New Disease Reports

First report of *Epicoccum nigrum* causing disease in *Lotus corniculatus* in Argentina

B. Colavolpe, J. Ezquiaga, S. Maiale* and O. Ruiz

IIB-INTECH (UNSAM-CONICET), Avenue Intendente Marino km 8.2, 7130 Chascomús, Argentina

*E-mail: smaiale@intech.gov.ar

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Lotus corniculatus is a species adapted to field conditions in the most important cattle production region in Argentina and constitutes a very valuable forage species. In January 2018, black leaf spots were observed on *L. corniculatus* (Fig. 1) in field plots in Chascomús, Buenos Aires. These spots were amphigenous, circular to irregular, 2-8 mm diameter, close to the leaf margin and reddish brown. Single lesions often coalesced to form larger lesions and became dark brown (Fig. 1).

Fungal isolates were obtained from the shoot lesions and were incubated on oatmeal agar, malt extract and potato dextrose agar (Crous *et al*., 2009) at 25°C in darkness. Macroscopic features of colonies on these media and mycelia characteristic were characterised under a microscope (Fig. 2) and conformed morphologically most closely to the genus *Epicoccum* (Chen *et al*., 2017). Total genomic DNA was extracted from fresh mycelia and PCR amplification was done using the ITS4 and ITS5 primers (White *et al*., 1990) designed to amplify the internal transcribed spacer (ITS) region. The amplified PCR products were purified and sequenced (GenBank Accession No. MH042300). A BLAST search revealed 99% identity to *E. nigrum* (KX815296 and MF510615).

An inoculation trial was done as follows. *Lotus corniculatus* plants were grown in a growth chamber at 25°C at 30-40% relative humidity with a 12 hr photoperiod. Thirty-day-old leaves were placed on Petri plates containing water agar medium. Leaves were sprayed with a conidia solution prepared by agitating the fungal lawn with distilled water in a petri dish culture, then suspending in 0.05% Tween 20 to a final concentration of 10^5 conidia/ml. Control leaves were sprayed with water containing 0.05% Tween 20. Petri plates were maintained at 25°C, 100% relative humidity for 16 days. The inoculated *L. corniculatus* plants were observed to be wilted with a yellow appearance and black spots (Fig. 3). The causal fungus was isolated and the macro- and microscopic characteristics were consistent with the inoculated fungus.

The genus *Epicoccum* was reported in Argentina as a seed pathogen in *Lotus* spp. (Sisterna & Lori, 2005). The isolate in the current study had 98% identity with *E. nigrum (*KC568289 and KY303832) that caused leaf spot in *Lablab purpureus* and brown leaf spot in loquat, respectively (Mahadevakumar *et al*., 2014; Wu *et al*., 2017). To our knowledge, this is the first report of *E. nigrum* causing leaf spot in *L. corniculatus* in Argentina.

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Figure 1

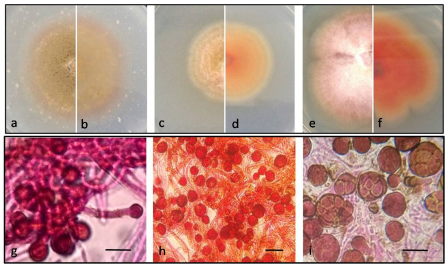

Figure 2

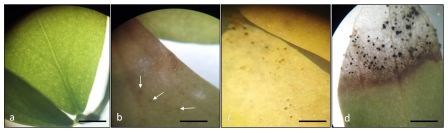

Figure 3

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