Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Stemphylium* Leaf Spot of Spinach (*Spinacia oleracea*) Caused by *Stemphylium beticola* in New York State

K. A. Spawton,¹ M. McGrath,² and L. J. du Toit^{1,†}

¹ Washington State University, Mount Vernon Northwestern Washington Research and Extension Center, Mount Vernon, WA 98273

² Cornell University, Long Island Horticultural Research and Extension Center, Riverhead, NY 11901

Plant Dis. 104:3068, 2020; published online as <https://doi.org/10.1094/PDIS-02-20-0343-PDN>. Accepted for publication 30 May 2020.

In June 2019, leaf spots were observed throughout a 0.02-ha certified organic crop of the spinach cultivars Acadia and Kookaburra on Long Island, Suffolk Co., NY. Older leaves had tan, papery lesions, each ≤ 15 mm in diameter with a diffuse margin and dark spores in the center that were typical of *Stemphylium*. Symptomatic leaf sections from four Acadia plants were surface-sterilized with 0.6% NaOCl for 90 s, triple-rinsed in sterilized, deionized water, dried, plated on water agar and half-strength PDA (each with 100 μ g of chloramphenicol/ml), and maintained at room temperature ($22 \pm 2^\circ\text{C}$) near a north-facing window for a natural diurnal cycle. Of 16 *Stemphylium* isolates, conidia of three selected randomly (St578, St582, and St588) were pigmented, muriform, and 14.0 to 32.0 μ m (20.7 ± 0.3 μ m, mean $\pm$ standard error) $\times$ 12.0 to 24.0 μ m (16.1 ± 0.2 μ m) ($n = 50$ /isolate). Sequences of the *glyceraldehyde-3-phosphate-dehydrogenase* gene (*GAPDH*) amplified from all 16 isolates (GenBank no. MN953009) using primers gpd1 and gpd2 (Berbee et al. 1999) were 100% identical to each other and to that of *Stemphylium beticola* isolate CBS 141024 (KU850667.1). Of the *calmodulin* gene (*cmdA*) sequences amplified from 16 isolates using primers CALDF1 and CALDR1 (Lawrence et al. 2013), eight were 100% identical to each other (MN953007) and to that of an onion strain of *S. beticola* from New York, On16-14 (MK675668.1). The other eight were 100% identical to each other (MN953008) and to that of a *Stemphylium* sp. isolate from sugar beet (*Beta vulgaris*) in Germany (KU850825.1), and 99.86% identical to that of the New York onion strain On16-14 of *S. beticola* (MK675668.1). New York isolates St578, St582, and St588 were each tested for pathogenicity on four 5-week-old plants of each of spinach cultivars Mandolin and Viroflay. Plants were enclosed in plastic bags

and covered with Remay for 24 h, atomized to runoff with a spore suspension of the relevant isolate (7×10^4 to 1×10^5 conidia/ml) prepared from 3-week-old cultures grown on clarified V8 agar as described for the original isolations, and then re-enclosed in bags for 24 h. Plants of both cultivars inoculated with sterilized water served as negative control treatments. Isolate St523 of *S. vesicarium* and St354 of *S. beticola*, which are pathogenic on Mandolin only and on Mandolin and Viroflay, respectively (Spawton et al. 2019), served as positive control treatments. Plants were placed in a greenhouse in a randomized complete block design and examined for 21 days after inoculation. The New York isolates and St354 caused tan lesions with diffuse margins on Mandolin and Viroflay that expanded rapidly (>10 mm diameter by 21 days). St523 only caused spots on Mandolin, as expected, and lesions remained <5 mm in diameter, each with a narrow, dark margin. Symptoms did not develop on noninoculated control plants. *Stemphylium* was reisolated from lesions on both cultivars inoculated with New York isolates. Sequences of *cmdA* and *GAPDH* of reisolates matched those of the original isolates. *Stemphylium* leaf spot of spinach was first described in California in 2001, with the causal agent identified as *S. botryosum* based on morphology (Koike et al. 2001). Recent molecular and morphological evidence indicated the historic isolates probably are *S. beticola*, but the predominant causal species in the southern United States is now *S. vesicarium* (Liu et al. 2020; Spawton et al. 2019). *S. beticola* also has been reported on spinach in Italy (Gilardi et al. 2018). Based on DNA sequences, morphology, and pathogenicity tests, the 2019 outbreak is the first confirmed report of *Stemphylium* leaf spot on spinach in New York, with the causal agent determined to be *S. beticola*. Identifying the causal species is important because cultivars differ in susceptibility to *S. beticola* versus *S. vesicarium*. This is particularly relevant for organic production due to a lack of adequately efficacious organic fungicides to manage *Stemphylium* leaf spot.

References:

- Berbee, M. L., et al. 1999. Mycologia 91:964.
- Gilardi, G., et al. 2018. Plant Dis. 102:2036.
- Koike, S. T., et al. 2001. Plant Dis. 85:126.
- Lawrence, D. P., et al. 2013. Mycologia 105:530.
- Liu, B. et al. 2020. Plant Dis. 104:1994.
- Spawton, K. A., et al. 2019. Phytopathology 109:S2.167.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: leaf spot, spinach, fresh market, *Stemphylium beticola*, *Stemphylium vesicarium*, cultivar susceptibility

[†]Indicates the corresponding author.
E-mail: dutoit@wsu.edu (L.J.duT.)