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The destructive invasive pathogen *Phytophthora lateralis* found on *Chamaecyparis lawsoniana* across the UK

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Summary

In 2010–2011, *Phytophthora lateralis* was isolated from diseased *Chamaecyparis lawsoniana* exhibiting dieback and mortality at eight geographically separate forest, parkland and shelterbelt locations in England, Scotland and Northern Ireland. In 2011, *P. lateralis* was also isolated from young symptomatic nursery plants of *C. lawsoniana* and *Thuja occidentalis* recently imported into Scotland from mainland Europe. These are the first findings of *P. lateralis* in the UK. At six of the field sites, only collar and root lesions were observed. However, at two sites, large stem and branch lesions unconnected to the collar region were also observed. *Phytophthora lateralis* was readily isolated from both aerial and basal lesions. In artificial inoculation experiments, two Scottish isolates of the pathogen caused lesions on *C. lawsoniana* shoots and were readily reisolated from the lesions, their pathogenicity being comparable to that of *P. lateralis* isolates originating from outside the UK. Isolates from six field sites and the two nursery interceptions exhibited ITS and *cox*II sequences identical to published sequences of French and North American isolates. However, the isolates from two field sites shared an ITS sequence with Taiwanese isolates and differed from North American, French and Taiwanese isolates by a single-base substitution in *cox*II, suggesting a separate evolutionary history. It is clear that *P. lateralis* now presents a significant threat to *C. lawsoniana* in Britain. The main source of the outbreaks is likely to be imported infested nursery stock.

1 Introduction

Phytophthora lateralis is an aggressive oomycete pathogen first described in the early 1920s causing mortality of nursery plants of *Chamaecyparis lawsoniana* (commonly known as either Lawson Cypress or Port Orford Cedar) near Seattle in the Pacific Northwest of North America (Zobel et al. 1985). The pathogen is now considered to have been unwittingly imported into the area on infected plants early in the 20th century where it came into contact with *C. lawsoniana,* a highly susceptible new host (Zobel et al. 1985; Hansen et al. 2000). In the 1950s, *P. lateralis* spread into the native range of *C. lawsoniana* in south-west Oregon and northern California, probably as a result of further nursery stock movement, where it has subsequently killed large stands of mature trees. These developments have virtually halted the trade in ornamental *C. lawsoniana* in the area (Hansen et al. 2000). Recently, *P. lateralis* has been isolated from soil and limited foliage infections associated with old-growth native yellow cedar (*Chamaecyparis obtusa* var. *formosana*) at several locations in Taiwan (Brasier et al. 2010; Webber et al. 2011). This suggests that Taiwan may lie within the geographical centre of origin of *P. lateralis* and that the pathogen has co-evolved on *C. obtusa* var. *formosana* (Brasier et al. 2010).

Sporangia and motile zoospores are the main dispersal propagules for *P. lateralis* (Tucker and Milbrath 1942; Hansen et al. 2000). Zoospores primarily initiate infection of the roots and root collar of *C. lawsoniana* in its native range in the USA, with infection resulting in orange/brown phloem lesions at the stem base of infected trees. When the phloem becomes disrupted, infected trees often die rapidly, the entire crown turning pale green at first and later becoming bronze as a result of rapid desiccation (Hansen et al. 2000). Sporangia of *P. lateralis* isolates from both Taiwan and Oregon are caducous with preformed pedicels, indicating adaptation for aerial dispersal (Brasier et al. 2010). Limited evidence of foliage infection has been observed in Taiwan and Oregon (Tirone and Roth 1957; Webber et al. 2011), and aerial bark infections have been observed in outbreaks of the pathogen in France (Robin et al. 2011). *Phytophthora lateralis* can also survive in the soil for many years in the form of thick-walled chlamydospores, which germinate to produce sporangia (Hansen et al. 2000). Generally, sporangial formation and infection of *C. lawsoniana* is favoured by cool, wet conditions. Over longer distances, in addition to possible wind dispersal, zoospores can spread along water courses, and chlamydospores can be carried in mud on feet and on vehicles associated with forestry operations and recreation (Hansen et al. 2000). Ornamental *Chamaecyparis* species form an important component of European amenity landscapes and the nursery trade (Woodhall and Sansford 2006). Until recently, *P. lateralis* was considered to be absent from Europe, except for four reported findings of the pathogen from an unknown origin on ornamental *C. lawsoniana* in nurseries: two in France in 1996 and 1998 (Hansen et al. 1999) and the other two in The Netherlands in 2004 and 2010 (Meffert 2007), which were subsequently believed to have been eradicated (Brasier et al. 2010). Owing to the potential threat posed to trees in Europe, *P. lateralis* was recommended for listing as an A1 quarantine organism by the European Plant Protection Organisation (EPPO) in 2006, but, despite this acknowledged threat, it has not been raised to quarantine status by the European Community.

Of considerable concern, therefore, was the recent recognition of another outbreak of *P. lateralis* in France, affecting thousands of shelterbelt *C. lawsoniana* over a 400-km^2 area of Brittany (Robin et al. 2011). Decline and mortality of these

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trees were first noticed in 2005 (Robin et al. 2011). An unexpected feature of this outbreak is the high number of aerial stem infections, which is thought to be due to a combination of wind dispersal of sporangia and locally mild, humid conditions enabling direct aerial infection of branches and stems (Robin et al. 2011). Such aerial dispersal is also likely to increase the risk of *P. lateralis* spreading over longer distances.

