Plant Health Centre
Scotland's Centre of Expertise

Scottish Government
Riaghaltas na h-Alba
gov.scot

# Understanding a new health threat to Caledonian Scots pine (*Pinus sylvestris*)

## Project Final Report

www.planthealthcentre.scot

Forest Research

Royal Botanic Garden Edinburgh

Scottish Forestry
Coilltearachd na h-Alba

This work was commissioned by Scotland's Centre of Expertise for Plant Health Funded by Scottish Government through the Rural & Environment Science and Analytical Services (RESAS) Division under grant agreement No PHC2022/07

**Authors:** Sarah Green[1*], Joanne E. Taylor[2], Maria Stanisz-Migal[1], Finnley Tierney-Kitchener[1], Steven J. Hendry[1], Stuart A'Hara[1], Katherine Lester[1], Molly Davidson[1], Laura Roehrig[1] and Ewan Purser[3]

[1]Forest Research, Northern Research Station, Edinburgh, UK, EH25 9SY
[2]Royal Botanic Gardens Edinburgh, UK, EH3 5LR
[3]Scottish Forestry, Edinburgh, UK, EH12 7AT
*Corresponding author

**Please cite this report as follows:** S. Green, J.E. Taylor, M. Stanisz-Migal, F. Tierney-Kitchener, S.J. Hendry, S. A'Hara, K. Lester, M. Davidson, L. Roehrig and E. Purser (2024). Understanding a new health threat to Caledonian Scots pine (*Pinus sylvestris*): Project Final Report. PHC2022/07. Scotland's Centre of Expertise for Plant Health (PHC). DOI: 10.5281/zenodo.10854119

Available online at: planthealthcentre.scot/publications

**Dissemination status:** Unrestricted



**Acknowledgements:** Ruth Holland and Cayla Smith at RBGE are thanked for assistance with the growth studies, and Joe Beesley at Forest Research for producing Figure 1.

# Content

# 1 Executive Summary

## *1.1 Background and objectives*

This study was initiated in response to a new health threat to Scots pine first observed in the Cairngorm region, which was brought to the attention of Forest Research pathology staff in late 2022. This new health threat is manifesting itself in the form of unusually abundant blackened cankers and dieback of shoots and branches, particularly in the lower crown. Numerous trees in the Cairngorm area were found to be affected and the pattern of symptoms appeared to be new. Scots pine, and particularly native 'Caledonian' Scots pine, is regarded as iconic in Scotland for a range of ecological, cultural and economic reasons. Therefore, it is a research priority to gain an understanding of the extent and causes of the canker disease for better protection of this species in the future. With this in mind, the objectives of this project were to:

- Determine the geographical extent of symptoms on Scots pine across Scotland.
- Identify and describe the agent/s responsible for the canker symptoms.
- Investigate historical, ecological and genetic information which may shed light on the drivers of this new health threat.

## *1.2 Research undertaken*

Surveys of Scots pine were carried out by the project team, Scottish Forestry tree health officers, Forestry and Land Scotland staff and other Scottish-based stakeholders, as well as staff based within Forestry Commission England and Natural Resources Wales. Samples of branch cankers were subject to morphological analyses of both host and fungal structures. Fungal isolates as well as adelgid insects were obtained from symptomatic material. The DNA of fungal isolates and adelgids was examined to confirm their taxonomic identity and genetic relatedness to known specimens. Literature searches were undertaken utilizing records dating back to the 1820s to better understand the fungi involved and the nature of any previous outbreaks.

## *1.3 Main findings*

Symptomatic Scots pine was found to occur in most regions of Scotland. Trees of all ages were affected across a range of site types including natural Caledonian pine forests, planted native woodland and commercial plantations. Across the 31 sites visited, canker symptoms and branch dieback were most frequently observed starting in the lower crown and appeared to progress upwards into the mid-crown area. Some of the worst affected sites in terms of visual prevalence of symptoms were new native woodland grant scheme plantings in wet, western, upland areas. In some cases, weak or suppressed young trees with severe cankering were killed.

One of the primary causal agents was found to be a previously obscure stroma-forming fungus currently named *Curreya pithyophila*. This fungus forms a black stroma which encircles young shoots, branches and main stems of Scots pine trees, most typically at branch junctions. Beneath the stroma are immature colonies of the native Scots pine woolly adelgid, *Pineus pini,* which feeds on the tree, initiating wounds. These wounds are then invaded by a longstanding fungal pathogen of pine, *Crumenulopsis sororia,* which causes the blackened, perennating cankers that expand, killing branches. Thus, we have identified three causative agents involved in this outbreak of canker disease of Scots pine; *C. pithyophila* in association with *P. pini* as the primary agents of bark killing, and *C. sororia* as a secondary invader of the damaged bark.

Using information gained from past literature, and our own morphological and genetic analyses of isolates, we surmise that the populations we assign to *C. pithyophila* in the UK

actually consist of two distinct but co-occurring species, at least one of which may be a recent introduction. Historical reports suggest that previous *C. pithyophila* outbreaks occurred on plantation Scots pine in Perthshire in the 1900s and north-east Scotland in the 1960s, with sporadic findings from the 1970s onwards. The relatedness of our specimens causing the current outbreak to the fungal specimens causing the previous outbreaks is unknown.

## *1.4 Recommendations*

Given the vast potential for inoculum production by all three main agents (*Curreya pithyophila, Pineus pini* and *Crumenulopsis sororia*), and the knowledge gaps that still exist in terms of understanding the drivers and longevity of this outbreak, it is difficult at this time to give a prognosis for the future development of the disease in Scotland or provide clearly informed management guidance. However, our research has demonstrated that the presence of the adelgid is required for colonisation by *C. pithyophila*, that there is almost certainly the potential to introduce the problem to new sites via infested planting material as young trees are susceptible, that weak or stressed trees appear most susceptible, and infestations typically start on the lower branches. We therefore recommend the following actions based on our current understanding of the disease to date, with more informed advice to come pending further research.

- Plant only healthy stock which is visibly free of adelgids or fungal infection.
- Source stock that has been propagated and grown in the UK by a reputable nursery, preferably one that is Plant Healthy certified Welcome to Plant Healthy - Plant Healthy.
- Only plant Scots pine on sites that are deemed suitable for this species. The Ecological Site Classification (ESC) tool can be used to assess site suitability Ecological Site Classification (ESC) - Forest Research.
- Match Scots pine provenance to site to ensure maximum vigour.
- Early brashing to remove susceptible lower branches may help prevent the disease establishing.
- Monitor Scots pine plantings for signs of *C. pithyophila* infestations and report any findings to the Tree Health Diagnostic and Advisory Service of Forest Research via Tree Alert Tree Alert (forestresearch.gov.uk).

