Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pseudomonas cichorii* Causing Bacterial Leaf Spot on Romaine Lettuce (*Lactuca sativa* var. *longifolia*) and Escarole (*Cichorium endivia*) in New Jersey

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We previously reported an outbreak of bacterial leaf spot (BLS) caused by *Pseudomonas cichorii* occurring on sweet basil in New Jersey during the summer of 2018 (Patel et al. 2019), a growing season characterized by increased leaf wetness due to high humidity and unusually high levels of summer rains. Leaf spot was also observed, as a one-time event, on older mature leaves on romaine and escarole lettuce during that same year. Symptoms on escarole were observed as grayish brown-to-black concentric lesions on leaf parenchyma tissue, ranging from 1 mm to 1 to 2 cm in diameter. In more severely diseased samples, lesions coalesced to form larger necrotic areas, giving a blight appearance. On occasion, infection was observed in leaf midveins as brownish gray necrosis. Symptoms on romaine lettuce were observed mostly as coalesced blackened lesions on leaf parenchyma tissue near margins with a rotted consistency that spread to the midvein in severe cases. Margins of leaf lesions were excised and macerated in sterile water before streaking onto nutrient agar (NA) and King's medium B agar (KMB) (Schaad et al. 2001). Growth on both NA and KMB was predominantly cream-colored circular bacterial colonies with undulated margins. Colonies on KMB fluoresced blue under 365-nm UV light. Two representative colonies isolated from each host were selected for further characterization. All isolates tested negative for levan production, positive for oxidase, negative for potato rot, negative for arginine dihydrolase, and induced a strong hypersensitive response on tobacco within

24 h, consistent with LOPAT descriptions for *P. cichorii* (Lelliott et al. 1966). A single strain from each host (ESC6F2 and Rom1-1) was further characterized genetically to confirm species. PCR analysis used two primer sets: 16S rRNA gene universal primers 27F/1492R and Hcr1 primers used to amplify a 520-bp region of the pathogenicity gene cluster *hrcRST* in *P. cichorii* (Cottyn et al. 2011; Patel et al. 2019). Partial 16S rDNA gene sequences were deposited in the GenBank database for each isolate (ESC6F2, MT974180; and Rom1-1, MT982172). Sequence comparisons of ESC6F2 and Rom1-1 shared 99% identity with each other and several *P. cichorii* strains within the GenBank database, including strain B5-2-1 isolated from sweet basil in New Jersey the same year (MK501753). The partial *hrcRST* locus (strain ESC6F2, MW048775; and Rom1-1, MW048774) shared 100% identity to each other and strain B5-2-1 (MK507764), and 99% identity with *P. cichorii* strain P18-1 (MH396007) isolated from *Ocimum basilicum* in Hawaii. Koch's postulates were performed on escarole variety Full Heart and romaine lettuce variety Ideal Cos to confirm pathogenicity of the isolated strains. Bacterial suspensions (1×10^7 CFU/ml) were syringe injected (0.1 ml) into the leaf midribs and pressure infiltrated into leaf parenchyma tissue of three plants. Control plants were inoculated with sterile water. Blackened necrosis developed within 72 h around bacteria-inoculation points, which expanded beyond inoculation points within a week. In contrast, control plants remained healthy and symptomless. Although significant crop loss occurred due to BLS on escarole and romaine lettuce, *P. cichorii* has not been isolated from diseased plant material since 2018. This suggests inoculum sources did not persist beyond 2018, or favorable environmental conditions for disease are inconsistent to cause noticeable damage to New Jersey crops.

References:

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