New Disease Reports

The occurrence of *Anthostoma decipiens*, the causal agent of '*Carpinus betulus* decline', in northern Iran

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Carpinus betulus (hornbeam) is a native tree growing in the Hyrcanian forests of northern Iran. Recently there have been increasing reports of hornbeam decline in this region. In 2014, we received, for the first time, reports of decline from Saad Abad Naharkhoran in the west of Golestan province. Subsequently symptoms of disease were observed in Koohmian and Daland forests during summer and autumn of that year (Fig. 1). Since then the disease has spread throughout the area, especially during the last summer (2017), when trees were subjected to a long period (50-60 days) of high temperatures (38-40°C) and drought conditions. Losses due to the decline were estimated to be about 10% in autumn. The infected trees showed large cankers with bright red resin-like clumps of conidia on the bark of the trunks and main branches (Fig. 2).

The red conidial masses were collected and transferred to the laboratory for fungus isolation. A transverse section of the infected tissues showed long conidiophores in the loculi inside the fungal stromata. The conidia were produced holoblastically and were unicellular, lunate, hyaline, 8-11 µm × 1.3-1.4 µm. The fungus grew rapidly on potato dextrose agar and produced cream-white to hyaline, moderate to profusely cottony aerial hyphae (Fig. 3). On the basis of morphological characteristics, the fungus was identified as *Cytospora decipiens* (teleomorph *Anthostoma decipiens*) (Rappaz, 1992).

In order to confirm the identity of the fungus, DNA was extracted and restriction fragments amplified using PCR. Total DNA was extracted from pure culture of 14 samples using the method described by Barnes *et al.* (2001). The characteristics of isolates were studied based on PCR amplification of the ITS region of rDNA, using ITS5 (forward) 5'-(GGAAGTAAAAGTCGTAACAAGG)-3' and ITS4 (reverse) 5'-(TCCTCCGCTTATTGATATGC)-3' universal primers. BLAST searches of the GenBank nucleotide database revealed 99% identity to *A. decipiens* (Genbank Accession No. KC774565) (Jaklitsch *et al.*, 2014). The sequence of *A. decipiens* isolate CBIR was deposited in Genbank (MG738274).

Pathogenicity tests were conducted on nine replicates of one-year-old hornbeam saplings, replanted in 3 l pots containing sterilised forest soil (95-102°C for 30 minutes). Mycelial plugs (5 mm) containing conidioma were removed from 21-day-old *A. decipiens* colonies grown on malt agar. The plugs were placed under the bark of stem wounds and wrapped with a piece of moistened, sterilised cotton and plastic film. Five saplings were used as control plants. These were inoculated in the same way except using a 5 mm plug of malt agar minus the fungus. The plants were maintained in the greenhouse and monitored for symptom development. After 30 days, 2-3 cm lesions appeared on the fungus-inoculated samples and the same fungus was re-isolated. No symptoms were observed on the controls.

It is thought that the spread of hornbeam decline caused by *A. decipiens* has accelerated during the last few years due to drought conditions and the warmer summers experienced in the Golestan forest region. Indeed, it has been suggested that climate change has also played a critical role in the spread of hornbeam decline in the different regions of Italy (Rocchi *et al.*, 2010; Saracchi *et al.*, 2015). This is the first report of '*Carpinus betulus* decline' caused by *A. decipiens* in Iran. However, reviewing the literature, there appears to be little information on the biology, host range and disease epidemiology of the fungus. Given this lack of information, as well as the possible importance of climate change in disease development, it is clear that this disease warrants further study.

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Figure 1

Figure 2

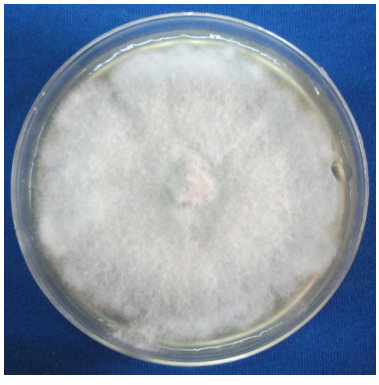

Figure 3

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