Assessment of pathogen threats to alternative forestry species and provenances in the UK, with a focus on local pathogen impacts at field trials in Scotland and England.

by

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Being a thesis submitted in partial fulfilment of the requirements for the MSc Degree in Plant Pathology

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RESEARCH PROJECT ASSESSMENT FORM

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Table of Contents

List of Tables

List of Figures

Summary

Invasive pathogens present a significant biotic threat to the UK forestry industry. Overreliance on a narrow range of tree species heightens the potential economic and ecological damage caused by tree disease epidemics. In 2009, new research efforts were initiated by Forest Research to investigate potential alternative species and provenances to expand the limited range currently in use. These efforts led to the establishment of several long term species and provenance trials located throughout the UK. This project sought to identify the key pathogen impacts on the range of species and provenances under assessment. This was achieved via analysis of the Forest Research Tree Health Diagnostic and Advisory Service database, so as to determine any recurrent pathogen threats, and by health surveys conducted at two of the species/provenance trials: one located at Glentress, Scotland and a second at Westonbirt, England. The database analysis highlighted *Cedrus atlantica* as a host species of concern, with 184 reported instances of infection by *Sirococcus tsugae*. These findings aligned with the observation of numerous symptomatic trees in each *C. atlantica* plot present at Glentress, with subsequent molecular identification of the pathogen. The health surveys detected significant impacts of *Dothistroma septosporum* on the range of *Pinus* spp. present at both Glentress and Westonbirt, with the shoot pathogen *Gremmeniella abietina* also contributing to observed damage on *Pinus pinaster* and *Pinus radiata*. In contrast, *Pinus strobus*, a potential alternative forestry species, appeared relatively unaffected and therefore may be of use in species diversification efforts to mitigate the impact of Dothistroma needle blight on pine plantations. However, this potential must be taken alongside the risk posed by *Cronartium ribicola*, causal agent of white pine blister rust. Whilst not statistically significant, mean foliage dieback and shoot mortality scores suggest that selection of native *Pinus sylvestris* provenances may be prudent in terms of reduced susceptibility to *D. septosporum*. The key pathogen impacts were summarised for each species present at the field trials, along with considerations for potential future threats due to changing climatic conditions and suggestions for future research efforts.

1. Introduction

1.1 Invasive Pathogens and Forestry in a Changing Climate

Invasive pathogenic organisms present a key biotic threat to forestry industries both in the United Kingdom and globally. Through infection and subsequent debilitation or mortality of their tree hosts, forest pathogen epidemics have the potential to inflict significant ecological and economic damage. Recent examples include the proliferation of tree diseases such as sudden larch death, caused by the oomycete pathogen *Phytophthora ramorum* and responsible for widespread devastation of larch (*Larix* spp.) (Webber, 2017), and Dothistroma needle blight, caused by the ascomycete *Dothistroma septosporum* and primarily affecting pines (*Pinus* spp.) (Mullett *et al.*, 2017). Both of these examples provide instances where forest pathogens have caused extensive damage to tree species utilised in commercial plantations, managed for the production of timber and numerous other wood products. In ecological terms, pathogens such as *Hymenoscyphus fraxineus*, causal agent of ash dieback, and *Ophiostoma novo-ulmi*, vectored by the elm bark beetle and cause of Dutch elm disease, illustrate how invasive pathogens can drastically alter host species population numbers, with attendant ramifications for supported biodiversity and woodland composition (Potter *et al.*, 2011; Broome and Mitchell, 2017). It is anticipated that both the frequency and intensity of forest pathogen epidemics will increase in the coming years, with a marked upward trend in the number of novel pathogen identifications since 2000 (Sturrock, 2012; Freer-Smith and Webber, 2017).

This increase and intensification can be attributed to two key factors: cross-border movement of infectious material via the international plant trade and changes in global mean temperatures as a result of rising atmospheric carbon levels (La Porta, 2008; Stenlid and Oliva, 2016). When confined to their native ranges, forest pathogens evolve alongside associated communities of trees and act as non-damaging constituents of ecosystems (Hansen, 2015). When introduced into new geographic areas, the situation is imbalanced from an evolutionary perspective and native tree species lack any coevolved defensive capacity. Highly damaging epidemics can then occur on species closely related to the pathogen's native host (Budde *et al.*, 2016). Continued expansion of the international plant trade, alongside globalisation more generally, has facilitated this displacement process, with live plants and timber acting as vectors for the entry of exotic pathogens into regions far removed from their native ranges (Liebhold *et al.*, 2012). The continued risk posed by this situation must be taken alongside indications that rising global temperatures will likely exacerbate the impact of forest pathogens (La Porta, 2008). This is due in part to increased abiotic stress placed on host species: extreme weather events which result in prolonged periods of drought or flooding can weaken forest trees and make them more susceptible to pathogen entry (Wainhouse *et al.*, 2016). In addition, warming temperatures may expand the distribution range of certain forest pathogens into previously unaffected areas and allow for successful overwintering of inoculum (Ghelardini *et al.*, 2016). The combined effects of increased warmth and humidity in particular can favour the sporulation and dispersal capacity of many pathogenic species (Seidl *et al.*, 2017).

1.2 Genetic Diversity as a Basis for Resilience

With globalised trade and climate change serving to intensify the continued threat posed by forest pathogens, the importance of adopting management practices aimed at reducing the potential damage inflicted by tree disease epidemics is clear. One such practice entails the implementation of species diversification, a key element of the UK Government's Tree Health Resilience Strategy (DEFRA, 2018). The reasoning for this lies in the fact that the UK forestry industry currently employs a limited range of primary tree species. As of March 2020, total woodland cover in the UK was estimated at 3.2 million hectares, roughly divided 50/50 between conifers and broadleaves (Forestry Commission, 2020). Of the area accounted for by conifers, 51% is comprised of a single species, Sitka spruce (*Picea sitchensis*), with Scots pine and larches covering 17% and 10% respectively. Overall, around 96% of coniferous woodland cover is comprised of just nine species (Reynolds *et al.*, 2021). In turn, nearly half of broadleaf woodland cover is dominated by just three species: birch, oak and ash at 18%, 16% and 12% respectively (Forestry Commission, 2020).

This lack of diversity denotes considerable risk in terms of potential future pathogen impacts. The integrity of the UK forestry industry, in both a commercial and ecological sense, could be greatly undermined if any of the primary species described above are found to be highly susceptible to a newly introduced exotic pathogen. Such a scenario has already been observed in the cases of ash, larch and pine. The overreliance on Sitka spruce appears particularly problematic in this sense. Improvement programmes have resulted in the widespread usage of clonally propagated stock (Lee and Watt, 2012); as a result, a significant proportion of Sitka spruce production stands comprise monocultures lacking any capacity for genetic adaptation to invasive pathogens. Identifying novel tree species with sufficient qualities to supplement the narrow range currently used in UK forestry will therefore be of considerable benefit in terms of enhancing resilience to future pathogen threats. This appears particularly important when considering the Committee on Climate Change's recommendation that the UK expands its woodland cover by 30 000 hectares per annum (CCC, 2020).

1.3 Species and Provenance Trials – A Valuable Research Opportunity

Species and provenance trials can play a key role in the identification of potential alternative forestry species. They allow for comparisons of performance to be made across a range of tree species and provenances grown together over long periods of time. Provenance refers to the geographic region from which seed was originally collected, with each provenance possessing a unique genotype with variable performance traits (Whittet *et al.*, 2019). As it stands, there is currently a lack of information regarding variation in levels of susceptibility to pathogen attack between species provenances (Cavers and Cottrell, 2015).

In 2009, the Forestry Commission funded the establishment of five field trials throughout the UK, designed to supplement the EU-funded REINFFORCE project (Reynolds *et al.*, 2021). This project will focus on two of these field trials – one located in Scotland and a second located in the south of England. The range of species under assessment are divided into four categories, reflecting the extent of their current usage in UK forestry: Principal, Secondary, Plot-Stage and Specimen (Jinks, 2017). Principal species are those already in widespread usage, whilst Secondary and Plot stage (collectively referred to as 'emerging' species) have demonstrated potential for wider adoption. Specimen species have rarely been tested for use

in forestry. Health surveys conducted at each site will determine the impact of existing local pathogens on the range of species and provenances being trialled, whilst also allowing for comparisons of symptomology to be made between the two geographic regions. The information obtained will provide insights into which species may be promising candidates for expansion of the narrow palette currently used in UK forestry, as well as identifying those which appear highly susceptible to existing pathogens and are therefore of questionable utility. Additional context will be provided via analysis of the Forest Research Tree Health Diagnostic and Advisory Service (THDAS) database. The database contains records detailing pathogen incidence by tree host dating back to the 1970s. Analysis will allow for identification of any recurrent pathogen threats relevant to the alternative species being trialled and will supplement findings obtained from the field surveys.

2. Literature Review

2.1 Current Pathogen Impacts on UK Forestry

To better understand the need for adoption of management strategies such as species and provenance diversification, this section will review the current impacts of two significant forest pathogens in the UK: *Phytophthora ramorum* and *Dothistroma septosporum*. These two species have been chosen due to their considerable impact over recent years, with the damage caused by each reflecting the potential economic and ecological consequences of tree disease epidemics in the UK.

In 2009, *Phytophthora ramorum* was identified as the causal agent of extensive dieback and mortality of Japanese larch (*Larix kaempferi*) at plantations in southwest England (Webber *et al.*, 2010). Symptomatic trees were found to exhibit needle discolouration, excessive resin bleed of outer bark and visible phloem lesions. Subsequent work confirmed European (*Larix decidua*) and hybrid (*Larix x eurolepis*) larch to be additional hosts, with inoculated needles producing thousands of spore-bearing structures known as sporangia (Harris and Webber, 2016). Between 2009 and 2016, approximately 20, 000 hectares of larch displayed symptoms of *P. ramorum* infection, with subsequent efforts to contain the disease resulting in the felling of millions of trees (Webber, 2017). Due to the prolific sporulation of infected larch trees, disease containment is achieved via the issuing of Statutory Plant Health Notices (SPHNs) by the Forestry Commission and other plant health authorities (UK Government, 2021). These notices require the site owner to destroy all infected host trees, with the area covered determined by a number of site characteristics (Welsh Government, 2019). The Forestry Commission's *Phytophthora ramorum in larch UK Situation Report* (2019) provides an insight into the current impact of sudden larch death: in 2018/19, 850 SPHNs were issued throughout the UK, surpassing the previous peak period of 2013/14, which saw the issuing of 550 SPHNs. As all three larches are categorised as Principal forestry species in the UK, the continued expansion of *P. ramorum* poses a significant threat.

Dothistroma septosporum, the ascomycete causal agent of Dothistroma needle blight, presents a further threat to UK forestry. Since the late 1990s, outbreaks of *D. septosporum* have caused significant damage to the three Principal pine species in the UK: Scots (*Pinus sylvestris*), lodgepole (*Pinus contorta*) and Corsican (*Pinus nigra* subsp. *laricio*) (Mullet *et al.*, 2017). Taken together, these species account for 29% of the total conifer area in the UK (Forestry Commission, 2020). Corsican pine in particular was found to be so badly affected that a moratorium on planting of the species was implemented in 2007 (Wainhouse *et al.*, 2016). *D. septosporum* infection occurs primarily during wet periods, via the action of splash-dispersed spores known as conidia (Coops *et al.*, 2003). Upon landing on a suitable host species, these spores germinate and enter the tree through needle stomata. Resultant symptoms can include the occurrence of raised black fruiting bodies within yellow/tan bands, which turn red over time, as well as premature needle cast (Forest Research, 2012). Severe cases of crown infection have the capacity to drastically reduce yield: in stands suffering from 70% mean crown infection, a 68% decrease in mean annual volume increment has been observed (Brown and Webber, 2008). Alongside this threat to commercial forestry, there are indications that Dothistroma needle blight may also pose a significant ecological risk to native Caledonian pinewood in Scotland (Forestry Commission Scotland, 2018).

