Journal of General Plant Pathology (2020) 86:157–161
https://doi.org/10.1007/s10327-019-00903-4

DISEASE NOTE

Leaf spot of *Polygonatum odoratum* caused by *Colletotrichum spaethianum*

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Received: 27 September 2018 / Accepted: 31 July 2019 / Published online: 16 December 2019

Abstract

In August 2014, leaf spots were found on *Polygonatum odoratum* in Jilin Province in China. The fungus isolated from the diseased leaves was identified as *Colletotrichum spaethianum* based on pathogenicity, morphology and molecular characterization. The fungus was re-isolated from lesions that developed on leaves after inoculation. This report is the first of a disease on *P. odoratum* caused by *C. spaethianum*.

Keywords Leaf spot · *Colletotrichum spaethianum* · *Polygonatum odoratum*

Polygonatum odoratum (Mill.) Druce (*Asparagaceae*), a flowering ornamental plant native to Asia and Europe, is used in traditional Chinese and Korean medicine. In August 2014, small brown spots were found on the edge of leaves of *P. odoratum* in a plant nursery at Jilin Agricultural University, Changchun, Jilin, China (125.24° E, 43.48° N). The lesions gradually enlarged to rounded brown spots (Fig. 1b), and leaf margins turned yellow (Fig. 1a).

For isolating the causal agent, small leaf pieces were removed from the lesion margins, then surface-sterilized for 1 min in 1.5% (v/v) NaOCl, washed twice with sterile distilled water, then plated onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) slants. Single spores were transferred with a needle using a microscope onto a PDA slant. A representative single-spore isolate was designated as CCPO34 and used in the following tests.

For confirming pathogenicity, a conidial suspension (1×10^6 conidia/ml) of isolate CCPO34 was sprayed onto 21 detached leaves and 10 leaves on young plants of *P. odoratum* following the method of Liu et al. (2013, 2017). Distilled water served as a negative control. These plants were covered with plastic bags for 6 days and kept in a greenhouse at 25–28 °C with a 12-h photoperiod using fluorescent light and 90% of relative humidity. This experiment was repeated twice. After 5, 7 and 9 days, all detached leaves (Fig. 1d–g) and intact leaves on inoculated plants (Fig. 1j) had symptoms but control plants had no symptoms (Fig. 1c, h, i). Isolate CCPO34 was consistently re-isolated from the lesions (Fig. 1g), but not from the control leaves (Fig. 1c, h, i). Brown spots on naturally diseased leaves of *P. odoratum* (Fig. 1b) and on the inoculated leaves were similar (Fig. 1d, f, g). By 14 days after inoculation with CCPO34, leaves on the plants had turned yellow and died (Fig. 1j).

Morphological characteristics of isolate CCPO34 were examined with a microscope after incubation on synthetic nutrient-poor agar (SNA) (Damm et al. 2009) at 25 °C with 12 h light/12 h dark period for 7 days. Conidia were 1-celled, hyaline, smooth-walled, aseptate, curved to slightly curved, with a more rounded or somewhat acute apex and a truncate base, $(15.5-)16-23.2(-28) \times (3.0-)3.5-4.1(-5.3)$ µm, mean ± SD = $20.5 \pm 2.8 \times 3.9 \pm 0.5$ µm, L/W ratio = 5.3 (Fig. 1o). Spore curvature ratios were calculated using the method of Sato et al. (2015): the average outer curvature of the conidia was 32.2, average inner curvature was 7.5, and average curvature deviation was 1.19. The colonies on PDA developed abundant white, aerial mycelium with olivaceous white mycelium below with a white margin (Fig. 1k, l). Appressoria were single or in loose groups, dark brown, irregular shapes, sometimes more or less lobed, smooth-walled, $(3.2-)8.1-9.4(-15.4) \times 3.2-6.2(-12.6)$ µm, mean ± SD = $9.3 \pm 2.5 \times 7.5 \pm 1.6$ µm, L/W ratio = 1.2 (Fig. 1p–t). Colonies on SNA were flat with an entire margin, having

