



First report of *Neofusicoccum parvum* causing shoot blight of pomegranate in Northern Greece

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Pomegranate (*Punica granatum*) is an important crop in Greece. In the spring of 2011, shoots of pomegranate cv. Wonderful wilted and were blighted, taking a distinct dark colour (Fig. 1) in commercial orchards located in Vrodou Katerinis, the Prefecture of Pieria, in northern Greece. Visual inspection showed that this pathogen infected about 2% of the shoots causing a 5% yield loss approximately. Black pycnidia were formed on the surface of blighted shoots. Conidia were hyaline, nonseptate, fusiform and measured 15-29 x 5-8 µm. Isolations from the lower margins of the cankers were made by plating tissue pieces (~ 3 mm) onto potato dextrose agar. The plates were incubated at 23°C for five days, and a fast-growing, mouse-grey coloured fungus was consistently isolated from these diseased stems. Reverse sides of the plates were initially white, and then became dark to olive green.

Genomic DNA (50 ng) was amplified using ITS4/5 with the following PCR run parameters: an initial preheating for three minutes at 95°C, followed by 34 cycles of denaturation at 94°C for one minute, annealing at 55°C for one minute, and extension at 72°C for two minutes, with a final extension at 72°C for eight minutes. An amplicon of approximately 700 bp was sequenced (GenBank Accession No. KR822222) and compared using a MEGABLAST search for highly similar sequences. Alignment data revealed the highest and most significant homology to *Neofusicoccum parvum* at 99% (KJ657701).

Koch's postulates were completed in the laboratory by inoculating 20 segments (6 cm in length and 1.5-2 cm in diameter) of one-year-old woody shoots of pomegranate cv. Wonderful. Using a cork borer, a 7 mm in diameter wound was created in the middle of each shoot segment by removing the bark and a 6 mm diameter agar plug bearing mycelia from a 15-day-old culture of *N. parvum* was inserted in the wound. The wound was covered with petroleum jelly and wrapped with adhesive tape to prevent desiccation. Ten control segments were similarly wounded and inoculated with an agar disk without fungal mycelium. All inoculated and non-inoculated shoot segments were incubated at 25°C in moist chambers, after which the resulting necrosis was recorded. Koch's postulates were satisfied after re-isolating the *N. parvum* from inoculated fruits that developed symptoms similar to those observed on shoots collected from orchards.

Although *Botryosphaeria dothidea* (syn. *Fusicoccum aesculi*) has been previously reported to cause fruit rots (Fu *et al.*, 2007) and shoot blight (Liu *et al.*, 2008) on pomegranate in China, to our knowledge, this is the first report of *N. parvum* associated with shoot blight on pomegranate in Greece. Pathogenicity tests conducted by Mohammadi (2013) showed that the fungus *N. parvum* isolated from grapevine was pathogenic to pomegranate. In Greece, the pathogen has also been associated with fruit rot and shoot blight of peaches (Thomidis *et al.*, 2011) and pistachio (Inderbitzin *et al.*, 2010).

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Figure 1

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