

Anthracnose of Christmas rose caused by *Colletotrichum* sp.

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Abstract Leaf spots were found on Christmas rose (*Helleborus niger*) in Yamagata Prefecture, Japan, in October 2006. The morphology of the causal fungus was very close to that of *Colletotrichum truncatum*. Classifying the species from the sequences of the internal transcribed spacer regions of ribosomal DNA was inconclusive, and the isolates were identified only as *Colletotrichum* sp. Artificial inoculation confirmed the pathogenicity of isolates to the host plant and some legumes. We propose the name anthracnose of Christmas rose for this disease by *Colletotrichum* sp.

Keywords *Helleborus* spp. · Anthracnose ·
Colletotrichum sp.

Introduction

The name Christmas rose is applied generally to species of the genus *Helleborus* (Ranunculaceae), perennial, ornamental plants native to the Mediterranean to West Asian regions. The name is also specifically applied to *Helleborus*

niger and, in Japan, to *Helleborus orientalis* and its hybrids. Christmas roses are popular in Japan because of the wide range of flower colors and their winter color. Leaf spots were observed on *H. niger* in Yamagata Prefecture, Japan, in October 2006. *Colletotrichum* sp. was isolated from the spots. We characterized the fungus and examined its pathogenicity to *H. niger* and some legumes.

Symptoms

Small, brown lesions were observed on mature leaves of *H. niger* in open fields in early October 2006. The lesions usually appeared at the leaf margin, but occasionally on the leaf blade, and became roundish and slightly concave (Fig. 1a). At later stages, numerous epidermal acervuli with many setae developed on the lesions (Fig. 1b). The lesions enlarged slowly, taking a month or more to cover the entire leaf blade. Because old or damaged leaves are removed by hand in November–December, the damage was not severe.

Isolation and identification

Diseased leaf sections were sterilized in 70% (v/v) ethyl alcohol for a few seconds. After two rinses with sterilized distilled water and removal of moisture with sterilized filter paper, the sections were incubated in 9-cm Petri dishes under moist conditions for several days at 25°C. Pale orange conidial masses appeared during incubation. The conidia were picked up with a platinum loop and streaked onto water agar. Monoconidial colonies were then isolated.

Colonies of two isolates, Ya457 and Ya458 (deposited as MAFF712313 and MAFF712314 in Genebank, National Institute of Agrobiological Science), that formed on potato