In October 2010, the Tree Health Advisory Service of Forest Research received a report of dieback and mortality of large *C. lawsoniana* in a woodland setting at Balloch Castle Country Park north of Glasgow (western Scotland). Subsequent investigations confirmed that a number of symptomatic trees at the site were infected with *P. lateralis*. Over the following year, dieback and decline of planted *C. lawsoniana* was reported at three more locations in the Glasgow area, several locations in Northern Ireland and two in England, and again the pathogen was isolated from symptomatic trees. Nursery plants of *C. lawsoniana* and *Thuja occidentalis* imported from continental Europe and showing foliage dieback were also intercepted at two locations in central Scotland and found to be infected with *P. lateralis*, with *T. occidentalis* being a new host record for the pathogen (Schlenzig et al. 2011). This paper describes the aetiology of the disease at these sites, including further evidence for the aerial spread of the pathogen, and reports the results of pathogenicity testing of British, Oregon and Taiwanese *P. lateralis* isolates on two genotypes of *C. lawsoniana*. The implications of these findings, including the role of the international nursery trade in introducing alien pathogens such as *P. lateralis* into the United Kingdom, are discussed.

2 Materials and methods

2.1 Media and isolation

SMA + MRP *Phytophthora* selective medium was based on the synthetic mucor agar of Elliot et al. (1966) with the addition of antibiotics as described in the study of Brasier et al. (2005). Carrot agar (CA) was prepared as described by Brasier (1967; see also Erwin and Ribeiro 1996) and carrot broth by the same method without the addition of agar. For attempted isolation of *Phytophthora* from necrotic phloem of *C. lawsoniana*, tissue samples of approximately 5 × 10 cm were excised from the margins of phloem lesions. For each sample, 30 small slivers of tissue, 10 per 9-cm Petri dish, were plated onto the selective medium and incubated at 18°C in darkness. Resulting colonies were subcultured onto CA.

At the Balloch Castle Country Park site, soil samples containing fine roots were also collected from around selected symptomatic trees; soil was collected at four cardinal points around each tree at approximately 10–20 cm depth and bulked. Each bulked soil sample was processed where possible within 48 h of collection by baiting with segments of rhododendron and *C. lawsoniana* foliage and plating necrotic bait pieces onto selective medium as described by Brasier et al. (2010). Isolations from nursery samples were carried out as described by Schlenzig et al. (2011).

Stock cultures were maintained on CA plates at 20°C in darkness and subcultured at 4-week intervals. Chlamydospores were observed on CA. To induce sporangial formation, 1-cm-diameter plugs from the edges of actively growing colonies growing on CA were flooded with unsterile pond water and incubated for 24–36 h at 20°C.

2.2 DNA extraction and amplification

To obtain DNA of *Phytophthora* species in phloem tissue, approximately 0.1 g of chopped phloem from the lesion margins was placed in an Eppendorf tube, frozen in liquid nitrogen and ground to powder in a bead mill (Retsch, Haan, Germany), and DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR was performed using the *Phytophthora*-specific forward primer Ph2 (Ippolito et al. 2002) and universal reverse primer ITS4 (Eurofins MWG Operon) at 10 mM in a total volume of 25 μl containing 0.45 mM $MgCl_2$, 5 μl of 5X buffer, 0.2 mM dNTPs, 0.625 U Taq and 1 μl of template DNA. Amplification was performed in a Biometra^R Tgradient thermocycler (Thistle Scientific, Glasgow, UK), with initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 min and a final extension of 72°C for 7 min. PCR products were purified and sequenced in both directions with the BigDye version 3.1 Ready Reaction Kit on an ABI Prism 3730 Capillary Sequencer (Applied Biosystems, Foster City, CA, USA). Raw sequences were aligned and edited using Sequencher version 4.8 for Windows, aligned with published ITS sequences in GenBank using BLAST (Altschul et al. 1990) and deposited in GenBank under accession numbers JQ582464–JQ582468.

For sequencing from pure cultures, isolates were grown for 7 days in 1 ml carrot broth in an Eppendorf tube and centrifuged for 5 min at 14 000 ***g***, the supernatant poured off and DNA extracted from mycelia as described above. Amplification, sequencing and editing of the ITS region were carried out as described above. Amplification of the cytochrome oxidase II (*cox* II) locus was carried out using primer pair FM 82 and Fm78 (Martin and Tooley 2003) at 0.5 μM with PCR conditions, sequencing and editing as described above. Edited sequences from pure cultures were aligned using ClustalW2 and deposited in GenBank under accession numbers JQ513966–JQ513970.

2.3 Pathogenicity testing

Zoospore suspensions of *P. lateralis* isolates P2202, P2426 (Table 1), P2397 (from Oregon) and P2291 (from Taiwan) were prepared using the method of B. Scanu (personal communication). Discs of 1 cm in diameter were cut from the edges of growing colonies on CA plates incubated in daylight. The discs were floated in plates containing sterile Petri solution for 48 h at 18°C under continuous daylight, and the Petri solution was then replaced with sterile deionized water and incubated for a further 48–72 h. Once sporangia were plentiful on the discs of mycelia, the plates were refrigerated for

Table 1. Details of *Phytophthora lateralis* isolates obtained from *Chamaecyparis lawsoniana* (unless otherwise stated) across the UK.

