

First Report of *Lasiodiplodia crassispora* as a Pathogen of Grapevine Trunks in South Africa

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In 2003 and 2004, a survey of grapevine (*Vitis vinifera* L.) trunk pathogens was conducted in 30 vineyards in the Western and Northern Cape and Limpopo provinces of South Africa. In each vineyard, 20 visually healthy plants were sampled randomly by removing the distal part of one cordon arm. Isolations were made onto potato dextrose agar (PDA) from the internal wood decay symptoms observed in the cordon samples. Seven *Botryosphaeriaceae* spp. were identified, including *Lasiodiplodia crassispora* (1). Other *Botryosphaeriaceae* spp. are known grapevine trunk pathogens (2). Species identity was confirmed by DNA sequence data of the partial translation factor 1- α gene (1) and sequences deposited in GenBank (GU233658 and GU233659). The *L. crassispora* isolates (CBS 125626 and 125627) were associated with brown internal necrosis, a known symptom of grapevine *Botryosphaeriaceae* spp. infection (3), in the cordon arms of Ruby Cabernet grapevines occurring in two vineyards in the Northern Cape Province. *L. crassispora* was described from cankered wood of *Santalum album* in Western Australia and endophytically from *Eucalyptus urophylla* in Venezuela (1). Its grapevine pathogen status was determined using both isolates in a repeated pathogenicity test that included three isolates each of *Botryosphaeria dothidea* and *Neofusicoccum australe* as positive controls (2), *Trichoderma harzianum* as a nonpathogen treatment, and an uncolonized agar plug as a negative control. The *Botryosphaeriaceae* spp. and *T. harzianum* were plated on PDA and incubated at 25°C for 7 days. Lignified, 6-month-old shoots of grapevine cv. Chardonnay were excised from grapevines with internodes 4 to 6 used for inoculations. Before wounding, shoots were disinfected by submersion for 1 min in a 1 ml/liter solution of a quaternary ammonium compound (Sporekill; ICA International Chemicals (Pty) Ltd, Stellenbosch, South Africa). Twelve shoots were used for each isolate or control treatment. Wounds were made 2 mm deep on the fifth internode of the shoots with a 5-mm flame-sterilized cork borer (2,3). Wounds were inoculated with a pathogen colonized agar plug (5 mm in diameter) or an uncolonized agar plug

and then covered with Parafilm (2,3). Inoculated shoots were incubated in the dark in moist chambers for 14 days at 25°C. After incubation, the bark of the shoots was peeled from the area around the wound and the lengths of any resultant lesions were measured under sterile conditions. The inoculum effect was assessed by analysis of variance and Student's *t*-test. Results showed that significantly ($P < 0.0001$) longer lesions were caused by *L. crassispora* (13.36 mm) compared with *N. australe* (9.27 mm) and *B. dothidea* (5.28 mm) and also significantly longer than lesions caused by the nonpathogen and negative controls (3.23 and 2.90 mm, respectively). To determine if lesions were caused by inoculated fungi, isolations were made from the tissue at the edges of the lesions by aseptically removing five 0.5×1 mm pieces of wood and placing them on PDA dishes amended with 0.04 g/liter of streptomycin sulfate. Dishes were incubated under normal fluorescent light at 25°C for 14 days before identifying isolated fungi based on morphological and cultural characteristics (1). To our knowledge, this is the first report of *L. crassispora* as a grapevine pathogen.

References: (1) T. I. Burgess et al. Mycologia 98:423, 2006. (2) J. M. van Niekerk et al. Mycologia 96:781, 2004. (4) J. M. van Niekerk et al. Phytopathol. Mediterr. 45:S43, 2006.