2.2 Species Diversification

In light of the damage caused by pathogens such as *P. ramorum* and *D. septosporum*, continued reliance on species such as Scots pine, lodgepole pine and larches presents a significant risk to the integrity of the UK forest industry. One possible outcome of species and provenance trials may be the identification of novel species with which to implement diversification efforts. This has become an important policy recommendation with regard to enhancing the resilience of UK forests (Forestry Commission, 2017; Defra, 2018) and this section will review the available evidence in support of species diversification as a viable means of reducing potential damage caused by invasive pathogens.

The rationale behind species diversification is straightforward: expanding the portfolio of tree species being used in a managed plantation or semi-natural woodland scenario will lower the proportion of damage caused by a species-specific pathogen (Ennos *et al.*, 2019). Whilst this theory appears sound, much of the evidence related to forests specifically has been anecdotal or extrapolated from assessments of agricultural systems (Pautasso *et al.*, 2005). However, a study conducted in Northern Ireland details a situation where the use of tree species mixtures conferred enhanced resistance to pathogen damage. McCracken *et al.* (2001) compared yield losses caused by the rust pathogen *Melampsora epitea* var. *epitea* on twenty willow (*Salix* spp.) species and variety mixtures against monocultural plantings of each constituent species. In all cases, a greater dry matter yield was obtained from the mixtures, with the species diversity serving to compensate for the loss of any single constituent due to disease impact.

From a broader, ecosystem perspective, Haas *et al.* (2011) examined the effects of species diversity on the behaviour of *P. ramorum* across a range of environmental conditions in California. Two possible scenarios were hypothesised: the observation of an amplification effect, whereby greater species diversity enhances the impact of *P. ramorum* due to the pathogen's broad host range, or a dilution effect, where the observed impact is reduced due to the lower competency of alternative hosts. Through long-term monitoring of a network of 280 plots randomly located across a 79 356-hectare study area, a consistent negative association between disease impact and species diversity was detected, providing evidence to support the dilution hypothesis. These findings align with a study conducted by Hantsch *et al.* (2014) to assess the effects of local tree diversity on foliar pathogen infestation of two common forestry species in Germany – small-leaved lime (*Tilia cordata*) and sessile oak (*Quercus petraea*). High levels of local species diversity were associated with reduced rates of pathogen infestation on both tree species, providing further evidence to support the dilution hypothesis.

More recently, Macpherson *et al.* (2017) devised a bioeconomic model to test the effect of production forest species composition on economic losses suffered due to pathogen damage. Whilst a reduction in economic loss was tied to species diversification, this effect was found to be dependent on a number of pathogen characteristics, including rate of infection spread, probability of pathogen arrival and time of pathogen arrival.

2.3 Provenance Selection

In addition to species diversification efforts, prudent selection of provenance can provide a further source of resilience against pathogen damage. Provenance refers to the geographical location of the stand of trees from which seed was originally collected, with the terms 'origin' and 'provenance' often used interchangeably (Hubert and Cundall, 2006). Species

provenances can vary considerably in their response to environmental conditions and biotic stresses, including susceptibility to pathogen attack (Whittet *et al.*, 2019). This section will review recent assessments of provenance trials which demonstrate the capacity for significant intraspecific variation in susceptibility to pathogen infection.

Fraser *et al.* (2015) assessed the susceptibility of six native Scottish provenances of *Pinus sylvestris* to *Dothistroma septosporum*. Two- and three-year old saplings were inoculated with conidial suspensions and incubated under optimal conditions for disease development. For the two-year old saplings, specimens originating from Amat were found to be significantly less susceptible than those originating from Glen Cannich and Glen Loyne. For the three-year old saplings, specimens from Beinn Eighe were significantly less susceptible than those obtained from Abernathy. Similar findings were obtained by Perry *et al.* (2016), who assessed the impacts of *D. septosporum* on a provenance trial comprising eight native provenances of *P. sylvestris*. Over two years, assessments of pathogen incidence were conducted by assigning survey trees a percentage score to reflect the proportion of infected current-year needles. Highly significant differences in susceptibility were detected between provenances, alongside an association between enhanced levels of disease resistance and high water availability at the site of origin. As frequent periods of rainfall have been tied to higher incidences of *D. septosporum* infection, it was theorised that resistant provenances have been subject to high levels of disease pressure over long periods of time and in response have developed coevolved resistance. In an assessment of swiss needle cast impacts, caused by the foliar pathogen *Phaeocryptopus gaeumannii*, on provenances of Douglas-fir (*Pseudotsuga menziesii*), Wilhelmi *et al.* (2017) also concluded that seed sources obtained from regions subject to high disease pressure were found to be the most tolerant.

A further example of variable susceptibility between provenances can be found in work undertaken by Jones *et al.* (2001). A local provenance of hawthorn (*Crataegus monogyna*) was compared against four British and four continental European provenances in experimental hedges located at two trial sites. Symptoms of powdery mildew, caused by the pathogen *Podosphaera clandestina*, were assessed over a two-year period by assigning survey plants a score denoting degree of infection. At both sites, the locally sourced provenance obtained significantly lower mildew scores when compared to the British and continental European provenances. More recently, Stocks *et al.* (2017) conducted assessments of ash (*Fraxinus excelsior*) provenance trials planted in the southeast of England in 2013. Survey trees were scored according to the level of damage inflicted by *Hymenoscyphus fraxineus*, causal agent of ash dieback. Significant differences were detected between source provenances, with trees originating from Scotland exhibiting greater levels of tolerance when compared to provenances obtained from Wales and Southeast England.

2.4 Potential Consequences of Species Expansion

Any consideration of species and provenance expansion must also address the potential risks of incorporating exotic species into UK forestry. Ennos *et al.* (2019) outline the key arguments against the introduction of non-native species. The first concerns the potential risk of endemic pathogens transferring onto newly introduced exotic tree species which lack any coevolved defensive capacity. An example of this can be found when considering the impact of *Gremmeniella abietina*, causal agent of Scleroderris canker of pines (known as Brunchorstia dieback in the UK and Europe), on lodgepole pine in Sweden. Karlman *et al.* (1994) explain that whilst *G. abietina* causes negligible damage on naturalised Scots pine, exotic plantations

of lodgepole pine have succumbed to widespread and severe decline. The likelihood of such an occurrence is increased when the introduced exotic species is closely related to a native species (Gilbert and Webb, 2007).

The second argument raised against the incorporation of non-native forestry species is the potential introduction of associated exotic pathogen species or strains into new geographical regions. A recent testament to this can be found when considering the introduction of two exotic strains of *D. septosporum* into the UK. Piotrowska *et al.* (2018) used microsatellite markers to identify three distinct strains of *D. septosporum* in Scotland: one of moderate genetic diversity and assumed to be endemic on native Caledonian Scots pine, a second exhibiting high levels of genetic variation and linked to the Dothistroma needle blight epidemic on Corsican pine and a third characterised by low genetic diversity and associated with introduced lodgepole pine. The implications of this are that the introductions of Corsican and lodgepole pine have resulted in the displacement of exotic strains of *D. septosporum* from continental Europe and North America respectively. Now present in Scotland, and with the potential for hybridisation, these strains present a distinct threat to Caledonian Scots pine.

Ennos *et al.* (2019) note that the risks described above are heightened in the absence of thorough species and provenance testing. This provides an opportunity for the current project. Health surveys conducted at each field trial may help to determine if any of the alternative species under assessment are subject to particularly severe damage from endemic pathogens. If this is the case, the viability of these species for incorporation into species diversification strategies may be called into question. Additionally, surveys of species and provenance trials can act as early warning systems for the inadvertent introduction of exotic pathogens associated with non-native tree species or provenances. However, even with these checks in place, incorporation of exotic species should only be undertaken after full consideration of the risks discussed above.

3. Aims and Objectives

3.1 Project Aims

1. Identify the key pathogen threats to a range of alternative forestry species and provenances in the UK.

2. Assess the potential for variable susceptibility to existing local pathogens between a range of alternative species and provenances being trialled by Forest Research.

3.2 Project Objectives

1. Analyse the Forest Research Tree Health and Diagnostic Advisory Service database records to identify any recurrent pathogen threats to the species under assessment.

2. Conduct health surveys at two Forest Research trial sites – one in Scotland and one in England – to determine the impacts of existing local pathogens by species and provenance.

3. Analyse data obtained from the health surveys to identify any significant differences in pathogen susceptibility between species/provenances.

4. Confirm identity of causal agents obtained from sample material collected at field trials via morphological and molecular analyses.

4. Materials and Methods

4.1 Analysis of THDAS Database

A master list of all tree species currently being trialled by Forest Research was obtained. Species in the Principal category, with the exception of *Acer pseudoplatanus*, were then omitted on the basis that extensive published evidence regarding pathogen threats was already available. The remaining species were in the Secondary or Plot Stage categories, with a total of 46 species chosen for analysis. The list, provided in Table 1, was then sent to the Head of Tree Health Diagnostic and Advisory Service (THDAS) at Forest Research, who obtained and returned the relevant records. Host species were ranked by total number of records and the most frequently reported causal agents were identified in each case. The number of reports per enquiry year was also assessed to determine any apparent trends in pathogen incidence. All graphs were produced in Microsoft® Excel for Mac (v. 16.51).

Table 1. Full list of host species selected for THDAS database analysis.

Host Species	**Category**
Acer pseudoplatanus	Principal
Abies grandis	Secondary
Abies procera	Secondary
Acer platanoides	Secondary
Alnus incana	Secondary
Carpinus betulus	Secondary
Eucalyptus gunnii	Secondary
Juglans nigra	Secondary
Juglans regia	Secondary
Nothofagus alpina	Secondary
Picea omorika	Secondary
Pinus radiata	Secondary
Populus balsamifera	Secondary
Quercus rubra	Secondary
Thuja plicata	Secondary
Tilia cordata	Secondary
Tsuga heterophylla	Secondary
Abies alba	Plot Stage
Abies amabilis	Plot Stage
Abies nordmanniana	Plot Stage
Alnus cordata	Plot Stage
Alnus rubra	Plot Stage
Betula lenta	Plot Stage
Calocedrus decurrens	Plot Stage
Cedrus atlantica	Plot Stage
Cedrus libani	Plot Stage

Cryptomeria japonica 'Elegans'	Plot Stage
Cryptomeria japonica	Plot Stage
Eucalyptus glaucescens	Plot Stage
Eucalyptus globulus	Plot Stage
Eucalyptus gundal	Plot Stage
Eucalyptus nitens	Plot Stage
Fagus orientalis	Plot Stage
Liriodendron tulipifera	Plot Stage
Nothofagus pumilio	Plot Stage
Picea orientalis	Plot Stage
Pinus peuce	Plot Stage
Pinus pinaster	Plot Stage
Pinus ponderosa	Plot Stage
Pinus strobus	Plot Stage
Quercus ilex	Plot Stage
Robinia pseudoacacia	Plot Stage
Sequoia sempervirens	Plot Stage
Sequoiadendron giganteum	Plot Stage
Sorbus torminalis	Plot Stage
Tilia platyphyllos	Plot Stage

4.2 Health Surveys of Species Trials

Health surveys were conducted at two Forest Research trial sites – Glentress, located near Peebles in the Scottish Borders, and Westonbirt, located near Tetbury in Gloucestershire, England. Both sites contained one to three provenances of 14 species. The selection of species differed between the two sites in certain cases – this is detailed in Table 2, along with species category and provenance information. The trials were arranged in a randomised block design comprising three replicated blocks. Each plot contained 49 trees (7 x 7 rows) planted at 2 x 2 metre spacing. Thirteen trees were assessed per plot (two per row except middle row where only central tree was assessed) by moving diagonally from the lower SW corner to the upper NE corner. In situations where accessibility did not permit movement through the plot, an alternative survey method scoring outer trees was adopted. For each surveyed tree, a percentage score was given for the following health variables: foliage discolouration, foliage dieback, defoliation, shoot mortality, branch mortality and main stem mortality. An additional column on the assessment sheet provided space for notes on any other observable symptoms, including the presence of obvious fruiting structures. Photographs were taken in cases of suspected infection, so as to illustrate the range of symptoms observed. Shoot and needle samples were also collected for morphological and molecular identification of causal agents.

Table 2. Full list of species and provenances present at Glentress (GT) and Westonbirt (WB).