Tree code and location	Date of sample	Stem diameter in cm	Sample material	*P. lateralis* isolate code[1]
BCH01, Balloch, Scotland	October 2010	70	Phloem, aerial stem lesion	P2202
BCH02, Balloch, Scotland	November 2010	na	Soil from base of tree	P2305
BCH14, Balloch, Scotland	November 2010	40	Phloem, root flare lesion	P2307
BCH18, Balloch, Scotland	December 2010	15	Phloem, basal lesion	P2311
BCH28, Balloch, Scotland	January 2011	60	Phloem, basal lesion	P2330
BCH32, Balloch, Scotland	January 2011	40	Phloem, basal lesion	P2331
BCH30, Balloch, Scotland	January 2011	30	Phloem, basal lesion	P2332
BCH21, Balloch, Scotland	January 2011	60	Phloem, basal lesion	P2333
BCH20, Balloch, Scotland	January 2011	40	Phloem, aerial stem lesion	P2334
BCH24, Balloch, Scotland	January 2011	60	Phloem, basal lesion	P2335
GC01, Greenock, Scotland	March 2011	25	Phloem, basal lesion	P2449
GC02, Greenock, Scotland	March 2011	40	Phloem, basal lesion	P2423
GC03, Greenock, Scotland	March 2011	20	Phloem, basal lesion	P2424
GC04, Greenock, Scotland	March 2011	70–80	Phloem, basal lesion	P2450
GC05, Greenock, Scotland	March 2011	60–70	Phloem,basal lesion	P2425
RG06, Rouken Glen, Scotland	March 2011	30	Phloem, basal lesion	P2426
Nursery, Central Scotland	April 2011	Nursery plant	Foliage	00971[2]
Nursery, Central Scotland	February 2011	Nursery plant	Foliage of *Thuja occidentalis*	01028[2]
KI3, Kilmaronock, Scotland	April 2011	25	Phloem, basal lesion	P2451
KI5, Kilmaronock, Scotland	April 2011	15	Phloem, basal lesion	P2452
KI7, Kilmaronock, Scotland	April 2011	45	Phloem, basal lesion	P2453
TFP1, Tollymore Forest Park, NI	June 2011	>100	Phloem, basal lesion	P2517
TFP2, Tollymore Forest Park, NI	June 2011	20	Phloem, basal lesion	P2518
TFP3, Tollymore Forest Park, NI	June 2011	25	Phloem, basal lesion	P2519
SFT01, Somerset Forest Park, Coleraine, NI	October 2011	15	Phloem, basal lesion	P2522
SFT02, Somerset Forest Park, Coleraine, NI	October 2011	40	Phloem, basal lesion	P2562
SFT03, Somerset Forest Park, Coleraine, NI	October 2011	30	Phloem, basal lesion	P2563
PLT03, Plympton, Devon, England	September 2011	20 (one of two forks)	Phloem, basal lesion	P2564
BLU03, Blubberhouses, Yorkshire, England	October 2011	40	Phloem, basal lesion	P2537
BLU04, Blubberhouses, Yorkshire England	October 2011	22 (one of two forks)	Phloem, basal lesion	P2538

na, not applicable.
[1]Isolate codes preceded with P deposited in the Forest Research Phytophthora culture collection.
[2]Isolates deposited in the SASA culture collection.

approximately 30 min and returned to room temperature, which stimulated the release of zoospores. Spore concentration was determined using a haemocytometer and adjusted to 2×10^3 ml^{-1} for inoculation.

Inoculation methods were based on those of Hansen et al. (2012). For each isolate, the cut ends of five 20–25 cm lengths of *C. lawsoniana* foliage of approximately 5 mm stem diameter were placed in a 500-ml beaker containing 60 ml of zoospore suspension (a resulting depth of approximately 1.5 cm) and left overnight. Each shoot was then transferred to a tube of sterile vermiculite medium, which had been moistened to saturation. The foliage was collected from two 35-year-old *C. lawsoniana* trees. The trees were of seed rather than clonal origin and showed minor differences in growth habit. It was therefore assumed that they represented two different genotypes. Five shoots of each genotype were challenged with four isolates of *P. lateralis.* Five control shoots of each genotype were prepared similarly but left in sterile water overnight before being transferred to the vermiculite. All shoots were then incubated in plastic chambers at 18°C for 21 days before the length of necrotic bark was measured on each one. Isolations were also made from the necrotic bark as well as from foliage that showed symptoms of blackening onto SMA + MRP medium to confirm the presence of *P. lateralis*.

3 Results

3.1 Disease distribution, symptoms and aetiology

Four sites in western Scotland, two in Northern Ireland and two in England at which decline and mortality of *C. lawsoniana* had been reported were investigated between October 2010 and October 2011 (Tables 1 and 2). Figure 1 shows the locations of the sample sites.

Table 2. Details of *Chamaecyparis lawsoniana* (LC) sites from which *Phytophthora lateralis* was isolated.

Site	Balloch Park	Greenock	Rouken Glen	Kilmaronock	Tollymore	Blubberhouses	Plympton	Somerset Park
Dates investigated [1]	October 2010–January 2011	March 2011	March 2011	April 2011	June 2011	September 2011	September 2011	October 2011
Approximate area (Ha)	80	30	92	Fragmented areas	630	2.2	70-m shelterbelt	37
No. of LC on site	~300	~730	>100	>40	>100	70	71	>50
Age of majority of trees (years)	20–100	50–100	30–90	10–100	50–100	70–80	~20	20–40
No. of symptomatic LC	109	247	15	~30	~30	23	23	~10
No. of LC from which *P. lateralis* isolated/total examined	10/10	5/5	1/3	3/4	3/3	2/4	1/3	6/10
Basal (B) or aerial (A) lesions observed	B + A	B	B	B + A	B	B	B	B
Introduced plants or soil within vicinity?	Soil and container grown stock	Soil and container grown stock	Adjacent to garden centre	Young LC hedge, recent soil movement	Container grown stock	Unknown	Adjacent to garden centre	Unknown

[1]Correspond closely to dates of first report of symptoms.

