New Disease Reports

First report of *Neonectria neomacrospora* on *Abies grandis* in Belgium

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In June 2017, symptoms of shoot dieback were detected on natural regeneration of an *Abies grandis* (grand fir) stand located in southern Belgium (Luxembourg province). The affected saplings were between five to ten years old and located in the understorey of grand fir planted in the early 1970's. Symptoms were observed on about 20% of the saplings which showed shoot tip necroses (Fig. 1) affecting lateral as well as terminal shoots. Bark of the necrotic areas displayed a purple to reddish colour and a reduction of shoot diameter was occasionally observed. Necroses were also associated with a browning and shedding of needles.

Isolations were made from the margin of necrotic tissues. Transverse sections of shoots were surface disinfected for 10 seconds in 70 % ethanol, 90 seconds in a sodium hypochlorite solution (0.25 % active chlorine) and rinsed three times in sterile distilled water. Debarked sections were plated onto potato dextrose agar (Difco, USA) and allowed to grow at 20°C in the dark. A fungus forming a white and felted mycelium (Fig. 2) was consistently isolated from the tissues. The colonies, observed from the reverse of the plate, were creamy white to beige. The fungus had a radial growth rate of 1.8 mm (+/- 0.04 mm) per day at 20°C.

Under humid conditions sporodochia bearing micro- and macroconidia formed readily on infected tissues at the margin of the necroses. Observed microconidia were ovoid to ellipsoid; their size was 5-10 x 2-4 µm. Macroconidia were straight to slightly curved with rounded ends. They were mostly 1-3 septate (1-septate: 12-26 × 4-6 µm; 2-septate: 20-34 × 4-6 µm; 3-septate: 31-51 × 4-6 µm), occasionally 4-septate.

DNA was extracted from mycelium and ITS sequencing was performed using the primers ITS4/ITS5 as recommended by EPPO (2016). Based on morphological features (Ouellette, 1972; Booth, 1979) and ITS sequences, the fungus was identified as *Neonectria neomacrospora*. A representative sequence was submitted to GenBank (Accession no. MG049669).

Pathogenicity tests were done by inoculating current-year excised shoots of *A. grandis* with the isolated *N. neomacrospora* strain (no. 5104 in the fungi collection of the Walloon Agricultural Research Centre). After surface disinfection with 70% ethanol, sixteen shoots of about 20 cm long were inoculated in their midpoint with a sterile map pin coated with mycelium. Sixteen other shoots were punctured with a sterile map pin and served as controls. The excised shoots were placed separately in flasks containing a small amount of sterile tap water. The shoots were maintained in a quarantine facility at 22°C under daylight and monitored weekly. After four weeks the first symptoms occurred on inoculated shoots. After six weeks, extended necroses had developed on all inoculated shoots (Fig. 3) while control shoots remained healthy. *Neonectria neomacrospora* was consistently re-isolated from all lesions.

Neonectria neomacrospora is known to cause damage on numerous *Abies* species in Denmark and Norway (EPPO, 2013), in Sweden (Pettersson *et al*., 2016) and in the United Kingdom (Pérez-Sierra *et al*., 2016), notably in Christmas tree plantations. This is to our knowledge the first report of *N. neomacrospora* in Belgium. The organism is mentioned on EPPO Alert List. The threat it might represent to the Belgian Christmas tree sector is unknown but its potential economic importance should be considered seriously.

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Figure 1

Figure 2

Figure 3

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