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New or Unusual Disease Reports

Cryptostroma corticale in Italy: new reports of sooty bark of *Acer pseudoplatanus* and first outbreak on *Acer campestre*

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Summary. Monitoring of emerging plant diseases in the Apennine mountains (central Italy) identified *Cryptostroma corticale* as the cause of the disease sooty bark of maple trees. The identified sites were located in rural or forested areas, next to buildings, in villages or suburbs, and one site was in a forest. Samples of symptomatic tissues were taken from *Acer pseudoplatanus* and/or *A. campestre*, as well as asymptomatic samples from *A. campestre* and *Aesculus hippocastanum*. All samples tested positive to a species-specific qPCR assay for the presence of *C. corticale*, indicating wide incidence of the disease in the northern Apennines of Italy, after attempted eradication of the first small group of infected plants were found in 2012.

Keywords. Maple, alien invasive species, emerging plant diseases, opportunistic pathogens, human health.

INTRODUCTION

Maple species (*Acer* spp.) are minority but important components of forest biodiversity (Spiecker *et al*., 2009), and are commonly used in urban settings (Pauleit *et al*., 2002; Sjöman *et al*., 2012) in Europe and in North America due to their environmental adaptability (Pasta *et al*., 2016). Maples are commonly planted in urban areas of Southern Europe, and the use of European maple species has been encouraged due to their low pollen allergenicity compared to *Acer negundo*, which is frequently found in cities (Acar *et al.*, 2007; Lacan and McBride, 2009; Chaparro and Terradas, 2010; Calatayud and Cariñanos, 2024). In Italy, maples trees are widely favoured as urban greenery, and are common in all major cities of the Alpine, Po Valley and Apennine regions, as well as in other Mediterranean areas (Bartoli *et al.*, 2021). For example, in Rome, use of maple trees has been encouraged because of their high rooting and carbon sequestration capacities and low ozone-forming potential. *Acer platanoides* is also valued for resistance to wind damage and air pollution, and *A. pseudoplatanus* has phytostabilising activity against soil contaminants (Mirabile *et al.*, 2015).

The health of maple trees is being increasingly challenged by *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller (Ellis and Everhart, 1889; Gregory and Waller, 1951), a pathogenic ascomycete considered non-native in Europe, and the causal agent of the disease sooty bark.

Cryptostroma corticale as first described by Ellis and Everhart (1889) as *Coniosporium corticale*. The first report of its presence in Europe was in 1945, in Wanstead Park in London, United Kingdom (Gregory and Waller, 1951). The fungus is known as a pathogen and saprophyte (Dickenson, 1980; Enderle *et al.*, 2020), and the endophytic stage was long assumed but only recently proven (Schlößer *et al.*, 2023). *Cryptostroma corticale* is opportunistic and causes symptoms when host trees suffer stress caused by abiotic factors such as high temperatures and drought (Dickenson, 1980; Enderle *et al.*, 2020). Reports of sooty bark periodically appeared after especially warm and dry summer periods as occurred in the 1960s and 1980s (Gregory and Waller, 1951; Moreau and Moreau, 1951; Townrow, 1953; Plate and Schneider, 1965; Young, 1978; Dickenson, 1980; Abbey and Stretton, 1985). Reports of *C. corticale* in Europe have increased since the drought years of 2003 and 2005 (Cech, 2004 ; Metzler, 2006 ; Robeck *et al.*, 2008; Langer *et al.*, 2013; Koukol *et al.*, 2014).

In Italy, the only published report of *C. corticale* was in 2012, when a small plantation of heavily damaged trees, clustered together, were identified at the forest edge on a mountain top in Bologna (Oliveira Longa *et al.*, 2016). No symptoms were observed on other *Acer* plants growing in the surroundings, and the outbreak was promptly eradicated.

Sooty bark symptoms include wilting, shoot dieback, greenish yellow wood discolouration, and development of blisters under host tree bark with subsequent heavy sporulation after the blisters burst (Gregory and Waller, 1951). Young's (1978) experimental evidence (reported in Dickenson, 1980) suggested that it takes a year or more for severe disease development, although small young trees may die within 10 months. The released spores of *C. corticale* can also cause hypersensitive pneumonitis in mammals (Towey *et al.*, 1932; Braun *et al.*, 2021). Humans with heavy exposure to the spores, such as forest or paper mill workers, as well as people with pre-existing lung diseases, are particularly at risk (Braun *et al.*, 2021).

