

First report of *Pleuroceras pseudoplatani* on *Acer rubrum*, *A. griseum*, *A. saccharinum*, *A. negundo*, *A. circinatum* and *A. macrophyllum* in Scotland

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ABSTRACT

In September 2016, large necrotic lesions were observed on the foliage of several maples in Aberdeenshire, Scotland. Symptoms resembled giant leaf blotch disease caused by *Pleuroceras pseudoplatani* (formerly *Gnomonia pseudoplatani*; *Ophiognomia pseudoplatani*) on sycamore maple (*Acer pseudoplatanus*), a common disease in Europe. Other than *A. pseudoplatanus*, no other *Acer* species have previously been reported as hosts for this pathogen. Symptomatic leaves were collected from *Acer rubrum*, *A. rubrum* var. *Somerset*, *A. griseum*, *A. saccharinum*, *A. negundo*, *A. macrophyllum*, and *A. circinatum*, and the known host of *P. pseudoplatani*, *A. pseudoplatanus*. Next generation sequencing was used to determine presence of the suspected *P. pseudoplatani* and other associated fungi in symptomatic leaves. *P. pseudoplatani* was predominant in all symptomatic samples with 99-100% identity with reference ITS sequences of *P. pseudoplatani* deposited in GenBank (NCBI). This is the first report of *P. pseudoplatani* associated with leaf blotch on *Acer* species other than *A. pseudoplatanus*. The newly reported damage on North American *Acer* species may be of particular concern because of the economic importance of maples for timber products, syrup production and as ornamental trees in urban landscapes. Precautions should be taken to limit trade of plants from infested areas in Europe.

Keywords: giant leaf blotch disease; *Pleuroceras pseudoplatani*; *Acer*; maple

RÉSUMÉ

En septembre 2016, on a observé que le feuillage de plusieurs érables de la région d'Aberdeenshire, en Écosse, portait de grandes lésions nécrotiques. Les symptômes ressemblaient à l'anthracnose géante causée par *Pleuroceras pseudoplatani* (anciennement *Gnomonia pseudoplatani*; *Ophiognomia pseudoplatani*) sur l'érable sycomore (*Acer pseudoplatanus*), une maladie répandue en Europe. Cet agent pathogène n'avait encore jamais été rapporté sur d'autres essences d'érable qu'*A. pseudoplatanus*. On a donc récolté des feuilles symptomatiques sur *Acer rubrum*, *A. rubrum* var. *Somerset*, *A. griseum*, *A. saccharinum*, *A. negundo*, *A. macrophyllum*, et *A. circinatum*, ainsi que sur *A. pseudoplatanus*, l'hôte connu de *P. pseudoplatani*. Grâce au séquençage à haut débit, il a été possible de vérifier si *P. pseudoplatani* et d'autres champignons associés se trouvaient sur les feuilles récoltées. On a ainsi pu confirmer avec 99-100 % de certitude que *P. pseudoplatani* prédominait dans tous les échantillons symptomatiques grâce aux séquences (ITS) de *P. pseudoplatani* contenues dans la GenBank (NCBI). Il s'agit de la première mention de *P. pseudoplatani* associé à l'anthracnose sur d'autres essences d'*Acer* qu'*A. pseudoplatanus*. Les dommages nouvellement rapportés sur des essences nord-américaines d'*Acer* devraient nous préoccuper en raison de l'importance économique de l'érable pour les produits du bois, la production de sirop et comme arbre d'ornementation en milieu urbain. Des mesures s'imposent donc pour limiter le commerce des plantes provenant des régions infestées en Europe.