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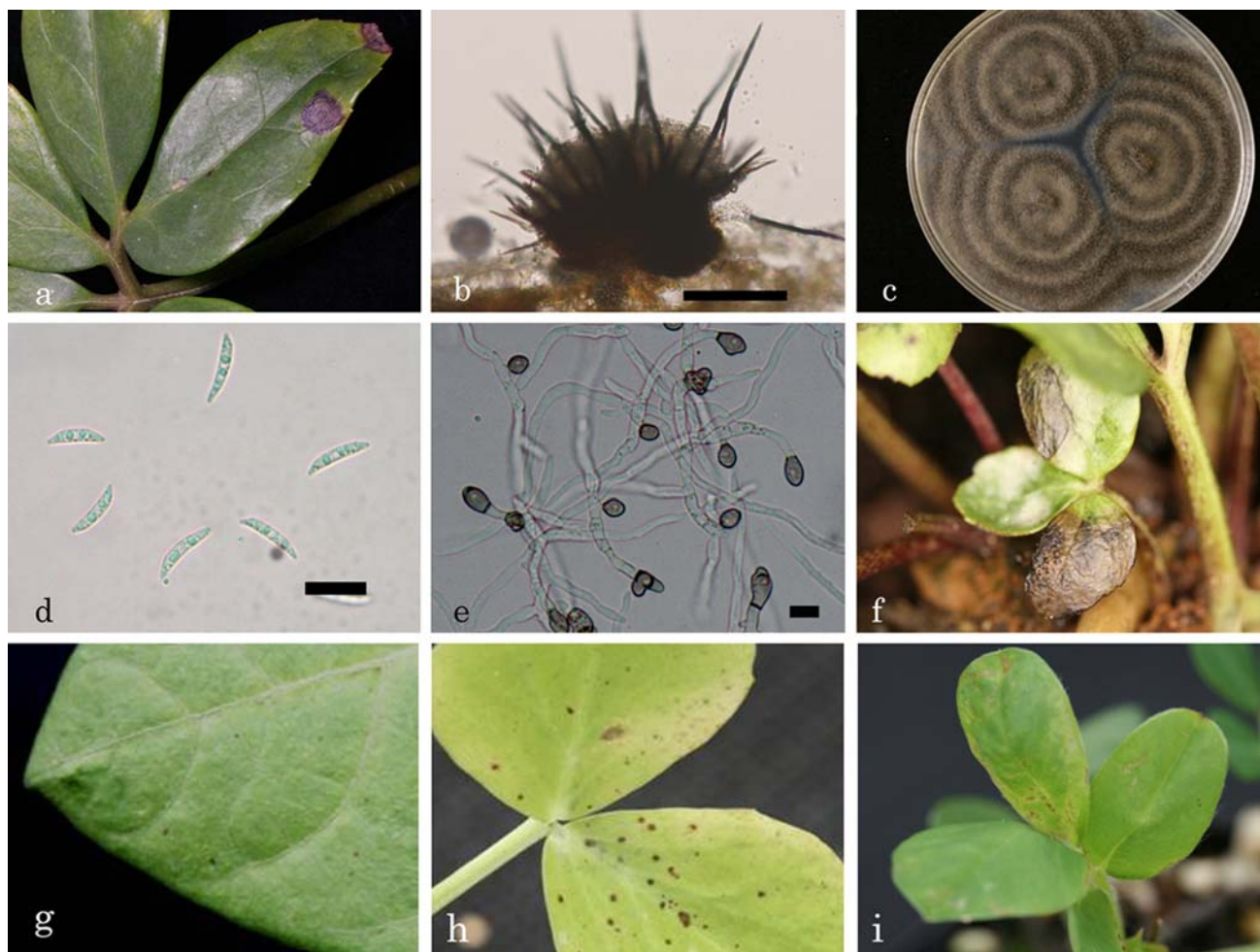


Fig. 1 Symptoms and causal organism of anthracnose on Christmas rose. **a** Naturally occurring leaf spots on *Helleborus niger*. **b** Epidermal acervulus produced on a leaf (cross-section; scale bar 100 μ m). **c** Colonies of isolate Ya457 on PDA plate. **d** Conidia of Ya457 produced on PDA plate (scale bar 20 μ m). **e** Appressoria produced on potato

carrot agar slide culture (scale bar 10 μ m). **f** Leaf spots and acervuli 14 days after inoculation with isolate Ya457. **g–i** Symptoms on legumes 12 days after inoculation with isolate Ya457 (**g** adzuki bean, **h** pea, **i** peanut)

dextrose agar (PDA) at 25°C under a 12 h black-light/12 h dark cycle for 10 days, were brownish gray with dark concentric circles (Fig. 1c). Conidial masses on the colonies were pale orange. Conidia of isolate Ya457 were falcate, slightly curved, occasionally scalene (asymmetric) at each end, aseptate, hyaline, with one or a few droplets of oil, 17.9–25.2 μ m long, and 2.6–4.2 μ m wide, with an obtuse apex and an abruptly truncate base (Fig. 1d). Appressoria that formed abundantly in slide cultures on potato carrot agar at 25°C in the dark for 2 days were one-celled, brown, thick-walled, clavate, ellipsoid to irregular, usually with an entire margin, 9.2–18.0 μ m long, and 6.2–12.3 μ m wide (Fig. 1e). Cultures of the isolates on PDA grew in the dark at 5–35°C with optimum growth at 25°C (55–57 mm diameter in 5 days). After more than 6 months incubation in a slant culture at 15°C in the dark, abundant black sclerotia formed; they were submerged and appeared

globose to irregular. Isolate Ya458 was the same. These morphological and cultural characteristics are mostly consistent with descriptions of *Colletotrichum truncatum* (Schwein.) Andrus & Moore (Table 1; Sutton 1980, 1992), especially the gradually tapering conidia with an obtuse apex and truncate base.

