

different fields in Shanghai, including Zhuanghang Comprehensive Experimental Station. The sampling sites included both greenhouse and open fields. Infected leaf sections (5 × 5 mm) were cut from the edge of the necrotic/healthy tissues, surface sterilized with 70% ethanol for 30 s and 2% sodium hypochlorite for 90 s, rinsed twice with sterilized distilled water, and placed on potato dextrose agar. Five morphologically similar fungal isolates were obtained in total. Fungal isolates developed white aerial mycelium that became dark brown after 7 to 9 days at 25°C. The mycelium was composed of branched, septate, hyaline, smooth, light to pale brown hyphae. Conidia were abundant, solitary, globose to subglobose, smooth, and aseptate, and the diameter was 12.5 to 14.5 μm. Cultural and morphological characteristics were identified according to protocols described by Wang et al. (2017), and isolates appeared to be morphologically similar to *Nigrospora oryzae*. Internal transcribed spacer (ITS) region and β-tubulin genes were amplified from DNA extracted from single-spore cultures, using ITS1/ITS4 primers (White et al. 1990) and Bt-2a/Bt-2b primers (Glass and Donaldson 1995), respectively, and then sequenced. Identical sequences were obtained from all isolates, and the sequences of representative isolate SH01 were submitted to GenBank (accession nos. MK262851 for ITS and MK262852 for β-tubulin). BLAST analyses showed sequences to share 99% identity with a sequence of *N. oryzae* for ITS (GenBank accession no. MH087476.1) and 99% with a sequence of *N. oryzae* (GenBank accession no. KY019613.1) for β-tubulin. According to Koch's postulates, pathogenicity tests were conducted through wound inoculation methods. Five-year-old blueberry plants were used, and the top five to six leaves of each branch were inoculated. Nine healthy potted plants of cultivar O'Neal were punctured with flamed needles and sprayed with a conidial suspension (10<sup>6</sup> conidia/ml). Nine plants similarly injured were sprayed with sterile distilled water and served as controls. Each plant was covered with a plastic bag for 72 h and incubated at 25 ± 2°C in humid chambers for 30 days with a 16-h/8-h light/dark cycle. After 10 days, symptoms on all wounded inoculated plants were similar to those previously observed with distinct dark brownish red borders. Symptoms were not observed on control plants. The same pathogen was reisolated from the spots. *N. oryzae* is known as a pathogen for several hosts worldwide (Farr and Rossman 2015). To our knowledge, this is the first report of *N. oryzae* causing leaf spot on blueberry in China. Because the disease could potentially adversely affect the future production of blueberries, it is important to develop control strategies against this disease in China.

#### References:

- Farr, D. F., and Rossman, A. Y. 2015. Fungal Databases, Syst. Mycol. Microbiol. Lab., ARS, USDA. Retrieved 18 August 2015 from <https://nt.ars-grin.gov/fungal-databases/>.
- Glass, N. L., and Donaldson, G. C. 1995. Appl. Environ. Microbiol. 61:1323.
- Wang, M., et al. 2017. Persoonia 39:118.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

e-Xtra

**Keywords:** leaf spot, blueberry, *Nigrospora oryzae*

#### First Report of Powdery Mildew Caused by *Erysiphe sedi* on *Sedum emarginatum* in China

Luchao Bai,<sup>1,2</sup> Yilin Li,<sup>2</sup> Xiao-ming Duan,<sup>2</sup> Guangjing Yin,<sup>3</sup> Qiang Lin,<sup>4</sup> Qingjiang Ma,<sup>3</sup> Changhong Shi,<sup>5</sup> and Guoxin Chen<sup>1,2,†</sup>

<sup>1</sup> State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, Qinghai 810016, China

<sup>2</sup> College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai 810016, China

<sup>3</sup> Forest Seedling Station of Qinghai Province, Xining, Qinghai 810000, China

<sup>4</sup> Forestry and Grassland Bureau of Qinghai Province, Xining, Qinghai 810000, China

<sup>5</sup> Makehe Forestry Bureau of Qinghai Province, Guoluo, Qinghai 814000 China

**Funding:** Funding was provided by Natural Science Foundation of Qinghai Province (grant no. 2017-ZJ-958Q) and National Natural Science Foundation of China (grant no. 31600513). Plant Dis. 103:2474, 2019; published online as <https://doi.org/10.1094/PDIS-02-19-0293-PDN>. Accepted for publication 1 May 2019.