This paper reports new outbreaks of the sooty bark identified in the northern Apennines in Central Italy.

MATERIALS AND METHODS

Study sites and sampling procedure

Diseased plants were observed between 2022 and 2024 at seven locations in or at the base of the Apennines (Figure 1). Details of these observations are listed below.

- About 15 symptomatic *A. pseudopatanus* trees (c. 15-years-old), in a mixed species plantation with *Fraxinus excelsior*, were found at Le Sassane (Gaggio Montano. 44.233890 N, 10.975204 E, 700 m a.s.l.) in the province of Bologna. The trees had been planted on a slight slope with a potentially wet depressed area at the slope base. The trees resembled those at the first outbreak in Montovolo. Most of the trees were severely damaged with typical sooty bark symptoms, and some were dead. Samples of bark, fungal stroma and wood were taken from five of the symptomatic trees.
- Two dead *A. pseudopatanus* trees with sooty bark symptoms were found on a road alongside a cultivated field near Riola (Vergato, 44.237902 N, 11.052955 E, c 420 m a.s.l.) in the province of Bologna. Wood and fungal stroma were sampled from both trees.
- Symptomatic *A. pseudoplatanus* trees were found in Castellonchio, in the province of Parma (44.546420 N, 10.004982 E, c. 910 m a.s.l.), which was followed by a report issued by the Regione Emilia Romagna (Ferrari and Bariselli, 2023). Here, in a small mountain village surrounded by forests, a group of young planted sycamores (circa hundred trees approx. 20-years-old) were heavily affected by the disease. The trees had severe crown dieback, bark cracking with abundant black stroma and fresh sporulation, and many were dead at the time of discovery.
- A mature *A. pseudoplatanus* plant (approx. 100 years old) with sooty bark symptoms (death of most of the crown and conspicuous stroma with active fungal