## *1.5 Next steps*

Further investigations are required to understand the drivers and timeframe of this widespread outbreak of *C. pithyophila* and *P. pini* on Scots pine in Scotland to allow an accurate prognosis for the future health of this species. These investigations will focus on determining whether one or both forms of *C. pithyophila* in Scotland represent new introductions into the UK or whether climatic factors may have triggered the outbreak. We will also try to determine when the outbreak might have started, through dating of cankers from key sites, including Caledonian pine sites in the east and west Highlands. This will allow an appraisal of the likely future impact on Scots pine.

# 2 Introduction

Scots pine (*Pinus sylvestris*) is a conifer species of significant economic value in Britain being widely planted in commercial forests and new native woodland schemes. Caledonian pine is the name given to the relict populations of endemic Scots pine which colonised Britain from refugia in Europe following the last ice age (Kinloch et al., 1986). These populations are found in the Scottish Highlands and despite their fragmented nature have huge ecological, conservation and cultural value due to the host of native flora and fauna which they support. For this reason, Caledonian pine is regarded as Scotland's most iconic tree species. Recently, however, attention has been drawn to its vulnerability to new biotic health impacts, for example the needle cast pathogen *Dothistroma septosporum* (Piotrowska et al., 2018).

In late 2022, a report was received by the Tree Health Diagnostic and Advisory Service (THDAS) of Forest Research of canker and dieback symptoms on Caledonian pine in the Cairngorm region. A subsequent site visit to the area confirmed the presence of multiple blackened cankers on shoots, branches and stems of Caledonian pine of all ages across a range of sites from Loch Garten to Insh, including Glenmore. Also observed on many symptomatic Scots pine trees was an unusual black stroma-forming fungus encircling shoots and branches apparently in association with the nymph stage of an adelgid species. The widespread nature of the symptoms was concerning but at this stage the factors responsible for causing the cankers, and the potential role and identity of the black stromatal fungus, were unknown.

This project was undertaken to investigate this apparently new health problem on Caledonian pine in response to the 2022 Scottish Plant Health Centre call entitled 'Enhancing preparedness against pests and diseases: plugging evidence gaps for Scotland'. In particular, the following research questions were addressed:

- Are the symptoms of black cankering present on native and non-native Scots pine in regions of Scotland other than the Cairngorm area?
- Can the agent/s responsible for the canker symptoms be identified and described?
- Can historical, ecological and genetic information shed light on the drivers of this new health threat?

# 3 Methods

## *3.1 Survey of Scots pine, sample collection and processing*

A request for samples of Scots pine with black cankers as well as specimens of the unusual black stroma-forming fungus was put out to Scottish Forestry tree health officers, Forestry and Land Scotland staff and other Scottish-based stakeholders, as well as staff based within Forestry Commission England and Natural Resources Wales. Samples were also collected from fourteen Scottish sites by members of the project team. This survey was not therefore restricted to Caledonian pine but included any natural or planted pine in the three home nations. Samples were sent with general site information (e.g. woodland type, approximate tree age), grid reference and collection date details to Forest Research either through the 'Tree Alert' THDAS portal or by direct communication with Forest Research pathologists. Sample collection was carried out between November 2022 and November 2023 (Appendix 1).

Samples, including any associated fungal and insect bodies, were examined under a dissecting microscope and micromorphology of the sporulating structures was assessed with a light microscope. Macro- and micromorphology were recorded and photographed, and measurements of asci, ascospores and conidia were made (minimum of 10 values) and the mean values ($\bar{x}$) are presented. Herbarium specimens were air dried and stored in paper envelopes at room temperature at Forest Research (accession numbers SP23-11 to SP23-76). Pieces of necrotic phloem, cortical and cambial tissues from cankers and visible lesions were plated onto malt extract agar (MEA) and incubated at room temperature for morphological and genetic assessments of developing colonies. For many of the cankers examined in more detail the underlying wood was found to be stained black and, in two samples each from a different site, isolations were attempted from the margin of the black staining.

For specimens of the black stroma-forming fungus, individual fruiting bodies were removed from the stroma, squashed in 10% glycerol solution on a microscope slide using a cover slip and spores observed under a light microscope. The spore suspensions in glycerol were then photographed for record-keeping purposes, spread onto MEA and incubated at room temperature for a few days. Individual germinating spores as visualised under a dissecting microscope were removed from the spread plates using a fine needle, transferred to fresh MEA and incubated at 15 °C to obtain single spore isolates.

## *3.2 Genetic analyses*

### *3.2.1 Fungal isolates*

DNA was extracted from the mycelium of fungal isolates from cankers and from single spore isolates of the black stroma-forming fungus that were actively growing on MEA. Isolates (1-3 per fungal specimen) were subject to PCR amplification of the internal transcribed spacer region of the ribosomal DNA (ITS) which is a DNA barcode region commonly used for fungal identification (Tekpinar & Kalmer, 2019). Up to three isolates from each stroma specimen (Appendix 1) were additionally tested using three further DNA barcode regions commonly used for genetic comparisons among fungal species. These were i) the translational elongation factor 1-alpha (TEF1-α) gene, ii) the β-tubulin (B-tub) gene and iii) the γ-actin gene (actin) (Tekpinar & Kalmer, 2019) (Appendix 1).

PCR amplification of DNA was carried out using the universal ITS1/ITS4 primers for the ITS region (White, 1990), EF1-728F/ EF1-986R primers for the TEF1-α gene (Carbone & Kohn, 1999), TUB2Fd/TUB4Rd primers for the B-tub gene (Aveskamp et al., 2009) and ACT-512F/ACT-783R primers for the actin gene (Carbone & Kohn, 1999). PCR products were verified by visualisation on an agarose gel and cleaned before Sanger sequencing.
Raw sequences were aligned and edited using Sequencher v. 5.4.6 for Windows and searched against published sequences in the GenBank NCBI nucleotide database using BLASTN+

(Altschul et al., 1990). Species identity was based on a 100% or 99% match across the entire sequence length to verifiable sequences derived from voucher specimens or published taxonomic papers. Sequence alignments of fungal isolates from stroma were carried out using MUSCLE Multiple Sequence Alignment tool (MUSCLE < Multiple Sequence Alignment < EMBL-EBI).

