Disease Notes

First Report of Anthracnose on Alfalfa Caused by *Colletotrichum linicola* in Serbia

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Abstract

Alfalfa (*Medicago sativa* L.) is economically the most important forage crop in Serbia. In July 2009, alfalfa plants showed symptoms characteristic of anthracnose disease ("shepherd's crook") including wilting and death of the upper portion of the stems. Anthracnose of alfalfa has been reported to be caused by *Colletotrichum trifolii* or *C. destructivum* (2). Alfalfa plants with anthracnose symptoms were collected in Srpska Crnja, South Banat District, Serbia. Infected tissue samples were surface disinfected with 5% sodium hypochlorite for 2 min and washed three times for 5 min in sterile distilled water. Surface sterilized tissue was transferred to sterile filter paper and placed on potato dextrose agar (PDA), and incubated at 24°C in the dark for 10 days (1). Developing colonies were light to dark olive green. In cultures on PDA medium, acervuli were formed. Conidia from acervuli were released in mucous masses that were orange to cream-pink in color. Conidia were hyaline, aseptate, straight with one end pointed and the other slightly rounded, measuring 12.5 to 25.0 × 2.5 to 7.5 µm (mean 19.83 × 4.42 µm). After 5 days,

numerous setae were formed. The setae were slightly darker at the bottom and lighter at the top, septate with 3 septa. Setae dimensions were 100 to 185.5 × 2.5 to 5 µm (average 160.9 × 3.12 µm). The isolated fungus was designated Coll-44. Stems of 30 7-week-old plants were spray-inoculated in the laboratory with an aqueous suspension of conidia (10^6 spores per ml; 10 ml per plant) harvested from 7-day-old cultures grown on PDA. The plants and two non-inoculated check plants were placed in a greenhouse and a cover with plastic bags at 25°C in darkness. After 48 h, plastic bags were removed from the plants. All plants were watered once a day. Symptoms were observed 10 days after inoculation. No symptoms were observed on non-inoculated plants. In the greenhou all 30 inoculated plants became diseased with anthracnose symptoms after 10 days. Coll-44 was consistently re-isolated from diseased stem tissue. Koch's postulates were fulfilled by re-isolation from inoculated alfalfa plants. Pure culture of the Coll-44 isolate was deposited in the public collection of CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (specimen no. CBS 3263). Partial sequences of the internal transcribed spacer regions-ITS (GenBank Accession No. JX908364) and betatubulin-TUB2 gene (KJ556347) were amplified and sequenced from extracted fungal DNA with primer pairs ITS1-ITS4 (4) and T1-Bt2b (3), respectively. ITS sequence of the Coll-44 isolate showed 100% nucleotide identity to the GenBank accessions JQ005765 and AB046609 of *C. linicola*. TUB2 sequence of isolate Coll-44 showed 99.6% nucleotide identity with the GenBank accession JQ005849 of *C. linicola* isolate CBS 172.51. To our knowledge, this is the first report of *C. linicola* causing alfalfa anthracnose in Serbia.

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