Phytophthora pseudosyringae on European beech and hornbeam in the UK

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Introduction

There is major concern about the spread and impact of *Phytophthora ramorum* and *P. kernoviae* on trees and plants in the UK. Annual national surveys monitoring their presence on *Rhododendron ponticum* are carried out and intensive spot surveys are done on sites of particular concern. At two such sites in Wales further inspections identified a few trees with bleeding cankers potentially caused by *P. ramorum*. These trees were investigated to determine the cause of the bleeding lesions.

Symptoms

The diseased trees were two very mature European beech (*Fagus sylvatica*) (Fig. 1) and a hornbeam (*Carpinus betulus*) on similar sites (Table 1).

Table 1. Environment and tree characteristics

Host species	Beech 1	Beech 2	Hornbeam
Soil type	Brown earth, stony	Brown earth, stony	Brown earth, gleyed
Soil pH	6.5	6.5	6.0
Site elevation	74m	74m	71m
Site features	Slope, water course 15m away, cycle path 3m from tree	Slope, water course 15m away, cycle path 2m from tree	Slope, stagnant stream/pool 10m above tree, foot path 3m from tree
Tree DBH (cm)	107.3	115.6	6 stems from 18-65; perimeter above fluting 5.6m
Tree height (m)	25.4	27.3	21.8
Estimated tree age	150-180 yrs	150-180 yrs	150-180 yrs

Fig. 1. Mature European beech tree (TLB 14, Beech 2 - Table 1) with bleeding cankers on root flares and stem

Beech trees

- Bleeding cankers on one of the two trees extended from the soil-surface interface upwards.
- Lesions were present on root flares of both trees extending up the trunk to 1.5 m and 2.5 m respectively.
- Numerous factors including damage to bark (engraving, lightning, mammal damage), loss of limbs and colonisation by wood rot fungi and *Armillaria* compromised tree health.
- The crown of the first beech tree was in very poor condition, extremely thin, holding only a few yellowed leaves. This tree has subsequently died.

Hornbeam

- This tree was a very mature coppice with 6 stems.
- Bleeds were present on most stems up to 6 m.
- Overall health was compromised (broken out limb, decay fungi present, *Armillaria*).
- The crown was in good condition.

Methods

- Panels of diseased material (3x5 cm^2) were excised from the tree over a dead-live junction, so that necrotic inner bark and a fresh margin of active lesion were obtained.
- The panels were washed and the pathogen isolated on Phytophthora selective medium (Ref. 3).
- Isolates were cultured on carrot agar (Ref. 1), exposed to pond water and natural light for 48 hours (Ref. 6) and morphological features examined using the microscope.
- DNA was extracted and the ITS regions amplified using primers ITS1 and ITS4. Amplicons were sequenced and compared to sequences in GenBank using BLAST.
- Log inoculation tests were carried out using mycelial plugs on wounded beech logs (ca. 20 cm diameter), incubated at 20°C for 5 weeks (Ref. 3). An isolate of *P. cambivora* (host non-specific, aggressive *Phythophthora*) and *P. alni* subsp *alni* (host specific to *Alnus*) were included for comparison. Paring away the outer bark exposed lesions (Fig. 2). Disease area was determined (Ref. 3).

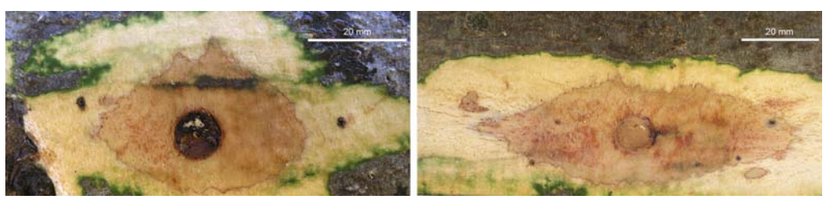


Fig.2. Exposed, inner bark lesions after 5 weeks on wound inoculated European beech caused by *P. pseudosyringae* (TLB 14A) (left) and *P. cambivora* (right)

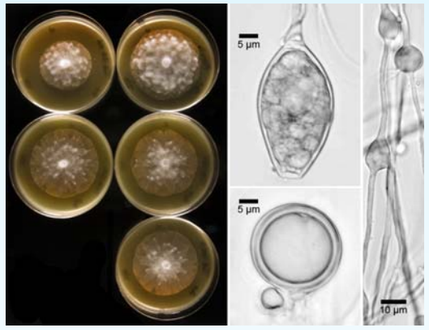


Fig. 3. Morphological features of UK isolates of *P. pseudosyringae* similar to German isolate PSEU 6.
(a) Colony morphology: (right, top to bottom) PSEU 6; TLB 105C; TLB 105D (left top to bottom) TLB 13A; MAR 13A
(b) Sporangium: Attached (although some caducous in colony), terminal, semi-papillate
(c) Oogonium with attached single celled, paragynous antheridium, and golden oospore
(d) Catenulate hypal swellings

