First Report of Target Spot Caused by *Corynespora cassiicola* on Strawberry in North America

R. B. Onofre,† C. S. Rebello, J. C. Mertely, and N. A. Peres,

Department of Plant Pathology, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598

Plant Dis. 103:1412, 2019; published online as https://doi.org/10.1094/PDIS-01-19-0057-PDN. Accepted for publication 2 February 2019.

Corynespora cassiicola has a wide geographic distribution and host range and is a threat to economically important crops such as cotton (*Gossypium hirsutum*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and soybean (*Glycine max*) in the Southeastern United States. Recently, *C. cassiicola* was reported on blueberry (*Vaccinium corymbosum*) in Florida (Onofre et al. 2016). In October 2018, severe leaf spotting and defoliation of strawberry (*Fragaria* × *ananassa* Duchesne) plants of cultivar Florida Radiance were observed in a shipment of plug plants received by a grower in Hillsborough County, FL. Leaf lesions were 3 to 5 mm in diameter, circular to irregular, with dark brown borders and beige centers. These symptoms were similar to the frogeye-type leaf spot recently described in a first report of *C. cassiicola* on strawberry in China (Zhang et al. 2018). Dark brown petiole lesions were also observed. Pieces of tissue from the leading edges of leaf lesions were disinfested in 0.625% sodium hypochlorite for 1 min, rinsed twice in sterile distilled water, placed on potato dextrose agar, and incubated for 10 days at 23°C with a 12-h photoperiod. Colonies were gray with velvety to hairy aerial mycelium. Conidia were obclavate to cylindrical, multiseptate, pale olivaceous to dark brown, smooth, 27 to 319 µm long, and 5 to 13 µm wide ($n = 150$). Based on the morphological characteristics described by Ellis and Holliday (1971), the pathogen was identified as *Corynespora cassiicola* (Berk & MA Curtis) C. T. Wei. The internal transcribed spacer (ITS) region and β-tubulin genes of three isolates were sequenced (Dixon et al. 2009; Shimomoto et al. 2011), and the sequences were deposited in GenBank (accession nos. MK333283 to MK333285 and MK335367 to MK335369). The β-tubulin sequences were 100% identical to *C. cassiicola* accession numbers KY290564.1 and KY082896.1, and the ITS region sequences showed 100% identity to *C. cassiicola* accession numbers MF320532.1 and JQ717069.1. Three single-spore isolates, obtained from different plants, were added to the University of Florida Gulf Coast Research and Education Center culture collection as accessions 18-664, 18-666, and 18-667; they were used for subsequent pathogenicity tests. Each isolate was evaluated for pathogenicity on five 35-day-old plants each of cultivars Florida Radiance, Florida Beauty, and Florida127. Each plant was spray inoculated with a suspension of 2×10^4 conidia/ml. As a control, five plants per cultivar were sprayed with sterile distilled water. Plants were covered with plastic bags immediately after inoculation to ensure high humidity and placed in the greenhouse. After 36 h, plants were removed from the plastic bags and maintained in the greenhouse. Initial symptoms were visible 2 days after inoculation; within 8 days, symptoms like those described above were observed on leaves, petioles, and fruit calyxes of all three cultivars. Control plants remained symptomless. *C. cassiicola* was readily isolated from lesions of inoculated tissues, fulfilling Koch's postulates. The experiment was repeated once. To our knowledge, this is the first report of *C. cassiicola* on strawberry in North America. Diseased plants were from a greenhouse nursery in Virginia, located near a soybean field. This raises a concern for the production of strawberry plants near other hosts that are susceptible to *C. cassiicola*. The establishment of the disease in the area could pose a serious threat for the strawberry industry in Florida.

References:

Dixon, L. J., et al. 2009. Phytopathology 99:105.
Ellis, M. B., and Holliday, P. 1971. *Corynespora cassiicola*. CMI Descriptions of Pathogenic Fungi and Bacteria, No. 303. Commonwealth Mycological Institute, Kew, U.K.
Onofre, R. B., et al. 2016. Plant Dis. 100:528.
Shimomoto, Y., et al. 2011. Plant Pathol. 60:253.
Zhang, M., et al. 2018. Plant Dis. 102:2037.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Corynespora cassiicola*, pathogen detection, small fruits, strawberry, North America

First Report of *Alternaria alternata* Causing Postharvest Rot on Stored Pomegranate in Pakistan

M. W. Alam,[1,†] A. Rehman,[2] S. Ahmad,[1] M. Sarwar,[1] S. Ali,[3] T. Shafeeq,[1] S. Aslam,[4] A. Mannan,[1] and H. Rosli[5]

[1] Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
[2] Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan
[3] Department of Horticulture, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
[4] Department of Ecology, College of Life Sciences, Beijing Normal University, China
[5] School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Plant Dis. 103:1412, 2019; published online as https://doi.org/10.1094/PDIS-01-19-0173-PDN. Accepted for publication 5 February 2019.

