First report of *Curvularia trifolii* causing Curvularia blight in *Agrostis stolonifera* in South of Portugal

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Algarve region (Portugal) has nearly 40 golf courses with a significant economic impact. Summer surveys on ten golf courses detected an unknown disease on one course in 2009, and on another course in 2012 and 2013 at 29-30ºC of daily average. The second course had symptoms on about 25% of the turf of two putting greens. Diseased bentgrass (*Agrostis stolonifera* L.) had a green dapple pattern with irregular patches of turfgrass on yellowed leaves. Prior to decaying affected leaves turned brown and then grey. Crown and leaf sheath infections resulted in dark brown dry rot. No lesions were observed on the roots. Leaves were surface-disinfected with 5% commercial bleach (0.225 % sodium hypochlorite) and cultured on Potato Dextrose

Agar (PDA). Ten fungal colonies grew from the leaf tissue, and brown mycelia, conidiophores and conidia were observed under a microscope. Conidia were ventricose pyriform, mostly abruptly curved, 20-36 (30 µm, sd = 4) x 7-12 µm (10.5, sd = 1.3) (n=50) predominantly 3- septate, with a prominent hilum and enlarged and darkened central cells. Colonies grown on PDA were black-brown with a black reverse side. Conidia differed in size 15.4-24.6 (19.99 µm, sd = 3.00) x 6-11 µm (8.68, sd = 1.54), n=50) and morphology (cylindrical or slightly curved). These characteristics were consistent with *Curvularia trifolii* (Kauffm.) Boedijn. (Ellis 1971; Falloon, 1976; Khadka, 2016). Species identification of the representative isolate A2 1.12 was confirmed by analysis of nucleotide sequences of the ITS1-5.8S-ITS2 region using primers ITS1 and ITS4 (White *et al*., 1990) and GPDH gene region with primers gpd (Koike et al., 2013). BLAST searches of GenBank showed a high similarity of the isolate ITS sequence (MG029439) to the reference sequence JN712458 of *C. trifolii* (99% identity) and GPDH sequence (MK570108) with LT715803.1 (97.88 % identity). The maximum likelihood phylogenetic tree shows that our isolate clustered with *C. trifolii*. The pathogenicity assay of this isolate was conducted in greenhouse on *A. stolonifera* 'Penncross'. The isolate was grown on PDA (25 °C, 10 days). Five pots (100 mL) were filled with a sand and peat mix (9:1 v/v) with 0.06 g seeds per pot, covered with a fine sand layer. Turfgrass was cut once a week from two weeks after seeding and fertigated with 0.5 g. L^{-1} Peter's foliar feed (27 + 15 + 12; N + P_2O_5 + K_2O; and micronutrients; Scotts, Heerlen, The Netherlands). To obtain a conidia suspension for inoculation, cultured plates were scraped with a sterilized spreader and water. The suspension was filtered through a sterile gauze. Conidia were counted under microscope (400x) with haemocytometer. The suspension was adjusted to $8x10^3$ conidia per mL and 10 mL were sprayed per pot. Pots maintained humidity for two days under micro-

tunnels. First disease symptoms appeared three days after inoculation. Bentgrass from the five pots developed Curvularia blight and rotted crown symptoms. Control plants (5 pots treated with water) did not display symptoms. This trial was repeated once. On PDA, *C. trifolii* was re-isolated from leaf lesions and morphologically identified confirming Koch's postulates. Ellis (1971) referred the presence of C. *trifolii* in Portugal, but no region, symptom description or grass species was detailed. Sivanesan (1987) reported *C. trifolii* in Portugal only on *Lolium multiflorum*. Therefore, this is the first report of *C. trifolii* in Algarve, affecting *A. stolonifera*. This disease can increase maintenance costs in greens of this area.

References

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