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DISEASE NOTES



First Report of Horseradish Leaf Spot Caused by *Alternaria brassicae* in Serbia

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Published Online: 29 May 2015 | <https://doi.org/10.1094/PDIS-11-14-1181-PDN>

In July 2014, a foliar spot disease of horseradish (*Armoracia rusticana*) was observed in the Bačka region of Serbia (45°14'17" N; 19°42'22" E). The disease was observed in several small vegetable farms and disease incidence approached 20 to 40%. The symptoms first appeared on the abaxial side of older leaves as light brown concentric spots with dark margins and sometimes haloes. The spots varied in size between 0.5 and 0.9 cm. The necrotic spots gradually enlarged, tore, and dropped out. Under high humidity and temperature in 2014 in that region, development of numerous necrosis spots resulted in a rapid reduction of leaves. Small pieces taken from infected leaf tissue of diseased horseradish were treated with 1% sodium hypochlorite for 1 min, rinsed with sterile distilled water (SDW), and placed on V8 agar. Isolates were grown in pure culture at 24°C for 5 days in 12 h light/dark photoperiod. Morphological characteristics of the colony and sporulation were determined for six representative isolates. Fungal colonies were smooth, color varied from white, off white to light brown with concentric zones and intensive sporulation. Conidia were light

brown, obclavate, produced singly or in short chains. Mature conidia were 16 to 38 μm wide and 73 to 158 μm long with a beak cell 79 to 120 μm long with 8 to 11 transverse septa and 0 to 4 longitudinal septa. Based on morphological characteristics, all isolates were identified as *Alternaria brassicae* (Berk.) Sacc (Simmons 2007). To confirm the pathogen's identity, DNA was extracted from the fungal isolates and subjected to PCR; the internal transcribed spacer region of rDNA was amplified using primers ITS1 and ITS4 (White et al. 1990). The resulting amplicons were sequenced by MacroGen Inc. (Seoul, South Korea) and deposited in NCBI GenBank (Accession Nos. KP115599 to KP115604). BLAST analysis of revealed 100% homology with a sequence of *A. brassicae* strain ATCC 58169 deposited in GenBank (JX499028). Pathogenicity of six representative isolates was tested on leaves of 2-month-old horseradish seedlings by spraying with aqueous conidial suspensions (10^5 conidia/ml) prepared from cultures grown on V8 agar at 24°C for 5 days. Negative control plants were sprayed with SDW. Plants were covered with plastic bags and placed for 48 h in a greenhouse at 20 to 22°C. For all isolates within 10 days, inoculated plants developed brown lesions on leaves. Plants treated with SDW were symptomless. To fulfill Koch's postulates, reisolations from all tested strains were done by streaking margins of necrotic leaf spot tissue onto V8 agar. Reisolated strains showed the same colony morphology as described above. Based on the pathogenicity test accompanied by completion of Koch's postulates, sequence analysis, and morphological and cultural characteristics, the strains were identified as *A. brassicae*. According to available literature data, this is the first report of this pathogen as the causal agent of brown leaf spot of horseradish in Serbia.



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