Species	Provenance Code	Category	Site	Provenance
Betula pendula	BEPE-NORD	Principal	WB	France-Nord
Betula pendula	BEPE-UNIT	Principal	GT + WB	Wales, UK
Larix decidua	LADE-THEI	Principal	GT + WB	Theil, France
Larix marschlinsii	LAEU-LAVE	Principal	GT	Lavercantière, France
Picea sitchensis	PISI-QSS	Principal	GT + WB	QSS.96(711) C4 Lot5
Picea sitchensis	PISI-USS	Principal	GT + WB	USS.09(OR18TE) Orchard 18
Pinus sylvestris	PISY-POLA	Principal	GT + WB	Eastern Poland race, France
Pinus sylvestris	PISY-SCOT	Principal	GT + WB	Scottish native population
Pinus sylvestris	PISY-VALS	Principal	GT + WB	Valsain, Spain
Pseudotsuga menziesii	PSME-ORCO	Principal	GT + WB	Oregon Coast, USA
Pseudotsuga menziesii	PSME-ORSI	Principal	GT + WB	Oregon Siskyou, USA
Pseudotsuga menziesii	PSME-WASH	Principal	GT + WB	Washington cascade, USA
Quercus robur	QURO-FRAN	Principal	WB	France
Quercus robur	QURO-UNIT	Principal	WB	New Forest, UK
Pinus radiata	PIRA-DOTH	Secondary	GT + WB	Doth resist, Amberly, UK
Pinus radiata	PIRA-WO	Secondary	GT + WB	09(450) F WO 828
Quercus rubra	QURU-FEST	Secondary	GT	France
Thuja plicata	THPL-BRIT	Secondary	WB	British Columbia, Canada
Thuja plicata	THPL-DARR	Secondary	GT	Darrington, Washington, USA
Thuja plicata	THPL-MONT	Secondary	WB	Montana, USA
Thuja plicata	THPL-OLYM	Secondary	WB	Washington State, USA
Abies amabilis	ABAM-P1	Plot Stage	WB	Oregon, USA
Abies nordmanniana	ABNO-AMBO	Plot Stage	GT	Ambrolauri/Nikordsminda
Abies nordmanniana	ABNO-APSH	Plot Stage	GT	Apsheronsk/Mezmai/Russia
Abies nordmanniana	ABNO-ARTV	Plot Stage	GT	Artvin-Savart-Yayla-Turkey
Abies nordmanniana	ABNO-BORJ	Plot Stage	GT	Borjomi/Tadzrici
Abies nordmanniana	ABNO-TLUG	Plot Stage	GT	Ambolauri/Tlugi
Cedrus atlantica	CEAT-VISF	Plot Stage	GT	VIS, France
Cryptomeria japonica	CRJA-P1	Plot Stage	WB	Kochi, Japan
Cryptomeria japonica	CRJA-P2	Plot Stage	WB	Ibaraki, Japan
Cryptomeria japonica	CRJA-P3	Plot Stage	WB	Iwate, Japan
Picea orientalis	PIOR-P2	Plot Stage	GT + WB	P2
Picea orientalis	PIOR TURK	Plot Stage	GT + WB	Turkey
Pinus pinaster	PIPT-CORD	Plot Stage	GT	Monfero, Spain
Pinus pinaster	PIPT-LACO	Plot Stage	GT	Landes and Corse
Pinus pinaster	PIPT-LAND	Plot Stage	GT	Landes, France
Pinus strobus	PIST-643	Plot Stage	GT	06(430) F 50708 (FORESTART)
Pinus strobus	PIST-CZRI	Plot Stage	GT	Czech Republic
Sequoia sempervirens	SESE-NOCA	Plot Stage	WB	Northern Coast, California, USA
Acer macrophyllum	ACMA-232	Specimen	WB	232-05

Quercus pyrenaica	QUPY-P1	Specimen	WB	Pirineo Navarro, Spain

4.3 Statistical Analysis of Health Scoring

All statistical analyses were performed in R v. 4.0.3 (R Core Team, 2020). Data were analysed using linear mixed models, so as to account for the random effect of repeated measurements within plots. All outcome variables were arcsine transformed to meet the assumptions of linear regression. Analysis of variance was used to assess the statistical significance of fixed effects: site and species-provenance. Adjusted marginal means and pairwise comparisons, with a Tukey correction for multiple comparisons, were obtained using the emmeans package (Lenth, 2020).

4.4 Morphological Identification of Causal Agents

Morphological identification of causal agents was performed via microscopic examination and photographing of fruiting bodies present on collected needle samples at x100 and x400 magnifications. Observed characteristics for both fruiting structures and spore types were then compared to accounts presented in published literature. In cases where no fruiting structures were initially evident, needles were placed on dampened paper towels and sealed in plastic bags for incubation at room temperature over seven days, with periodic checks for the presence of fruiting bodies.

4.5 Isolations from Shoot Samples

Isolations were performed on all collected shoot samples. Using sterile technique, outer bark was removed from shoot sections displaying visible lesions. Tissue shavings from across the live/dead junction were then plated onto Malt Extract Agar (MEA) or, in cases of suspected *Phytophthora* infection, Synthetic Mucor Agar (SMA). Plates were then incubated in the dark at room temperature. Subcultures were produced to obtain pure cultures of the resultant colony types on MEA, and these were incubated at 17°C.

4.6 Molecular Identification of Causal Agents

Molecular analysis was performed on a range of shoot, needle and fungal samples, with pathogen identification achieved via sequence analysis of the ITS (internal transcribed spacer) gene region. Lesion material from all collected shoot samples was used for analysis, whilst only needle samples which did not present any characteristic fruiting structures after incubation were chosen. All fungal subcultures were first inspected by host species, with representative colony types chosen for molecular analysis based on frequency of isolation. All shoot and needle samples were lyophilised overnight using an Alpha 1-2 LDplus freeze drier (Martin Christ, Osterode). Homogenisation was then performed by adding three steel beads to each sample tube and grinding in a mixer mill (MM 400, Retsch, Haan) for 60 seconds. DNA extractions and PCR inhibitor removal was performed using the NucleoSpin® Plant II kit (Macherey-Nagel, Düren) and the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, California), as per the manufacturer's instructions. All DNA samples were amplified using the ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers

(White *et al.*, 1990). PCR mixtures (50µl) contained: 10µl of 5x Colourless GoTaq® Flexi Buffer (Promega, Madison), 29.75µl of H_2O, 2µl of each primer, 3µl of $MgCl_2$, 1µl of dNTPs, 0.25µl of *Taq* polymerase and 2µl of DNA sample. Negative controls contained 2µl of H_2O in place of the DNA sample and positive controls contained 2µl of *Chalara fraxinea* gDNA. For all fungal cultures, a direct colony PCR method was used in place of DNA extraction: for each sample, a sterile pipette tip was used to scrape fragments of mycelium from the colony surface. The pipette tip was then placed directly into the PCR mixture and rotated for approximately 2-5 seconds. All samples were then amplified using a Veriti™ 96 Well Thermal Cycler (Thermo Fisher Scientific, Waltham) programmed with the following cycling parameters: 95°C for 5 min followed by 30 cycles of 35 s at 95°C, 60 s at 55°C and 45 s at 72°C, with a final extension step of 5 min at 72°C and cooling at 4°C. Gel electrophoresis was carried out in 1% agarose gels stained with GelRed® Prestain Plus 6X DNA Loading Dye (Biotium, Fremont). PCR products (approximately 650 bp) were viewed on a UVP 2UV Benchtop Transilluminator (Thermo Fisher Scientific, Waltham) under UV light. DNA samples were then cleaned up using an ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham) as per the manufacturer's instructions before being sent to The James Hutton Institute (Invergowrie, Dundee) for sequencing. Returned sequences were cleaned up using Sequencher® (Gene Codes Corporation, Ann Arbor) before being matched in GenBank® to voucher specimens, culture collection sequences or sequences from published taxonomic papers at 99% identity or higher via BLAST® (NCBI, Bethesda).

5. Results

5.1 THDAS Database Analysis

Of the 46 host species selected for database analysis, 12 returned no reported instances of pathogen infection: *B. lenta*, *C. japonica* 'Elegans', *E. glaucescens*, *E. globulus*, *E. gundal*, *E. nitens*, *J. nigra*, *N. alpina*, *N. pumilio*, *P. peuce*, *P. ponderosa* and *S. torminalis*. For the remaining 34 species, a total of 567 records were obtained. These records dated back to 1982, with the most recent entries added in 2020. A marked increase in the number of reported instances was apparent from 2010 onwards, with significantly more records obtained in 2016 than other enquiry years. This is illustrated in Figure 1.

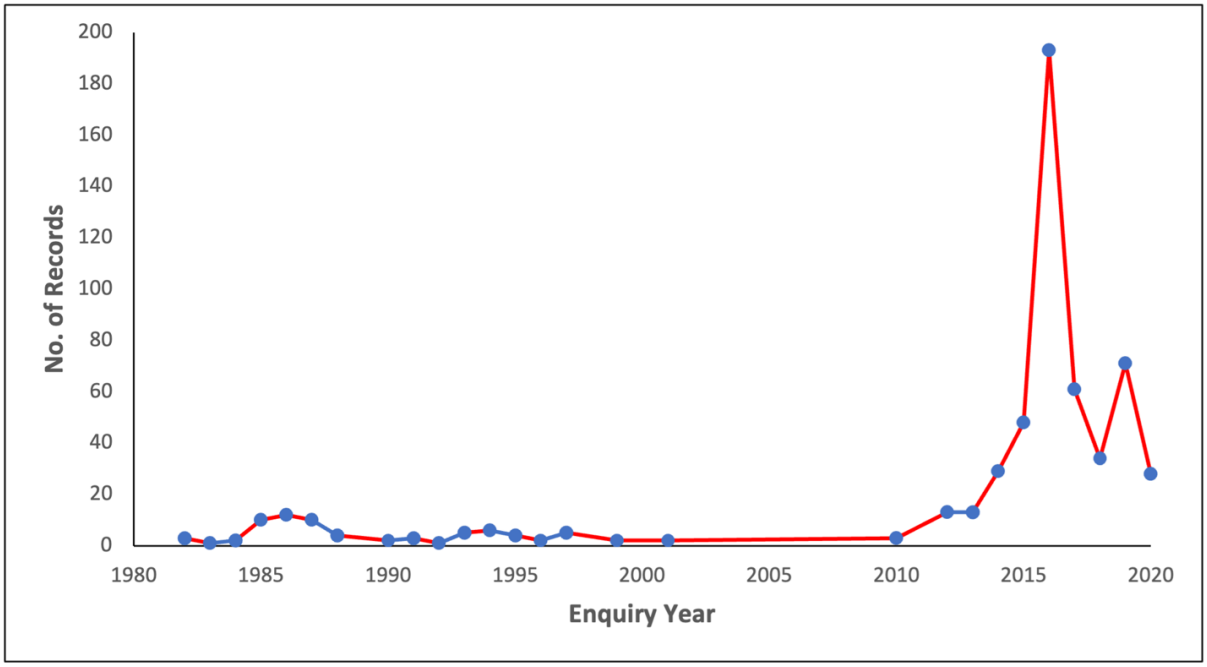

Figure 1. Total number of THDAS database entries per enquiry year.

A total of 193 entries were recorded in 2016 and 108 of these can be attributed to a single pathogen: *Sirococcus tsugae*. Ninety-seven of these entries reported instances of infection on *Cedrus atlantica*, with the remaining 11 entries detailing *S. tsugae* infection of *Cedrus libani*. Overall, *C. atlantica* was the most reported host species, with a total of 255 records. Figure 2 illustrates the total number of records per host species.

