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NEW OR UNUSUAL DISEASE REPORT

Cryptostroma corticale in the northern Apennines (Italy)

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Summary. *Cryptostroma corticale* was observed on declining trees of *Acer pseudoplatanus* L. at Montovolo, a mountain site located in the northern Apennines, Italy. Morphological and biomolecular analyses confirmed the presence of the fungus in affected trees, which has not yet been officially described as occurring in Italy. No damage by the pathogen was observed on other species of *Acer* in the affected area, while drought–related decline symptoms were present on *Quercus pubescens* and *Ostrya carpinifolia* near the affected site, confirming the possible role of climate and especially repeated drought periods in the appearance of the problem.

Key words: *Acer pseudoplatanus*, climate change, forest workers health.

Introduction

In June 2013, declining maple trees (*Acer pseudoplatanus* L.) were observed at Montovolo (Grizzana Morandi, Bologna, Italy, 44.215232 N, 11.093391 E) in the Northern Apennines. The affected stand was located in a small valley at 910 m above sea level, on the top of the mountain. Trees 20 to 35 years old showed reduced foliage and dead branches (Figure 1), while the bark of branches and stems peeled off, exposing massive black stromae, sometimes covering the tree trunks (Figure 2). These released clouds of brownish spores when contacted. All of the 20 plants in the group of declining trees showed the same symptoms. The affected trees were clustered together at the edge of the wood while no symptoms were observed on other *Acer* growing 50 to 100 m from this disease focus.

Materials and methods

Infected bark was collected from three affected trees and the morphological characteristics of stromae and conidia were examined under a dissecting microscope.

Nucleic acids were extracted directly from fresh conidia using a commercial kit (NucleoSpin® Plant II, MN GmbH & Co. KG), and were amplified using primers ITS 5 (GGAAGTAAAAGTCGTAACAAGG) and ITS 4 (TCCTCCGCTTATTGATATGC) (White *et al.*, 1990). PCR products, after purification with ExoSAP-IT (USB Corporation), were sequenced using the Big Dye terminator v3.1. cycle sequencing kit (Applied Biosystems) on ABI Prism 3130xl Genetic Analyzer (Applied Biosystems). BLASTN comparison of the sequences was performed using the NCBI database to confirm the identity of fungal conidia.

Results and discussion

The observed symptoms and morphological characteristic of both stromae and conidia were similar to those described for *Cryptostroma corticale* (Ellis & Everh.) P.H.Greg. & S. Waller (Gregory and Waller, 1951).

The conidia collected from the stromae (Figure 3) were aseptate, smooth, pale brown, globose, cylindrical or obovoid, ranging from 4-7 µm in length ×

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Figure 1. Declining maple trees in the affected maple stand at Montovolo, Italy.

Figure 2. Characteristic bark peeling and black fungal stroma on a declining maple.

3–4 µm width (mean = 6.0 ± 0.78 × 3.7 ± 0.34 µm (variation expressed as standard deviations). The mean length to width ratio of the conidia was 1.6 µm. These dimensions fall within the ranges reported for *C. corticale*, 4-6 × 3.5-4 µm (Ellis and Ellis, 1997) and 5-12 × 3.5-4.0 µm (Lohrer, 2010).

BLAST analysis of the ITS sequences revealed a 100% sequence homology with *C. corticale* HG934110-HG934114, isolated from *A. pseudoplatanus* in the Czech Republic (Koukol et al, 2014) and with the sequences KP114070 and KP114090, isolated from a decaying *Acer* sp. tree in Geneva (Cochard et al, 2015), published in GenBank database. One sequence obtained in this study was deposited in GenBank with accession number KR870994.

The whole wooded area of Montovolo (about 600 ha) has been surveyed during 2014 and 2015. To date, no similar symptoms have been observed on other *Acer* species (*Acer opulifolium, A. campestre* and *A. monspessulanus*) growing sporadically in the woods nearby the identified foci. Suffering trees of *Quercus pubescens* and *Ostrya carpiniflolia*, with evident dieback of the crown, were scattered in the whole area, suggesting a general decline probably due to repeated drought periods. Meteorological data indicate that severe droughts have occurred in the summers of 2011 and 2012, while normal or abundant rains occurred in 2013 and 2014. The repeated drought stress situations could have enhanced the colonization of maples, as has been reported in other parts of Europe (Gregory and Waller, 1951; Koukol *et al.*, 2014). Till now, surveys carried out in another 25 sites in the Bologna and Modena Apennines where *Acer* presence was reported have not recorded any sympto-

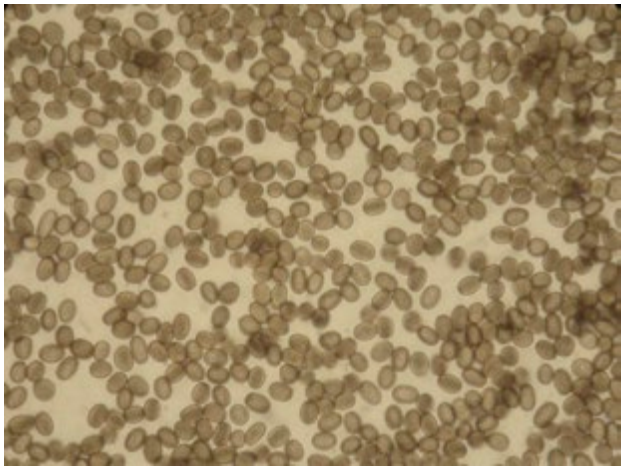

Figure 3. Micrograph of fresh conidia of *Cryptostroma corticale* (×100 magnification).

matic trees. The surveyed sites range from 5 to 50 km distance from the identified disease focus.

Cryptostroma corticale was recorded on maple in Italy in 1952 in an official report of the EPPO technical working group (Wilkins, 1952), without any additional information. No other records of this fungus from Italy were found in literature before or after the cited paper. Other authors (Gibbs, 1997; Koukol *et al*., 2014) did not confirm the presence of *C. corticale* in Italy, and the fungus was not described in Italian forest pathology texts (Capretti and Ragazzi, 2009).

This report confirms that *C. corticale is* present in Italy, at least in Apennine woods. Furthermore we have shown complete homology of the fungus with strains recently obtained from central Europe (Koukol *et al*., 2014). Other recent records were from the Netherlands (Anonymous, 2014), Bulgaria (Bencheva, 2014) and Switzerland (Cochard *et al*., 2015), suggesting possible spread of the pathogen in Europe. If the appearance or the reappearance of this disease in Italy is due to different climatic conditions, or is related to an accidental introduction of the pathogen, remain pending questions. The presence of new foci of this fungus also need to be reported as it causes hypersensivity pneumonitis in humans, known as the "Maple bark stripper's disease" (Gregory and Waller, 1951; Gibbs, 1997).

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