First report of *Leptosphaeria biglobosa* ´brassicae´ as causal agent of Phoma leaf spot in *Brassica napus* (Canola) in Argentina.

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Canola (*Brassica napus* L.) is the second largest oilseed crop in the world providing 13% of the world's oil supply. This crop has been grown in Argentina since the 1930s, and the area devoted to its cultivation varies every year, reaching a maximum of 95000 Ha in the 2012/2013 growing season.

Phoma stem canker is considered the most important and devastating disease in *Brassica napus* and other *Brassicae* species [1]. The causal agent is a complex of two closely related fungal species, *Leptosphaeria maculans* and *L. biglobosa*. In Argentina, the presence of *L. maculans* in canola plants was reported for the first time in 2004 [2], but the existence of *L. biglobosa* has not been recorded so far.

During the 2015/2016 season, we collected several leaf samples with typical Phoma leaf spot symptoms obtained from canola crops from the north and northeastern regions of the *Buenos Aires* province. The necrotic lesions were circular to irregularly oval, 8 to 15 mm in diameter, pale brown in the center, grayish green at the margin and characterized with the presence of pycnidia. Several leaf pieces with lesions were rinsed twice with deionized sterile water and placed in a humid chamber during 2-3 days to induce the pycnidia to exude cirri of conidia. After this period, one cirrus per sample was transferred onto PDA plates supplemented with antibiotics (15 mg/L STR, 15 mg/L GEN and 12 mg/L TET) using an inoculation needle under stereoscopic microscope. Thus, several isolates were obtained, some of them showing rapid mycelial growth rate and pigment production on PDA medium, as showed by the isolate Tapidor of *L. biglobosa* used as control (kindly provided by Professor Bruce Fitt, University of Hertfordshire-UK). Microscopic analysis showed hyaline conidias, subcylindrical with an average size of 4-5 x 1.5-2 µm [3]. In order to confirm the identity of these isolates, a PCR assay using genomic DNA as template was performed to distinguish *L. maculans* from *L. biglobosa* with the species-specific primers L*mac*R, L*mac*F, and L*big*F in a three-primers strategy [4]. These reactions gave a 444-bp amplicon as expected for *L. biglobosa* ´brassicae´. In addition, these results were confirmed by sequencing the nuclear ribosomal internal transcribed spacer (ITS) region, which showed a 99% of identity with the sequence of *L. biglobosa* ´brassicae´ deposited at the GenBank database (FO905468). *L. biglobosa* isolates were then tested for pathogenicity on the canola cultivars Westar and Bioaureo 2286 (Nuseed). With this purpose, cotyledons of 10-day-old seedlings were pricked with a needle, and each wound inoculated with 10 µl of a conidial suspension (10^7 conidia/ml). Fourteen days after inoculation, irregular and brown necrotic lesions were visible at the site of inoculation. These cotyledons were detached and placed in a humid chamber to induce pycnidia formation. After three days cirri of conidia were transferred to a plate with PDA supplemented with antibiotics as mentioned above. The identity of these isolates of *L. biglobosa* were confirmed by pigment production on PDA medium and by PCR assay using species-specific primers. To our knowledge, this is the first report of *L. biglobosa* ´brassicae´ as a pathogen of canola in Argentina. This finding shows that not only *L. maculans* but also *L. biglobosa* are the causal agents of Phoma leaf spot in Argentina´s canola cropping areas. Therefore, precise molecular phenotyping techniques are necessaries to identify the causal agent of this disease.

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