Pomegranate (*Punica granatum*) is an important crop in Pakistan and is predominantly planted in Muzaffargarh (30°4′27.7572″N, 71°11′4.7544″E) and Multan (30°7′40.9296″N, 71°23′11.1984″E), which are the primary pomegranate producing areas of the Punjab Province, Pakistan. During November 2017, postharvest fruit rot was observed on 'Kandhari' pomegranate after 35 days of storage at 6°C. The external symptoms consisted of small, circular, reddish brown lesions (3 to 9 mm in diameter), which rapidly enlarged (22 to 29 mm), resulting in fruit rot. Internal symptoms consisted of a dark rot of the fruit core. Disease incidence was estimated at 20%. Symptomatic tissues (3 to 4 mm^2) were cut from the fruit surface and sterilized in 75% ethanol for 30 s, 1% sodium hypochlorite for 90 s, rinsed three times with sterile distilled H_2O, plated onto potato dextrose agar, and incubated at 25°C for 7 days. A dark olivaceous fungus with abundant, branched, brown to black, and septate hyphae was consistently isolated (85% isolation frequency) from 20 fruit samples. Conidia were produced in long chains, typically ovoid or ellipsoidal with a short conical beak at the tip. Conidia were dark brown, septate, 11 to 45 × 7 to 21 µm in size, and had two to six horizontal septa and zero to four vertical septa. Conidiophores were straight, septate, light to olive brown, and measured 24 to 58 µm long × 2 to 4 µm wide. The morphological characteristics matched the description of *Alternaria alternata* (Simmons 2007). For molecular identification, DNA of one (PD20183) single-spore isolate was extracted using a Qiagen DNA extraction kit. The internal transcribed spacer (ITS) region and a fragment of the RNA polymerase II (RPB2) gene and endopolygalacturonase (endoPG) gene were amplified with primers ITS1/ITS4 (White et al. 1990), fRPB2-7cF/fRpb2-7cR (Liu et al. 1999), and PG3/PG2b (Andrew et al. 2009), respectively. The amplicons were sequenced and submitted to GenBank (accession nos. MK392017, MK400690, and MK105919). BLAST analysis of the ITS rDNA revealed 99% identity with that of *A. alternata* isolate MG717290. Moreover, the RPB2 and endoPG sequences were 99 and 100% identical with *A. alternata* ex-type strain CBS 916.96 (KC584375 and JQ811978). For pathogenicity tests, 10 surface-sterilized Kandhari fruit were sprayed with a conidial suspension (10^5 conidia/ml) using a handheld sprayer. Ten fruit sprayed with sterile distilled water served as controls. After 15 to 17 days at 6°C, all inoculated fruit exhibited symptoms similar to those observed in storage. No symptoms were observed in control fruit. *A. alternata* was consistently (95% isolation frequency) reisolated from inoculated fruit, confirming Koch's postulates. Pathogenicity tests were repeated twice. Fruit rot of pomegranate caused by *Alternaria* sp. has been reported in the United States, Mexico (Farr and Rossman 2018), and Greece (Tziros et al. 2008). To our knowledge, this is the first report of postharvest fruit rot on pomegranate caused by *A. alternata* during cold storage in Pakistan. Further research is needed on management options to combat the disease.

References:

Andrew, M., et al. 2009. Mycologia 101:95.
Farr, D. F., and Rossman, A. Y. 2018. Fungal Databases, Syst. Mycol. Microbiol. Lab., ARS, USDA.
Liu, Y. L., et al. 1999. Mol. Biol. Evol. 16:1799.
Simmons, E. G. 2007. *Alternaria*: An Identification Manual. CBS Fungal Biodiversity Center, Utrecht, the Netherlands.
Tziros, G. T., et al. 2008. Plant Pathol. 57:379.
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, fruit, pathogen detection

First Report of Leaf Necrosis Caused by *Alternaria alternata* on *Ceratostigma willmottianum* in Italy

A. Garibaldi,[1] D. Bertetti,[1] S. Matić,[1] I. Luongo,[2] and M. L. Gullino[1,2,†]

[1] Centre of Competence AGROINNOVA, University of Torino, 10095 Grugliasco, Italy
[2] DISAFA, University of Torino, 10095 Grugliasco, Italy

Plant Dis. 103:1412, 2019; published online as https://doi.org/10.1094/PDIS-02-19-0408-PDN. Accepted for publication 3 March 2019.