*Sedum emarginatum* Migo is native to China and also commonly grown as an ornamental plant. In August 2018, severe powdery mildew was

observed on almost all *S. emarginatum* in the south mountain forest park of Xining (36.7°N, 101.7°E, altitude 2,360 m), China. A voucher specimen was deposited in the Herbarium of Plant Pathology at Qinghai University under accession number QHU2018074. This powdery mildew is morphologically characterized as follows. White powdery mildew colonies developed on infected plants and subsequently covered surfaces of leaves, petioles, and stems, which resulted in leaf desiccation and defoliation. Fungal mycelia were amphigenous, white, effuse or in patches, and persistent, with lobed hyphal appressoria. Conidiophores were erect and up to 100 μm long, foot cells straight, cylindrical, and 21 to 52 × 8 to 10 μm, followed by one to three shorter cells. Conidia were ellipsoid-ovoid, subcylindrical, and 24 to 40 × 10 to 17 μm. Chasmothecia were scattered to gregarious, depressed globose, and 75 to 110 μm in diameter. Peridium cells were irregularly polygonal and 6 to 23 μm in diameter; appendages mostly five to 18, mycelium-like, unbranched, and usually interwoven with the mycelia. Asci four to 10, ellipsoid-obovoid, saccate, sessile, or short-stalked, 45 to 85 × 30 to 60 μm with three to five spores. Ascospores broadly ovoid to subglobose, faintly yellow and 18 to 29 × 12 to 19 μm. The fungus was identified as *Erysiphe sedi* based on morphology of its asexual and sexual morphs (Braun and Cook 2012). To confirm identification, DNA was extracted from the fungus and the ITS region amplified using primers ITS5/P3 and PM5/ITS4. The cloned fragments were then sequenced. The ITS region sequences were deposited in GenBank with accession number MK340819. A BLAST analysis of several sequences revealed 100% identity with *E. sedi* infecting *Sedum bulbiferum* Makino in South Korea (MK511841), *E. aquilegiae* infecting *Caltha palustris* Linn. in the United Kingdom (KY653199), *Pseudoidium hortensiae* infecting *Hydrangea serrata* (Thunb.) Ser. in South Korea (MG654731), and so on. Based on the ITS rDNA phylogenetic tree, the sequences clustered within a strongly supported clade with three sequences mentioned above. Phylogenetic trees were constructed using the neighbor-joining method with the Kimura two-parameter substitution model in MEGA 5.0. Branch robustness was assessed via bootstrap analysis with 1,000 replicates. ITS sequences from this group are almost identical but include many *Erysiphe* species isolated from a wide range of host plants. Based on the host plant, we think the species is *E. sedi* (Meeboon and Takamatsu 2015; Takamatsu et al. 1999, 2009, 2015). Pathogenicity tests were performed by rubbing infected leaves onto healthy leaves and stems of four *S. emarginatum* seedlings. Four healthy seedlings were kept separately as noninoculated control plants. Inoculated and noninoculated plants were maintained separately in different rooms of a greenhouse held at 22 to 25°C. Inoculated plants developed powdery mildew signs and symptoms after 14 days, whereas control plants remained symptomless. Fungus morphology on inoculated plants was identical to that originally observed on naturally diseased plants. To our knowledge, this is the first report of the powdery mildew fungus *E. sedi* infecting *S. emarginatum* in China and also worldwide. We believe this disease has the potential to cause significant losses and become a limiting factor in production of *S. emarginatum*.