### *3.2.2 Adelgids*

Adelgid nymphs were removed from beneath the fungal stroma on three Scots pine samples from i) Assynt, Highland; ii) Bush Estate, Midlothian; and iii) Cumnock, East Ayrshire. DNA was extracted from the nymph bodies and the mitochondrial cytochrome c oxidase subunit I (COI) region amplified using the universal primers, LCO1490/HCO2198 (Folmer et al. 1994). All subsequent steps for PCR verification, clean-up, sequencing and sequence analysis were as described above for the fungal isolates.

## *3.3 Temperature growth-rate studies of* Curreya pithyophila

Isolates from eight specimens were included in the growth studies (Appendix 1). For each specimen three single spore isolates were used with three replicates per isolate. The experiment was set up by plating 5 mm diameter mycelial plugs cut from the margin of actively growing colonies onto MEA at the centre of cross hairs marked on the bottom of each plate. Plates were incubated at 5, 10, 15, 20 and 25 °C for eight weeks and colony diameter measured weekly. At the end of the experiment mean daily growth rate was calculated for each isolate.

# 4 Results

## *4.1 Survey of Scots pine*

Samples of Scots pine containing cankers and the black stroma-forming fungus harbouring adelgids were collected or received from 31 sites across Scotland (Figure 1) plus one site in Devon, England. Canker symptoms and branch dieback were most frequently observed starting in the lower crown and appeared to progress upwards into the mid-crown area. Sampled sites comprised native Caledonian pine forests (7) where trees of all ages were found to be affected, new native woodland plantings dating back to the 1990s and early 2000s (9), young to thicket-stage commercial plantations (12) as well as amenity and shelterbelt trees (3) of approximate ages ranging from 8-50 years (Figure 1). Some of the worst affected sites in terms of visual prevalence of symptoms were new native woodland grant scheme plantings in wet, western, upland areas. At some sites, including Caledonian pine sites, native woodland plantings and commercial plantations, a small number of weak or suppressed young trees with severe cankering were killed.

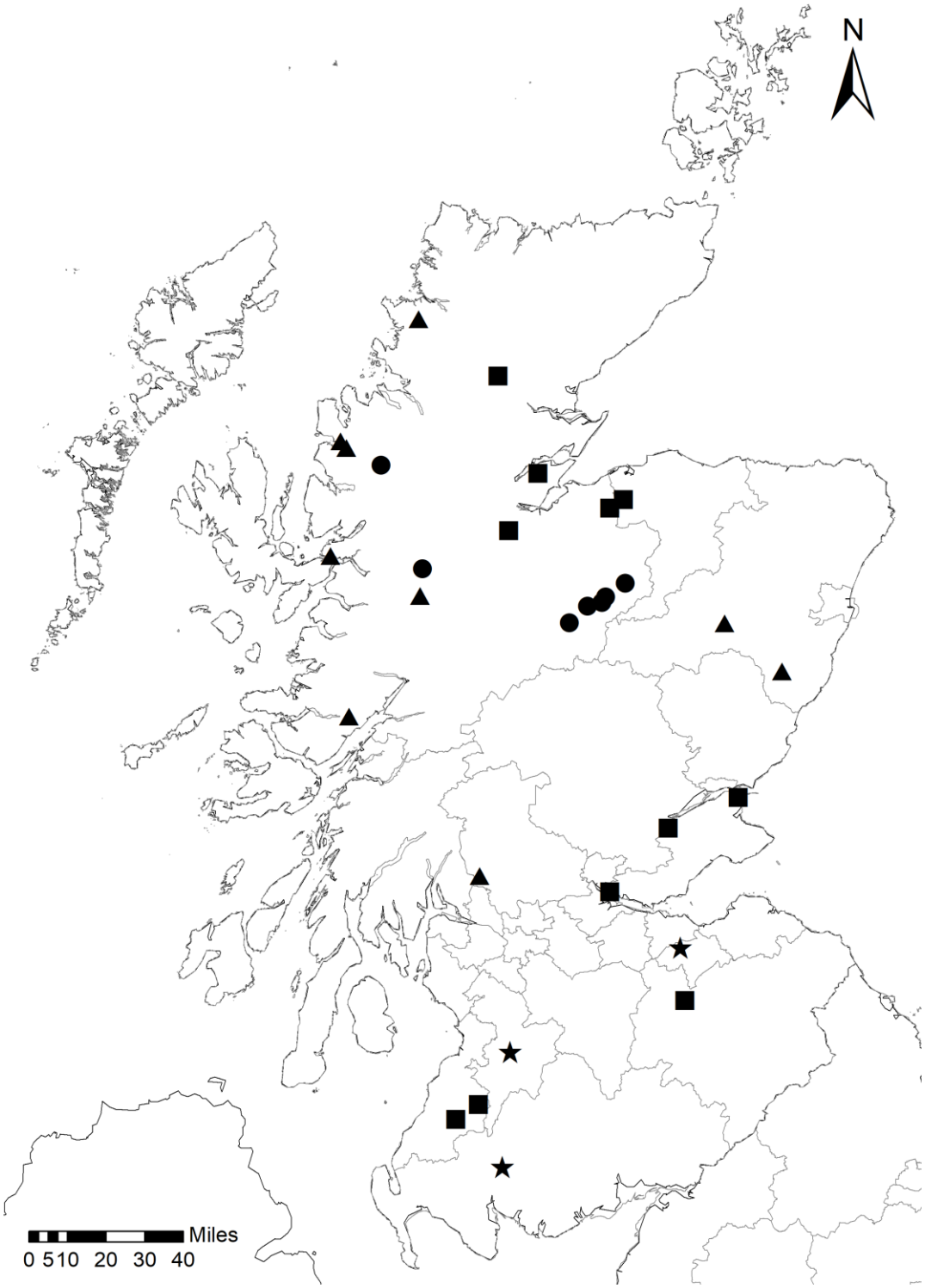


*Figure 1. Location of 31 sites in Scotland from which samples were received of Scots pine containing the black stroma-forming fungus and adelgids (subsequently identified in this study as* Curreya *pithyophila and* Pineus pini*). Symbols represent Caledonian pinewood (circle), native woodland planting (triangle), commercial plantation (square) and amenity/shelterbelt planting (star).*

Typical symptoms included blackened cankers on shoots and branches, with many samples displaying multiple cankers on a single branch, which were often located at shoot junctions (Figure 2). These cankers varied in depth from a few millimetres (mm) to half the thickness of the affected branch or shoot with exposure of the underlying wood. The cankers were predominantly found in the lower to mid crown region of the affected trees, many of which

appeared to be suffering progressive branch dieback from the lower crown upwards. Cankers on samples from five different sites were found to contain fruiting bodies and spores of the canker-causing pine pathogen *Crumenulopsis sororia* (CABI, 2009).