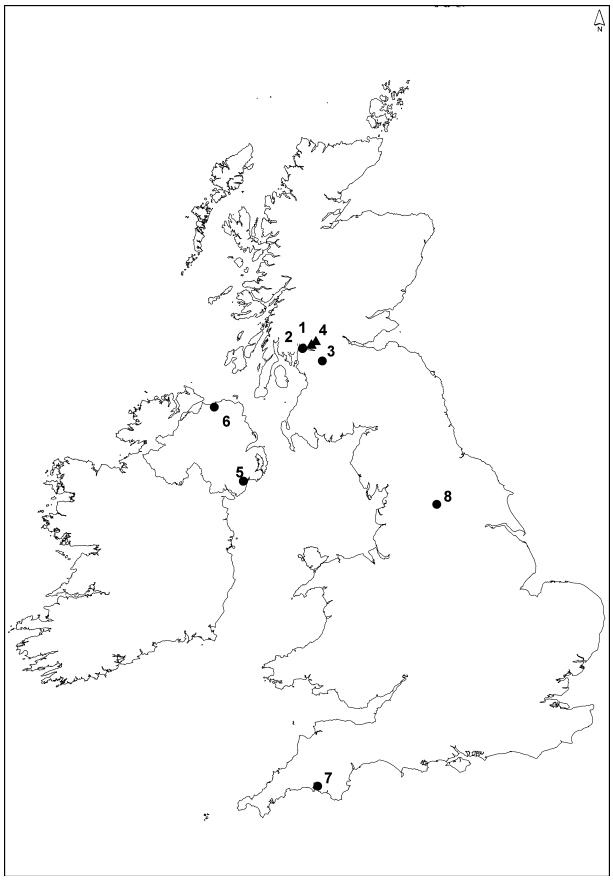

Fig. 1. Locations of *Phytophthora lateralis* outbreaks in the UK. 1. Balloch; 2. Greenock; 3. Rouken Glen; 4. Kilmaronock; 5. Tollymore Forest Park; 6. Coleraine; 7. Plympton; 8. Blubberhouses. ● Indicates sites infected with the Oregon genotype of *P. lateralis*, and ▲ indicates sites infected with the rare genotype of *P. lateralis*.

3.1.1 Balloch Castle Country Park

The trees at Balloch Castle Country Park, the first site investigated, were examined in considerable detail. The site contained mixed ornamental woodland including approximately 300 open-grown *C. lawsoniana* aged between 20 and 100 years. A survey in November 2010 revealed 82 recently dead or dying *C. lawsoniana* throughout the stands, including scattered individuals as well as groups of trees (I. Murgatroyd, personal communication). The *C. lawsoniana* exhibited a range of symptoms including subtle crown thinning and discolouration indicating early stages of infection, occasional or frequent dieback of individual branches within the crown indicating aerial infections (Fig. 2a) sometimes accompanied by resin bleeding on the main stem or side branches, and sudden overall reddening or bronzing and desiccation of the foliage suggesting girdling of the lower stem (Fig. 2b). Some branches with dieback exhibited bark cracking. Resin bleeding was only found in association with aerial lesions on the stems or branches. There was no evidence of direct infection of foliage similar to that observed in Oregon and Taiwan (Tirone and Roth 1957; Webber et al. 2011).

Three trees exhibiting dieback of individual branches (BCH14, BCH 20 and BCH21; Table 1) were felled, and another (BCH01; Table 1) had a large branch removed to allow a destructive examination of their root flares, main stems and branches as follows:

BCH01 was 20 m tall and 70 cm in diameter. Two side branches of approximately 30 cm diameter near the bottom of the crown exhibited foliar senescence and resinosis. One branch was found to have an orange-to-cinnamon-brown girdling lesion 53 cm long with scattered resin pockets within it and up to 23 cm above it (Fig. 3a). The bottom edge of the lesion was 2.6 m from the base of the tree. The other branch had a similar lesion 1.3 m long that occupied approximately 75% of the 30 cm circumference. The bottom edge of this lesion was 2 m from the base of the tree. For both lesions, the phloem between the bottom of the lesion and the collar and root flare area was healthy, showing that neither lesion had a connection to the base of the tree, which did not exhibit phloem necrosis. The two lesions therefore appeared to be a result of independent 'aerial infection' events. *Phytophthora lateralis* was subsequently isolated from multiple points on both lesions (Table 1).

BCH14 was 23 m tall and 40 cm in diameter and exhibited scattered branch dieback of approximately 50% of the crown (Fig. 2a). A 75-cm-long lesion apparently coming from below ground level was found on one large root flare (Fig. 3b). A 10-cm-long orange-to-cinnamon-brown lesion, also apparently coming from below ground level, was found on another root flare. The remaining two root flares were healthy above ground level. BCH14 also had a very resinous phloem lesion 2.2 m long on the main stem extending from 10.7 m above ground level and associated with a fork. At least 50% of the stem circumference was affected. Again, there was no connection to the tree base. *Phytophthora lateralis* was isolated from multiple points within the lesions on the root flares (Table 1), but was not confirmed by isolation from the aerial lesion although serological tests (Pocket Diagnostic®; Forsite Diagnostics Ltd, York, UK) gave positive results for *Phytophthora* along its entire length. The tree therefore appeared to have independent root or collar and aerial stem infections.