Ceratostigma willmottianum Stapf, a member of Plumbaginaceae family, is a perennial shrub with a long summer flowering period, commonly grown in public and private gardens. During the autumn of 2018, leaf necrotic areas were observed primarily on lower leaves of 12-month-old plants of *C. willmottianum* growing either in pots or in the ground, in a private garden located in Biella province (northern Italy). Fifteen of 20 plants were affected (average disease severity 5%). Necrotic areas were light brown, irregularly polygonal, 1 to 4 mm in size, and surrounded by extensive chlorosis. Symptomatic leaves were washed for 1 min with sterile water and blotted dry, and small pieces of tissue were taken from the margins of necrotic areas and placed on potato dextrose agar for 7 days, at temperatures from 20 to 25°C. The developing dark green colored fungal colonies were transferred on the same medium, at the same temperatures, to obtain pure cultures. Successively, pure cultures were grown on sterilized leaves of *C. willmottianum* placed on potato carrot agar (Simmons 2007), under a light/dark regime of 10 h/14 h, from 20 to 23°C. On this medium, colonies produced branched conidiophores (up to two branches), supporting conidia borne in chains. Conidia were light gray-green, ovoid to obclavate, multicellular, and measured 13 to 43 × 4 to 12 µm (average 23 × 9 µm, $n = 50$). Conidia contained one to seven transverse and zero to three longitudinal septa. When present, beaks were 2 to 5 µm long. Based on these morphological characteristics, the fungus was identified as *Alternaria* sp. (Simmons 2007). DNA from one fungal isolate was extracted using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek, Darmstadt, Germany), and a polymerase chain reaction was performed by amplification of the internal transcribed spacer (ITS) region of rDNA, the *endoPG*, the *tub2*, and the *cmdA* gene portions (Lawrence et al. 2013; Woudenberg et al. 2015). Four sequences with 529 (ITS), 476 (*endoPG*), 994 (*tub2*), and 671 bp (*cmdA*) (GenBank accession nos. MK204576, MK558219, MK558217, and MK558221, respectively) were obtained. A BLASTn search of these sequences showed 100% homology with *Alternaria alternata* (Fries) Keissler (accession nos. KU933199, MH728996, MH560610, and MG736308). A spore suspension of 0.8×10^5 CFU/ml in sterile water was obtained from the pure cultures used for the observations described above. This suspension was sprayed (10 ml/plant) onto leaves of three healthy plants of *C. willmottianum*. Three plants treated with sterile water served as controls. All plants were placed in moistened plastic bags for 7 days and maintained in a greenhouse in which temperatures ranged from 20 to 22°C. The first leaf necrosis appeared 10 days after inoculation, causing an irregular necrotic area up to 4 mm in size. *A. alternata* was reisolated from affected leaves and identified by the amplification of the *endoPG*, *tub2*, and *cmdA* gene portions (GenBank accession nos. MK558220, MK558218, and MK558222). Control plants remained healthy, and the attempt to reisolate the pathogen failed. To our knowledge, this is the first report of *A. alternata* infecting *C. willmottianum* in Italy, as well as worldwide. This disease could become important owing to the reduction of the ornamental value of affected plants of *C. willmottianum*, the use of which is increasing in the landscape.

References:

Lawrence, D. P., et al. 2013. Mycologia 105:530.
Simmons, E. G. 2007. *Alternaria*: An Identification Manual. CBS Fungal Biodiversity Centre, Utrecht, the Netherlands.
Woudenberg, J. H. C., et al. 2015. Stud. Mycol. 82:1.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: fungi, ornamentals, epidemiology

First Report of *Colletotrichum gloeosporioides* Causing Anthracnose on *Sorbaria sorbifolia* in China

X. Y. Li,[1] Q. H. Wang,[2] S. S. Fan,[1] P. B. Feng,[1] Y. H. Yang,[1] X. Y. Zhang,[3] and Z. Y. Liu[1,†]

[1] College of Plant Protection, Shandong Agricultural University, Tai'an, Shandong, 271018, China
[2] Shandong Provincial Academy of Forestry, Jinan, Shandong 250014, China; and
[3] Research Institute of Forestry New Technology, Chinese Academy of Forestry, Beijing, China

Funding: Funding was provided by National Natural Science Foundation of China (grant nos. 31770685 and 31270687). Plant Dis. 103:1413, 2019; published online as https://doi.org/10.1094/PDIS-06-18-1011-PDN. Accepted for publication 26 January 2019.

