Diseases Caused by Oomycetes

First Report of Strains Within the *Pythium spinosum* Species Complex Causing Carrot Cavity Spot in California

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Carrots (*Daucus carota* L. subsp. *sativus* [Hoffm.] with typical symptoms of cavity spot, that is, sunken, round to elliptical lesions 2 to 5 mm long (Hiltunen and White 2002), were collected from two locations in California, Bakersfield and Riverside, in January and July 2019, respectively. Carrots were rinsed in tap water, and 4-mm² lesion fragments were pressed into selective corn meal agar (CMA-PARP; Schrandt et al. 1994) and incubated at 23°C in the dark for 4 days. Identification of pure cultures was performed via amplification and sequence analysis of two genomic regions, the Internal Transcribed Spacer 1-5.8S-ITS2 (ITS) region and the cytochrome C oxidase subunit 1 (*COI*) gene, using the universal primers UN-UP18S42/UN-LO28S576B (Schroeder et al. 2006) and OomCOXI-Levup/OomCOXI-Levlo (Robideau et al. 2011), respectively. Via BLAST, two isolates from organically grown carrots in Bakersfield (MCIF19) and Riverside (JSCS19), with identical ITS sequences (GenBank accession nos. MZ799354 and MZ799355, respectively), showed 99.61% similarity (1,021/1,025 bp) to that of *Pythium spinosum* (AY598701.2). However, the *COI* of MCIF19 (MZ803207) showed 98.72% similarity (692/701 bp) to that of *P. paroecandrum* (GU071818.1), while the *COI* of JSCS19 (MZ803208) was identical (701/701 bp) to that of *P. kunmingense* (GU071820.1), a rarely isolated species considered within the species complex of *P. spinosum* (Robideau et al. 2011). According to these results, the isolates were identified as belonging to the *P. spinosum* species

complex, part of *Pythium* Clade F (Lévesque and De Cock 2004; Robideau et al. 2011). Further research is needed to clarify the exact taxonomic status of both isolates. Koch's postulates were completed using two different assays. Each assay was done twice with carrots of the cultivar 'Maverick.' Surface-sterilized, freshly harvested, mature carrots in a plastic box lined with moistened sterile paper towels were each inoculated with four CMA plugs (5-mm diameter) with actively growing mycelium of each isolate. CMA plugs, non-inoculated or colonized by a known pathogenic *P. violae* strain, were used as the negative and positive control, respectively. Boxes were closed to maintain humidity and incubated at 23°C in the dark. Lesions similar to the ones caused by *P. violae* were observed at day 3 for all plugs of both isolates. No symptoms were observed for the negative control, even after extending the incubation to 7 days. In a more natural assay, four nontreated carrot seeds were planted in tree seedling pots (25 × 6.5 cm) containing sterilized 50/50 peat moss/sand combined with 15-ml V8 broth (Schrandt et al. 1994) with densely grown mycelium. The same inoculation treatments were used as for the carrot disk assay. Plants (one plant/pot, four plants/treatment) were maintained at 23°C under a 16 h photoperiod with daily watering (20 ml). At 14 weeks, the carrots inoculated with *P. violae* and the two test isolates showed cavity spot lesions while no symptoms were observed on carrots growing in noninoculated medium. For both assays, pathogens were reisolated from rinsed symptomatic tissue and their identity was confirmed using the molecular analysis described above. No oomycetes were recovered from the noninoculated carrots. Although several *Pythium* species have been associated with cavity spot before, this is, to our knowledge, the first report of strains within the *P. spinosum* species complex causing carrot cavity spot in California and elsewhere.

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