Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria japonica*, a Causal Agent of Black Spot, on Kale in South Carolina, U.S.A.

A. P. Keinath,^{1,†} S. M. Toporek,¹ V. B. DuBose,¹ S. H. Zardus,¹ and J. B. Ballew²

¹ Coastal REC, Clemson University, Charleston, SC

² Clemson Cooperative Extension, Lexington, SC

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In January 2020, charcoal gray, dull lesions were observed on leaves of organic kale (*Brassica oleracea* var. *acephala*) cultivar Darkibor in two fields in Lexington County, South Carolina, the county with the most acres of leafy brassicas in the state. Leaf spots, also visible on the leaf underside, covered <5% of the leaf area. No spores were present. Portions of leaf spots from eight leaves, four per field, were cultured on one-quarter-strength potato dextrose agar (PDA/4). Eleven isolates of *Alternaria* spp. were recovered. Isolates ALT12 and UL3 were cultured in *A. solani* medium, and DNA was extracted (Maiero et al. 1991). The internal transcribed spacer (ITS) region, translation elongation factor 1- α (*tef1*), RNA polymerase second largest subunit (*rpb2*), and *Alternaria* major allergen (*Alt a 1*) genes were amplified with the primer pairs V9G/ITS4, EF1-728F/EF1-986R, RPB2-5F2/FRPB2-7cR, and Alt-for/Alt-rev, respectively, and sequenced (Woudenberg et al. 2014). Sequences for isolates ALT12 and UL3, collected from different leaves in the same field, were identical to each other and to isolate AC97 (accession nos.: ITS, LC440597; *tef1*, LC482018; *rpb2*, LC476803; *Alt a 1*, LC481628) of *A. japonica* Yoshii (Nishikawa and Nakashima 2020). ITS, *tef1*, *rpb2*, and *Alt a 1* sequences for each isolate were deposited in GenBank under the accessions MW374952, MW389653, MW389655, and MW389657 for ALT12 and MW374951, MW389652, MW389654, and MW389656 for UL3, respectively. Conidia of *A. japonica* (20 of ALT12, 10 of UL3) produced by 7-day-old cultures on Spezieller Nährstoffarmer agar measured $62.1 \pm 11.4 \times 18.8 \pm 2.2$ μ m (standard deviation). Median numbers of transverse and longitudinal septae were six (four to eight) and two (one to three), respectively. Conidia formed

singly or in chains of two. Cells were constricted around the transverse septae (Nishikawa and Nakashima 2020; Woudenberg et al. 2014). Chlamydospores were present in cultures of ALT12. ALT12 was pathogenic on kale cultivars Darkibor and Winterbor inoculated in a greenhouse following the procedures of Al-Lami et al. (2019). Four replicate pots with two plants each were used; plants were 6, 9, and 5 weeks old in trials 1, 2, and 3, respectively. The oldest three leaves of each plant were spray inoculated with a suspension of 5×10^5 conidia/ml; noninoculated control plants were sprayed with water. All plants were kept for 48 h at 100% relative humidity and then moved to a bench in a greenhouse held at 21/16°C day/night temperatures. The second and third oldest leaves were rated 13 days after inoculation. Small gray or black spots developed on inoculated leaves and petioles in all trials, and on one noninoculated leaf in trial one. Disease incidence on inoculated leaves (73.1%) was greater than on noninoculated leaves (0.05%) ($P < 0.0001$). Cultivars did not differ in susceptibility ($P = 0.12$). Portions of lesions on inoculated leaves and portions of noninoculated leaves were cultured on PDA/4 amended with antibiotics (Keinath 2013). *A. japonica* was reisolated from 46 of 50 inoculated leaf blades; 22 of 28 inoculated petioles; and one of eight, zero of eight, and zero of seven noninoculated leaves in the three trials, respectively. Growers in South Carolina consider black spot, or *Alternaria* leaf spot, the most important fungal disease on organic kale. The presence of a second causal agent in addition to *A. brassicae* may increase disease occurrence. *A. japonica* previously was reported on arugula in California (Tidwell et al. 2014). This is the first report of *A. japonica* in the eastern United States.

References

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[†]Indicates the corresponding author.
A. P. Keinath; tknth@clemson.edu