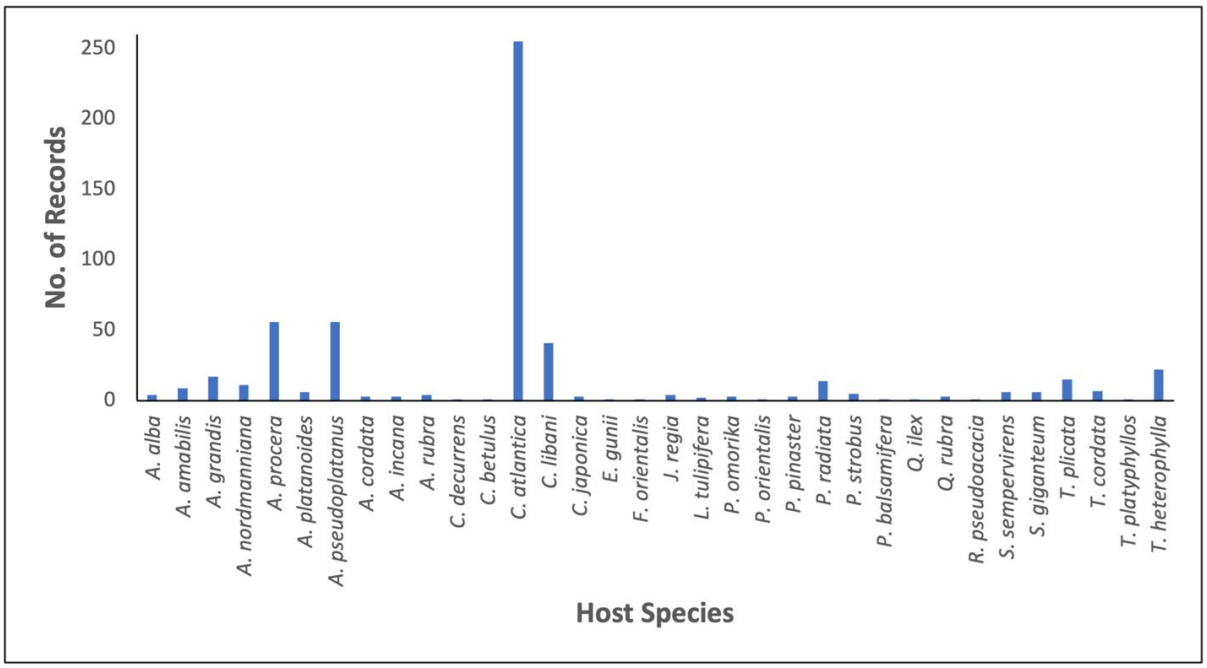

Figure 2. Total number of THDAS database entries per host species.

With a total of 209 records, the most frequently reported causal agent, by a considerable margin, was *S. tsugae*. A range of 89 distinct pathogens were reported, with 71 returning less than five records each. Table 3 illustrates the top five most frequently recorded pathogens which, when taken together, account for 333 of the 567 obtained records.

Table 3. The five most frequently reported causal agents from the THDAS database, with affected host species and number of records.

Pathogen	**No. Records**	**Host Species + No. Records**
Sirococcus tsugae	209	*C. atlantica* (184), *C. libani* (21), *T. heterophylla* (4)
Neonectria neomacrospora	44	*A. procera* (33), *A. grandis* (4), *A. amabilis* (3), *A. nordmanniana* (3), *A. alba* (1)
Sirococcus conigenus	29	*C. atlantica* (16), *C. libani* (12), *T. heterophylla* (1)
Heterobasidion annosum	26	*T. plicata* (6), *A. procera* (6), *A. grandis* (4), A. amabilis (3), *T. heterophylla* (3), *A. pseudoplatanus* (1), *C. atlantica* (1), *P. omorika* (1), *P. strobus* (1)
Diaporthe sp.	25	*C. atlantica* (15), *T. heterophylla* (6), *A. amabilis* (1), *C. libani* (1), *C. japonica* (1), *S. sempervirens* (1)

5.2 Pathogen Impacts at Glentress and Westonbirt

This section will summarise the observed pathogen impacts for each species present at Glentress and Westonbirt. Photographs of symptomatic trees, characteristic fruiting structures and spore types will be presented where available, with brief descriptive text. Three provenances (ABAM-P1, THPL-BRIT and THPL-OLYM) were missing from their plots,

possibly due to failed establishment, and therefore could not be assessed. All plots containing provenances of *Abies nordmanniana* (ABNO-AMBO, ABNO-APSH, ABNO-ARTV, ABNO-BORJ AND ABNO- TLUG) and *Picea orientalis* (PIOR-P2 and PIOR-TURK) displayed no indications of pathogen infection but were all very small and generally struggling to establish. The plots at Westonbirt containing *Acer macrophyllum* (ACMA-232) also displayed no signs of infection but were well established and appeared generally healthy. The remaining species all presented symptoms of pathogen infection and will be described below in alphabetical order.

Betula pendula

All plots at both Glentress and Westonbirt containing the *Betula pendula* provenance BEPE-UNIT were found to display symptoms characteristic of infection by the fungal pathogen *Marssonina betulae*. These symptoms included sunken black cankers (Panel A of Figure 3), characteristic leaf spots (Panel B of Figure 3) and progressive dieback of shoots and branches, evident on the central tree with notably sparser foliage illustrated in Panel C of Figure 3. These symptoms align with descriptions provided by Green (2005), Green and MacAskill (2007) and De Silva *et al*. (2008). Similar symptoms were also observed on the BEPE-NORD provenance, only present at Westonbirt, but to a lesser degree.

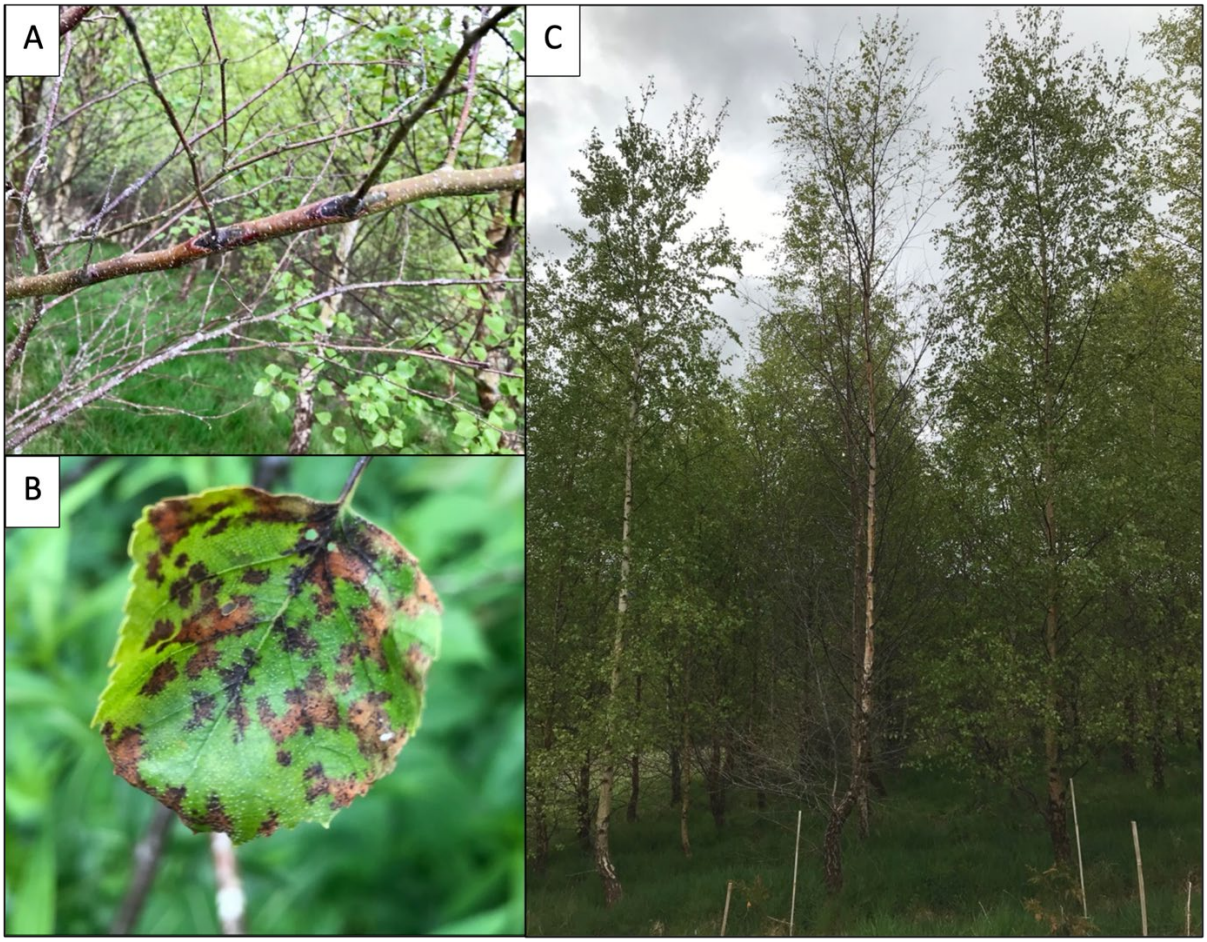

Figure 3. Symptoms of M. betulae infection on BEPE-UNIT provenance. Panel A: sunken cankers at Glentress. Panel B: leaf spots at Westonbirt. Panel C: progressive shoot and branch dieback on central tree at Glentress.

Cedrus atlantica

Cedrus atlantica was only present at Glentress and was represented by a single provenance: CEAT-VISF. All three plots containing CEAT-VISF displayed symptoms indicative of *Sirococcus tsugae* infection. These included characteristic pink to red needle discolouration and shoot tip wilting (Panels A and B of Figure 4), aligning with published descriptions provided by Sancisi-Frey (2017A) and Schmitz (2018). These symptoms were found scattered throughout foliage on afflicted trees (Panel C of Figure 4).

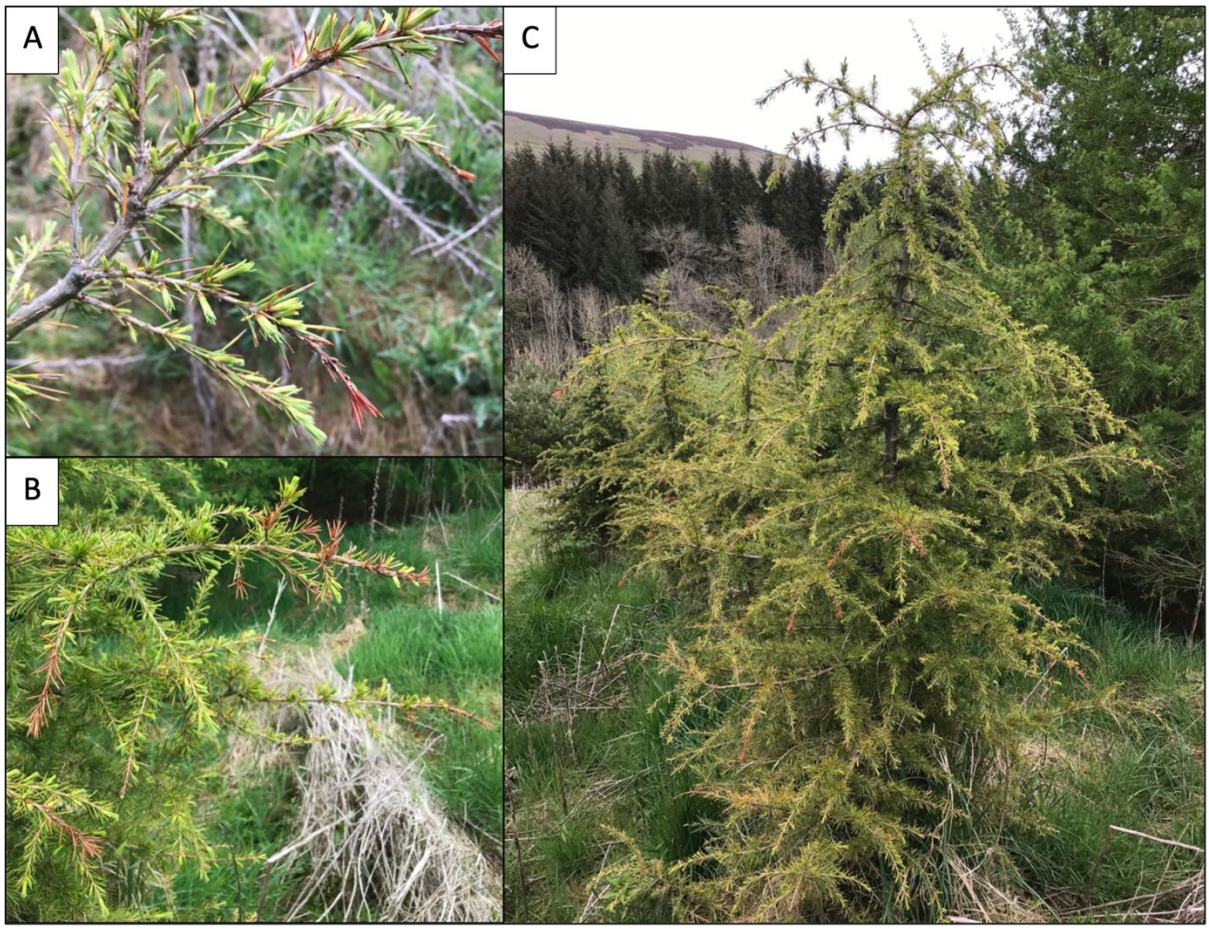

Figure 4. Symptoms of S. tsugae on CEAT-VISF provenance. Panels A and B: close ups of characteristic pink needle discolouration and shoot tip wilting. Panel C: scattered symptoms throughout foliage of symptomatic tree.