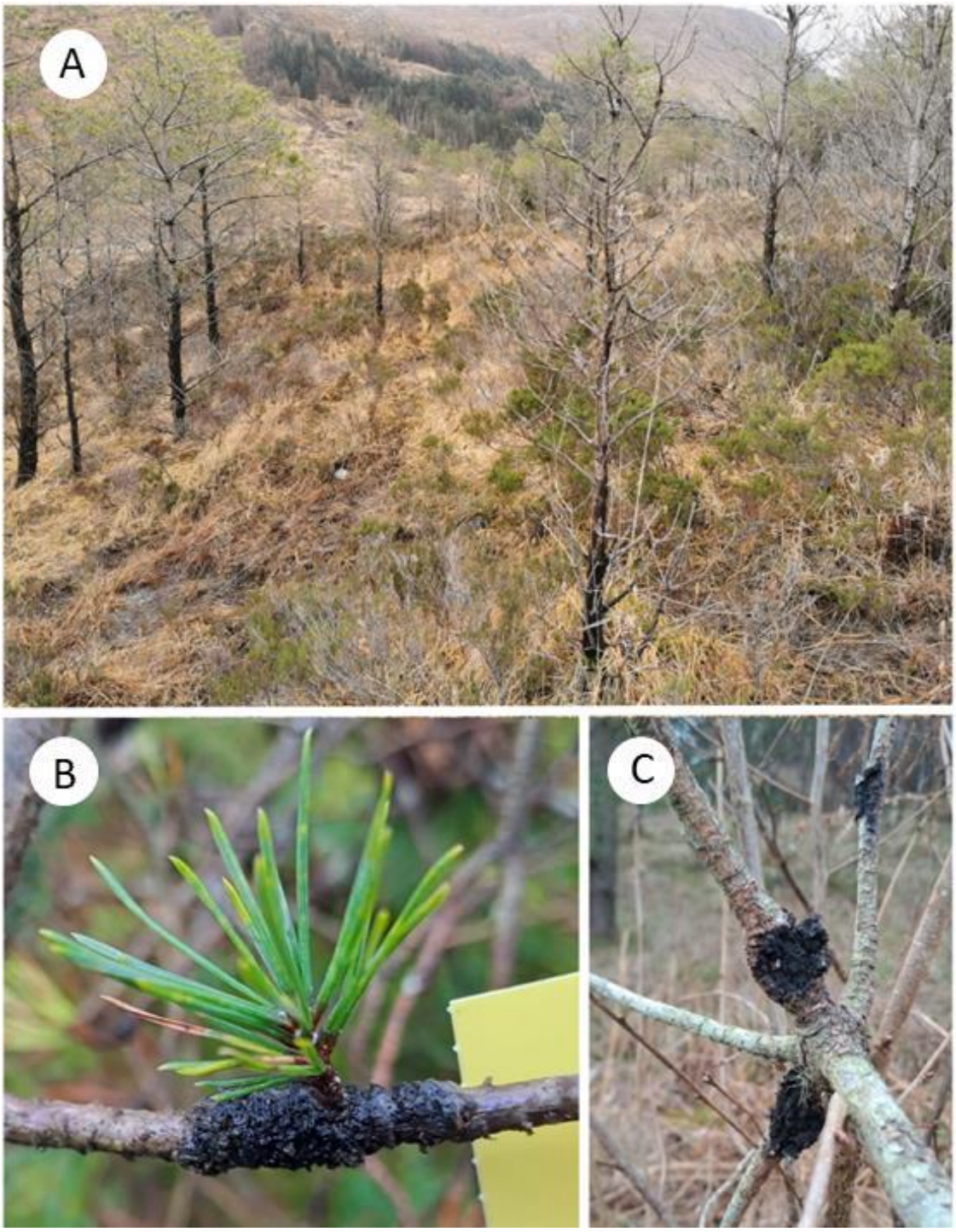


*Figure 2. A, symptoms of dieback in Scots pine infested with black stroma and cankers. B, black fungal stroma encircling shoot. C. typical multiple, blackened cankers.*

From the 31 sampled sites, a total of 76 herbarium specimens of the black stroma-forming fungus were obtained. In all specimens, the fungus was found to be growing over adelgid nymph colonies. The fungus was never observed in the absence of the adelgid. Correspondence with other stakeholders, including mycologists and naturalists with decades-long history of studying fungi in Scotland, including a longstanding specialist in micro fungi on Scots pine,

failed to yield an identity for, nor indeed any recognition of, this fungus. A report on the Assynt Field Club website by local photographer David Haines Rare Assynt record of fungus Chaetosphaeria myriocarpa - Assynt Field Club (assyntwildlife.org.uk) revealed that the same fungus, wrongly identified at the time, had been present on a small number of planted Scots pine at Little Assynt in 2014. Otherwise, no information could be gleaned from local experts or other forestry stakeholders about past findings. Herbarium specimens resulting from this survey were maintained at Forest Research for subsequent examination, sequencing and identification as detailed below.

## *4.2 Description of the black stroma-forming fungus, its association with adelgid nymphs and impact on the host*

The stroma initially develops at the base of needles (dwarf shoots) and at branching junctions of long shoots of Scots pine. It is possibly initially associated with scale leaves and bracts. Stroma can also form on the main stems. The stroma expands longitudinally and tangentially, forming a cylindrical stroma that girdles the shoots and branches (Figure 2) often resulting in dieback of the distal portion of the tree. When girdling happens on the main stems of young trees the dieback can affect all tree parts above the stroma. On larger diameter stems the stroma will often only develop as patches on one side.

The stroma of the fungus has a 'caviar' appearance (Figures 3 and 4) caused by the spherical fruiting structures of the fungus embedded in the stroma. These include both sexual structures (ascomata) and asexual (conidiomata). Consistently observed beneath the fungal stroma at its expanding margins are dense clusters of live adelgid nymphs located between the fungal stroma and the inner bark of the tree (Figure 3). Away from the expanding stroma margins, but still encapsulated within it, the nymphs are dead and appear shrivelled and dark. The adelgids are parthenogenic and propagate beneath the expanding stroma. It is assumed that the fungus thrives on the honeydew extruded by the adelgids and possibly on the dead adelgids. Of note is the fact that the fungus is never observed without the adelgid, but free living adelgids are frequently observed without the fungus in tufts of white woolly wax at needle bases.

