Diseases Caused by Oomycetes

First Report of *Pythium spinosum* as a Causal Agent of Crown and Root Rot in Greenhouse Cucumber Cultivation in Italy

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The origin of cucumber (*Cucumis sativus* L.) has been attributed to Africa, China, India, or the Near East. Cucumbers are now grown throughout the world using fields or greenhouse culture. In Italy, cucumbers are mainly cultivated in Sicily and central-northern Italy. During the spring of 2019, in the province of Latina (central Italy), mature cucumber plants of variety Ekron F1 grown in a greenhouse showed symptoms of yellowing and wilting of the leaves and brown necrosis on roots. The estimated severity of the disease in the greenhouse was 15% of plants. Fragments of symptomatic tissues cut from the root lesions were placed on potato dextrose agar (PDA) amended with 50 ppm of chloramphenicol and 50 ppm of streptomycin at 24 ± 2°C incubation temperature. A *Pythium* species was consistently isolated from semiselective medium with heavy aerial mycelia, and three pure cultures were obtained by hyphal tip transfers on PDA for further investigations. Main hyphae were nonseptate, globose or limoniform, thin-walled, ranging in size from 2.7 to 4.8 µm in diameter with terminal and intercalary hyphal swellings. Hyphae occasionally had two to three digitate protuberances. Oogonia were produced terminally, globose, and 15 to 20 µm in diameter. Oospores were plerotic, rarely aplerotic, thin-walled, and 16 to 19 µm wide. On the basis of morphological features, the isolates were identified as *Pythium spinosum* Sawada (Sawada and Chen 1926). The identity was confirmed by internal transcribed spacer (ITS) sequence with 99% homology with KU208359 and by cytochrome oxidase subunit 1 (COI) gene with 99% homology with HQ708835 GenBank sequence. The sequences of the three isolates (CREADC-

Om303, 304, and 305) were deposited in GenBank with accession numbers MN988848, MN988849, and MN988850 and numbers MN990462, MN990463, and MN990464 for ITS and COI, respectively. Pathogenicity tests were conducted in the greenhouse on variety Ekron F1 plants at the third to fourth leaf stage (Sinclair and Dhingra 1985). For each isolate, 10 plants were inoculated with a 5-mm mycelial plug cut from the margins of actively growing 4-day-old PDA cultures. Plugs were inserted through the epidermis to the phloem at the collar level and wrapped in parafilm to prevent dehydration and contamination. Five plants were used as controls and inoculated as described above using a sterile PDA plugs. Inoculated plants were kept under controlled conditions (humidity of 70%; 12-h/12-h day/night photoperiod) in a greenhouse at 25 ± 2°C. After 1 week all inoculated plants showed stem necrotic streaks extending downward to the crown and roots. Most of the plants died, after developing symptoms like those originally observed on plants naturally infected, thus fulfilling Koch's postulates. Control plants remained symptomless. Oomycete colonies isolated from the lesions and cultured on PDA morphologically resembled the inoculated isolates. *P. spinosum* has been shown to cause damping-off and root rot of many plants. It was originally isolated from snapdragon seedlings in Taiwan (Sawada and Chen 1926). It is a cosmopolitan species. It has been associated with damping-off of watermelon in the United States (Middleton 1943), on pumpkin in Queensland, Australia (Teakle 1960), and with vine decline of cucumber in Oman (Al-Sadi et al. 2011). More recently, *P. spinosum* has been associated with root rot of chili in Pakistan (Nawaz et al. 2015). To our knowledge, this is the first report of *P. spinosum* as the causal agent of crown and root rot of cucumber plants in Italy. More careful water management in the greenhouse would avoid the occurrence of *Pythium* root rot in this crop system production.

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