Cryptomeria japonica

At Westonbirt, plots containing provenances of *Cryptomeria japonica* (CRJA-P1, CRJA-P2 and CRJA-P3) were found to exhibit symptoms characteristic of Pestalotiopsis foliage blight, caused by the fungal pathogen *Pestalotiopsis* sp. These symptoms included initial yellowing before browning and withering of foliage at shoot tips (Panels A and B of Figure 5). Inspection of foliage samples via microscopy revealed the presence of characteristic spore masses which are exuded from foliage as tendrils under prolonged wet conditions (Panel C of Figure 5), aligning with descriptions provided by Thrush *et al.* (2021). The observed spore morphologies (Panel D of Figure 5) aligned with descriptions provided by Enebak (2012): conidia are five-

celled with brown median cells and branched appendages at one end and a single appendage at the other.

Figure 5. Symptoms of Pestalotiopsis sp. infection on C. japonica provenances. Panels A and B: initial yellowing and subsequent browning/withering of foliage. Panel C: black spore masses exuded from foliage. Panel D: characteristic Pestalotiopsis conidia.

Larix spp.

At both Glentress and Westonbirt, plots containing *Larix decidua* (LADE-THEI) displayed scattered instances of minor shoot dieback (Panel A of Figure 6) with visible lesions apparent when removing the outer bark from afflicted shoots. Panel B of Figure 6 illustrates the border between brown necrotic tissue and healthy green/white tissue. Based on the host species, an absence of any apparent pest damage and previous descriptions of disease symptomology (Sancisi-Frey, 2017B), it was theorised that this damage may be due to the sudden larch death pathogen, *Phytophthora ramorum*. However, efforts to isolate the causal agent from shoot samples onto SMA did not yield *P. ramorum*, with molecular analysis of the resultant fungal cultures on MEA (presented in section 5.4) returning equal sequence matches for two *Nectria* species: *Nectria nigrescens* and *Nectria cinnabarina*. As *N. cinnabarina* has occasionally been noted as a pathogen of trees (Hirooka *et al.*, 2011), it is possible that this species may be responsible for the observed damage. Similar instances of scattered, minor shoot tip wilting

were also observed on *Larix marschlinsii* (LAEU-LAVE), present only at Glentress, but to a lesser degree.

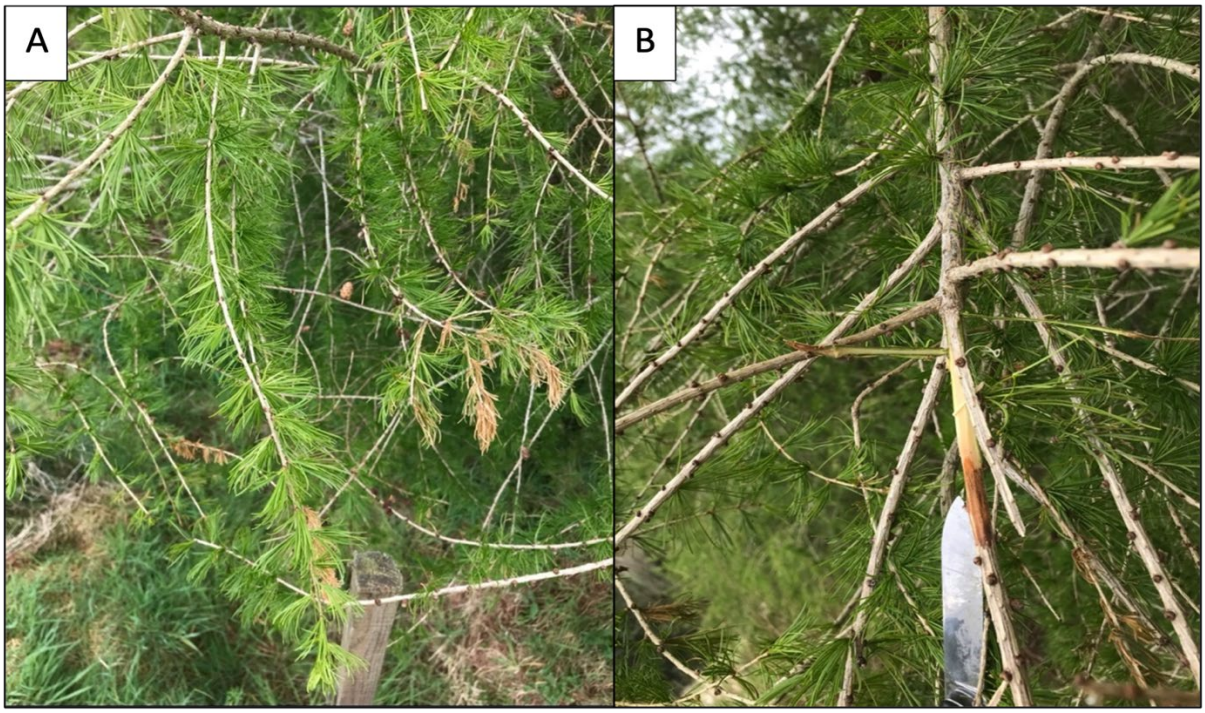

Figure 6. Shoot wilting and dieback on LADE-THEI provenance. Panel A: scattered shoot wilting and foliage discolouration. Panel B: visible lesion, displaying brown necrotic tissue and healthy white/green tissue.

Picea sitchensis

At Westonbirt, plots containing both provenances of *Picea sitchensis* (PISI-QSS and PISI-USS) were found to display symptoms indicative of Rhizosphaera needle cast, caused by the fungal pathogen *Rhizosphaera kalkhoffii*. These symptoms included characteristic rows of black pycnidia on infected needles, with spore morphologies inspected via microscopy aligning with illustrative figures provided by Watt (2018). No indications of infection by *R. kalkhofii* were detected on either *P. sitchensis* provenance present at Glentress.

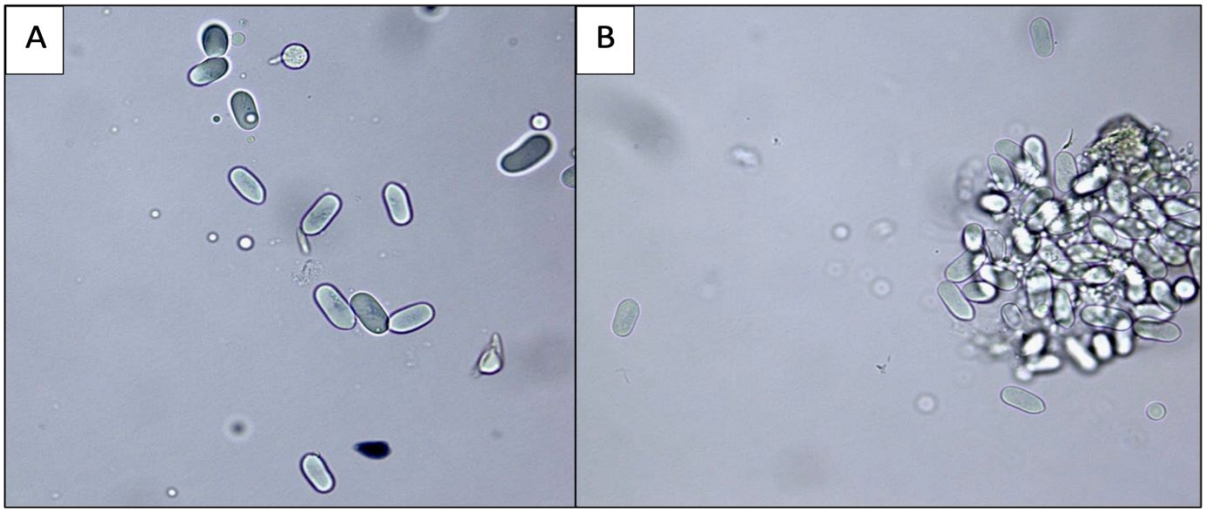

Figure 7. R. kalkhoffii spores present on PISI-QSS needle samples. Panels A and B: detail of spores examined on microscope slide.

Pinus spp.

Symptoms of *Dothistroma septosporum* infection were detected on all pine species and provenances at both Glentress and Westonbirt, with the exception of *Pinus strobus* (PIST-CZRI and PIST-643). Significant variation within plots was evident, with Panel A of Figure 8 illustrating two adjacent survey trees (PISY-VALS) at Glentress displaying notable differences in symptom expression. Panels B and C of Figure 8 provide examples of characteristic red banding and needle discolouration observed on a provenance of *Pinus pinaster* (PIPT-LACO) at Glentress, aligning with descriptions provided by Bulman *et al*. (2004). Needle samples collected from all provenances of *Pinus sylvestris* (PISY-POLA, PISY-VALS and PISY-SCOT), *Pinus radiata* (PIRA-WO and PIRA-DOTH) and *Pinus pinaster* (PIPT-CORD, PIPT-LACO and PIPT-LAND) at Glentress were inspected via microscopy to confirm the presence of *D. septosporum* fruiting bodies (Panel A of Figure 9). In addition, measurements of conidia (Panel E of Figure 8) were performed for PIPT-LACO, PIPT-LAND and PIPT-CORD to check consistency with those provided by Markovskaja and Treigienė (2009). Fruiting bodies of *Cyclaneusma minus* were also detected on needle samples collected from PIRA-DOTH, PIRA-WO, PISY-SCOT and PISY-POLA, with the observed ascus (Panel B of Figure 9) aligning with illustrative figures provided by Cech (2012). Shoot samples obtained from PIPT-CORD, PIPT-LACO, PIRA-DOTH and PIRA-WO yielded cultures of *Gremmeniella abietina* (presented in section 5.4). *Gremmeniella abietina* is the causal agent of Brunchorstia dieback (Brown and MacAskill, 2005), a shoot disease of pines, and will therefore have contributed significantly to the observed damage on *P. pinaster* and *P. radiata*. A shoot sample obtained from PIPT-LAND also yielded *Cenangium ferruginosum*, an additional shoot dieback pathogen of pines (Ryu *et al.*, 2018).