Mots-clés : anthracnose géante; *Pleuroceras pseudoplatani*; *Acer*; érable

Introduction

Pleuroceras pseudoplatani (von Tubeuf) Monod (syn: *Ophiognomonia pseudoplatani* (von Tubeuf) Barrett and Pearce, *Gnomonia pseudoplatani* von Tubeuf; anamorph: *Asteroma pseudoplatani* Butin & Wulf) is a necrotrophic parasite causing giant leaf blotch disease of sycamore maple. The pathogen was first described from sycamore (*Acer pseudoplatanus* L.), a species native to the mountainous areas of central Europe, by von Tubeuf (1930) as *G. pseudoplatani* from Germany, and

later from Denmark (Buchwald 1957). The widespread occurrence of the disease in Britain in the Oxford area in 1973 (Barret and Pearce 1981) and in Central Europe (Germany and neighbouring countries) by 1986, led to more investigations of the disease and the causal agent (Butin and Wulf 1987; Wulf 1988). However, the most recent reports of the disease in Europe are limited to occurrence notes, primarily from England (Lonsdale *et al.* 2002) and Austria (Kirisits 2007). While the fungus has been known as a leaf pathogen

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on sycamore maple for many years (Philips and Burdekin 1992, Butin 1995), no other *Acer* species have previously been reported as hosts for this pathogen.

The pathogen has a scattered distribution throughout the U.K. but is particularly prominent in central and northern Scotland, extending north of Inverness, yet only associated with sycamore maple. Fyvie Castle (Aberdeenshire, Scotland) is a 13th century castle located approximately 40 km north of Aberdeen, with a landscaped parkland designed in the early 19th century, and a collection of ornamental tree and shrub species including several non-native *Acer*. In September 2016, symptoms resembling giant leaf blotch disease caused by *P. pseudoplatani* were observed on the foliage of several maple species, including the known host, *A. pseudoplatanus*, but also other *Acer* spp. The purpose of this study therefore was to assess whether the observed necrotic lesions were associated with *P. pseudoplatani*, as identified by Illumina MiSeq sequencing of ITS2 amplified DNA from different species of *Acer*.

Materials and Methods

Sampling

Leaves of several *Acer* species growing on the grounds of Fyvie Castle (57° 26' 35.88" N – 2° 23' 41.64" W) and exhibiting conspicuous leaf symptoms (Fig. 1, 2) were collected (a minimum of five and up to ten leaf samples per tree) in September 2016. Samples were collected from the following species: *Acer rubrum* L. (origin: central and eastern North America), *A. rubrum* var. Somerset, *A. griseum* (Franch.) Pax (origin: central China), *A. saccharinum* L. (origin: central and eastern North America), *A. negundo* L. (origin: Central and North America), *A. macrophyllum* Pursh (origin: western North America), and *A. circinatum* Pursh (origin: western North America). Leaf samples were also collected from the known host of *P. pseudoplatani*, *A. pseudoplatanus* L. (origin: northwest/central Europe and western Asia) for comparison. Although *A. platanoides* (origin: eastern and central Europe and western Asia) and *A. palmatum* (origin: Japan, Korea) were also present in the gardens, no similar lesions were observed on those species.