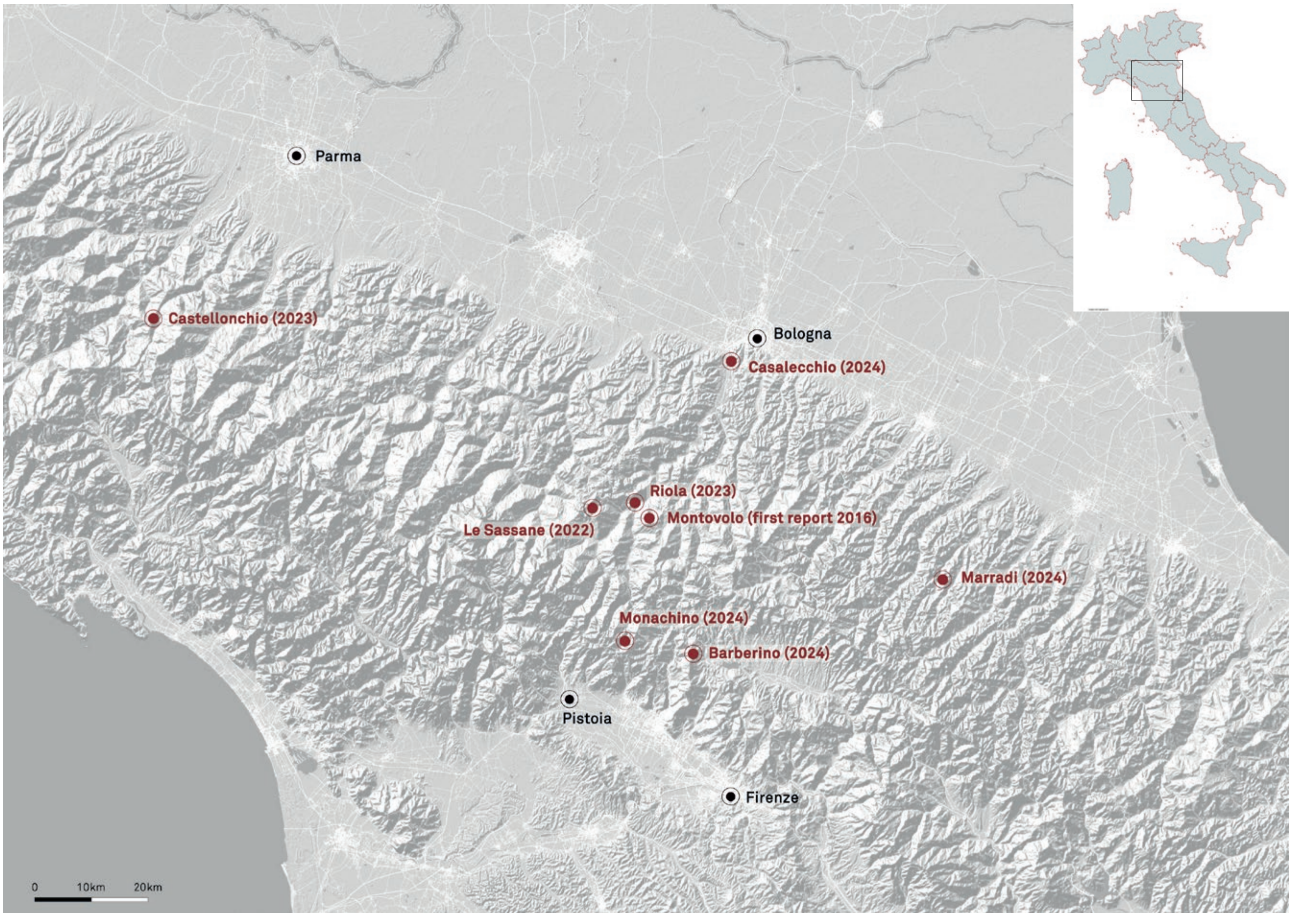

Figure 1. Infection sites (red dots, with year of discovery in parentheses) along the Appenines in Emilia Romagna (Bologna and Parma) and Tuscany (Pistoia and Firenze). The geographical image is a shaded relief from Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community (modified). The insert map of Italy was created at https://www.mapchart.net/italy.html.

sporulation on the stems and branches) was identified in Monachino, a group of houses in a mountain forest area in the province of Pistoia (44.02122 N, 11.03270 E, c. 710 m a.s.l.). The record was reported by the Phytosanitary service of Tuscany (Regione Toscana, 2024). Bark and fungal stroma were sampled from the main stem of the plant. Next to the diseased *A. pseudoplatanus,* an asymptomatic *Aesculus hippocastanum* L. tree was growing. Since *A. hippocastanum* is a known host of *C. corticale* (Young, 1978), and a recent report from Germany showed that *C. corticale* also caused symptoms on *Ae. hippocastanum* (Brenken *et al*., 2024), twig samples from this tree were taken and analysed.

– In the vicinity of Marradi, province of Firenze (44.103722 N, 11.73475 E, c. 515 m a.s.l.), five symptomatic *A. pseudoplatanus* trees of unknown origin in the private garden of an isolated country house were affected. Samples of the bark with stroma were taken from all the symptomatic trees as well as twigs of one asymptomatic *A. campestre* tree growing among the diseased trees.
– In Barberino di Mugello, province of Firenze (43.997781 N, 11.181664 E, c. 695 m a.s.l.), approx. 15 symptomatic *A. pseudoplatanus* trees with 3- to 4-year-old sooty bark symptoms were observed in a forested area close to the ancient monastic settlement of Montecuccoli. Symptomatic bark tissue was sampled from three of these trees.
– In Casalecchio di Reno (44.473327 N, 11.283565 E, c. 70 m a.s.l.), 42 *A. campestre* trees of putative natural origin were found to be dead or symptomatic (extensive crown dieback and visible fungal stroma from bark cracks on the main stems and branches) in a forested area, part of a large historic garden which is transformed into a peri-urban park. Three addi-

tional symptomatic *A. pseudoplatanus* trees were also surrounding private buildings in the immediate vicinity. Bark and stroma were sampled from five of the *A. campestre* trees.

Except in Riola and Barberino del Mugello, where it was not known how long the maple trees had been showing sooty bark symptoms, in the other cases decline and death of the plants was rapid, as reported by the locals or as observed by the authors of the present paper, i.e. over one or two growing seasons.