Necrosis is observed in the tree's tissues underlying each stroma (Figure 3), often down to the cambium. Attempts to isolate fungi from necrotic phloem, cortical and cambial tissues were unsuccessful suggesting that the death of the host tissues was caused by the adelgid feeding and not by the fungus which itself only grows between the inner and outer bark layers (Figure 4) and is not pathogenic. The action of the adelgids causes the host tissue to callous and distort in the proximity of the stroma. The stroma can be entirely fungal or is often partially covered in disrupted layers of thin bark. If the adelgids die below the stroma, the stroma also dies and falls off.

Despite its conspicuous nature it was very difficult to identify the stroma-forming fungus due to its lack of coverage in the mycological and tree pathology literature. Records of this fungus and its association with adelgids in the UK are scant. There are brief records in the standard UK forest pathology and mycological textbooks (Peace, 1962; Ellis & Ellis, 1997) and websites (https://fungi.myspecies.info/all-fungi/cucurbidothis), with some reports in the Fungal Records Database of Great Britain and Ireland (FRDGBI). However, we concluded it was the ascomycete species *Curreya pithyophila* due to the conspicuous nature of the stromata, the unique association with the adelgids, and the corresponding micromorphological features such as the dimensions and morphology of ascospores (20.8 × 6.9 µm) (Murray & Parry, 1969) (see section 4.4 *Morphology and taxonomy of UK specimens*). To better understand this fungus, a comprehensive literature review was undertaken, by identifying all previous names (synonyms) and finding all the references associated with these names using Index Fungorum (https://www.indexfungorum.org/) and Google Scholar. This review revealed three previous

published records of its occurrence in the UK (McIntosh, 1915; Holm, 1967; Murray & Parry, 1969). The literature review is detailed in chronological order below.

## 4.3 *Literature review*

The fungus that is currently known as *Curreya pithyophila* was first described in **1823** (Fries, 1823) on the living trunk and branches of *Pinus sylvestris* during the spring in Germany and named *Sphaeria pithyophila* J.C. Schmidt & Kunze [as '*pityophila*']. Only the stroma symptoms were described, with no discussion of the adelgids or spore descriptions. The taxonomy of this fungus has been very confused due to the presence of two spore 'types' with differences recognised in the literature as variations within a species, or as two different formal varieties. Spores are brown and have cross walls called septa. The spores are described as dictyospores when they have both transverse (horizontal) and vertical (longitudinal) septa (e.g. Fig. 3J) and phragmospores when they only have transverse (horizontal) septa (e.g. Fig. 4J, I, F, K).

In **1863** there were two treatments of this fungus both giving it different names; *Cucurbitaria pithyophila* (J.C. Schmidt & Kunze) De Not. (phragmospores illustrated) (De Notaris, 1863) and *Diplodia pityophila* (J.C. Schmidt & Kunze) Fuckel. Fuckel (1863) then reported the fungus as being very rare on dry branches of pine in the autumn and described the spores as 'septate'. Neither author mentions adelgids and it is quite feasible that this is because specimens were studied from dry branches where the adelgids would have died, and become unrecognisable, due to lack of resources. In **1873** *Cucurbitaria pithyophila* var. *cembrae* Rehm was described (Rehm, 1873) representing the phragmospore variety to distinguish it from the dictyospore type. In addition, this description is the first to mention the asexual stage which was described as *Phragmotrichium* (Rehm, 1873).

The first probable UK record of this fungus was in Wales in **1876** when the fungus *Sphaeria parmeliarum* W. Phillips & Plowr. was described as a parasite of the lichen *Parmelia sexatilis*, on a living 'spruce fir', which was possibly Norway spruce (*Picea abies*). In none of these descriptions was the presence of adelgids noted and neither by Holm (1967).

In North America in **1876** this fungus was described on *Pinus strobus* in Massachusetts under the name *Melogramma spraguei* Berk. & M.A. Curtis and synonymised in the same year as *Thyridium spraguei* (Berk. & M.A. Curtis) Sacc., although neither of these names are included in the list of synonyms for *Curreya pithyophila* (Holm, 1967).

Cavara (**1897**) recorded *Cucurbitaria pithyophila* var. *cembrae* on *Abies* in Italy, attributing the callousing (hypertrophy) to the fungus but not mentioning the insects (Cavara, 1897). The description of symptoms of dieback on the *Abies* trees were very similar to those described on Scots pine in our study and include the subsequent formation of cankers and the infestation of these by wound pathogens. Cavara (1897) indicated that the disease occurred on young and old firs both at altitude and lower levels in the Apennines.

The first record of this fungus in Scotland occurs in **1907** when McIntosh (1915) found the disease on Scots pine plantations in Perthshire. The affected trees were assumed by the author to have originated from 'foreign seed' due to their uncommon growth form and were located near Inver on the Dunkeld Estates. There was no mention of adelgids here and no micromorphological details.

In **1921** the name *Cucurbidothis pithyophila* (J.C. Schmidt & Kunze) Petr., was given to this fungus by Petrak (1921) when the new genus *Curcubidothis* was introduced. No mention was made of the spore types or of the adelgids. The first time that adelgids ('Chermes') were noted were on collections made in North America in the **1920**'s and **30'**s (see GBIF using the search term *Cucurbitaria pithyophila*). These were examined on pine hosts by Boyce (**1952**) who referred to the fungus as *Cucurbitaria pithyophila* (after *Cucurbitaria* was treated by Welch

in 1929 and *Cucurbidothis* synonymised) and who was the first to note the association with adelgids, describing the fungus as 'entomogenous'. Again, no mention is made of the spore types.

In Europe, Franz (**1955**) recorded the phragmosporus form of this fungus as *Cucurbitaria pithyophila*, on the bark of 'fir' (*Abies alba*) associated with the adelgid *Adelges piceae*. This was the first published record of the fungal/adelgid association in Europe. Peace (**1962**) mentions the McIntosh (1915) report of *Cucurbitaria pithyophila* on Scots pine in Scotland and states that 'it has attracted little attention in Britain since'. This author cites Boyce (1952) in reference to the reported association with 'scale insects' on *P. monticola* in the USA and suggests that the fungus may 'behave similarly in Britain but is not regarded as a serious source of damage'. Peace (1962) also reports *Cucurbitaria pithyophila* as occurring on silver fir (*Abies alba*) and 'other conifers' in Britain but offers no source of reference.

The most comprehensive discussion on the taxonomy of *Cucurbidothis pithyophila* was by Holm (**1967**), much of which has already been discussed. Significantly, at no point does Holm (1967) mention the adelgids, even though there are by now two published records of the adelgid association (Boyce, 1952; Franz, 1955). Holm (1967) notes the different spore sizes as well as the different spore morphology of the two types and outlines how the two types should be recognised as two varieties of *Cucurbidothis* with the dictyosporus *Cucurbidothis pithyophila* var. *pithyophila* (J.C. Schmidt & Kunze) Petr and the phragmosporous *Cucurbidothis pithyophila* var. *cembrae* (Rehm) L. Holm.