#### References:

- Braun, U., and Cook, R. T. A. 2012. Taxonomic Manual of the Erysiphales (Powdery Mildews). CBS Biodiversity Series No. 11. CBS, Utrecht, the Netherlands.
- Meeboon, J., and Takamatsu, S. 2015. Mycoscience 56:230.
- Takamatsu, S., et al. 1999. Mycoscience 40:259.
- Takamatsu, S., et al. 2009. Mycol. Res. 113:117.
- Takamatsu, S., et al. 2015. Mycologia 107:475.

The author(s) declare no conflict of interest.

e-Xtra

**Keywords:** powdery mildew, *Erysiphe sedi*, *Sedum emarginatum*, China

#### First Report of *Rhizoctonia solani* AG-4 HGI Causing Crown and Stem Rot on Purple Coneflower (*Echinacea purpurea*) in Italy

A. Garibaldi, D. Bertetti, G. Gilardi, S. Matic, and M. L. Gullino<sup>†</sup>

Centre of Competence for the Innovation in the Agro-Environmental Sector (AGROINNOVA) and DISAFA, University of Torino, 10095 Grugliasco, Italy

Plant Dis. 103:2474, 2019; published online as <https://doi.org/10.1094/PDIS-01-19-0223-PDN>. Accepted for publication 1 May 2019.

In July to August 2018, crown and stem rot symptoms were observed on purple coneflower (*Echinacea purpurea* L.) grown as an ornamental in a garden located near Biella, 45°36'00"N 8°03'00"E (northern Italy). Water-soaked lesions developed on the crown directly above the soil line in 5 to 10% of 60-day-old plants grown in 25-liter pots. The disease was more common and severe in shaded or very humid areas, leading plants to death.

Diseased tissue was surface disinfested for 10 s in 1% NaOCl, rinsed with sterile water, and plated on potato dextrose agar (PDA) amended with 25 mg/liter of streptomycin sulfate. A fungus with the morphological characters of *Rhizoctonia solani* (Sneh et al. 1991) was recovered from 70% of the samples, transferred, and maintained in pure culture. Mycelium of 10-day-old cultures grown on PDA, at 22 ± 1°C, was light brown, compact, with radial growth. Sclerotia were not present. DNA was extracted from a pure culture of a single isolate coded 43 with the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek, Darmstadt, Germany). The internal transcribed spacer (ITS) region of rDNA was amplified by using the primers ITS1/ITS4 (White et al. 1990). The purified 668-bp PCR product was sequenced at the BMR Genomics Centre (Padova, Italy) and deposited in GenBank under accession number MK430998. A BLASTn search of this sequence showed 100% identity with the reference sequence KF907733 of *R. solani* AG-4 HGI isolated from *Brassica oleracea* (Hua et al. 2014). Anastomosis group was defined by pairing one strain of *R. solani* from *E. purpurea* with *R. solani* isolates belonging to already known anastomosis groups (AG1, AG2, AG4, AG7, and AG11) and observing microscopically the hyphal fusions. Three replicates for each pairing were made. *R. solani* from *E. purpurea* anastomosed (fusion frequency < 30%) with *R. solani* isolate AG4 (Sneh et al. 1991). This characterization was in accordance with the features of the isolates described above and with characteristics reported for AG4 group. Moreover, the ITS analysis permitted identification of the subgroup HGI of *R. solani* AG4. Five pots of 30-day-old plants of *E. purpurea* were used to evaluate the pathogenicity of isolate 43 of *R. solani*. The test was repeated once. Healthy plants were transplanted into steam-disinfested peat substrate (Cyclamen, Turco S.p.a., Italy) artificially infested by mixing 1 g/liter of *R. solani* grown on autoclaved wheat kernels at 25°C for 10 days. Plants transplanted in noninoculated substrate and maintained in the same greenhouse at 20 to 25°C served as a control. The first symptoms, similar to those observed in the garden, developed 10 to 12 days after inoculation, and 60 to 80% of plants died in both replications. Control plants remained healthy. *R. solani* was always reisolated from affected crowns and stems. Although *R. solani* was reported on *E. purpurea* in Canada (Chang et al. 1997), this is the first report of the disease in Italy and in Europe. The impact of the disease is unknown because it was observed for the first time in a private garden.