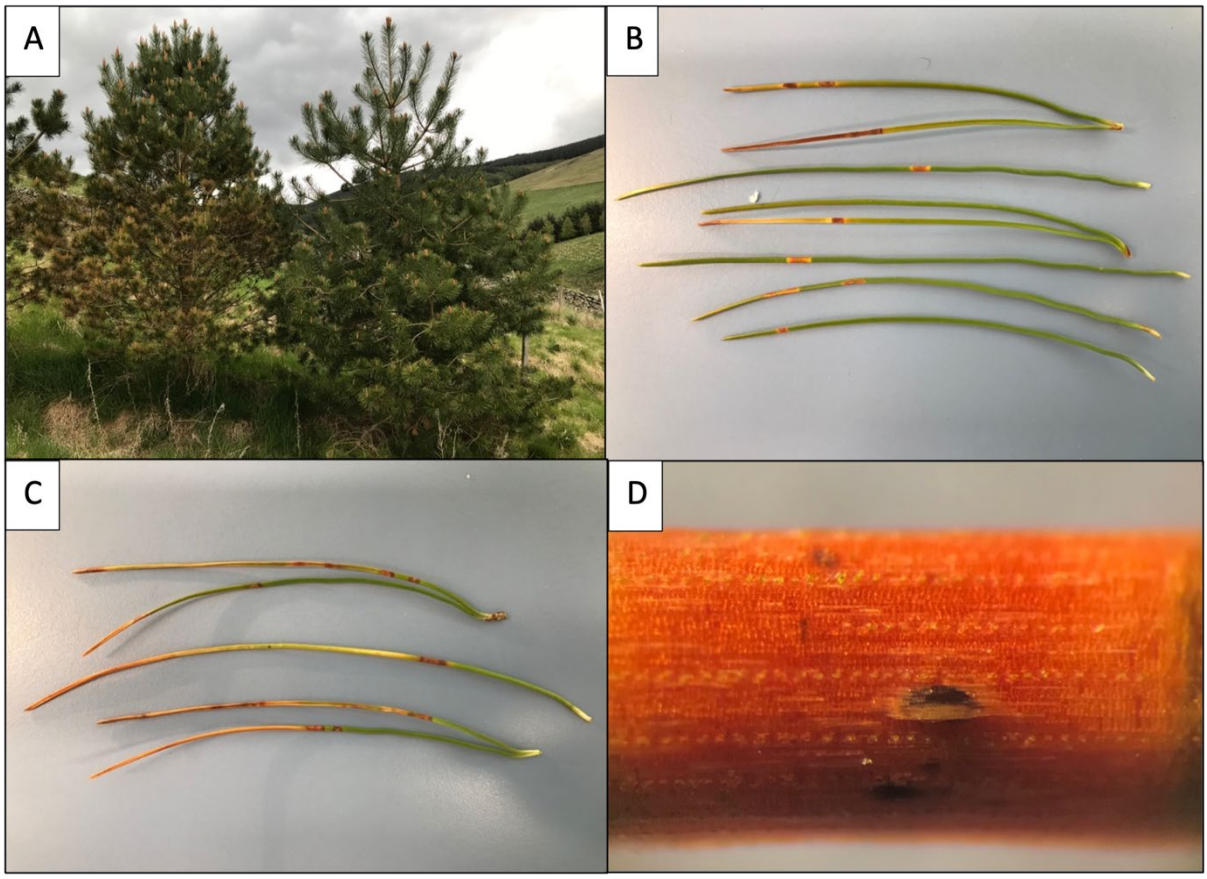

Figure 8. Symptoms of D. septosporum infection on pine species at Glentress. Panel A: evidence of variation within plots of PISY-VALS. Panels B and C: characteristic red banding and discolouration on PIPT-LACO needle samples. Panel D: D. septosporum fruiting body rupturing through PIPT-LACO needle epidermis.

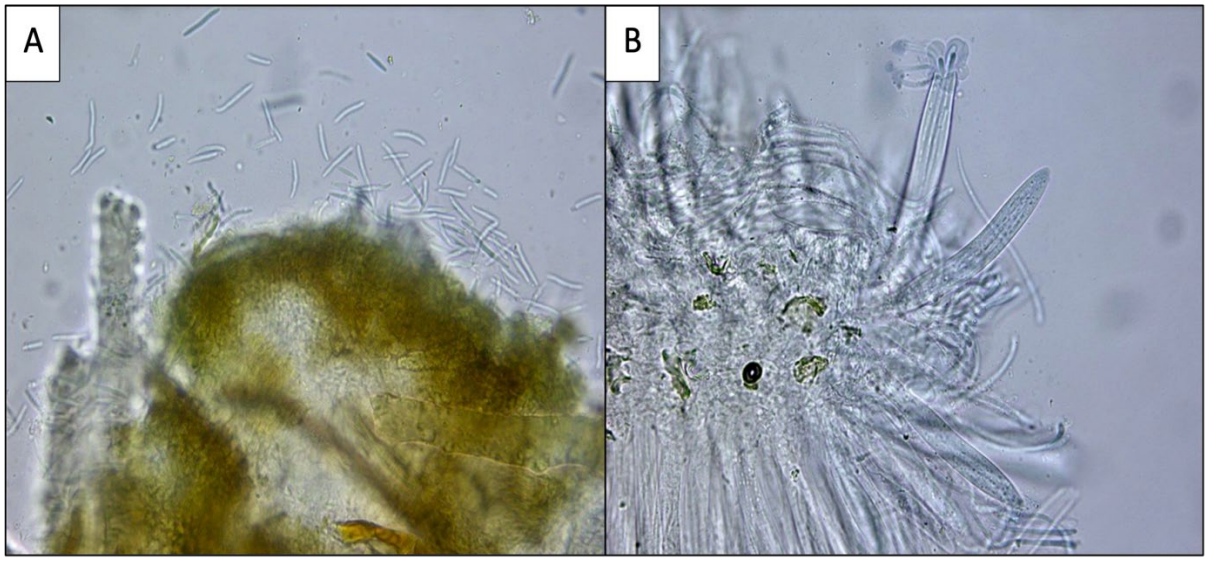

Figure 9. Spore types present on PISY-SCOT needle samples collected from Glentress. Panel A: D. septosporum conidia. Panel B: C. minus ascus.

Pseudotsuga menziesii

A plot at Glentress containing a provenance of *Pseudotsuga menziesii* (PSME-ORSI) was found to exhibit symptoms characteristic of *Sirococcus tsugae* infection, in addition to the symptoms observed on *Cedrus atlantica.* Panel A of Figure 10 depicts a shoot sample collected from the symptomatic tree, with examined conidia (Panel B of Figure 10) found to be consistent with descriptions provided by Schmitz *et al.* (2018): one-septate, fusiform and hyaline. At Westonbirt, all provenances of *P. menziesii* (PSME-ORSI, PMSE-ORCO and PSME-WASH) displayed symptoms of Swiss needle cast, caused by the fungal pathogen *Phaeocryptopus gaeumannii*. Infected needles displayed rows of black pseudothecia, aligning with symptom descriptions provided by Montwé *et al.* (2021). No indications of Swiss needle cast were detected at Glentress.

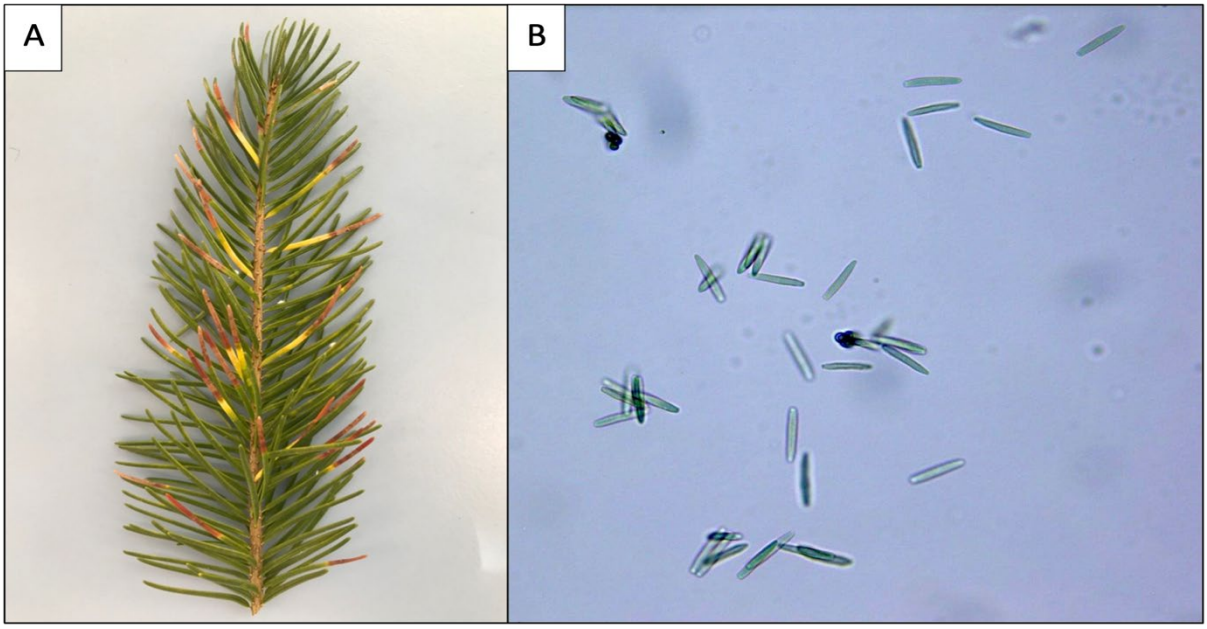

Figure 10. Symptoms of S. tsugae infection on PSME-ORSI at Glentress. Panel A: Symptomatic shoot sample. Panel B: Conidia inspected via microscopy.

Quercus spp.

Plots at Glentress containing *Quercus rubra* (QURU-FEST) (Panel A of Figure 11) were found to contain scattered instances of fine shoot dieback and tip withering. Isolations from shoot samples yielded cultures with equal sequence matches (see section 5.4) for *Apiognomonia errabunda* and *Fusicoccum quercus*, both of which are known as pathogens of oak (Butin, 1981; Brazee, 2014). At Westonbirt, plots containing *Quercus pyrenaica* (QUPY-P1) and *Quercus robur* (QURO-FRAN and QURO-UNIT) displayed numerous instances of powdery mildew, with the severity ranging from limited to moderate (Panel B of Figure 11). A possible causal agent may be the fungal species *Erysiphe alphitoides* (Forest Research, 2021).

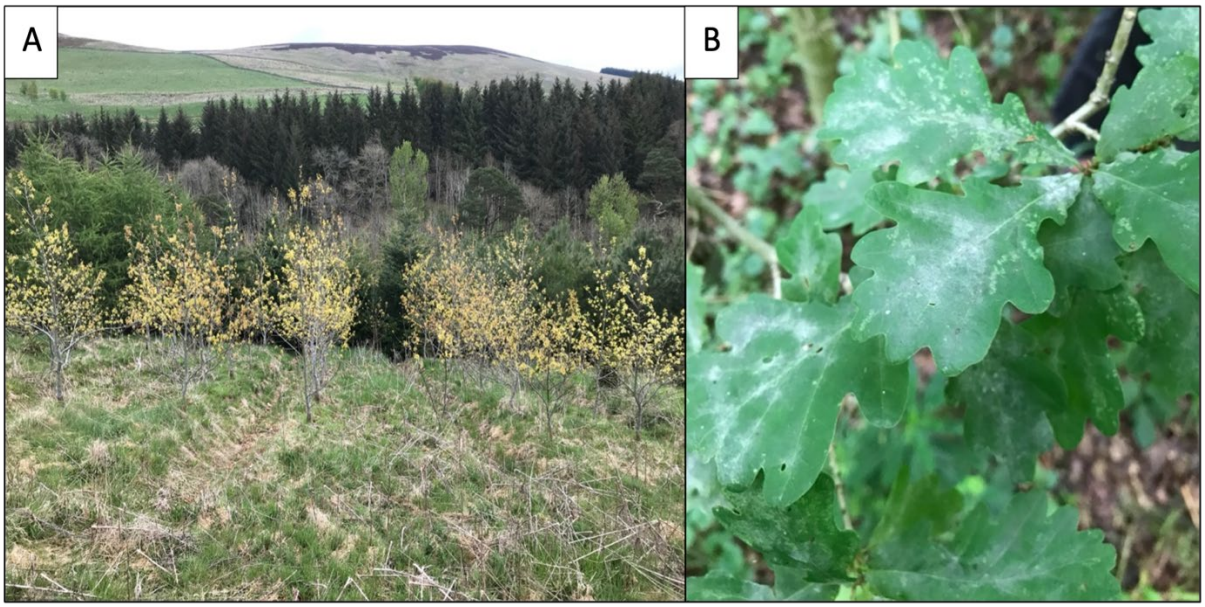

Figure 11. Quercus species at Glentress and Westonbirt. Panel A: overview of Q. rubra (QURU-FEST) plot at Glentress. Panel B: Symptoms of powdery mildew on Q. robur (QURO-UNIT) at Westonbirt.

Sequoia sempervirens

At Westonbirt, a plot containing *Sequoia sempervirens* (SESE-NOCA) displayed symptoms similar to that of Pestalotiopsis foliage blight. Panel A of Figure 12 provides an illustration of the observed foliage discolouration and shoot tip dieback. However, microscopic examination of shoot samples revealed the presence of fruiting bodies which do not resemble those produced by *Pestalotiopsis* sp. (Panel B of Figure 12), with the causal agent unable to be identified in the current project.

