Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Godronia cassandrae* as a Major Cranberry Fruit Rot Pathogen in Eastern Canada

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The complex etiology of cranberry fruit rot (CFR) (Oudemans et al. 1998) has made it difficult to precisely identify the fungi involved in CFR and their relative importance in North America. To remedy this situation, a multiplex polymerase chain reaction (PCR) approach targeting the 12 most commonly reported fungi in CFR was recently developed (Conti et al. 2019). However, in surveys conducted in Eastern Canada, the molecular tool revealed the presence of an unknown fungus in more than 30% of the collected samples. Analyses were thus undertaken to identify this species. From 117 rotten fruit collected at harvest in 2017, 34 samples of the unknown fungus, all morphologically similar, were isolated but not detected using the molecular tool. Their internal transcribed spacer (ITS) ribosomal regions were sequenced using universal primers (Vilgalys and Hester 1990; White et al. 1990) and searched against the GenBank database using the BLASTn tool (Altschul et al. 1990). The top match was obtained with *Godronia cassandrae* (anam. *Fusicoccum putrefaciens*) (accession no. MH855281 [Vu et al. 2019], 98 to 100% identity and an *E* value of 0.0), even though some isolates had minor nucleotide differences, as presented in the tree. Sequences were deposited in GenBank as accession numbers MT599989 to MT600022. Because *G. cassandrae* had been reported, albeit rarely, on cranberry in Michigan (Olatinwo et al. 2003), it was supposed to amplify with the molecular tool developed from the strain DAOM C216021 (Agriculture and Agri-Food Canada, Ottawa, ON) identified in 1993 on *Vaccinium angustifolium* as *G. cassandrae*. Analysis of the sequences used to build the specific primers from this strain confirmed the DAOM strain as being *Neocucurbitaria juglandicola*, which was never diagnosed in our cranberry samples. To confirm this revised diagnosis, a multisequence

alignment (MSA) was performed on the ITS regions of the isolates from rotten cranberries and sequences available for the genus *Godronia* in the NCBI nucleotide database (NCBI txid269064). This MSA allowed us to find discriminant regions between *Godronia* spp. A pair of PCR primers specific to *G. cassandrae* found on cranberry fruit was then designed (the forward and reverse sequences are AATCAGTGGCGGTGCCTGTC and TACCGCTTCACTCGCCGTTAC, respectively), generating 196-bp amplicons, with an annealing temperature of 65°C. The diagnosis of 7,835 fruit sampled at three timepoints (harvest, after 3 and after 6 weeks of storage) in 2018, from four cranberry farms located in Québec (CA) and Nova Scotia (CA), detected *G. cassandrae* in 2,350 samples (30%). To assess the pathogenicity of four specimens from 2017, Koch's postulates were completed on two healthy fruit per isolate. The fruit were wounded with a sterilized tooth pick and individually inoculated; two fruit were used as a control. Based on our observations, the fungi isolated from cranberry fruit displayed a pale lemon-yellow mycelium and black pycnidia. Conidia are hyaline, cylindrical, and divided by a single septum. These morphological characters are similar to the ones described in the literature for *G. cassandrae* (Polashock et al. 2017). Rot symptoms appear as a discoloration from the firm, red, and healthy cranberry fruit to a yellowish-orange softer fruit. Molecular characterization of the reisolated fungus confirmed the presence of *G. cassandrae*. We report *G. cassandrae* for the first time as a major cause of CFR in Eastern Canada. Its prevalence in cranberry fields of Québec and Nova Scotia suggests that it supplants *Physalospora* as the main fungus involved in CFR in Eastern Canada.

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