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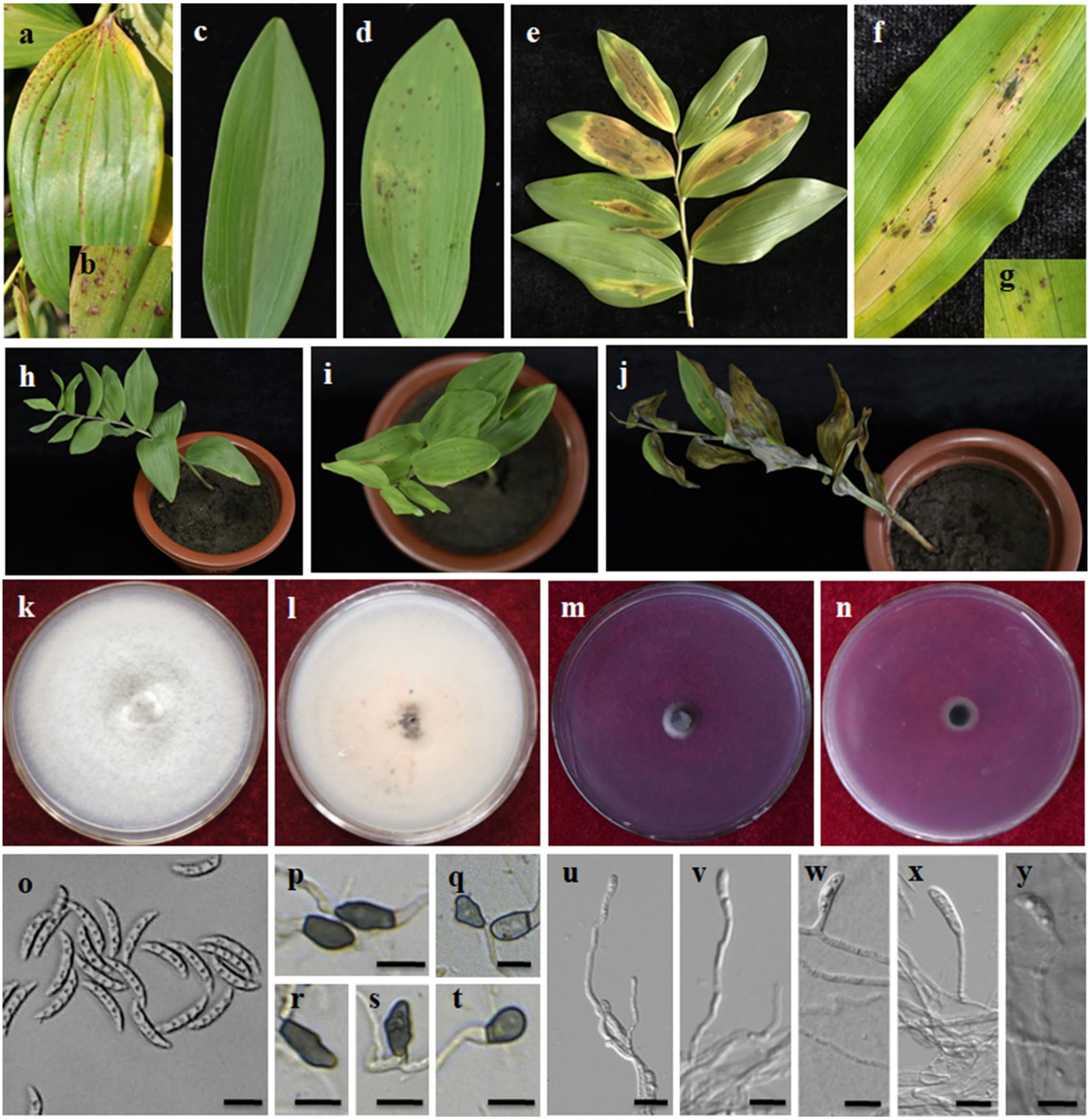