Murray & Parry (**1969**) described the dictyosporus form of the fungus ('*Cucurbitaria pithyophila*') and its association with adelgids (*Pineus pini*) on Scots pine in Scotland with 'many cases' in Deeside and Morayshire. Simultaneously, Casagrande (1969) published the most detailed study to date on the biology of the fungus, in addition to a survey on Scots pine, and its geographical distribution. Casagrande (**1969**) considers that there is only one species and states that the description needs to be updated to include both spores with transverse and longitudinal septa.

Takahashi & Saho (**1972**) report the dictyosporous form of *Cucurbitaria pithyophila* on various species of plantation and native pines and fir in Japan, describing the disease symptoms on fir as common on young trees, especially in cold regions. No mention of adelgids is made.

## *4.4 Morphology and taxonomy of UK specimens*

The fungus is identified as *Curreya pithyophila* (J.C. Schmidt & Kunze) Arx & E. Müll., Stud. Mycol. 9: 80 (1975) with the following position in classification: *Cucurbitariaceae, Pleosporales, Pleosporomycetidae, Dothideomycetes, Ascomycota, Fungi*. Occurring on living branches and bark of species of *Pinaceae* in Europe, North America and Asia. Always associated with adelgids (woolly aphids, family *Adelgidae* order *Hemiptera*) (Casagrande, 1969).

Spore type could be determined for *C. pithyophila* samples from 27 of the 31 sites, with dictyospore-only specimens found at seven sites, phragmospore-only specimens found at thirteen sites and both dictyospore and phragmospore specimens found at a further seven sites. There was no indication that geographical location of collection determined spore type, and it should be noted that the surveys were not exhaustive, and that further sampling may yield both spore types at more sites. When found at the same sites, dictyospore and phragmospore specimens were often found on the same trees and even on the same branches. For both spore types the sexual fruiting bodies known as ascoma (plural; ascomata) are around 500 µm in size, black, smooth, often partially embedded in the stroma. Separate descriptions are given for the two spore types below.

The dictyospore type (Figure 3) has cylindrical asci (spore sacs) (136.5 × 10.5 µm) containing 8 sexually produced ascospores which are mid-yellowish brown in colour with 3-6 transverse septa and 1 or 2 vertical septa mainly in the middle cells, but sometimes additionally in other cells. Ascospores (19.5 × 8.5 µm) are constricted at the septa, smooth and partially overlapping in the asci, forming germ tubes mainly from the end cells. The fruiting bodies (conidiomata, plural or conidioma, singular) of the anamorph (asexual form), which produce spores known as conidia, are observed on the stroma tightly clustered between ascomata or sometimes dominating smaller stroma. Conidia are round to slightly ellipsoidal, smooth, thin walled, mid-yellow brown (4.6 × 3.6 µm) and germinate with a single germ tube (Figure 3). Colonies derived from ascospores growing on MEA at 15°C in the dark reach 40 mm diameter after 56 days (a growth rate of 0.7 mm/day). Colonies derived from conidia are slightly slower growing (0.65 mm/day). Colonies are various shades of grey occurring often in concentric rings, often paler in the centre, felty and dense. When viewed from below the colonies are cream coloured becoming dark brown in the centre (Figure 5).

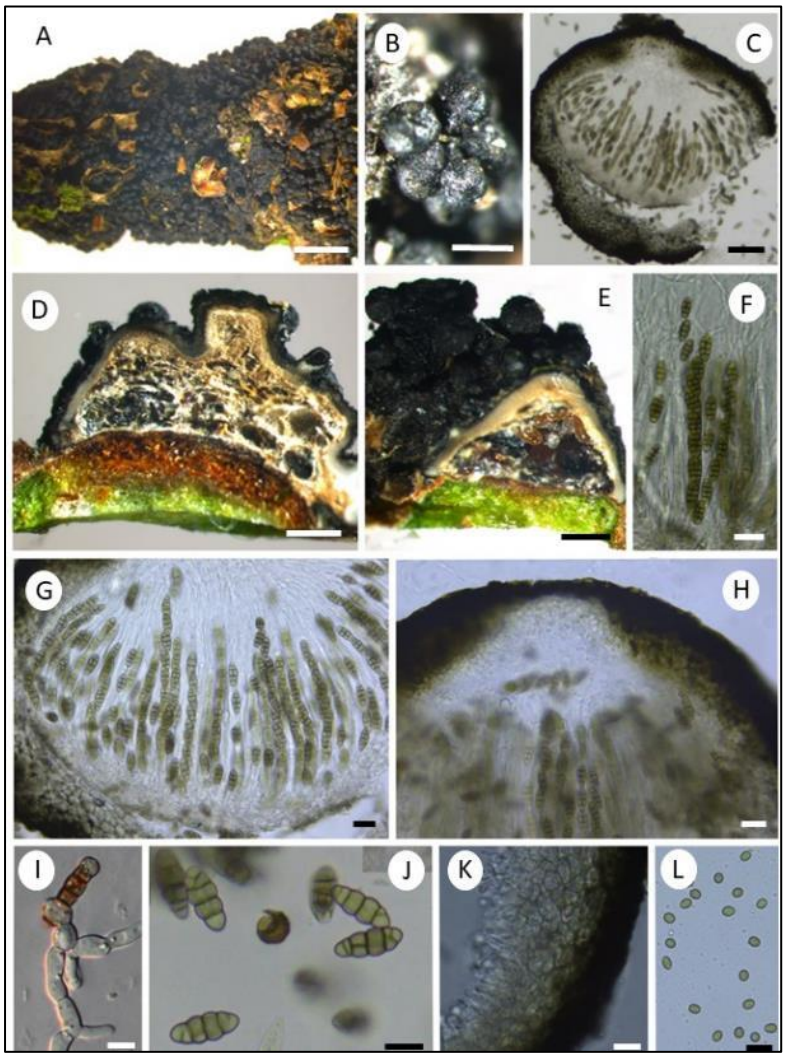


*Figure 3.* Curreya pithyophila *dictyospore type. A, stroma on Scots pine branch. B, ascomata; C, section of ascoma showing asci in which the spores are formed. D, section through stroma in the area where the tissue is calloused and the adelgids are dead. E, section through expanding edge of the stroma with live adelgids and no evidence of callousing. F, G, formation of asci and ascospores within ascoma. H, section through ascoma showing ostiole through which spores are exuded. I, germinating ascospore. J, dictyosporous ascospores. K, conidioma wall and cells. L, conidia. Scales: A = 4 mm; B, E = 500 µm; C = 50 µm; D = 1 mm; F–H = 20 µm; I–L = 10 µm.*