Sorbaria sorbifolia (L.) A. Braun is commonly used for landscape plantings in China, Japan, Korea, Russia, North America, and Europe. The tree is generally disease-free; however, leaf spots were observed on *S. sorbifolia* in Tai'an city (N36°11′39″, E117°06′45″) in June 2007 and were then found in other areas of Shandong Province, China. Disease incidence was above 60%. Early symptoms appeared as circular, necrotic areas about 1 mm in diameter and then developed into circular or irregular, brown to reddish brown or black spots (3-5 mm in diameter). The centers of the lesions were gray-white with some surrounded by a yellow halo. In late summer and autumn, most lesions eventually dried, and the leaves withered and curved. To isolate the pathogen from infected leaves, small sections (3 to 4 mm^2) were excised from the lesions and surface sterilized with 70% ethanol and 1% NaClO for 30 s, rinsed three times with sterile distilled water, and then plated on potato dextrose agar (PDA). Cultures were incubated at 25°C for 3 to 5 days, and pure cultures were obtained. Cultures on PDA were initially white with abundant aerial mycelium. After 5 to 7 days, cultures turned gray to grayish black with orange conidial masses. Conidia were hyaline, straight, cylindrical, apex obtuse, base truncate, and ranged from 14.8 to 17.7 × 4.5 to 6.5 µm ($\bar{x} = 16.1 \times 5.4$, $n = 50$). Appressoria were brown, ovoid to irregularly shaped, 6.8 to 7.5 × 5.1 to 5.8 µm ($\bar{x} = 7.3 \times 5.5$, $n = 50$). The morphological and cultural characteristics were consistent with descriptions of *Colletotrichum gloeosporioides* (Cannon et al. 2008). Isolate CGSS0709 was further studied by phylogenetic analysis of the ribosomal internal transcribed spacer (ITS1-5.8S-ITS2) and partial sequences of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), chitin synthase (CHS-1), and β-tubulin (TUB2) (Weir et al. 2012). The ITS, GAPDH, ACT, CHS-1, and TUB2 sequences (GU289402, MH433626, MH433625, MH433628, and MH433627) were compared with sequences in Q-Bank. The similarity of ITS and CHS-1 was 100% between isolate CGSS0709 and *C. gloeosporioides* ex-type culture IMI 356878. CGSS0709 ACT and GAPDH sequences showed 99.1 and 97.8% with IMI 356878, and TUB2 100% with *C. gloeosporioides* ZJUC12. A maximum parsimony phylogenetic tree was constructed grounded on combining all sequenced loci in MEGA7. Isolate CGSS0709 was in the same cluster with *C. gloeosporioides* with 100% bootstrap support. Pathogenicity tests were conducted in June to July by spraying conidial suspensions (10^5 conidia/ml) onto 20 leaves of *S. sorbifolia* grown outdoors; dH_2O served as a control. All inoculated leaves were wrapped with black plastic bags to keep relative humidity high for 2 days. All inoculated leaves developed brown spots 7 days postinoculation, but no symptoms occurred on the controls. Koch's postulates were fulfilled with the reisolation from symptomatic leaf tissues. The results of morphological characteristics, molecular data, and pathogenicity testing confirmed that *C. gloeosporioides* caused leaf spot of *S. sorbifolia*. To our knowledge, this is the first report of *C. gloeosporioides* causing anthracnose on *S. sorbifolia*. The result provided crucial information for management of this disease.

References:

Cannon, P. F., et al. 2008. Mycotaxon 104:189.
Weir, B. S., et al. 2012. Stud. Mycol. 73:115.

The author(s) declare no conflict of interest.

Occurrence of Verticillium Wilt Caused by *Verticillium dahliae* on Okra (*Abelmoschus esculentus*) in North China

W. B. Zhai, M. Z. Zhang, H. Gao, J. Meng, J. Y. Shi, W. W. Zhang, and F. J. Qi[†]

The State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, China

Funding: This work was supported by the National Natural Science Foundation of China (31371898). Plant Dis. 103:1413, 2019; published online as https://doi.org/10.1094/PDIS-07-18-1279-PDN. Accepted for publication 30 January 2019.

Okra (*Abelmoschus esculentus* L. Moench) belongs to the Malvaceae family, which is a fairly new vegetable crop currently grown in a large area in China from Hainan to Hebei province. During 2015 to 2017, wilted okra plants were observed from May to September in an okra seed breeding base located in Beijing's Fangshan District. The affected plants showed typical wilt symptoms, including gradual leaf yellowing, wilting, and dark brown discoloration of the vascular tissue. The affected plants later died as the disease progressed. Field surveys were conducted to investigate the disease in okra in north China from early June to late July 2018. The disease was found sporadically but had spread to every okra-growing planting area in north China. To identify the pathogen associated with this disease, basal stems of diseased plants were washed under tap water, and then the discolored vascular tissue was cut into fragments of 0.5 cm in diameter,