BCH20 was 22 m tall and 40 cm in diameter with scattered branch dieback of approximately 50% of the crown. The root flares were healthy. The main stem divided into four stems 7 m above ground level. One had a girdling lesion extending from 11 to 13 m above ground level with heavy associated resinosis and no connection to the tree base. Above 13 m, the phloem was moribund and the foliage dead and dying. The other three stems also exhibited resinosis and dieback, but these were not investigated. *Phytophthora lateralis* was isolated from the lesion (Table 1).

BCH21 was 25 m tall, dividing into four stems at 6 m. The largest stem and a second smaller stem had resinosis from 10 to 12 m above ground level and associated dieback of side branches. Only the smaller stem was investigated. This had a

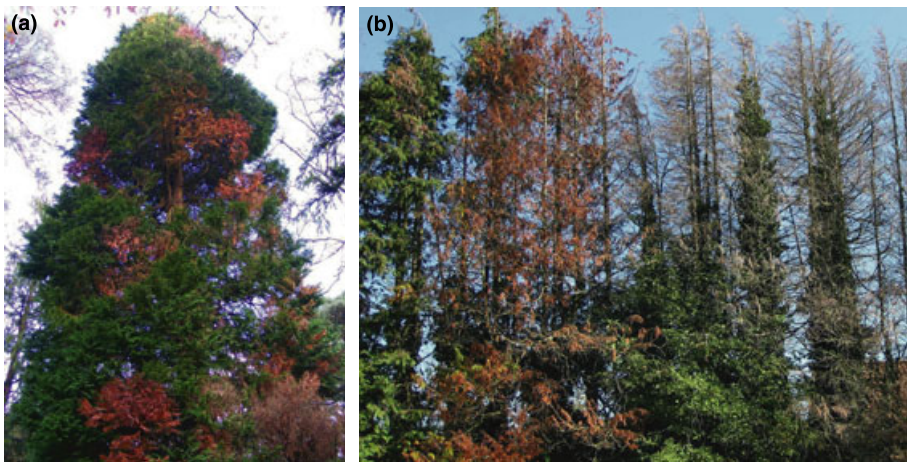

Fig. 2. (a) Dieback of individual branches of *Chamaecyparis lawsoniana* associated with aerial stem lesions caused by *Phytophthora lateralis.* (b) Crown decline along a row of *C. lawsoniana* owing to collar infection by *P. lateralis.*

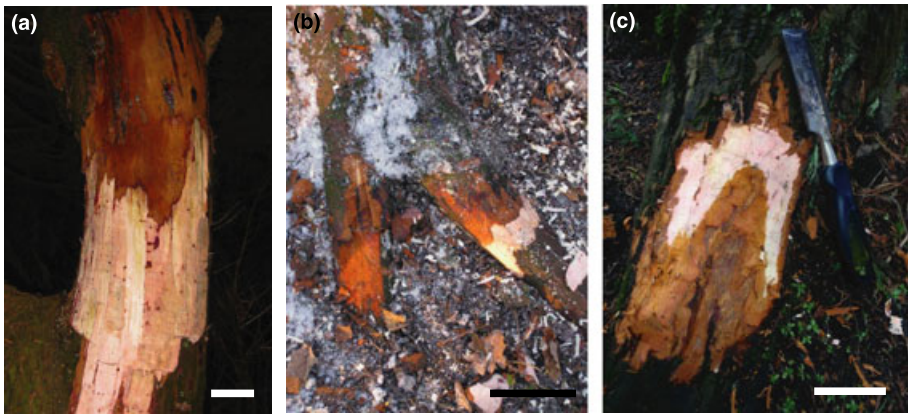

Fig. 3. Phloem lesions on *Chamaecyparis lawsoniana* caused by *Phytophthora lateralis.* Scale bars = approximately 10 cm. (a) Girdling branch lesion with resin pockets. (b) Root flare lesions, with lesion on left-hand side extending below ground. (c) Collar lesion showing extending 'tongue' of infection.

stem lesion extending from 10 to 11 m above ground level with no connection to the bottom of the tree. *Phytophthora lateralis* was isolated from the lesion (Table 1).

A further six trees at Balloch with crown symptoms were subject to a more limited investigation (BCH02, BCH18, BCH24, BCH28, BCH30 and BCH32; Table 1). All had phloem lesions on the root flares or collar, which extended below soil level and from which *P. lateralis* was isolated (Table 1). Some basal lesions extended as far as 1 m up the stem. On an additional two symptomatic trees, the presence of *P. lateralis* was confirmed by PCR conducted on DNA extracted from necrotic phloem, but the pathogen could not be isolated. On several other symptomatic *C. lawsoniana* at Balloch, either no active *Phytophthora* lesions were found at ground level or the lesions may have been overgrown by *Armillaria*, which was found infecting the upper root phloem of three trees.

Overall, between January and May 2011, 103 symptomatic *C. lawsoniana* were recorded at Balloch Castle Country Park, 28 of which displayed dieback of individual branches consistent with aerial infection and the remainder exhibiting sudden decline of the entire crown, indicating that the lower stems had been girdled. During August 2011, a further six trees developed symptoms, of which two died rapidly (I. Bain, personal communication).