The phragmospore type (Figure 4) has cylindrical asci (159 × 9.8 µm) containing eight ascospores which are mid yellowish brown (21.2 × 7.4 µm), with 1-4 transverse septa and slight constrictions at septa. Ascospores form germ tubes from each cell. Conidiomata of the asexual anamorph form are also observed among ascoma on the stroma and produce round to slightly ellipsoidal conidia which are smooth, thin walled, mid yellow brown (6.6 × 4.3 µm) and germinate with a single germ tube. Colonies derived from ascospores growing on MEA at 15°C in the dark reach 40 mm diameter after 56 days (0.7 mm/day). Colonies derived from conidia are slightly faster growing (0.75 mm/day). Colonies are peach coloured, often slightly paler towards the centre, felty to woolly. When viewed from below the colonies are peach becoming yellow-brown in the centre (Figure 5).

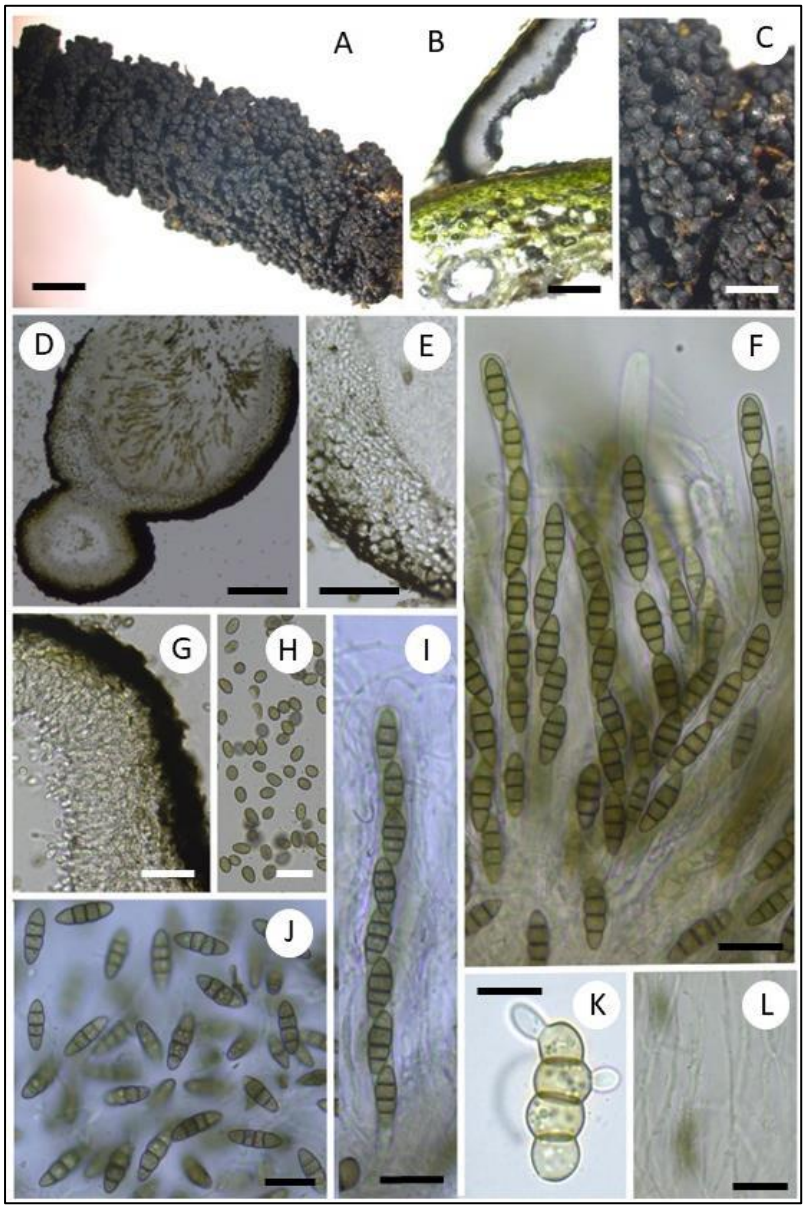


*Figure 4.* Curreya pithyophila *phragmospore type. A, stroma on Scots pine branch. B, section through the expanding edge of the stroma showing no evidence of penetration of the host tissues by the fungus. C, ascomata. D, section through and ascoma and adjacent conidioma. E, section though the ascoma wall. F, I & L, asci and surrounding tissues. G, conidioma wall and cells. H. conidia. J, phragmosporous ascospores. K, germinating ascospore. Scales: A = 4 mm; B, D = 100 µm; C = 2 mm; E = 50 µm; F, G, I, J, L = 20 µm; H, K = 10 µm.*