The sequences of the internal transcribed spacer regions of ribosomal DNA (rDNA-ITS) were analyzed to confirm species identification. Sequences of isolates Ya457 and Ya458 (deposited in DDBJ/EMBL/GenBank as AB443951 and AB443952) were coincident with previous sequences (AJ301985 and AJ301976) classified as *C. truncatum* (Nirenberg et al. 2002), but they also had 99.8% homology with *C. dematium* (AF411773; Vinnere et al. 2002). Japanese *Colletotrichum* species can be grouped into 20 ribosomal groups (RGs) based on the ITS1 sequence (Moriwaki et al. 2002), and *C. truncatum*

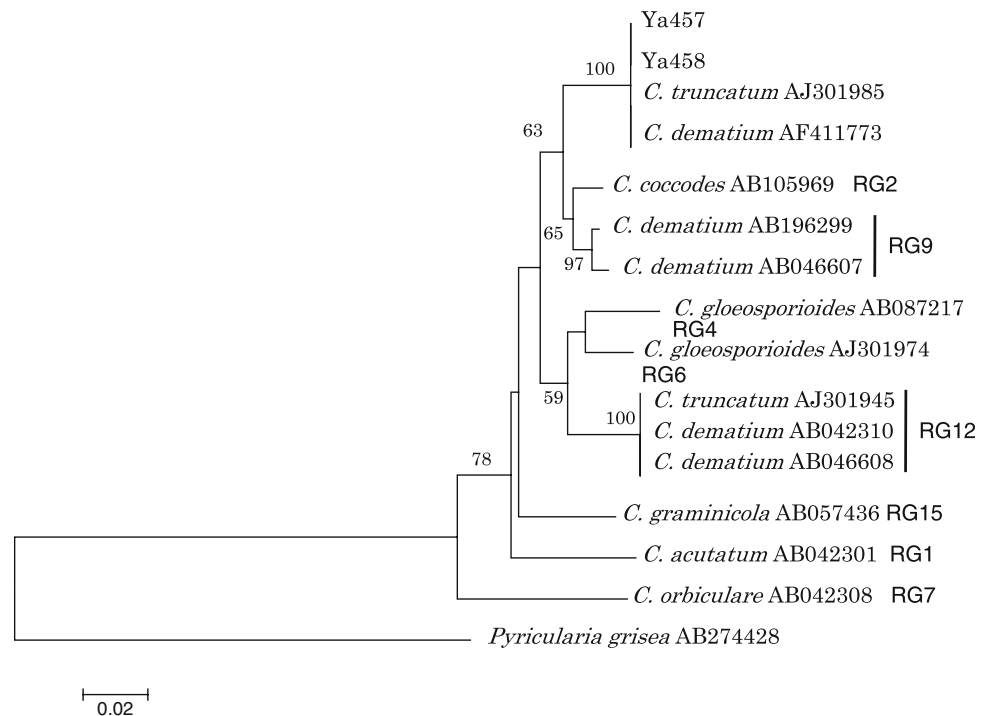
Table 1 Comparison of morphological characteristics of isolate causing anthracnose of Christmas rose with reference isolates of *Colletotrichum* species

Trait	Ya457	Ya458	<i>C. truncatum</i> ^a	<i>C. truncatum</i> ^b	<i>C. dematium</i> ^a
Colony	Brown-gray with dark concentric rings	Brown-gray with dark concentric rings	Pale mouse-gray to mouse gray, salmon, ochraceous to cinnamon	Dark brown to black, with a felt of grayish mycelium	White to pale mouse gray or gray, reverse dark brown
Conidial masses	Pale orange	Pale orange	Saffron to orange	Honey	Olive gray to light vinaceous salmon
Sclerotia	Abundant, black, globose to irregular	Abundant, black, globose to irregular	Scattered irregular	Abundant	Abundant black, conical
Conidia	Falcate, gradually curved, obtuse apex, abruptly truncate base, 17.9–25.2 × 2.6–4.2 μm	Falcate, gradually curved, obtuse apex, abruptly truncate base, 19.9–26.6 × 3.0–4.0 μm	Falcate, fusiform, tapered gradually toward, abruptly truncate base, 12–16 × 4–6 μm	Falcate apices obtuse, guttulate, 15.5–24 × 3.5–4 μm	Falcate, fusiform, gradually tapered to each end, 18–26 × 2–3 μm
Appressoria	Clavate, ellipsoid to irregular, margin usually entire, 9.2–18.0 × 6.2–12.3 μm	Clavate, ellipsoid to irregular, margin usually entire, 8.6–20.2 × 6.3–10.9 μm	Clavate to irregular, 6–12 × 6–12 μm	Clavate, circular to irregular, 11–16 × 8–9.5 μm	Clavate, ovate, irregular, margin entire or slightly lobed, 7.5–18 × 4–12.5 μm

