New Disease Reports

Stem die-back of highbush blueberries caused by *Neofusicoccum parvum* in China

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In September 2010, symptoms of blueberry stem blight were observed on highbush blueberries (*Vaccinium corymbosum*) in Lijiang and Qujing, Yunnan province (southwestern China). Symptoms included stem dieback, twig blight (Fig. 1) and extensive dull brown vascular discolouration (Fig. 2A), with crop damage ranging from 10 to 17%. These symptoms were similar to but distinctive from those caused by *N. vitifusiforme,* confirmed in 2008 as a pathogen of blueberry in China (Kong *et al*., 2010). *N. vitifusiforme* causes characteristic reddish-brown discolouration of the vascular system as well as dieback and bud and branch blight (Fig. 2B). Samples from plants with symptoms were washed with running tap water, surface sterilised with 2% sodium hypochlorite and then 70% ethanol, rinsed in sterile distilled water, plated on potato dextrose agar (PDA) and incubated at 26°C. Fungal isolates developed copious white aerial mycelium that became dark grey after five to six days, and formed black pycnidia after 21 days. Single hyphal tip cultures of putative isolates were stored in the culture collection (CMW) of the Urban Modern Agriculture Engineering Research Center at the Kunming University.

Conidia forming on PDA were one-celled, fusiform to ellipsoidal, externally smooth and thin walled, with dimensions of 12.5-21.5 x 4.6-7.0 (average 17.3 x 5.6 µm). Fungal morphology differed from that of *N. vitifusiforme* with conidia hyaline, granular, fusoid to ellipsoid, widest in the upper third with an obtuse apex and flattened, sub-truncate base (dimensions 18-21 x 4.5-8 µm) (Kong *et al*., 2010). *N. parvum* and another closely related species *N. ribis* cannot be distinguished based on internal transcribed spacer (ITS) rDNA sequences (Zhou & Stanosz, 2001). Partial sequences of the elongation factor 1-α (EF1-α), a portion of RNA polymerase II subunit, and slight differences in conidial morphology are used to distinguish the two species (Pavlic *et al*., 2009). Identity was confirmed by analysis of the rDNA ITS region (ITS1-5.8S -ITS2) and the translation elongation factor 1-alpha (EF1-α).BLAST searches at GenBank showed highest nucleotide sequence identity with *N. parvum* reference sequences (ITS: > 99%, GQ471815; EF1-α: 99-100%, FJ900658, GU064943). Representative sequences of isolates from both regions were deposited in GenBank (ITS: Accession Nos. JX096632, JX096634, for isolates LIJING22, LIJIANG23 respectively; EF1-α: JX096636, JX096637 for the same isolates). Morphological and molecular results confirmed this species as *N. parvum*.

Pathogenicity tests were conducted on two-year-old blueberry seedlings (highbush blueberries). Mycelial plugs (2-3 mm in diameter) from actively growing colonies of *N. parvum* (PDA) were applied to same-size bark wounds in the centre of the stems. Inoculated wounds were wrapped with Parafilm. Control seedlings received sterile PDA plugs. Inoculated and control seedlings (five each) were kept in a greenhouse and watered as needed. After 10 days, all of the inoculated but none of the control blueberry seedlings showed dark vascular stem tissue. *N. parvum* was re-isolated from symptomatic tissues, thus fulfilling Koch's postulates. No symptoms were visible in the control seedlings. *N. parvum* has been reported as a pathogen causing branch canker on avocado (McDonald *et al*., 2009), and has also been confirmed on blueberry in Argentina (Wright *et al*., 2012) and Korea (Choi *et al*., 2012). To our knowledge, this is the first report of *N. parvum* on blueberry in China.

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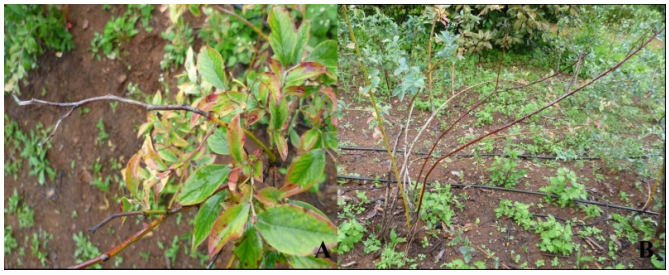

Figure 1

Figure 2

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