#### References

- Liang, K. F., et al. 1997. Plant Dis. 81:1461.  
 Hua, G. K., et al. 2014. PLoS One 5:e111750.  
 Sneh, B., et al. 1991. Identification of *Rhizoctonia* Species. APS Press, St Paul, MN.  
 White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

**Keywords:** fungi, ornamentals, soilborne pathogens

### First Report of Leaf Black Spot on White Chrysanthemum (*Chrysanthemum morifolium*) Caused by *Phoma bellidis* in China

Y. H. Liu,<sup>1</sup> C. Q. Zhang,<sup>1†</sup> and D. J. Dai<sup>2</sup>

<sup>1</sup> Department of Crop Protection, Zhejiang Agriculture and Forest University, Lin'an 311300, China

<sup>2</sup> Institute for the Control of Agrochemicals of Zhejiang Province, Hangzhou 310020, China

**Funding:** Funding was provided by Zhejiang Public-Interest Technology Application Study (grant no. 2016C32002). Plant Dis. 103:2475, 2019; published online as <https://doi.org/10.1094/PDIS-03-19-0611-PDN>. Accepted for publication 4 May 2019.

White chrysanthemum (*Chrysanthemum morifolium*) is one of the most important Chinese medicinal plants in Zhejiang province. In Tongxiang city of Zhejiang province, which produces 60% of white chrysanthemum in China, leaf black spot (LBS) disease of *C. morifolium* has become more and more problematic. In 2016, LBS disease incidence on *C. morifolium* in Tongxiang city was more than 50%. Symptoms on leaves were initially round or oval puce spots, which then coalesced into larger black lesions. A total of 47 infected leaves collected from nine different fields in Tongxiang city were surface sterilized with 1.5% sodium hypochlorite for 2.5 min, rinsed twice in sterilized water, plated on 2% potato dextrose agar (PDA), and incubated at 25°C in the dark. Colonies of the fungus on PDA were initially yellowish and later became grayish-white. After 25 days on 3% oatmeal agar, black spherical pycnidia produced light pink cirrhi containing single-celled, colorless, long oval conidia (3.3 ± 0.3 × 1.9 ± 0.2 µm) for 35 tested isolates. These characteristics were consistent with *Phoma bellidis*. To confirm the identification based on morphological traits, the ribosomal

internal transcribed spacers (ITS), large subunit (LSU), and *tub2* of three isolates were amplified from DNA extracted from mycelium produced on PDA and analyzed (Pearce et al. 2016), respectively, with the primers ITS1/ITS4, LR0R (5'-GTACCCGCTGAACCTAAGC-3')/LR7 (5'-TACTACCACCAAGATCT-3') and Btub2Fd (5'-GTBCACCTYCARACC GGYCARTG-3')/Btub4Rd (5'-CCRGAYTGRCCRAARACRAAGTTGT C-3'). The sequences were deposited in NCBI with accession numbers of ITS (MK419331, MK419332, and MK419333), LSU (MK419337, MK419338, and MK419339), and *tub2* (MK572949, MK572950, and MK572951). BLAST analysis of the ITS (525-bp) segments showed 99% identity with the ITS sequence of *P. bellidis* isolate 346 (GenBank accession no. KM507775.1). The LSU (1,046-bp) segments showed 99% identity with that of *P. bellidis* isolate CBS 714.85 (GU238046.1), and the *tub2* (499-bp) gene segments showed 95% identity with that of *P. bellidis* (KR818909.1). Results of multilocus phylogenetic analyses based on ITS, LSU, and *tub2* suggested the pathogen was *P. bellidis*. Uninfected healthy plants of *C. morifolium* were sprayed either with a conidial suspension of 5 × 10<sup>6</sup> conidia/ml in distilled water as the inoculum or with distilled water only to provide an uninoculated control and were incubated in pots (4 cm diameter by 6 cm) in growth chambers at 25°C under an 8-h/16-h day/night regime with 90% relative humidity for 24 h. For each isolate, 20 whole plants were inoculated. After 10 days, 24 of 25 isolates caused lesions on all inoculated plants, whereas no symptoms developed on the non-inoculated controls. Cultures reisolated from lesions and cultured on PDA exhibited morphological characteristics identical to those of *P. bellidis* (Chen et al. 2015; Lv et al. 2011), confirming Koch's postulates. Inoculation tests were conducted twice. To our knowledge, this is the first report of LBS disease in white chrysanthemum, which is a major factor limiting its production. Corresponding measures must be adopted to manage this disease in a timely manner.