Samples from all sites, consisting of a part of fungal stroma, symptomatic shoots and wood samples, were brought to the University of Florence for morphological characterisation, and CNR laboratory facilities for molecular characterisation. Samples were taken from trees with stromatal spots on the trunks. Trunk tissues were scraped to obtain spores, or pieces of bark with stroma were excised with an axe and collected in sterile plastic bags. A branch (approx. 2 cm at the base), with multiple twigs, was cut from the asymptomatic *Ae. hippocastanum* (in Monachino), and from the asymptomatic *A. campestre* (in Marradi), to determine endophytic presence of *C. corticale*.

Morphological and molecular identifications of samples

Spore samples were microscopically examined. Parts of these samples were also inoculated onto malt extract agar (3% MEA, Biotec) and then incubated at room temperature with ambient light in order to isolate the fungus. Isolation of *C. corticale* was attempted for all samples including spores.

DNA extraction

When isolations were achieved, isolates were grown until they reached the margins of 90 mm diam. Petri dishes containing 3% MEA covered by cellophane discs (Celsa) of the same diameter. The mycelium was then scraped from the cellophane and placed in 2 mL capacity Eppendorf tubes each containing two tungsten beads (3 mm, Qiagen) and placed at -80°C for 20 min. DNA was likewise extracted from wood, bark and spore samples. These samples were then ground using a Retsch Mill (MM 400, Retsch), set to 25 oscillations sec^{-1} for 2 min. DNA was extracted from ground mycelium using the EZNA Plant DNA Kit (Omega Bio-tek), according to the manufacturer's protocol. Total DNA concentrations were estimated using the Tecan Infinite M Plex (NanoQuant Plate™). Eluted DNA samples were kept at -20°C until analysis.

DNA amplification

PCR was conducted only on the mycelium DNA, using the ITS1 and ITS4 primer set (White *et al.*, 1990) to amplify internal transcribed spacers 1, 2 and the 5.8S gene. The PCR products were purified using the mi-PCR Purification kit (Metabion International,), and were sent for sequencing to Macrogen (Milan, Italy). The acquired sequences (PQ339922, PQ339923, PQ339924) were analysed using the BLASTn function on www.ncbi.nlm.nih.gov.

DNA was also extracted from all samples, including wood, spores and mycelium, and analysed by qPCR, using the species-specific primers for *C. corticale* described by Kelnarová *et al.* (2017) and Muller *et al.* (2023). Asymptomatic samples taken from *A. campestre* collected from Marradi and *Ae. hippocastanum* at the Monachino site, were also analysed using qPCR.

RESULTS AND DISCUSSION

The spores observed with a microscope were morphologically identified as *C. corticale*. The spores were dark brown and ovoid, with average size of 5.7 × 3.9 µm (5.1-6.7 µm × 3.4-4.4 µm; n = 20; Figure 2), which is consistent with the descriptions of *C. corticale* by Gregory and Waller (1951) and Ellis and Everhart (1889). The outgrowing mycelium from spores placed on MEA was initially white and later turned brownish, as is characteristic for this fungus. *Cryptostroma corticale* cultures were successfully obtained from symptomatic trees of *Acer pseudoplatanus* in Monachino and Marradi, and of *A. campestre* in Casalecchio di Reno. For all the other field sites, it was not possible to obtain cultures because the stroma were dried. The BLASTn results for amplified DNA extracts showed a 100% identity and query cover matches for *C. corticale* strain CBS 216.52 (MH857008), and for several other *C. corticale* strains.

The qPCR yielded positive results for *C. corticale* for all the tested samples, including plant material from asymptomatic maples and from *Ae. hippocastanum*. This confirms presence of the fungus in the sampled asymptomatic woody tissues. Although no symptoms were observed on *Ae. hippocastanum*, the present results confirm previous observations that *C. corticale* can colonize alternative hosts. Young (1978) reported saprophytic infections by *C. corticale* on *Ae. hippocastanum*, and Dickenson (1980) reported that *C. corticale* can sporulate on sterile autoclaved wood of several plant species, including *Ae. hippocastanum*. In 2022 a diseased *Ae. hippocastanum* tree, exhibiting black spore fissures, loss