Figure 12. Possible symptoms of Pestalotiopsis foliage blight on SESE-NOCA at Westonbirt. Panel A: characteristic foliage discolouration and shoot tip dieback. Panel B: Fruiting structures of unknown causal agent.

Thuja spp.

The plots at Glentress containing *Thuja plicata* (THPL-DARR) were generally found to be struggling or, in many cases, dead, suggesting an unsuitable planting site. Plots at Westonbirt containing the THPL-MONT provenance displayed relatively minor instances of Thuja blight (Panels A and B of Figure 13), caused by the fungal pathogen *Didymascella thujina*. The observed symptoms – dead/desiccated leaf scales with cavities left by fallen fruiting bodies – aligned with descriptions of Thuja blight (RHS, 2021).

Figure 13. Symptoms of Thuja blight on THPL-MONT at Westonbirt. Panels A and B: characteristic dead leaf scales, with cavities left by fallen fruiting bodies.

5.3 Statistical Analysis of Health Scoring

Two of the scored health variables were identified as being most representative of pathogen impacts at both sites: foliage dieback, to reflect damage caused by foliar pathogens, and shoot mortality, to reflect pathogens causing fine shoot dieback. For foliage dieback, a highly significant effect of species-provenance was detected ($p<0.002$), whilst the effect of site was non-significant ($p=0.454$). For shoot mortality, a highly significant effect of species-provenance ($p<0.002$) and a significant effect of site ($p=0.018$) were detected, with mean values across all species recorded at Westonbirt (8.28) being higher than those reported at Glentress (5.84). No significant differences were detected between provenances within the same species, except in the case of *B. pendula*. For shoot mortality, the BEPE-UNIT and BEPE-NORD provenances differed significantly (Tukey multiple comparisons test, $p=0.001$). As the pines (*Pinus* spp.) provided the greatest number of both species and provenance comparisons within a single genus, the mean scores and HSD groups have also been summarised in Table 4 below. For foliage dieback, the *Pinus strobus* provenances (PIST-CZRI and PIST-643) displayed significantly lower mean scores when compared to many of the other pine species, being in a distinct HSD group to PISY-POLA, PISY-VALS, PIPT-LAND, PIRA-WO and PIRA-DOTH. For shoot mortality, the *P. strobus* provenances are in a distinct HSD group to PISY-POLA, PISY-VALS and PIPT-LAND.

Table 4. Mean foliage dieback and shoot mortality scores for all species and provenances within the Pinus genus. Letters denote Honestly Significant Differences: any provenances with shared letters are not statistically different from one another.

Species	Provenance	Foliage Dieback		Shoot Mortality	
		% Disease Score	HSD Group	% Disease Score	HSD Group
Pinus sylvestris	PISY-POLA	26.10	g	23.84	fg
	PISY-VALS	24.83	g	12.83	defg
	PISY-SCOT	11.01	bcdefg	8.80	cdef
Pinus pinaster	PIPT-LAND	20.70	efg	20.67	efg
	PIPT-CORD	17.02	cdefg	10.88	bcdefg
	PIPT-LACO	8.85	abcdefg	4.58	abcde
Pinus radiata	PIRA-WO	19.28	fg	3.47	abcde
	PIRA-DOTH	19.23	fg	4.15	abcde
Pinus strobus	PIST-CZRI	0.21	abcd	0.13	abc
	PIST-643	0.09	abc	0.08	abc

Figure 14 displays mean foliage dieback and shoot mortality scores across all species-provenances (both sites) with 95% C.I.

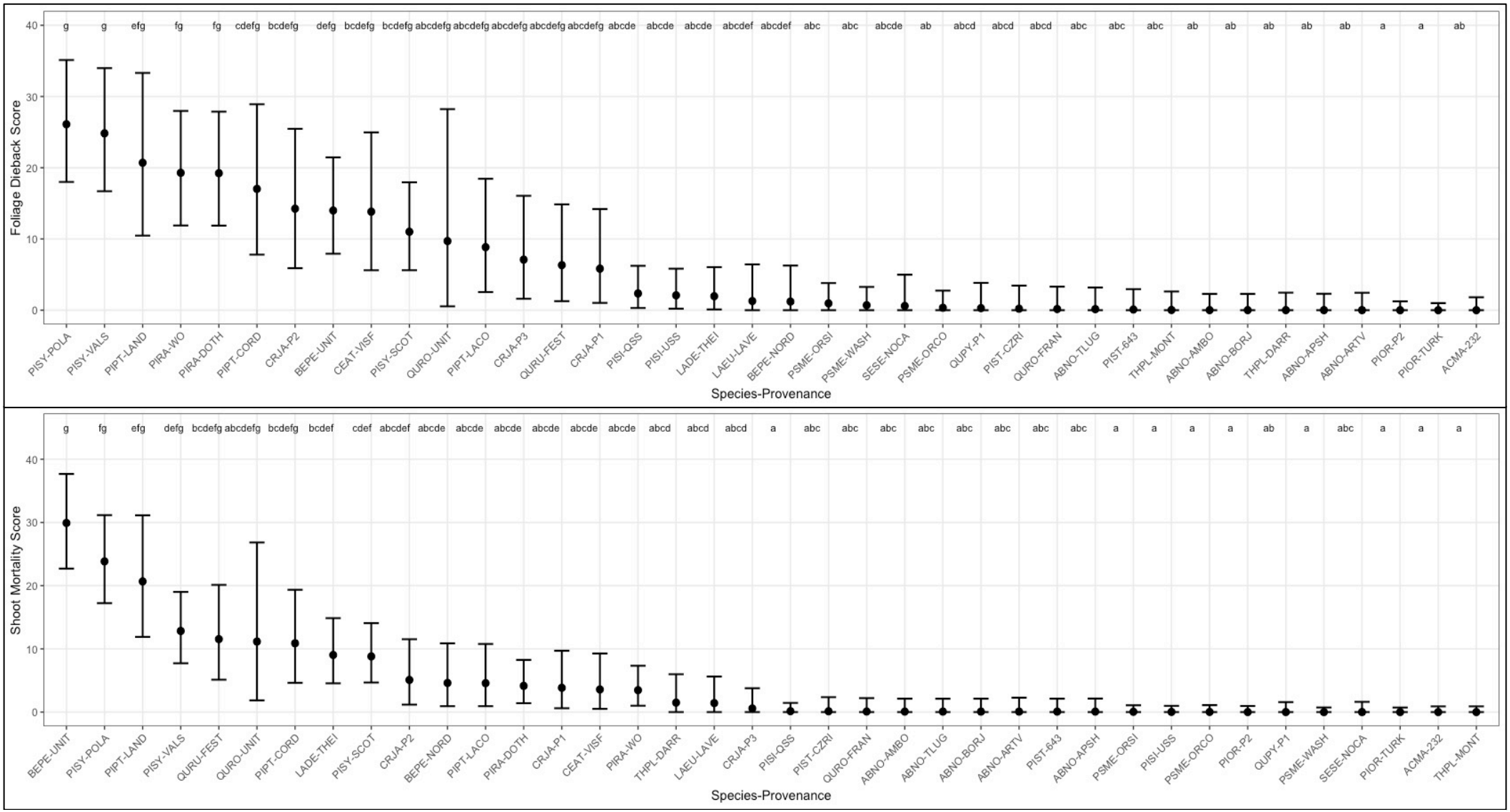

Figure 14. Mean foliage dieback and shoot mortality scores across all species-provenances at both trial sites. Error bars represent 95% confidence intervals. Letters denote Honestly Significant Differences: any species-provenances with shared letters are not statistically different from one another.

5.4 Molecular Analysis of Sample Material

Table 5 displays the sequence matches obtained after BLAST analysis of all samples. A range of fungal species were identified, including confirmation of *S. tsugae* on CEAT-VISF and *D. septosporum* and *C. minus* on PIRA-WO, with many endophytic species appearing across a number of host species. An additional shoot pathogen – *Gremmeniella abietina* – was also confirmed on PIPT-CORD, PIPT-LACO, PIRA-DOTH and PIRA-WO. For samples 38 and 6M, equal matches were obtained for *Apiognomonia errabunda* and *Fusicoccum quercus*, and for sample 54, equal matches were obtained for *Nectria nigrescens* and *Nectria cinnabarina*.

Table 5. All samples identified via BLAST analysis of returned sequences.

Sample ID	**Species-Provenance**	**Sample Type**	**Sequence Match (99% or higher)**
1M	CEAT-VISF	Fungal Culture	*Coniothyrium lignorum*
3M	CEAT-VISF	Fungal Culture	*Sirococcus tsugae*
4M	CEAT-VISF	Fungal Culture	*Cosmospora berkeleyana*
5M	CEAT-VISF	Fungal Culture	*Phacidium* sp.
2M	CEAT-VISF	Fungal Culture	*Diaporthe* sp.
25M	CEAT-VISF	Fungal Culture	*Coniothyrium lignorum*
22	CEAT-VISF	Shoot	*Phacidium* sp.
54	LADE-THEI	Shoot	*Nectria nigrescens/Nectria cinnabarina (equal matches)*
9M	LADE-THEI	Fungal Culture	*Coniothyrium lignorum*
25	LAEU-LAVE	Shoot	*Coniothyrium lignorum*
89	PIPT-CORD	Shoot	*Gremmeniella abietina*
15M	PIPT-CORD	Fungal Culture	*Diaporthe sp.*
3	PIPT-LACO	Shoot	*Gremmeniella abietina*
13M	PIPT-LACO	Fungal Culture	*Gremmeniella abietina*
93	PIPT-LAND	Shoot	*Cenangium ferruginosum*
75	PIRA-DOTH	Needle	*Gremmeniella abietina*
73	PIRA-WO	Shoot	*Gremmeniella abietina*
16M	PIRA-WO	Fungal Culture	*Gremmeniella abietina*
23M	PIRA-WO	Fungal Culture	*Coniothyrium lignorum*
73N	PIRA-WO	Needle	*Dothistroma septosporum*
7	PIRA-WO	Needle	*Cyclaneusma minus*
35	PISY-SCOT	Shoot	*Coniothyrium lignorum*
14M	PISY-SCOT	Fungal Culture	*Fusarium* sp.
26M	PISY-SCOT	Fungal Culture	*Fusarium* sp.
5	PSME-ORSI	Shoot	*Diaporthe* sp.
18M	QURO-FRAN	Fungal Culture	*Diaporthe* sp.
65	QURU-FEST	Shoot	*Cryptosporiopsis diplodioides*

38	QURU-FEST	Shoot	*Apiognomonia errabunda/Fusicoccum quercus* (equal matches)
7M	QURU-FEST	Fungal Culture	*Pezicula neocinnamomea*
6M	QURU-FEST	Fungal Culture	*Apiognomonia errabunda/Fusicoccum quercus* (equal matches)

Figure 15 illustrates the range of colony types identified via BLAST analysis of returned ITS sequences.