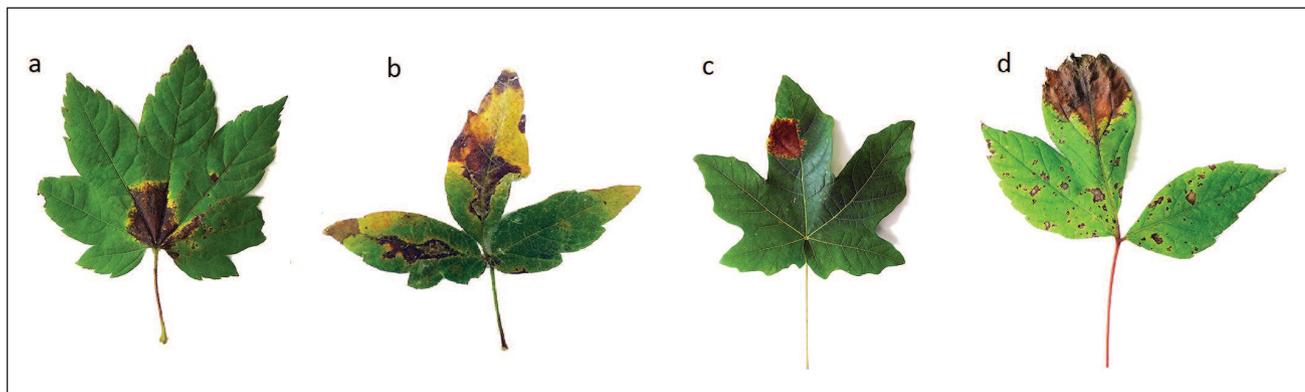


Fig. 1. Symptoms of giant leaf blotch disease caused by *Pleuroceras pseudoplatani* on (a) *Acer circinatum*; (b) *A. griseum*; (c) *A. macrophyllum*; and (d) *A. negundo*.

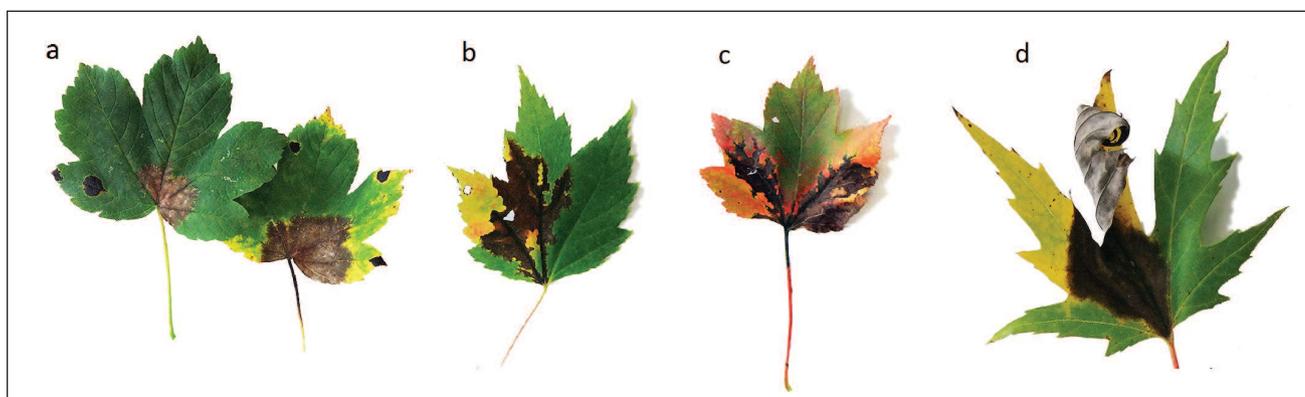


Fig. 2. Symptoms of giant leaf blotch disease caused by *Pleuroceras pseudoplatani* on (a) *Acer pseudoplatanus* showing characteristic brown necrotic patches on the leaf lamina and also black stroma of *Rhytisma acerinum*, cause of tar spot of sycamore; (b) *A. rubrum*; (c) *A. rubrum* Somerset; and (d) *A. saccharinum*.

Identification of the causal agent

We opted to use next generation sequencing (NGS) to determine presence of the suspected *P. pseudoplatani*, as well as other associated fungi in symptomatic leaves (the latter results characterizing the fungal community are presented in a separate manuscript currently in preparation). Five necrotic leaf samples from each *Acer* spp. were placed in 50 ml Falcon tubes and lyophilized for 48 hours. Freeze-dried samples (30 – 50 mg) were transferred to 2 ml Eppendorf tubes and pulverized to powder in a ball mill (MM 200, Retsch GmbH and Co., KG, Haan, Germany). Thereafter, DNA was extracted from 30 mg homogenized powder using the E.Z.N.A. SP Plant DNA Kit (Omega Bio-tek, Doraville, Georgia) following the protocol for dry samples.

