Occurrence of soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora* in the southern part of Buenos Aires province, Argentina

P. E. Grijalba^{A,C} and E. Guillin^B

^AFacultad de Agronomía Universidad de Buenos Aires, San Martin 4453, Argentina. ^BInstituto de Genética, INTA Castelar-Buenos Aires, Argentina.

^CCorresponding author. Email: grijalba@agro.uba.ar

Abstract. *Diaporthe phaseolorum* var. *caulivora*, the causal agent of soybean stem canker, was detected in Necochea (Buenos Aires province, Argentina), which is the southernmost record of the pathogen in South America.

Soybean stem canker (SSC) has been a problem in Argentina since 1996–97. Yield losses for 1998 were estimated at 128 800 tons, due mostly to *Diaporthe phaseolorum* var. *meridionalis* (Dpm). During 2000–01, *D. phaseolorum* var. *caulivora* (Dpc) was widespread in Santa Fe province, but was restricted to certain areas in Buenos Aires province (Pioli *et al.* 2002; Grijalba and Guillin 2005).

During a survey conducted in 2004-05 in Buenos Aires province, we found that SSC had already reached the city of Necochea (38°00'S, 59°20'W), with an incidence of 35% in one field. There were black lesions, 1-8 cm long on the stems and side-branches of infected plants, some of which surrounded the stem (Fig. 1a, b). Diseased stem pieces 3-5 mmlong were surface disinfected by immersion in 2% NaOCl for 2 min, rinsed in sterile distilled water and placed on 2% potato dextrose agar (pH 5). Cultures were incubated in the dark at $25 \pm 2^{\circ}$ C for 48 h and then under near-ultraviolet light with a 12 h photoperiod for 35-40 days to induce the development of the anamorph and teleomorph. A pathogenicity test was conducted using a colonised toothpick method (Hildebrand 1953) on the same cultivar where the disease was found. This cultivar, like many others, was advertised as resistant to canker without specifying the variety of the pathogen. Inoculated plants were kept in wet plastic bags (near 100% relative humidity) at $25 \pm 2^{\circ}$ C for 72 h then transferred to a glasshouse at the same temperature. Pure cultures of the fungus were used for molecular characterisation by PCR-RFLP analysis (Zhang et al. 1997).

Plants displayed symptoms identical to those of field-infected plants 14 days after inoculation. Control plants inoculated with uncolonised toothpicks remained healthy.

Isolates from the field-infected plants and artificially inoculated plants were similar. The isolate produced white colonies with compact, cottony tufts of mycelium. Stromata were infrequent, small, and did not produce pycnidia, but long-beaked, dark perithecia were produced in clusters. The mean ascus length was $26.5 \pm 0.6 \,\mu\text{m}$, while the bicellular, bigutulate ascospores were $8.25 \pm 0.6 \,\mu\text{m}$ long. Isolates from the field-infected plants were identical to the Dpc standard using the



Fig. 1. Field plant showing (*a*) internal symptoms reaching the medulla and (*b*) external symptoms on the stem.

PCR-RFLP assays (Alu I, *Hha*I, Mse I, Dde I, Rsa I) (Fig. 2). The symptoms, teleomorph morphology and PCR-RFLP patterns were coincident with those expected for Dpc (Fernandez and Hanlin 1996; Pioli *et al.* 2002).

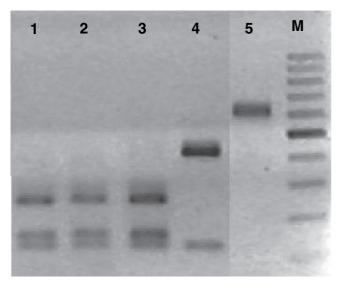


Fig. 2. Alu I–PCR-RFLP. Lanes 1–2, field samples; 3, *D. phaseolorum* var. *caulivora*; 4, *P. longicolla*; and 5, *D. phaseolorum* var. *meridionalis*. M, 100 bp marker.

This work reports the southernmost latitude for South America at which soybean stem canker, caused by *D. phaseolorum* var. *caulivora*, was detected.

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