#### References

- Chen, Q., et al. 2015. Stud. Mycol. 82:137.  
 Lv, R., et al. 2011. Plant Dis. 95:1190.  
 Pearce, T. L., et al. 2016. Plant Pathol. 65:1170.

The author(s) declare no conflict of interest.

**Keywords:** fungi, ornamentals, herbaceous/flowering plants, etiology

### First Report of Stem Rot Disease of Rose Mallow (*Hibiscus rosa-sinensis*) Caused by *Sclerotium rolfsii* in India

Amar Bahadur,<sup>1</sup> Deeba Kamil,<sup>2†</sup> Prasenjit Debnath,<sup>1</sup> Anjali Kumari,<sup>2</sup> Akanksha Tyagi,<sup>2</sup> and T. Prameela Devi<sup>2</sup>

<sup>1</sup> College of Agriculture, Lembucherra, Agartala, Tripura, India

<sup>2</sup> Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi, Delhi, India

Plant Dis. 103:2475, 2019; published online as <https://doi.org/10.1094/PDIS-09-18-1480-PDN>. Accepted for publication 4 May 2019.

Rose mallow (*Hibiscus rosa-sinensis*) is an ornamental plant that produces colorful flowers and is native to China. Generally, *Hibiscus* sp. is not affected by many devastating diseases, but during January 2017, four plants out of 20 plants in the farm of the College of Agriculture, Lembucherra, in Agartala, Tripura (India), exhibited wilting and dieback owing to rotting of the lower trunk or crown tissues. Further disease incidence was recorded between 5 and 20% in an agricultural field of Agartala region. White mycelium with sclerotia was observed on the outer surface of the lower stems, and sclerotia were also found inside the stems. Symptomatic stem sections and sclerotia from two plants were surface disinfested in 0.5% sodium hypochlorite for 1 min, washed thrice with sterilized distilled water, air dried, and placed in Petri dishes containing water agar and incubated at room temperature (25°C). The hyphae were subsequently transferred to potato dextrose agar. Pure cultures were obtained with abundant, aerial, and white mycelia. Tan to dark brown sclerotia 0.5 to 1.5 mm in diameter developed after the mycelium covered the entire plate. The pathogen was tentatively identified as *Sclerotium rolfsii* Sacc. based on the presence of clamp connections in the hyphae and ultrastructure of sclerotia (Aycock 1966). The ultrastructure of sclerotia revealed the presence of four separate cell layers (i.e., outer thick skin, a broad rind that was two to four cells thick, a cortex of thin-walled cells, and a medulla made of loosely arranged, ordinary filamentous hyphae). To confirm the identification, the genomic DNA was extracted from the mycelial mat of *S. rolfsii* using a ZR fungal/bacterial DNA miniprep kit (Irvine, CA), and the internal transcribed spacer region of rDNA was amplified using primers ITS1 and ITS4 (White et al. 1990). A PCR product of 550 bp was sequenced. The sequence (GenBank accession no.