Results

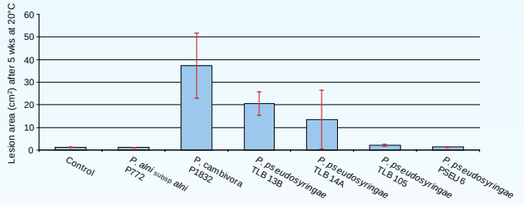
- Cultural and morphological features were in close agreement with those described for *P. pseudosyringae* (Ref. 6) (Fig. 3).
- The ITS BLAST searches showed >99% homology to sequences of *P. pseudosyringae*, confirming the morphological identification.
- There was much variation amongst the isolates of *P. pseudosyringae* in the lesion area formed in 5 weeks. One isolate was highly aggressive, one moderately so and two were benign (Fig. 4).
- Only the isolates originally obtained from beech caused moderate to aggressive lesions (Table 2; Fig. 4).
- On inoculated logs all isolates except *P. alni* subsp. *alni* caused lesions greater than the controls and the pathogen was re-isolated.
- *P. cambivora* was the most aggressive *Phytophthora* species tested (Fig. 4).

Table 2. Phytophthora species and isolates used in pathogenicity tests and details of original host and country isolates were obtained from.

Species	Isolate number	Host of origin	Country of origin
P. alni subsp *alni*	P772	*Alnus glutinosa*	England, UK
P. cambivora	P1832	*Fagus sylvatica*	England, UK
P. pseudosyringae	PSEU 6*	*Quercus robur*	Germany
P. pseudosyringae	TLB 14	*Fagus sylvatica*	Wales, UK
P. pseudosyringae	TLB 13	*Fagus sylvatica*	Wales, UK
P. pseudosyringae	TLB 105	*Carpinus betula*	Wales, UK

* *Isolate kindly supplied by T. Jung*

Fig. 4. Relative aggressiveness of *Phytophthora pseudosyringae* to inner bark of *Fagus sylvatica* using wound inoculation tests



Discussion

This is a first record of *P. pseudosyringae* being found in the UK and hornbeam is a new host for this pathogen. Although our isolates closely matched previous descriptions (Ref. 6), since *P. pseudosyringae* is recently described and very similar morphologically to *P. syringae* it is possible that it has been misidentified previously in the UK (Ref. 6).

Initial pathogenicity tests on beech indicate that *P. pseudosyringae* is less aggressive than *P. cambivora* corroborating previous work (Refs. 2, 5, 6).

A large range in aggressiveness of isolates was evident in our pathogenicity tests with some isolates hardly causing lesions. Similar epidemiological behaviour was observed in pathogenicity tests on English holly (Ref. 8). Others comment that there may be a relationship between host specificity and isolate aggressiveness (Refs. 2, 6). More work is required to resolve this issue.

Frequently more aggressive *Phytophthora* species are isolated together with *P. pseudosyringae* (Refs. 4, 5, 6). *P. pseudosyringae* has a different pathogenic effect on beech seedlings than some of the more aggressive *Phytophthora* species (Ref. 5). We also isolated another *Phytophthora* sp. simultaneously (*cf. P. drechsleri*) but need to carry out more detailed investigations of the diseased trees and further isolations to determine the ecological and pathogenic roles of the various Phytophthoras involved.

The site details reveal that the diseased trees in Wales were close to pathways and a source of water. This could suggest a connection with human recreational activity either through introducing the pathogen to the area or through stress imposed on the tree by activity around its roots thus altering its susceptibility. This has also been noted in Italy (Ref. 4). Brasier (personal communication) has also commented that the pathways could alter the drainage of the soil making conditions conducive to disease.

Much remains unknown about the ecological role and behaviour of *P. pseudosyringae*. In Europe it is regarded as a pathogen causing fine root rot and bleeding stem cankers of native tree species. However, in the USA it is isolated from necrotic leaves and twig cankers frequently from bay laurel (*Umbellularia californica*) with only a few records of it being associated with oak bleeding stem cankers (Ref. 9). It is suggested that the wide distribution and reduced virulence of this pathogen in the USA may indicate that it is endemic there (Ref. 7). Addressing population structure and epidemiological issues is important so that the status of this pathogen as a threat to forests and other plants can be determined and proactive steps taken to prevent damage.

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