).

Pathogenicity

In the inoculation experiment, the burnt and inoculated parts of leaves and shoots discolored to pale brown after 1 month, and the periphery of lesion turned yellow. After 6 months, black capitiform to setiform synnemata with conidial head were formed on the diseased parts (Figs. 16–19). The same fungus could be reisolated from the conidia. However, disease symptoms stopped developing further, and no typical twig blight was observed as in nature.

Discussion

Synnemapestaloides rhododendri is characterized by a black determinate synnema, (1–)3-level verticillately branched



Figs. 16–19. Symptoms of the leaf and shoot of *R. hybridum* Hort. "Pink Dream" in the inoculation experiment. **16** Lesion on leaf. **17** Lesion on branch. **18** Close-up of leaf lesion (note black columnar synnemata). **19** Close-up of shoot lesion (note black columnar synnemata). *Bars* **16, 17** 5 mm; **18, 19** 500 μm

conidiophores, and *Pestalotia*-like conidia formed in a black conidial head (Figs. 3–12). It comes superficially close to *Pycnostysanus azaleae* on the same host genus (Ellis 1976; Kaneko et al. 1988; Sutton 1973). However, *S. rhododendri* is different from *P. azaleae*, which produced aseptate blastoconidia. In conidial morphology, *S. rhododendri* closely resembles *Pestalotia pezizoides* De Not., the type species of the genus. However, *P. pezizoides* produces conidia over the surface of pezizoid stroma. Further, important differences are seen on appendages between the two species: i.e., *P. pezizoides* has 3–9 apical appendages and a single centric basal one, whereas *S. rhododendri* has a single apical appendage and a single excentric basal one (Guba 1961; Nag Raj 1993; Sutton 1969).

Bud blight disease by *P. azaleae* is common on several Ericaceaous plants (Davis 1939; Kaneko et al. 1988; Schmitz 1920). The symptom caused by *P. azaleae* was mainly bud blight, whereas that by *S. rhododendri* was typically twig blight. Therefore, we propose here to call this disease synnemapestaloides twig blight (see Fig. 1). The pathogenecity of the fungus was demonstrated on inoculated leaves and shoots of *R. hybridum* Hort. "Pink Dream," although we were unsuccessful in reproducing the typical disease as seen on *R. brachycarpum* in nature (Figs. 1, 2). Some factors might be involved in the disease: (1) the *Rhododendron* cultivar used in this inoculation experiment was somewhat resistant to the fungus; (2) the inoculation was not timely done; or (3) hard environmental conditions in the mountains during the winter (e.g., low temperature, heavy snow).

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