Fig. 1 Symptoms on leaves of *Polygonatum odoratum* and morphology of causal agent, *Colletotrichum spaethianum*. **a** Anthracnose and **b** brown spots after natural infection. **c** Water-inoculated leaf with no symptoms 9 days after inoculation. **d**–**f** Lesions on detached leaves 5 (**d**), 7 (**e**) and 9 (**f**) days after inoculation (dai) with isolate CCPO34. **g** Brown spots on inoculated detached leaf 9 dai. **h**–**j** Lesions on intact leaves 14 dai with CCPO34. **h** Mock-inoculated, nonwounded plant. **i** Mock-inoculated plant, wounded with a fine sterile needle before inoculation with sterile water. **j** Plant that was wounded before inoculation with CCPO34. **k**–**y** Morphology of *C. spaethianum*. **k**, **l** Colonies on PDA (**k**, **l**) and on SNA(**m**, **n**). **o** Conidia (**o**), appressoria (**p**–**t**) and conidiophores (**u**–**y**) on SNA. Scale bars = 10 µm

short grayish white, aerial mycelium (Fig. 1m, n). The conidiophores formed directly on hyphae (Fig. 1u–y). Morphological and cultural characterizations were almost consistent with the description of *Colletotrichum spaethianum* (Allesch.) Damm et al. (2009). Setae were not observed.

For molecular support of these observations, genomic DNA of isolate CCPO34 was extracted using the method of Liu et al. (2013). The 5.8S nuclear ribosomal gene with

Table 1 Strains and isolates of *Colletotrichum* spp. and the accessions used in this study

Species	Strain or Isolate	GenBank accession					References
		ITS	GAPDH	ACT	CHS-1	TUB2	
Colletotrichum americae-borealis	CBS 136232	KM105224	KM105579	KM105434	KM105294	KM105504	Damm et al. (2014)
Colletotrichum antirrhinicola	CBS 102,189	KM105180	KM105531	KM105390	KM105250	KM105460	Damm et al. (2014)
Colletotrichum bryoniicola	CBS 109849	KM105181	KM105532	KM105391	KM105251	KM105461	Damm et al. (2014)
Colletotrichum coccodes	CBS 164.49	JQ005775	—-	JQ005838	JQ005796	JQ005859	O'Connell et al. (2012)
Colletotrichum dematium	CBS 125.25	GU227819	GU228211	GU227917	GU228309	GU228113	Damm et al. (2009)
Colletotrichum dematium	CBS 115524	GU227826	GU228218	GU227924	GU228316	GU228120	Damm et al. (2009)
Colletotrichum destructivum	C8	MF033896	MF033897	MF033900	MF033898	MF033901	Xue et al. (2018)
Colletotrichum fuscum	CBS 200.54	KM105179	KM105529	KM105389	KM105249	KM105459	Damm et al. (2014)
Colletotrichum fuscum	CBS 133703	KM105175	KM105525	KM105385	KM105245	KM105455	Damm et al. (2014)
Colletotrichum higginsianum	Abo 1–1	KM105185	KM105537	KM105395	KM105255	KM105465	Damm et al. (2014)
Colletotrichum lilii	CBS 109214	GU227810	GU228202	GU227908	GU228300	GU228104	Damm et al. (2009)
Colletotrichum liriopes	CBS 122747	GU227805	GU228197	GU227903	GU228295	GU228099	Damm et al. (2009)
Colletotrichum pisicola	CBS 724.97	KM105172	KM105522	KM105382	KM105242	KM105452	Damm et al. (2014)
Colletotrichum spaethianum	CDLG2	JQ400003	JQ400010	JQ399989	JQ399996	JQ400017	Yang et al. (2012)
Colletotrichum spaethianum	**CCPO34**	**MH020771**	**MH020772**	**MH045677**	**MH020773**	**MH045678**	**This study**
Colletotrichum spaethianum	CSSX5	GQ485586	GQ856747	GQ856769	GQ856718	GQ849426	Yang et al. (2009)
Colletotrichum spaethianum	CBS 167.49	GU227807	GU228199	GU227905	GU228297	GU228101	Damm et al. (2009)
Colletotrichum tabaci	CBS 124249	KM105206	KM105560	KM105416	KM105276	KM105486	Damm et al. (2014)
Colletotrichum tabaci	N150	KM105204	KM105557	KM105414	KM105274	KM105484	Damm et al. (2014)
Colletotrichum verruculosum	IMI 45525	GU227806	GU228198	GU227904	GU228296	GU228100	Damm et al. (2009)
Colletotrichum vignae	CBS 501.97	KM105183	KM105534	KM105393	KM105253	KM105463	Damm et al. (2014)
Colletotrichum tofieldiae	CBS 130851	KR003335	KR003360	KR003345	KR003355	KR003340	Unknown