^a Sutton (1992)

^b Sutton (1980)

Fig. 2 Phylogenetic tree of the isolates and related species based on ITS1, 5.8S, and ITS2 regions by the neighbor-joining analysis. Numbers on each branch represent bootstrap values obtained from 1000 replications (only values >50 are shown). *Pyricularia grisea* was used to root the tree. Bar distance corresponding to two base changes per 100 nucleotide positions. RG ribosomal group (Moriwaki et al. 2002)



separated into RG11 and RG12. However, our isolates, at 171 bp, were different from members of RG11, at 182 bp (J. Moriwaki of National Agricultural Research Center, Hokuriku Research Center, personal communication), and did not belong to other RGs in a phylogenetic analysis (Fig. 2).

Pathogenicity

Two healthy plants of 2-year-old *H. niger* about 10 cm tall were inoculated with one of the isolates (Ya457 or Ya458). Each isolate was grown on V-8 juice agar at 25°C for 14 days under 12 h black-light/12 h darkness to obtain

Table 2 Pathogenicity of *Colletotrichum* isolates on leaves of species of legumes

Plant	Present isolates	
	Ya457	Ya458
Adzuki bean (<i>Vigna angularis</i>)	±	±
Common bean (<i>Phaseolus vulgaris</i>)	±	±
Milk vetch (<i>Astragalus sinicus</i>)	–	–
Pea (<i>Pisum sativum</i>)	+	+
Peanut (<i>Arachis hypogaea</i>)	+	+
Soybean (<i>Glycine max</i>)	–	–
Winged bean (<i>Psophocarpus tetragonolobus</i>)	–	–

+: brown spots were observed; ±: slightly browned; –: symptomless

spores. A conidial suspension (10^5 conidia/ml sterile distilled water) was sprayed onto the plants without wounding. After inoculation, the plants were held in a transparent plastic case in a greenhouse for 48 h at 25°C, then were uncovered and grown for 14 days. As a negative control, plants were sprayed with sterilized water. Symptoms similar to the natural lesions appeared only on leaves that developed in the previous year or on the current year's small, young leaves (Fig. 1f). The pathogen was recovered from diseased plants, but not from control plants.

We also examined the pathogenicity of the isolates on seedlings of seven legume species: adzuki bean (*Vigna angularis*), common bean (*Phaseolus vulgaris*), milk vetch (*Astragalus sinicus*), pea (*Pisum sativum*), peanut (*Arachis hypogaea*), soybean (*Glycine max*) and winged bean (*Psophocarpus tetragonolobus*). A conidial suspension (10^6 conidia/ml sterile distilled water) was sprayed onto the plants without wounding. After 14 days, brown lesions 1–2 mm in diameter appeared on pea and peanut. Leaves of common bean and adzuki bean were slightly browned (Table 2; Fig. 1g–i). Leaves of winged bean, milk vetch, and soybean were symptomless.

Name of the disease

Colletotrichum truncatum causes anthracnose in members of the Leguminosae (Sutton 1980; von Arx 1987). It is pathogenic to species in six genera and is highly virulent to

most cultivars of pea (Weidemann et al. 1988). On the other hand, *C. truncatum* was isolated from 14 nonleguminous weeds, and some isolates were pathogenic to soybean (Hartman et al. 1986). Perhaps *C. truncatum* can infect a wide range of plant species other than legumes. The Christmas rose isolates were also pathogenic to the host plant and some of the tested legumes, including pea. The isolates resembled *C. truncatum* based on the morphological and cultural characteristics described in the present study, but they were not clearly distinct from some isolates identified as *C. dematium*, on the basis of the ITS1, 5.8S, and ITS2 sequences. Therefore, so far, we can identify them only as *Colletotrichum* sp. that produce falcate spores. Because no disease of Christmas rose caused by *Colletotrichum* sp. has ever been reported elsewhere, we propose to name the disease anthracnose (tanso-byo in Japanese) of Christmas rose.

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