3.1.2 Other sites in Scotland, Northern Ireland and England

Scotland: During 2011, declines of *C. lawsoniana* were investigated at three sites close to Glasgow: Greenock, Rouken Glen and Kilmaronock (Fig. 1; Tables 1 and 2). At Greenock and Rouken Glen, all declining trees exhibited pale or bronzed foliage across the entire crown, symptomatic of root or collar infections. *Phytophthora lateralis* was isolated from basal lesions on five trees at Greenock (GC01-05) and from one tree at Rouken Glen (RG06) (Tables 1 and 2). BLAST analyses of DNA sequences obtained directly from basal lesions on two other sampled *C. lawsoniana* at Rouken Glen gave 99% identity to *Phytophthora cambivora*. At Kilmaronock, all trees showed overall foliage bronzing, symptomatic of root/collar infections except for a single mature tree exhibiting scattered branch dieback indicative of aerial infection. Approximately 0.5–1 m up the main stem of this tree were patches of resinosis and bark cracking associated with small, discrete lesions in the phloem surrounding resin pockets. Although an isolate was not obtained, BLAST analysis of a DNA sequence from this lesion gave 99% identity with *P. lateralis*. A further three trees at the site were sampled, and *P. lateralis* was isolated from basal lesions (Ki3, Ki5, Ki7) (Tables 1 and 2).

Northern Ireland: At Tollymore Forest Park in County Down, Northern Ireland (Fig. 1), decline of *C. lawsoniana* was reported in the spring of 2011. An on-site investigation revealed overall foliage bronzing and desiccation, affecting numerous *C. lawsoniana* scattered among six plantation areas, five of which were classed as conifer high forest. A further twelve similarly symptomatic trees were located in an adjacent formal parkland setting. Seven of these trees were considered over 100 years old and were multistemmed, with an average of six stems per tree (I. Irwin, personal communication). *Phytophthora lateralis* was isolated from basal lesions on three trees at this site, including one mature parkland specimen (TFP1) and two plantation trees (TFP2 and TFP3) (Tables 1 and 2).

At Somerset Forest Park, Coleraine (Fig. 1), similar dieback of *C. lawsoniana* was reported in summer 2011. The site is a forest and meadow recreation area with a large number of *C. lawsoniana* planted as a component of mixed conifer–broadleaf woodland or along paths and streams as an ornamental. In October 2011, recently dead *C. lawsoniana* and trees with crown symptoms ranging from overall or partial pale to bronze foliage were present at multiple locations. *Phytophthora lateralis* was isolated from basal lesions on three trees (SFT01-SFT03) (Tables 1 and 2). When six symptomless trees in the same area were investigated subsequently, three were also found to have basal lesions and *P. lateralis* was again isolated from all three trees (L. Quinn, personal communication).

England: In September 2011, dieback of *C. lawsoniana* was reported at two locations in England: Plympton in Devon and Blubberhouses in Yorkshire (Fig. 1; Tables 1 and 2). The Plympton site involved a closely planted shelterbelt of 10- to 12-

m-tall *C. lawsoniana* lying alongside and within 3–4 m of a garden centre. Thirteen trees, all in the centre of a 'run' of symptomatic trees, were dead and defoliated (i.e. Fig. 2b). Another nine trees at the margins had either pale foliage or partially or entirely bronzed foliage. A single symptomatic outlier with bronze foliage lay 8 m from the main 'run'. *Phytophthora lateralis* was isolated from basal lesions on three marginal trees (PLT01-PLT03) (Tables 1 and 2). The distribution of symptomatic trees at this site was consistent with spread from tree to tree via soil or roots.

The Blubberhouses site comprised part parkland and part woodland adjacent to a village cricket pitch with many large mature *C. lawsoniana*. Crown symptoms ranged from full or partial pale to red brown foliage. *Phytophthora lateralis* was isolated from basal lesions on two symptomatic trees (BLU03 and BLU04) (Tables 1 and 2).

3.2 Isolations

Twenty-seven symptomatic *C. lawsoniana* trees at the above sites yielded isolates of *P. lateralis* from phloem lesions, as summarized in Table 1. Symptomatic foliage of two young nursery plants in central Scotland also yielded *P. lateralis:* one a *C. lawsoniana* imported 7 months earlier from The Netherlands and the other a *T. occidentalis* recently imported from France (Table 1).

At the Balloch site, baiting of soil samples from around affected trees was also carried out. Based on culture morphology and sequencing of the ITS region, isolates identified as *P. lateralis*, *P. cinnamomi*, *P. gonapodyides*, *P. hibernalis* and *P. syringae* were obtained from the soil. Also at Balloch, *Phytophthora ramorum* was isolated from an aerial stem lesion on a large 25-m-tall *C. lawsoniana* and from several shoot lesions on *Rhododendron ponticum*.

3.3 Identification of *Phytophthora lateralis* isolates by phenotype and DNA sequence

All the isolates of *P. lateralis* (Table 1) conformed morphologically to previous descriptions of *P. lateralis* (Tucker and Milbrath 1942; Brasier et al. 2010). They were sexually sterile but produced large, thin-walled, laterally attached chlamydospores on solid medium and non-papillate sporangia with short preformed pedicels in liquid culture. Some isolates were further identified as *P. lateralis* based on BLAST analysis of their partial ITS1-5.8S-ITS2 sequences.