CBS culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, *IMI* culture collection of CABI Europe UK Centre, Egham, UK.

the two flanking internal transcribed spacers (ITS), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), a partial sequence of the actin (*ACT*), chitin synthase 1(*CHS-1*) and beta-tubulin (*TUB*2) genes were amplified and sequenced using primer pairs ITS-1 + ITS-4 (Gardes and Bruns 1993; White et al. 1990), GDF1 + GDR1 (Guerber et al. 2003), ACT-512F + ACT-783R (Carbone and Kohn 1999), CHS-345R + CHS-79F (Carbone and Kohn 1999), and T1 (O'Donnell and Cigelnik 1997) + Bt-2b (Glass and Donaldson 1995) (the PriMicro database (https://primicro.jpn.org/)), respectively.

The ITS, *GAPDH*, *ACT*, *CHS-1* and *TUB*2 sequences of isolate CCPO34 were deposited in GenBank (accession MH020771 for ITS, MH020772 for *GAPDH*, MH045677 for *ACT*, MH020773 for *CHS-1*, and MH045678 for *TUB*2). A BLAST search revealed that all the sequences had more than 99% identity to *C. spaethianum* (isolate CBS 167.49; GU227807 for ITS, GU228199 for *GAPDH*, GU227905 for ACT and GU228297 for *CHS-1*, and GU228101 for *TUB*2). Phylogenetic trees were constructed using MEGA7 (Kumar et al. 2016) for the combined data set of the ITS, *GAPDH*, *ACT*, *CHS-1* and *TUB*2 genes for 22 sequences from *Colletotrichum* (Table 1). Percentage bootstrap support > 60% (1000 replications) by the maximum parsimony (MP) methods are shown on the respective branch. Isolate CCPO34 of *P. odoratum* clustered in the *C. spaethianum* clade (Fig. 2).

Isolate CCPO34 was placed in the Herbarium of Mycology of Jilin Agricultural University. This pathogen has been previously reported on *Peucedanum praeruptorum* Dunn, *Lilium lancifolium* Thunb and *Atractylodes japonica* Koidzumi in China (Guan et al. 2018; Guo et al. 2013; Zhao et al. 2016); *Hemerocallis flava* Linn and *Allium fistulosum* L. in Brazil (Santana et al. 2016; Vieira et al. 2014); *A. fistulosum*, *Crinum latifolium* L., *Iris germanica* L. and *Raphanus sativus* L. in Japan (Sato et al. 2005, 2015); and on *Hosta plantaginea* Aschers in Korea (Cheon and Jeon 2016). In May 2001, *C. spaethianum* was also found on potted plants of *Polygonatum falcatum* A.Gray in Japan (Sato et al. 2015; Tomioka et al. 2008). This study on the etiology of leaf spot on *P. odoratum* based on pathogenicity experiments, morphological and molecular characterization is the first report of *C. spaethianum* causing leaf spot on *P. odoratum* in China.