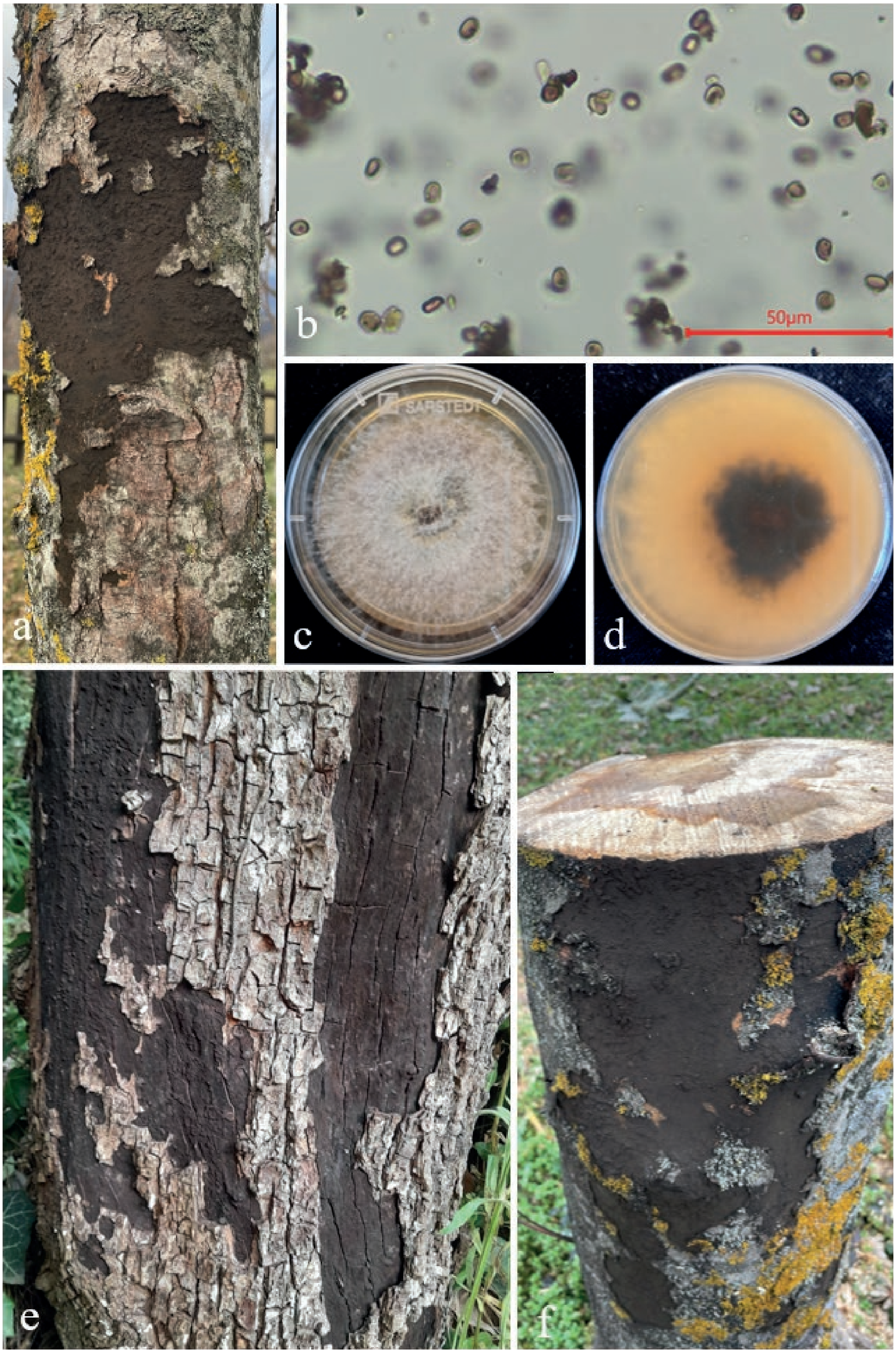

Figure 2. a) Sporulation on an *Acer pseudoplatanus* tree (photo credit: Stefano Romei). b) Spores of *Cryptostroma corticale* obtained during sampling. c) Culture of *C. corticale* isolated from material from Marradi (Accession number: PQ339924). d) View of the underside of the same culture. e) Symptoms of *C. corticale* infection on *Acer campestre*. f) *Acer pseudoplatanus* trunk with sporulation of *C. corticale* and cut surface with typical staining and white rot (photo credits: Stefano Romei).