A previous study showed that Taiwan *P. lateralis* isolates differed from North American and early French isolates by two bases and two deletions at the ITS locus and by seven bases at *cox*II (Brasier et al. 2010). ITS sequences of isolates from the more recent French outbreak in Brittany were also found to be identical to those of North American and earlier French isolates but to differ by at least two bases from those of Taiwanese isolates (Robin et al. 2011). Partial ITS1-5.8S-ITS2 and *cox*II sequences were obtained for all the isolates of *P. lateralis* from the field in Scotland, England and Northern Ireland and from the nursery *T. occidentalis* and *C. lawsoniana* (Table 1) and compared with those of isolates of *P. lateralis* originating from Taiwan (GQ381313–22) and Oregon (AF266804; AY369360; Brasier et al. 2010) and with a sequence (AF287256) for an isolate collected in France in the late 1990s (Winton and Hansen 2001).

Isolates from the same geographical location shared 100% sequence similarity across both ITS and *cox*II loci. For ITS, those from the Greenock, Rouken Glen, Tollymore, Somerset Forest, Plympton and Blubberhouses sites and from the nursery plants exhibited the same profile as the Oregon isolates. However, the isolates from the Balloch and Kilmaronock sites exhibited the same profile as the Taiwan isolates, with a C rather than a T at positions 312 and 417 on the alignment. With *cox*II, the isolates from Taiwan differed from all other isolates at seven bases as reported previously. The isolates from Greenock, Rouken Glen, Tollymore, Somerset Forest, Plympton and Blubberhouses and the nursery plants again exhibited an identical profile to the Oregon isolates. However, the isolates from Balloch and Kilmaronock differed from all other isolates by a single base: an A rather than a T at position 487.

3.4 Pathogenicity testing

The pathogenic potentials of two Scottish isolates P2202 (Balloch) and P2426 (Rouken Glen) were compared with those of isolates from Taiwan (P2291) and Oregon (P2397) using cut stems of *C. lawsoniana*. There was no lesion development associated with the control treatments, but all *P. lateralis* isolates produced lesions with mean lengths ranging from 2.8 to 5.2 cm (Fig. 4). Isolate and host genotype effects were analysed by a two-factor analysis of variance (ANOVA), which revealed significant differences in lesion lengths among the four isolates ($p < 0.001$) and between the two host genotypes ($p < 0.05$). The mean length of lesions produced by the Taiwanese isolate (P2291) was significantly smaller than the mean lengths of lesions caused by the other three isolates (Fisher's protected least significant difference test). Isolate × host genotype interactions were not significant at $p < 0.05$. *Phytophthora lateralis* could also be isolated readily from all the phloem lesions, regardless of isolate, thereby satisfying Koch's postulates for the UK isolates. Moreover, not only was the phloem on the lower portion of the shoots colonized, but the lesions extended into fronds of foliage causing blackening and necrosis. Again, *P. lateralis* could be readily isolated from this necrotic foliage.

4 Discussion

Chamaecyparis lawsoniana is an important component of the urban landscape, parks and amenity woodlands in the British Isles. This study reports the first outbreaks of the destructive introduced pathogen *P. lateralis* on *C. lawsoniana* in the UK. *Phytophthora lateralis* has already been found at eight widely separated locations, seven of them located in cool, wet western regions of the UK, potentially more climatically suitable for the pathogen. The dieback and mortality observed at the

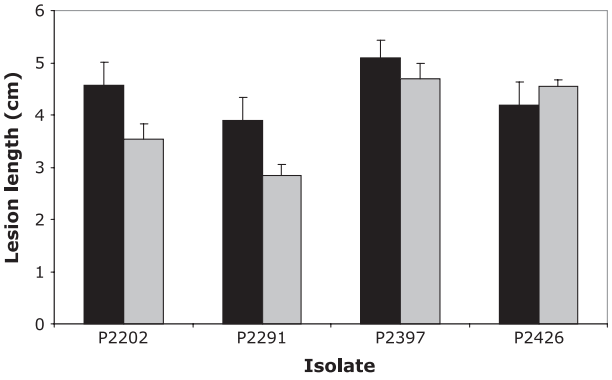

Fig. 4. Mean lesion sizes produced in the phloem of *Chamaecyparis lawsoniana* shoots inoculated with zoospores of *Phytophthora lateralis*. The two *C. lawsoniana* genotypes are indicated by the differently shaded blocks, and error bars indicate ± standard error of the mean.

sites are heavy, and more outbreak sites are being reported. It is therefore clear that *P. lateralis* presents a significant threat to *C. lawsoniana* in Britain.

Although long recognized as a major threat (Hansen et al. 2000), together with many other such organisms that pose a risk to plant health, *P. lateralis* was regrettably not accorded official quarantine status in Europe (Brasier 2008). Currently, however, *P. lateralis* is being treated as a regulated organism by UK Plant Health services, and the outbreaks are being dealt with in a similar way to those being applied to the recently introduced *P. ramorum*: infected trees and diseased plants in nurseries are destroyed on site, and movement of infested soil is being restricted. All the UK outbreak sites appeared to have been infected within the previous 2–6 years as judged by the current progressive decline of most symptomatic trees and the absence of many long-dead individuals at each site. Although the Balloch and Kilmaronock sites had areas of impeded drainage and waterlogging, the other sites appeared to be generally well drained, suggesting that site conditions were not the primary reason for the establishment of *P. lateralis*. There were no obvious climate or site factors to explain why aerial infections were observed only at the Balloch and Kilmaronock sites, although a detailed analysis of site factors was not undertaken.