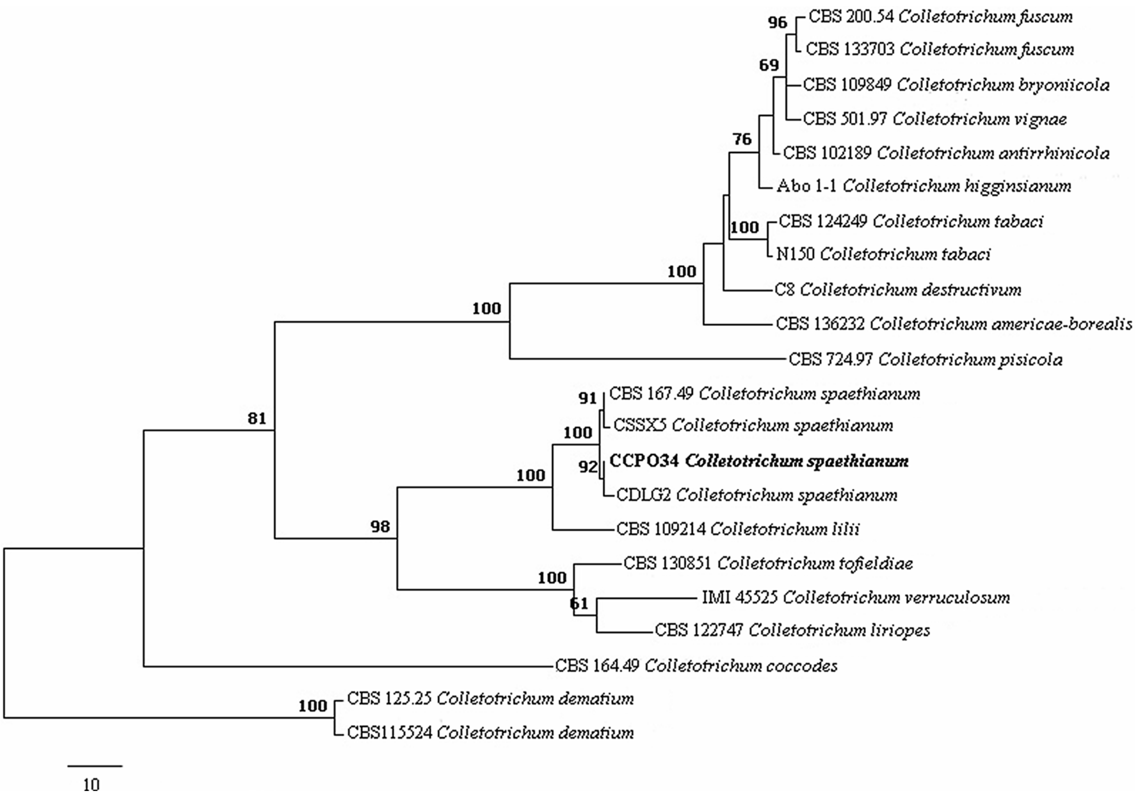

Fig. 2 Phylogenetic tree constructed using combined data set for ITS, *GAPDH*, *ACT*, *CHS-1* and *TUB*2 sequences from 22 accessions of *Colletotrichum*. Percentage bootstrap support > 60% (1000 replications) using maximum parsimony (MP) methods are shown on the respective branches. Isolate CCPO34 from leaf spots on *Polygonatum odoratum* clustered with *Colletotrichum spaethianum*

Acknowledgements We highly appreciate Prof. Hsiang and Dara Muammad Zulqar Nain for careful review of this manuscript. This study was supported by the National Natural Science Foundation of China (No. 31700010), Phylogeny and taxonomy of *Colletotrichum* species by using a polyphasic approach on medicinal plants in northeast China. This work was also supported by 111 Project (No. D17014) and the Research Fund (No. 2018YFD0201100).

Compliance with ethical standards

Conflicts of interest We have no conflict of interest.

Human and animal rights statement This article does not contain any studies with human participants or animals.

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