Disease Notes

First Report of *Cercospora carotae*, Causal Agent of Cercospora Leaf Spot of Carrot, in Serbia

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Published Online: 7 Jul 2014 | https://doi.org/10.1094/PDIS-08-13-0858-PDN

Abstract

Carrot (*Daucus carota* L. subsp. *sativus* [Hoffm.] Arcang.) is an important vegetable in Serbia, where it is grown on nearly 8,000 ha. In August 2012, ~1,500 ha of carrot fields were inspected in southern Bačka in North Serbia. In nearly 40% of the fields, severe foliar and stem symptoms characteristic of cercospora leaf spot of carrot, caused by *Cercospora carotae* (Pass.) Solheim (3), were observed. Lesions on stems were oblong, elliptical, and more or less sunken, while those on the leaves were amphigenous, subcircular, light brown in the center, and surrounded by a dark brown margin. Conidiophores emerging from the lesions formed very loose tufts but sometimes were solitary. Conidiophores were simple and straight to subflexuous

with a bulbous base (17 to 37 × 3 to 5 µm). Conidia were 58 to 102 × 2 to 4 µm, solitary, cylindrical to narrowly-obclavate, and hyaline to subhyaline with 2 to 6 septa. To obtain monosporial isolates, the conidia from one lesion were placed on water agar plates at 25°C in the dark for 24 h, after which single germinated conidia were selected and each placed on a petri dish containing potato dextrose agar (PDA). To confirm pathogenicity of three of the isolates, Koch's postulates were tested on carrot seedlings (3-true-leaf stage of growth) of a Nantes cultivar, SP-80, with 12 plants tested/isolate and 12 non-inoculated plants used as a control treatment. The leaves were atomized until runoff with the appropriate *C. carotae* spore suspension (10^4 conidia/ml sterilized water), while control plants were atomized with sterile water. All plants were then incubated in a dew chamber for 72 h, then transferred to a greenhouse at 25 ± 2°C. After 2 weeks, characteristic symptoms resembling those observed in the field developed on all inoculated plants; control plants were asymptomatic. The pathogen was re-isolated from all inoculated plants, and identity of the re-isolated fungi confirmed morphologically as described above, and molecularly as described below. The pathogenicity test was repeated with no significant differences in shape and size of lesions, or dimensions of conidiophores and conidia among isolates. To verify the pathogen identity molecularly, the 28S rDNA was amplified and sequenced using the V9G/LR5 primer set (2,4) as well as internal primers OR-A (5′-ATACCCGCTGAACTTAAGC-3′) and 2R-C (5′-AAGTACTTTGGAAAGAG-3′); the ITS region of rDNA using the ITS1/ITS4 universal primers (5); and histone H3 gene (H3) using the CylH3F/CylH3R primers (1). The sequences for the three isolates were deposited in GenBank as Accession Numbers KF468808 to KF468810, KF941306 to KF941308, and KF941303 to KF941305 for the 28S rDNA, ITS and H3 regions, respectively. BLAST results for the ITS sequences indicated 94% similarity to the ITS sequence of an isolate of *Pseudocercosporella capsellae* (GU214662) and 92% similarity to the ITS sequence of an isolate of *C. capsici* (HQ700354). The H3 sequences shared 91% similarity with that of several *Cercospora* spp., e.g., *C. apii* (JX142548), *C. beticola* (AY752258), and *C. capsici* (JX142584), all of which shared the same amino acid sequence of the encoded H3 protein. Also, the 28S rDNA sequences had 99% similarity (identity of 318/319, with 0 gaps) with the single sequence of *C. carotae* available in GenBank (AY152628), which originated from Norway. This is, to our knowledge, the first report of *C. carotae* on carrot crops in Serbia as well as southeastern Europe.

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