Diseases Caused by Fungi and Fungus-Like Organisms

Cladosporium Leaf Spot Caused by *Cladosporium variable* in Winter High Tunnel Production of Spinach (*Spinacia oleracea*) in Maine, United States

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In January 2021, leaf spots were observed on 1 of 22 proprietary spinach cultivars in a trial under high tunnels in Albion, ME. Approximately 1% of plants were symptomatic with <30% leaf area affected. Some spinach growers reported ≤100% incidence in high tunnels in the northeastern United States. Leaf spots on the cultivar in Albion were <5 mm in diameter and tan-colored, with dematiaceous spores in short chains on long conidiophores at the center of older lesions. Lesions were surface-sterilized in 0.6% NaOCl for 90 s, rinsed in sterile water, dried, and placed on clarified V8 agar medium amended with chloramphenicol (100 µg/ml). Three *Cladosporium* isolates (Cds053, Cds055, and Cds057) produced olive-green colonies with dematiaceous conidia that were zero- to two-septate, catenulate, and 8.0 to 28.0 µm (16.6 ± 0.3 µm, mean ± standard error) × 4.0 to 12.0 µm (8.3 ± 0.1 µm) ($n = 50$ conidia/isolate). The partial internal transcribed spacer (ITS) region of rDNA, translation elongation factor 1- α gene (*tef1*- α), and actin gene (*act*) were amplified and sequenced with primer sets UNUP18S42/UNLO28S576B (Bakkeren et al. 2000), EF1-728F/EF1-986R (Carbone and Kohn 1999), and ACT-512F/ACT-783R (Carbone and Kohn 1999), respectively. The sequence of each locus was identical among the isolates. The *tef1*- α (GenBank accession no. MZ905363) and *act* (MZ905362) sequences were identical to accessions of *tef1*- α (EF679480.1) and *act* (EF679556.1) of *Cladosporium variable* (Cooke) G. A. de Vries, whereas the ITS rDNA sequence (MZ893461) was identical to that of many *Cladosporium* spp.,

including *C. variable*. Each isolate was inoculated onto four replicate 42-day-old plants of each of the spinach cultivars Viroflay and Mandolin. The plants were enclosed in plastic bags for 24 h, atomized with a spore suspension (10⁶ conidia/ml) prepared from potato dextrose agar colonies of each isolate, and returned to the bags for 24 h. Four plants of each cultivar were atomized with deionized water as a control treatment. Plants were arranged in a randomized complete block design. By 5 days, tan spots had developed on *Cladosporium*-treated plants of each cultivar. By 14 days, Mandolin had developed more severe lesions (20.0 ± 2.8% mean ± standard error of leaf area with symptoms) than Viroflay (5.4 ± 0.4%). Control plants treated with water did not develop lesions. After 15 days, a leaf from a plant of each treatment was incubated in a moist chamber at 20°C with a natural diurnal light cycle. Sporulation was observed at the center of lesions from *Cladosporium*-inoculated plants <24 h after incubation, and *Cladosporium* was reisolated. The *act* locus amplified and sequenced from the reisolates, as described above, had an identical sequence to that of the original isolates, fulfilling Koch's postulates. Empirically, *Cladosporium* leaf spot has established in winter high tunnel production of spinach in the northeastern United States (R. Katz, *personal observation*). To our knowledge, this is the first official documentation of the disease in Maine. The disease is favored by cool, humid conditions (Inglis et al. 1997). Management strategies under these conditions in Maine and greater New England include foliar applications of effective fungicides (du Toit et al. 2004), selection of partially resistant cultivars, cultural practices to reduce relative humidity and durations of leaf wetness, and planting pathogen-free or treated seeds (du Toit and Hernandez-Perez 2005; Hernandez-Perez and du Toit 2006).

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