of bark and death of crown parts, was identified in Trier, Germany (Brenken *et al.*, 2024). This report showed that in central Europe *C. corticale* is also causing isolated damage on *Ae. hippocastanum*, posing a threat to urban areas in central Italy. During the monitoring of the present study, symptomatic (Casalecchio di Reno, Figure 2) and asymptomatic (Marradi) *A. campestre* trees were found to be infected by *C. corticale*. From the present study, *Acer campestre* hence appears to be a similarly common host for *C. corticale*. Dickenson (1980) showed that *A. campestre* was similarly susceptible to *C. corticale* infections as *A. pseudoplatanus*, but this host has rarely been observed to show natural symptoms in Europe (Moreau and Moreau, 1951; Anon, 1952 cited in Dickenson, 1980). This is most likely due to the sporadic occurrence of *A. campestre* in central Europe.

Cryptostroma corticale usually causes disease when trees are stressed by drought and above average temperatures (Gregory and Waller, 1951; Dickenson, 1980; Enderle *et al.*, 2020). Over the course of the monitoring carried out in the present study, recent disease outbreaks with viable spores were found in Sassane, Castellonchio, Monachino and Casalecchio di Reno. The trees in Casalecchio di Reno had died over the course of the previous year. The symptoms observed in Riola and Barberino di Mugello were not fresh, and very few spores could be found. The symptoms from these two regions were estimated to be approx. 2–4 years old. The disease in Barberino di Mugello could have been due to a change in the forest structure, due to establishment of a fire protection strip, where few solitary trees were left standing. The sudden change from a closed forest to open field conditions is likely to have triggered the sooty bark outbreak in this area. The report in Barberino del Mugello is the only report of this disease in a forested area and not close to buildings. Outbreaks in urban areas would generally be unsurprising due to increased temperatures, harsh insolation, pollution and soil compression conditions, and trees standing at large distances from each other. This combination of conditions is known to cause severe water deficit in crowns of maple trees (Close *et al.*, 1996).

Sooty bark outbreaks are currently found along a portion of the northern Apennines in central Italy. Further spread of this disease is likely along the Apennines and to urban areas. Spores of *C. corticale* are estimated to disperse within a radius of at least 300 km (Muller *et al.*, 2023). Burgdorf *et al.*, (2022) reported a *C. corticale* spore count of 277 spores cm^{-2} d^{-1} during July and August 2019 in northern Bavaria, Germany, a year with many reported infections. Fifty-four percent of sycamore trees in the surveyed stand had visible sporulating trunk fissures. Numbers of counted spores varied over the year and were greatest during summer and early autumn (Burgdorf *et al.*, 2022). These results indicate potentially high infection pressure during summer period in northern Bavaria, where trees are stressed due to high temperatures and drought.

Identifying the pathway for introduction and the local sources of inoculum will be challenging, since *C. corticale* can survive endophytically for long periods before becoming pathogenic (Kelnarová *et al.*, 2017), and infection pressure can be high when bark fissures are present (Burgdorf *et al.*, 2022). The potentially infested area is assumed to be large, especially regarding the reports of *C. corticale* spread by Muller *et al.*, (2023). Often outbreaks are not detected or identified due to lack of knowledge and awareness, or effective controls, and are not eradicated prior to spore production, furthering spread of the fungus.

Emergence of sooty bark poses a threat for trees in the Mediterranean region. A large part of Italy has climatic characteristics favourable to the establishment of *C. corticale*. Furthermore, summer drought is forecast to occur during the next few decades in the Mediterranean region and parts of temperate Europe (Kottek *et al.*, 2006). This and the popularity of maples as urban greenery (Pauleit *et al.*, 2005; Augustinus *et al.*, 2024) increases the importance of selecting suitable provenances and genotypes of *A. pseudoplatanus* and *A. campestre,* which can adapt to the increasingly challenging environmental conditions that are likely to occur.

This emphasizes the need for awareness and caution regarding the disease sooty bark of maple trees. Especially for urban areas, this disease poses threats for conservation of established and valuable trees, but potentially also for human health.

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