The ITS2 region of the rDNA was amplified by PCR using the primers fITS7 and ITS4 (White *et al.* 1990, Ihrmark *et al.* 2012). Reactions were carried out in 50 µl volumes each containing: 5 ng µl⁻¹ template DNA, 200 µM of dNTPs; 750 µM of MgCl₂; 0.025 µM Phusion Hot Start polymerase (Thermo Scientific), and 200 nM of each primer in 1× buffer. All amplification reactions were performed in an Eppendorf® Master Cycler®. The PCR program started with denaturation at 98 °C for 3 min, followed by 31 cycles of 98 °C for 30 sec, annealing at 57 °C for 30 sec and 72 °C for 30 sec, followed by a final extension step at 72 °C for 7 min.

PCR products were purified using Agencourt AMPure XP (Agencourt Bioscience Corp, Massachusetts USA) and quantified using a Qubit 3.0 Fluorimeter with the Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA). After quantification, PCR products were pooled in an equimolar mix and sent for Illumina MiSeq sequencing (Mr DNA molecular research lab, Texas, USA).

The data derived from the sequencing process were processed using a proprietary analysis pipeline (www.mrd-nalab.com, MR DNA, Shallowater, TX). Sequences were depleted of barcodes and primers, before removing short sequences < 200 bp, sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp. Sequences were then denoised and chimeras removed. Operational taxonomic units (OTUs) were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity; Dowd *et al.* 2008a, 2008b; Edgar 2010, Eren *et al.* 2011, Swanson *et al.* 2011). OTUs were taxonomically classified using BLASTn against a curated GreenGenes/RDP/NCBI derived database (DeSantis *et al.* 2006).

Results and Discussion

After stringent quality sequence curation, a total of 2 553 687 sequences were parsed and 2 118 874 clustered. The 1 215 238 sequences identified within the fungi domain were utilized for final microbiota analyses. The average number of reads per sample was 32 844. Among these sequences, *Pleuroceras pseudoplatani* was predominant among all other fungal taxa detected (Blast search for the *Pleuroceras* spp. sequences extracted from the representing OTUs showed 99-100% homology with reference sequences of *P. pseudoplatani* deposited in GenBank (NCBI [GenBank Accession Nos. KY621262 – KY621295, inclusive]). Although *P. pseudoplatani* was detected in foliage of all *Acer* species exhibiting large blotchy lesions, the relative abundance of *P. pseudoplatani*

reads varied greatly among *Acer* species (between 1.0% and 96.3%; average 62.7%). Lesions caused by *P. pseudoplatani* on *A. griseum* were smaller (and correspondingly had the lowest abundance of *P. pseudoplatani* reads) than on other *Acer* species.

This is the first report of *P. pseudoplatani* associated with leaf blotch on *Acer* species other than *A. pseudoplatanus* – *A. rubrum*, *A. griseum*, *A. saccharinum*, *A. negundo*, *A. circinatum*, and *A. macrophyllum*. The symptoms share many characteristics with those on *A. pseudoplatanus*, particularly the large, necrotic lesions that appear dark brown from above and pale greyish-brown below (Barrett and Pearce 1981), and which may occupy large proportions of the leaf lamina by autumn (Fig. 1, 2). The smaller lesions on *A. griseum* compared to other *Acer* species may reflect a lower susceptibility to the disease in *Acer* species of Asian origin.

The newly reported damage caused by *P. pseudoplatani*, especially on North American *Acer* species, is of particular concern since several species of maple comprise a significant component of the mixed deciduous forests in northern North America, some having high economic value for timber products, syrup production and as ornamentals in urban landscapes. At the moment, the fungus is only known in Europe. Further work should be done to isolate and test *P. pseudoplatani* in controlled pathogenicity tests with other important North American *Acer* spp. and exotic ornamentals commonly used as landscape trees and precautions taken to limit the movement of this pathogen in nursery trade beyond the current zone of infestation in Europe.

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