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Additional Information

First report of *Campylocarpon fasciculare* causing black foot disease of grapevine in Spain. S. Alaniz, C. Agustí-Brisach and D. Gramaje, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain; and M.I. Aguilar, Laboratorio de Producción y Sanidad Vegetal de Almería, Autovía del Mediterráneo Sal. 420, Camino de San Nicolás nº 1, 04745-La Mojonera, Spain; and A. Pérez-Sierra and J. Armengol, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.

In May 2008, symptoms of black foot disease were observed on 8-year-old grapevines (*Vitis vinifera* L.) cv. Garnacha in Albuñol (Granada province, southern Spain). Affected plants showed delayed budding with low vigor. Roots showed black discoloration and necrosis of wood tissues. Root fragments were cut, washed under running tap water, surface-sterilized for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Small pieces of discoloured or necrotic tissues were plated onto potato dextrose agar (PDA) supplemented with 0.5 g L⁻¹ of streptomycin sulfate. Plates were incubated at 25°C in the dark for 10 days, and all colonies were transferred to PDA. A *Cylindrocarpon*-like fungus was consistently isolated from necrotic root tissues. Single conidial isolates were obtained and grown on PDA and Spezieller Nährstoffarmer Agar (SNA) and incubated at 25°C for 10 days in darkness. On PDA the isolates developed white, thick, and cottony to felty abundant mycelium. On SNA, all isolates produced slightly to moderately curved one-septate (22.5-) 25.6 (-27.5) × (5-) 5.63 (-6.25) µm, two-septate (30-) 36.1 (-45) × (6.25-) 7.08 (-7.5) µm, three-septate (37.5-) 47.9 (-52.5) × (6.25-) 7.5 (-8.75) µm, four-septate (47.5-) 53.3 (-62.5) × (7.5-) 7.89 (-8.75) µm, and five-septate (52.5-) 61.8 (-67.5) × (7.5-) 8 (-8.75) µm macroconidia. Microconidia were not observed. DNA sequence of the rDNA internal transcribed spacer region (ITS) was obtained for isolate Cf-270 and deposited in GenBank (Accession No. HQ441249). This sequence showed high similarity (99%) to the sequence of *Campylocarpon fasciculare* Schroers, Halleen & Crous (GenBank Accession No. AY677303), in agreement with morphological features (1). Pathogenicity tests were conducted with inoculum produced on wheat (*Triticum aestivum* L.) seeds that were soaked for 12 h in flasks filled with distilled water. Each flask contained 300 mL of seeds that were subsequently autoclaved three times after excess water was drained. Two fungal disks of a 2-week-old culture of *C. fasciculare* (isolate Cf-270) grown on PDA were placed aseptically in each flask. The flasks were incubated at 25°C for 4 weeks, and shaken once a week to avoid clustering of inoculum. Plastic pots (220 cc) were filled with a mixture of sterilized peat moss and 10 g of inoculum per pot. One-month-old grapevine seedlings were planted individually in each pot and placed in a greenhouse at 25 to 30°C in a completely randomized design. Control plants were inoculated with sterile uninoculated seeds. Six replicates (each one in individual pots) were used, with an equal number of control plants. The experiment was repeated. Symptoms developed in all plants 20 d after inoculation and consisted in reduced vigor, interveinal chlorosis and necrosis of the leaves, necrotic root lesions with a reduction in root biomass, and plant death. The fungus was re-isolated from the roots of affected seedlings and identified as *C. fasciculare*, completing Koch's postulates. No symptoms were observed on the control plants. Black foot disease of grapevines can be caused by different species of *Cylindrocarpon* and *Campylocarpon*. *Campylocarpon fasciculare* was first reported in South Africa in 2004 (1). To our knowledge, this is the first report of *C. fasciculare* causing black foot disease of grapevine in Spain as well as other countries in Europe.

References: (1) F. Halleen et al. Stud. Mycol. 50: 431, 2004.