Diseases Caused by Bacteria and Phytoplasmas

First Report of *Dickeya dianthicola* Causing Blackleg on New Guinea Impatiens (*Impatiens hawkeri*) in New York State, U.S.A.

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New Guinea impatiens (NGI), *Impatiens hawkeri*, has a \$54 million wholesale market value in the United States (National Agricultural Statistics Service 2019) and is highly resistant to impatiens downy mildew (*Plasmopara destructor*) according to growers' experience. In March 2019, NGI cultivar Petticoat White in a New York greenhouse showed wilting, black stem streaks, and vascular discoloration, with a 20% disease incidence. Symptomatic tissue pieces were added to sterile water in a test tube and streaks made on potato dextrose agar (PDA). After incubation at 26°C for 2 days, the most abundant colony type (mucoid, pale yellow) was transferred to PDA. One representative colony was selected and labeled as isolate 67-19. A single colony of isolate 67-19 was transferred to lysogeny broth (Bertani 1951) and cultured at 28°C. Genomic DNA was extracted and polymerase chain reaction (PCR) performed using the 16S rRNA gene universal primers fD2 and rP1, resulting in a partial 16S rRNA amplicon (Weisburg et al. 1991). BLASTn analysis showed 99% identity with sequences of species belonging to *Dickeya*. Different primer sets have been developed to detect and identify the genus *Dickeya* and its various species (Pritchard et al. 2013). The primer sets used for genus identification, dnaX (Ślawiak et al. 2009), Df/Dr (Laurila et al. 2010), and ADE1/ADE2 (Nassar et al. 1996), resulted in 500-, 133-, and 420-bp amplicons, respectively. Results suggested the bacterium was a *Dickeya* sp. To determine whether the species could be *Dickeya dianthicola* causing blackleg on NGI (*I. hawkeri*), the specific primer set DIA-A was used (Pritchard et al. 2013), and the expected product of 150 bp was obtained. BLASTn results showed

that the partial *dnaX* sequence (MT895847) of isolate 67-19 had 99% identity with the sequence of *D. dianthicola* strain RNS04.9 isolated in 2004 from potato (*Solanum tuberosum*) in France (GenBank CP017638.1). Therefore, isolate 67-19 was designated as *D. dianthicola*. The complete genome of *D. dianthicola* strain 67-19 was generated using Nanopore and Illumina sequencing (CP051429) (Liu et al. 2021). Average nucleotide identity determined by FastANI (version 1.1) (Jain et al. 2018) showed 97.43% identity between the genome of *D. dianthicola* strain 67-19 and that of *D. dianthicola* strain NCPPB 453 (GCA_000365305.1), isolated in 1957 from carnation (*Dianthus caryophyllus*) in the United Kingdom. The pathogenicity of *D. dianthicola* strain 67-19 was shown on NGI cultivars Petticoat White and Tamarinda White. In July 2020, sterile toothpicks were used to make wounds and to transfer bacteria from a 48-h PDA culture of *D. dianthicola* strain 67-19 to the stems of four plants of each cultivar. Four plants of each cultivar were mock inoculated similarly, and all wound sites were wrapped with Parafilm before placing plants on a greenhouse bench. Ten days later, stems inoculated with *D. dianthicola* strain 67-19 showed necrotic lesions similar to the original symptoms, whereas control plants did not show symptoms. One month after inoculation, bacteria were reisolated from all symptomatic stems. PCR was performed on the reisolated bacteria as described. The *dnaX* sequence (MT895847) was confirmed to match that of *D. dianthicola* strain 67-19 (CP051429) 100%, and fragments of the expected size were amplified (Liu et al. 2021). Stab inoculations of strain 67-19 into potato stems and tubers also resulted in blackleg and soft rot symptoms at the sites of inoculation, whereas mock-inoculated stem and tuber showed no symptoms. The sequence of the *dnaX* gene of the reisolated bacterium from inoculated potatoes was confirmed to match that of *D. dianthicola* strain 67-19. To our knowledge, this is the first report of blackleg of NGI caused by *D. dianthicola* in the United States and worldwide. Because the disease caused by *D. dianthicola* poses a significant threat to the ornamentals and potato industries (Charkowski et al. 2020), further research on genome biology, epidemiology, and management options is needed.

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