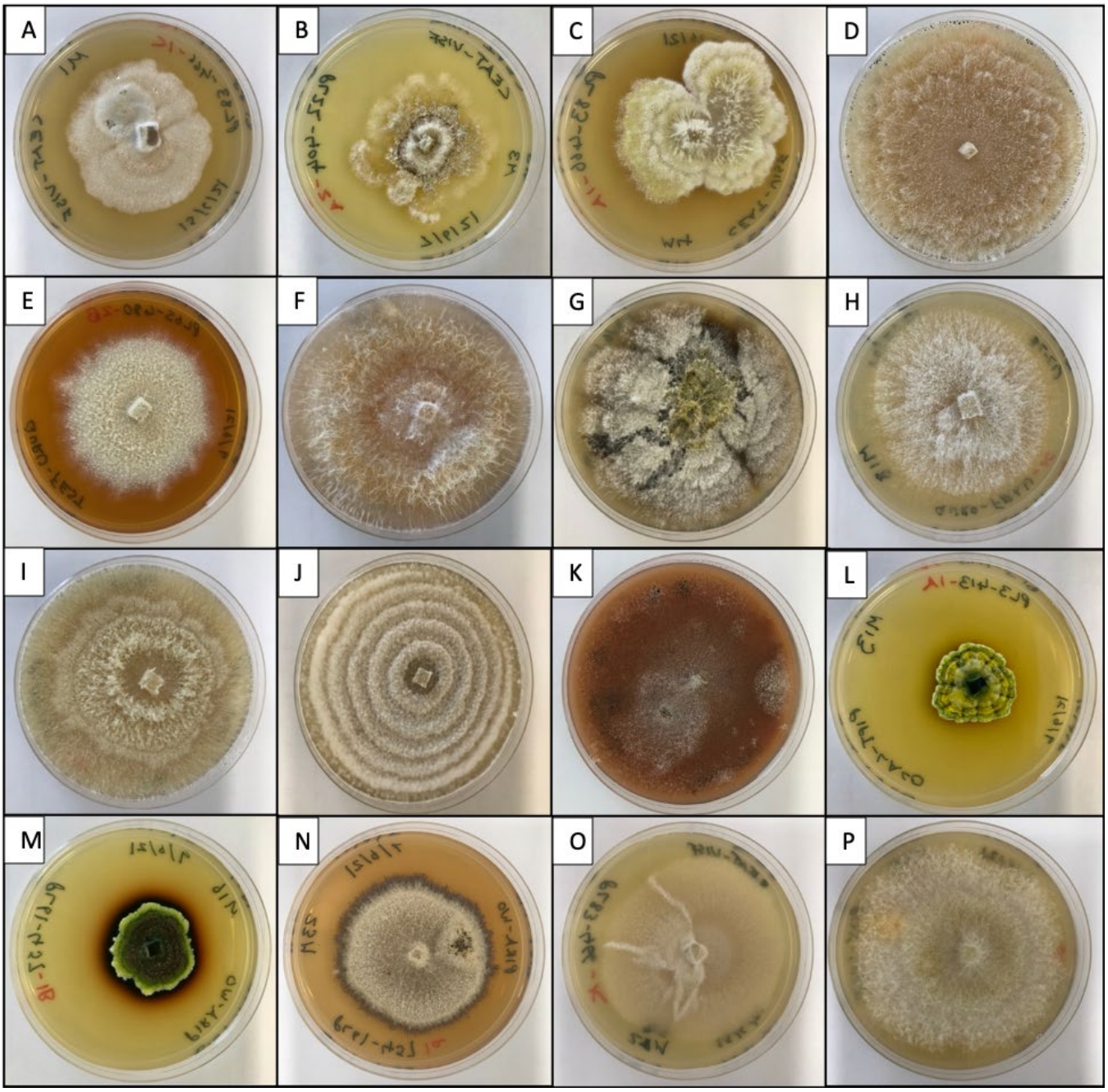

Figure 15. All fungal isolates identified via analysis of the ITS region. Al cultures on MEA and incubated at 17°C. A: Coniothyrium lignorum (1M), B: Sirococcus tsugae (3M), C: Cosmospora berkeleyana (4M), D: Phacidium sp. (5M), E: Pezicula neocinnamomea (7M), F: Fusarium sp. (14M), G: Diaporthe sp. (15M), H: Diaporthe sp. (18M), I: Diaporthe sp. (2M), J: Apiognomonia errabunda or Fusicoccum quercus (6M), K: Coniothyrium lignorum (9M), L: Gremmeniella abietina (13M), M: Gremmeniella abietina (16M), N: Coniothyrium lignorum (23M), O: Coniothyrium lignorum (25M) and P: Fusarium sp. (26M).

6. Discussion

The results of the Tree Health Diagnostic and Advisory Service database analysis clearly identify *Cedrus atlantica* as a host species of concern, with 184 reported instances of infection by *Sirococcus tsugae*. This aligns with the observation of numerous symptomatic trees in all three *C. atlantica* plots at Glentress and subsequent molecular identification of the pathogen (sample 3M). As the species was absent at Westonbirt, *C. atlantica* is represented in the current study by a single provenance: CEAT-VISF. Future work may therefore seek to assess potential variation in susceptibility to *S. tsugae* in terms of both provenance and geographical location. As a Plot-Stage species, *C. atlantica* has been highlighted for potential use on drier site types in eastern and southern Britain (Pérez-Sierra *et al.*, 2015). Determining the extent to which disease pressure from *S. tsugae* may limit the viability of *C. atlantica* as a candidate alternative species would therefore be of value. In addition, further analysis may be undertaken to ascertain if the substantial spike in reported instances of *S. tsugae* infection observed in 2016 may be linked to climatic conditions specific to that enquiry year.

The health scoring results highlight three pine species – *Pinus sylvestris*, *Pinus pinaster* and *Pinus radiata* – as being particularly heavily impacted, with provenances from each species being found toward the upper end of the recorded mean foliage dieback and shoot mortality scores. These findings attest to the widespread presence of *Dothistroma septosporum* at both trial sites, as well as the impact of *Gremmeniella abietina* and *Cenangium ferruginosum* on *P. pinaster* and *P. radiata* at Glentress. Whilst not statistically significant, the differences in mean foliage dieback and shoot mortality scores do allow for some provisional recommendations to be made with regard to provenance selection. For *P. sylvestris*, the native PISY-SCOT provenance obtained a mean foliage dieback score of 11.01, less than half that of the PISY-POLA (Polish) and PISY-VALS (Spanish) provenances, with scores of 26.10 and 24.83 respectively. A similar disparity is evident for the shoot mortality scores: 8.80 for PISY-SCOT, 23.84 for PISY-POLA and 12.83 for PISY-VALS. It may be the case that these differences in susceptibility can be attributed to coevolution of the native PISY-SCOT provenance with *D. septosporum* pathotypes endemic to the UK, aligning with observations made by Perry *et al.* (2016B). The Polish and Spanish provenances, in contrast, have had no opportunity to develop natural resistance to UK *D. septosporum* strains and therefore exhibit higher levels of susceptibility. Selection of exotic *P. sylvestris* provenances would therefore appear to be inadvisable in terms of the risk posed by *D. septosporum*. For *P. pinaster*, the PIPT-LACO provenance (hybrid of populations from Landes and Corse) appeared to exhibit reduced susceptibility when compared to the PIPT-LAND (Landes, France) and PIPT-CORD (Monfero, France) provenances, whilst no differences were apparent between the two *P. radiata* provenances PIRA-WO and PIRA-DOTH.

Whilst each of the pine species described above were heavily impacted by *D. septosporum*, it is notable that *Pinus strobus*, a Plot-Stage species, appeared unaffected, with significantly lower mean foliage dieback and shoot mortality scores when compared to many of the *P. sylvestris*, *P. pinaster* and *P. radiata* provenances. This suggests that, in terms of resilience to Dothistroma needle blight, *P. strobus* may represent a promising alternative forestry species. However, this potential must be considered alongside the risk posed by white pine blister rust, caused by the pathogen *Cronartium rubicola*. In environmentally favourable conditions and in the presence of its alternate host (*Ribes* spp.), this pathogen has caused widespread and significant damage to *P. strobus* in North America (Kinloch, 2003). In the UK, *P. strobus* was discontinued as a forestry species in the late 1800s due to damage caused by *C. rubicola*

(Forest Research, 2021B). Nonetheless, it may still be the case that in certain site types, and with the use of *C. rubicola*-resistant stock, integration of *P. strobus* may serve to mitigate the impact of *D. septosporum* on pine plantations.

With regard to Principal species, the significant difference in mean shoot mortality observed between the BEPE-UNIT and BEPE-NORD provenances of *Betula pendula* suggest that provenance choice may play a significant role in mitigating damage caused by *Marssonina betulae*. Interestingly, this represents an instance where a non-native provenance (BEPE-NORD, France) was found to be significantly less susceptible when compared to a native provenance (BEPE-UNIT, Wales). For *Pseudotsuga menziesii*, the possibility for infection by *S. tsugae* has been previously noted (Pérez-Sierra *et al.*, 2017) and it is possible that the observed symptoms at Glentress could be due to high inoculum loads produced by nearby *C. atlantica* plots. It currently appears unlikely that *S. tsugae* will become a major pathogen of *P. menziesii* – a recent pathogenicity test on Belgian forestry species found that *S. tsugae*-inoculated seedlings of *P. menziesii* produced significantly smaller necrotic tissue areas when compared to *C. atlantica* seedlings (Pirronitto *et al.*, 2021). Similarly, the detection of Swiss needle cast symptoms on *P. menziesii* at Westonbirt does not currently represent a major pathogen threat. The causal agent, *Phaeocryptopus gaeumannii*, is known to be widespread but is considered to be insignificant (Forest Research, 2021C), aligning with the low foliage dieback and shoot mortality scores obtained for both provenances (PSME-ORSI and PSME-ORCO). However, this situation may change: two studies have highlighted rising winter temperatures as a key determining factor in the severity of Swiss needle cast outbreaks on *P. menziesii* (Stone *et al.*, 2007; Henry Lee *et al.*, 2017). It is therefore possible that climate change will result in Swiss needle cast becoming a disease of some significance for *P. menziesii* plantations in the UK. In contrast, *Rhizosphaera kalkhoffii*, detected on *Picea sitchensis* at Westonbirt, is currently considered to be a minor pathogen (Reeb and Shaw, 2015; Tuffen and Grogan, 2019) and there are no indications that climate change may exacerbate its impact.

A recent report produced by the Welsh Government compiled a list of the top 5 alternative forestry species for future use in UK forestry (Peters *et al.*, 2021). The selections were based on analysis of a range of attributes, including suitability for timber production, resilience to pests/pathogens and adaptability to changing climatic conditions. Three species present in the current study were included in the list: *Sequoia sempervirens* at number 1, *Cryptomeria japonica* at number 2 and *Thuja plicata* at number 3. There is therefore likely to be increased interest in identification of potential pathogen threats to these species specifically. The observation of Pestalotiopsis foliage blight on *Cryptomeria japonica*, a Plot-Stage species, may present an area for further research: the impact of the pathogen was not insignificant, with the health scores obtained for the CRJA-P2 provenance in particular appearing in the upper range of recorded foliage dieback and shoot mortality values. Further work is required to identify the causal agent responsible for the observed damage on *S.* sempervirens. For *T. plicata,* Thuja blight had previously been detected at Westonbirt in 2017 and remedied through removal of heavy weed growth, a contributing factor to highly humid conditions conducive to the pathogen (Reynolds *et al.*, 2021). A recent report from central Scotland of *T. plicata* infection by *Phytophthora lateralis*, a pathogen more frequently seen on Lawson cypress, may suggest a future pathogen threat (Wilson *et al.*, 2016)

In terms of differences between Glentress and Westonbirt, the heavy weed growth mentioned above may have contributed to the significant effect of site on shoot mortality. In general, the plots at Westonbirt displayed more vigorous growth, contributing to potential

issues of crowding, and were subject to the proliferation of thick, woody weed growth between plots. Both of these factors may have contributed to humid conditions favoured by fungal pathogens. In addition, Westonbirt is known to have fertile soils, a factor which has been linked to the increased activity of pathogens such as *D. septosporum* (Lambert, 1986). These site characteristics may explain the higher levels of shoot mortality observed at Westonbirt. For foliage dieback, the non-significant effect of site suggests generally consistent levels of foliar damage at both Glentress and Westonbirt.

With regard to molecular analysis of sample material, the two most frequently identified fungal species were *Gremmeniella abietina* and *Coniothyrium lignorum*, with six sequence matches each. The contribution of *G. abietina* to the observed levels of damage on *P. pinaster* and *P. radiata* can therefore be considered as significant. As no reported instances of pathogenic activity could be found for *C. lignorum*, it is most likely an endophyte. Endophytes are non-pathogenic members of diverse fungal communities within tree species (Sieber, 2007). For samples 38 and 6M, equal matches were obtained for *Apiognomonia errabunda* and *Fusicoccum quercus*. A literature search identified a paper containing an illustration of an *A. errabunda* culture closely resembling the concentric colony morphology of sample 6M (Vainio *et al.*, 2017). As *A. errabunda* is the causal agent of oak anthracnose (Brazee, 2014), it is therefore probable that the observed symptoms of fine shoot dieback at Glentress were due to *A. errabunda.*

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