As with other introduced *Phytophthora* pathogens of trees, importation of infested nursery plants is the most likely mode of arrival of *P. lateralis* in the UK, and movement of both infested plants and soil is a likely mode of local spread (Brasier and Jung 2006; Brasier 2008; Moralejo et al. 2009). Indeed, two UK outbreak sites were adjacent to garden centres, and a further three sites had been subject to recent landscaping and replanting schemes involving imported ornamentals. Outbreaks of *P. lateralis* have occurred in Europe since the 1990s, and in recent decades, enormous volumes of ornamental nursery stock have been brought into the UK from Europe. The most likely source of the UK outbreaks is therefore importation of infested plants from neighbouring European countries, as already demonstrated by the finding of *P. lateralis* on imported *T. occidentalis* (Schlenzig et al. 2011). However, all conifer plant imports from North America to Europe are either banned or strictly controlled, raising the further question of how *P. lateralis* arrived in the earlier outbreaks in Europe.

Further evidence for the origins of the UK and wider European outbreaks may come from molecular fingerprinting. Based on ITS sequence, French isolates examined to date have been identical to the published sequences of North American isolates but distinct from those of Taiwanese isolates, indicating a possible origin via importation of nursery stock from the USA (Robin et al. 2011). A similar analysis of ITS and *cox*II sequences of UK isolates has shown that the majority, including those from six different outbreak sites and those from two recent interceptions of *P. lateralis* on nursery stock imported from France and the Netherlands, are also similar to those of French and North American isolates. However, those from two sites, Balloch and Kilmaronock, are distinct and exhibit a different phylogenetic profile, sharing an ITS sequence with that already published for Taiwanese isolates and differing from North American and French isolates by a single-base substitution in *cox*II. This indicates that they have a separate evolutionary history from the other UK isolates. Moreover, the Balloch and Kilmaronock sites are only 10 km apart, suggesting local spread of this rare genotype. A detailed phenotypic and molecular comparison of a large collection of Taiwanese isolates with isolates from North America and Europe, including those from the UK, is now in progress and may shed further light on the Balloch–Kilmaronock outbreak source.

At Balloch, the regulated quarantine pathogen *P. ramorum* was also isolated both from infected rhododendron foliage and from an aerial stem lesion on *C. lawsoniana*, the latter being only the second recorded finding of *P. ramorum* on this host (Brasier and Webber 2012). While *P. cinnamomi* is a warm-temperature pathogen not considered to be widespread in Scotland, it was found to be infecting many recently planted European yews (*Taxus baccata*) at Balloch (J. F. Webber and S. Sancisi-Frey, unpublished data). Many mature yews on the site were also dying although *P. cinnamomi* was not confirmed as the cause of these symptoms. In addition, the exotic *P. hibernalis*, a pathogen of citrus, was found in soil sampled from the site (J. F. Webber and S. Sancisi-Frey, unpublished data). The Balloch site has recently undergone a £1.8-million landscape regeneration programme. This included bringing in thousands of tonnes of bulk soil and hundreds of containerized shrubs and trees from more than 30 nurseries, including 14 nurseries in continental Europe (I. Bain, personal communica-

tion). It is possible therefore that *P. lateralis*, *P. ramorum, P. cinnamomi* and *P. hibernalis* were introduced to the Balloch site on the plants and soil brought in from these other locations.

Phytophthora lateralis was considered to be mainly a root and collar pathogen, but recent observations that the species has caducous sporangia (Hansen et al. 1999; Brasier et al. 2010; Robin et al. 2011) and evidence for foliar infection in Taiwan (Webber et al. 2011) indicate that aerial infections can occur. Indeed, *P. lateralis* is highly unusual in having sporangia that are both non-papillate and caducous, a combination that may reflect adaptation to both soil and aerial habitats (Brasier et al. 2010). The recent observation of aerial lesions on stems and branches as well as collar lesions at the new French outbreak sites (Robin et al. 2011) provides further evidence for aerial infection. Additionally, in the present study, the observation at Balloch of large aerial stem and branch lesions on trees apparently unconnected to ground level suggests that aerial stem infections that are not extensions of root infections may occur directly through penetration of intact bark and that infection propagules must have been generated above ground, most likely from infected foliage.

To better understand the risk posed to woodland, plantation and ornamental *Chamaecyparis* across the UK and Northern Ireland and the effectiveness of current eradication measures, more field studies on the pathogen's behaviour are needed: in particular, studies on the potential for sporulation and dispersal from foliar infections; the conditions required for aerial infection including whether wounding is needed; the influence of site conditions such as drainage, rainfall and temperature on infection; and whether the pathogen is especially favoured by the climatic conditions prevalent in western regions of the UK.

In North America, *P. lateralis* is not confined to *C. lawsoniana* in the field but causes occasional root disease on *Taxus brevifolia* (Pacific yew) at sites with high concentrations of infected *C. lawsoniana* (Hansen et al. 2000). It also infects other *Chamaecyparis* nursery stock (Tucker and Milbrath 1942). In Taiwan, the host range of *P. lateralis* is unknown beyond its association with the indigenous *C. obtusa* and *C. formosana* (Brasier et al. 2010; Webber et al. 2011). The majority of confirmed *P. lateralis* infections in the UK, France and the Netherlands to date have been on *C. lawsoniana*. However, in view of its recent isolation from *T. occidentalis* nursery plants (Schlenzig et al. 2011), the possibility that *P. lateralis* poses a threat to a wider range of hosts in the UK, including the native yew *T. baccata* and the common ornamental × *Cupressocyparis leylandii*, requires investigation.

The spread of *P. lateralis* to the UK demonstrates yet again the threat to urban and rural ecosystems posed by the burgeoning global trade in live plants and the failure of current European biosecurity protocols (Brasier 2008).

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