First Report of Leaf Spot of Pumpkin Caused by *Curvularia hawaiiensis* in Pakistan

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Pumpkin (*Cucurbita pepo*) is a well-known edible plant with many medicinal properties. In Pakistan, this highly consumable crop has a great impact in the local markets. In October 2018, pumpkin plants (cv. Autumn Gold), growing on the premises of University of Agriculture, Faisalabad (31°26′14.3″N, 73°04′28.6″E) suffered a disease outbreak that resulted in severe leaf necrosis occurring on more than 51% of all plants in the 2-ha experimental field. Leaves displayed circular necrotic lesions, minor leaf tip necrosis, and leaf spotting (6 to 9 mm in diameter) covering approximately 20 to 30% of the leaf blade. To isolate the causal agent, the margins of infected leaves were cut into small pieces and surface sterilized with 0.1% $HgCl_2$ for 1 min followed by 70% ethanol for 30 s and rinsed twice in sterile distilled water. The samples were blotted dry on sterilized filter paper, placed on the Petri plates containing potato dextrose agar medium, and incubated for 1 week at 25 ± 1°C with 12-h light/dark photoperiod. Initially, mycelium showed light-brown edges and dark brown pigmentation at the center of the Petri plate. Fungal colonies also formed two to three concentric rings on the reverse of the plate. Conidia were characterized as solitary, obovoid to fusiform, round at both ends, three to six distoseptate (16.53 to 27.50 × 6.31 to 7.87 µm). Conidiophores were simple or branched, multiseptate, straight to flexuous, smooth to verruculose, and cylindrical. The morphological characteristics were typical of *Curvularia hawaiiensis* (Kusai et al. 2016; Manamgoda et al. 2012). Sequence identification was performed using the D1 to D3 domains of the large subunit (LSU) and the internal transcribed spacer region (ITS) of the nuclear rDNA, and the fragments of translation elongation factor 1-α (*tef1*) and partial DNA-directed RNA polymerase II second largest subunit (*rpb2*) genes, which were amplified using primers LROR-F/LR5-R (Schoch et al. 2012), ITS1F/ITS4R (White et al. 1990), EF1-983F/EF1-2218R, and 5F2/7CR (O'Donnell et al. 2007), respectively. The sequences of the ITS (615 bp), *rpb2* (771 bp), *tef1* (918 bp), and LSU (912 bp) gene regions were deposited in GenBank with accession numbers MN053866, MN067868, MN067869, and MN055715, respectively. A BLASTn search of the derived sequences resulted in 99% nucleotide similarity of the ITS and *rpb2* sequences and 100% similarity of *tef1* and LSU genes with the corresponding sequences of *C. hawaiiensis* (GenBank accession nos. MH864413, HG779167, KM196587, and JN941532, respectively). Leaves of 3- to 4-week-old pumpkin plants (cv. Autumn Gold) were inoculated by spraying a conidial suspension of 10^6 conidia/ml prepared from 14-day-old culture of *C. hawaiiensis*. Plants were incubated at 25 ± 1°C and covered with plastic bags for the maintenance of relative humidity. After 8 to 10 days, plants showed the characteristic symptoms the same as the original, and no symptoms were observed on control plants sprayed with distilled water. The fungal pathogen (*C. hawaiiensis*) was successfully reisolated from the symptomatic tissues of the inoculated plants, compared with the original, and found identical, fulfilling the Koch's postulates. To the best of our knowledge, this is the first report of leaf spot of pumpkin caused by *C. hawaiiensis* in Pakistan and in the world. The identification of this pathogen is critical to ensuring maintained productivity of an important vegetable crop in Pakistan and should lead to the development of management strategies to combat the disease.

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First Report of Leaf Spots Caused by *Alternaria arborescens* on *Symphyotrichum novi-belgii* in Italy

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Traditional Michaelmas daisy (*Symphyotrichum novi-belgii*, earlier *Aster novi-belgii*), Compositae family, is an herbaceous, perennial plant producing rose-purple flowers, grown in gardens as well as for cut flower production. During the spring of 2019, leaf spots were observed on leaves of 10-month-old potted plants of *S. novi-belgii* growing in a nursery at Agroinnova Centre (Torino, northern Italy). Ten of 30 plants were affected. The disease was also observed on 10 of 100 plants growing in a private garden located in Biella province (northern Italy). Necrotic areas were irregular, light brown spots, surrounded by a dark halo, 0.5 to 5.0 mm in size. The affected plants lost their ornamental value. Affected leaves were washed with sterile water and dried. Then, small pieces of tissue were taken from the margins of the necrotic areas and plated on potato dextrose agar, at temperatures ranging from 20 to 25°C under a light/dark regime of 16 h/8 h. Dark green fungal colonies were obtained that produced branched conidiophores with chains of ovoid to ellipsoid, tan to brown conidia. Conidia had one to five transverse septa and zero (rarely one to two) longitudinal or oblique septa and measured 11 to 45 × 6 to 17 µm (average 26 × 11 µm) (n = 50). These morphological characteristics indicated that the fungus isolated from *S. novi-belgii* was an *Alternaria* sp. (Simmons 2007). The fungal DNA from one isolate was extracted using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek, Darmstadt, Germany), and a PCR reaction was performed using the internal transcribed spacer (ITS) (White et al. 1990), the rpb2, the endoPG, the Alt a 1, and the OPA10-2 primers (Woudenberg et al. 2015). Sequences with 549 bp (ITS), 809 bp (rpb2), 464 bp (endoPG), 498 bp (Alt a 1), and 702 bp (OPA10-2) (GenBank accession nos. MN183754, MN185003, MN185001, MN185002, and MN185004, respectively) were obtained. A BLASTn analysis of these sequences showed the highest identity with *Alternaria arborescens*. The identities with the reference strain CBS 102605 of *A. arborescens* were 100% for ITS, 99.74% for rpb2, 99.57% for endoPG, 99.15% for Alt a 1, and 99.68% for OPA10-2 (accession nos. AF347033, KC584377, KP124712, AY563303, and AY295028, respectively). In the pathogenicity test, a spore suspension of 1.0 × 10^5 CFU/ml was obtained from a culture of the isolate used for molecular analysis and sprayed onto leaves of three healthy plants of *S. novi-belgii*. Three control plants were treated with sterile water. All plants were maintained at high relative humidity in plastic bags for 7 days, at temperatures ranging from 18 to 25°C. About 10 days after the inoculation, the first leaf necrosis appeared, only on inoculated plants. *A. arborescens* was reisolated from symptomatic leaves. Control plants remained symptomless. *Alternaria zinniae* is recorded on several hosts belonging to the Asteraceae family, including the *Aster* genus (David 1991). To our knowledge, this is the first report of *A. arborescens* affecting *S. novi-belgii* in Italy, or anywhere in the world. This disease could become important because of the increasing use of *S. novi-belgii* both in the landscape and in cut flower production.

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Binucleate *Rhizoctonia* (*Ceratobasidium*) AG E Affecting Red Raspberry (*Rubus idaeus*) Plants in Idaho

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