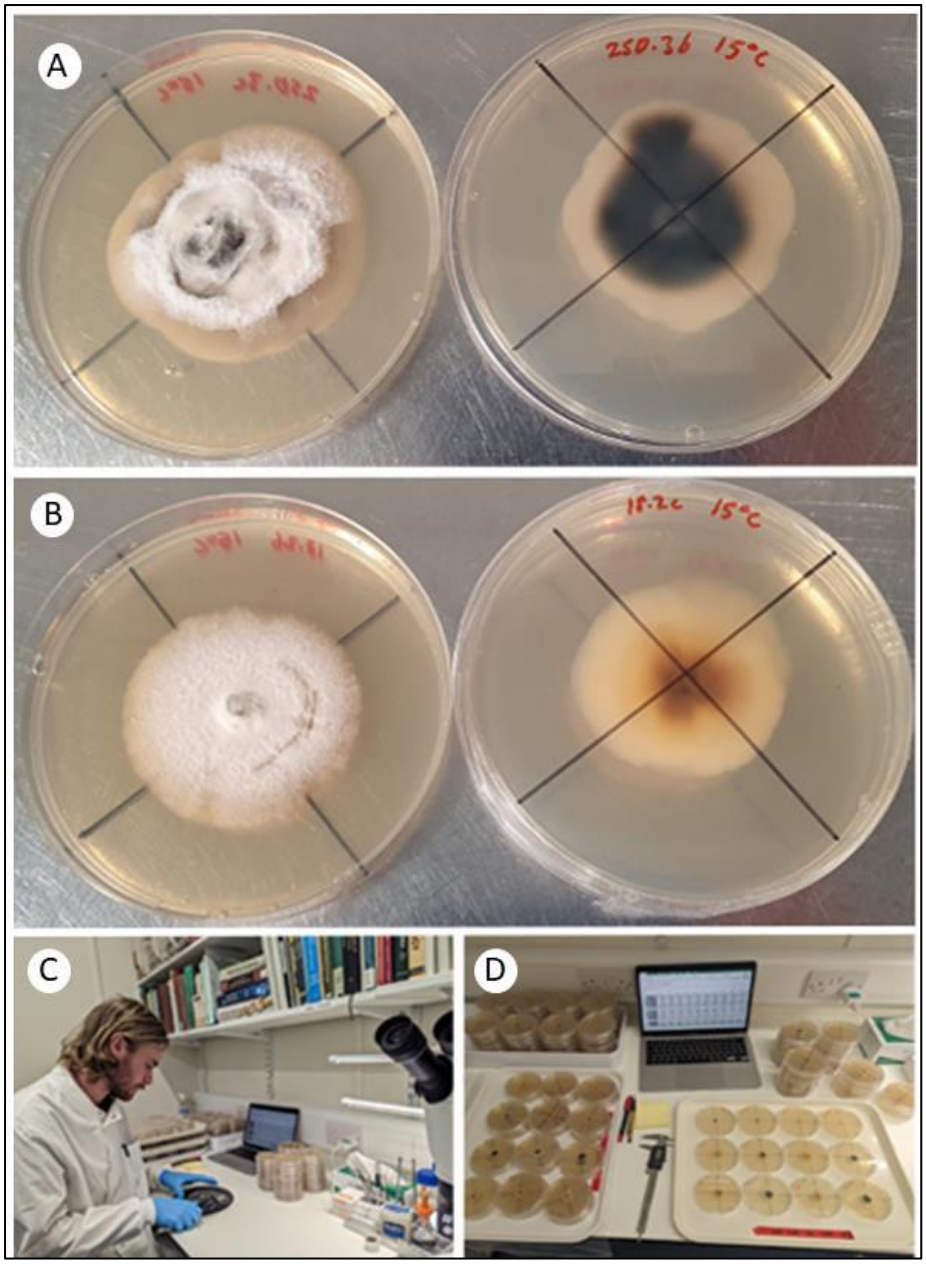


*Figure 5. A & B.* Curreya pithyophila *culture morphology after 8 weeks at 15°C in the dark. A, dictyospore type. B, phragmospore type. C & D, growth study experimental set up.*

## *4.5 Genetic analyses*

### *4.5.1 Curreya pithyophila*

ITS sequences from phragmospore and dictyospore specimens of *Curreya pithyophila* collected in this study (Appendix 1) were 603 base pairs (bp) in length with the closest match in the GenBank NCBI nucleotide database being the 'type' material of a species recently described from China, *Alloleptosphaeria shangrilana* (Thiyagaraja et al., 2021), which had 94% identity (534 identical nucleotides out of a 567 bp alignment) and 95% identity (534 identical nucleotides out of a565 bp alignment) to UK phragmospore and dictyospore 'types', respectively.

ITS sequences were obtained for 22 UK phragmospore specimens. Isolates from 21 phragmospore specimens conformed to a single dominant sequence, whereas an isolate from phragmospore specimen SP23-48 (Appendix 1) differed by one bp. ITS sequences were

obtained for 14 dictyospore specimens (Appendix 1). Ten dictyospore specimens conformed to a single dominant sequence. For two dictyospore specimens (SP23-53D and SP23-53A; Appendix 1), one isolate per specimen differed from the dominant sequence by the same two bp whereas the other two isolates of each specimen were identical to the dominant sequence. All three isolates of specimen SP23-71 also had this sequence (Appendix 1). Additionally, all three isolates of specimen SP23-25 (Appendix 1) differed from the dominant sequence by another one bp. UK phragmospore and dictyospore ITS 'dominant type' sequences differed at six positions across 603 bp.

TEF1-α sequences were obtained for five phragmospore specimens and seven dictyospore specimens (Appendix 1). Three phragmospore specimens had the same sequence whereas the other two sequenced specimens (SP23-45P and SP23-48, Appendix 1) differed by the same one bp. All dictyospore TEF1-α sequences were identical. Phragmospore and dictyospore TEF1-α sequences differed at 11 positions across 242 bp.

B-tub sequences were obtained for seven phragmospore specimens and all were identical, as were all seven dictyospore specimens (Appendix 1). Phragmospore and dictyospore B-tub sequences differed at 21 positions across 348 bp. Actin sequences were obtained for two phragmospore specimens and three dictyospore specimens, with sequences identical within each spore type. Phragmospore and dictyospore actin sequences differed at 6 positions across 270 bp.

### 4.5.2 *Fungi isolated from cankers*

Fungal isolates from cankers were identified based on their closest ITS sequence matches in the GenBank NCBI nucleotide database. These included four isolates of the canker-causing pathogen *Crumenulopsis sororia* which were obtained from black stained wood in two samples from two different sites. Other fungi isolated included *Crumenulopsis pinicola, Heterotruncatella spartii, Therrya pini, Microsphaeropsis olivacea, Coniothyrium lignorum* and *Sarea difformis* which are all either endophytic, saprobic or resinicolous fungi and therefore not considered important in the disease.

### 4.5.3 *Adelgids*

COI sequencing of both single and multiple adelgid samples yielded 620 bp of clean sequence. There were two single base differences in sequences which varied across the three sites. The closest match in the GenBank NCBI nucleotide database was to a *Pineus pini* (pine woolly adelgid) voucher specimen, with 100% identity over 618 bp. There were also some matches at the same level of identity with *Pineus orientalis* (the spruce-pine adelgid) although genetic studies indicate that *P. pini* and *P. orientalis*, which are morphologically indistinguishable, are likely to be the same species adapted onto different hosts (Havelka et al., 2021).

## *4.6 Temperature growth-rate studies of* Curreya pithyophila

All isolates grew fastest at 15 °C (Figure 5). Growth was minimal at 5 °C and was inhibited at 25 °C. The isolates at 5 °C resumed growth after returning them to 15°C whereas those that were incubated at 25 °C were dead and did not grow. Dictyospore isolates showed the widest range of growth rates at 15 °C (0.64–0.82 mm/d) and less so at 10 °C (0.41-0.59 mm/d), whereas at 20 °C phragmospore isolates were more variable (0.44-0.71 mm/d). The growth rates of the anamorphs, 32A(P) and 25A(D), showed no consistent pattern (Figure 6).

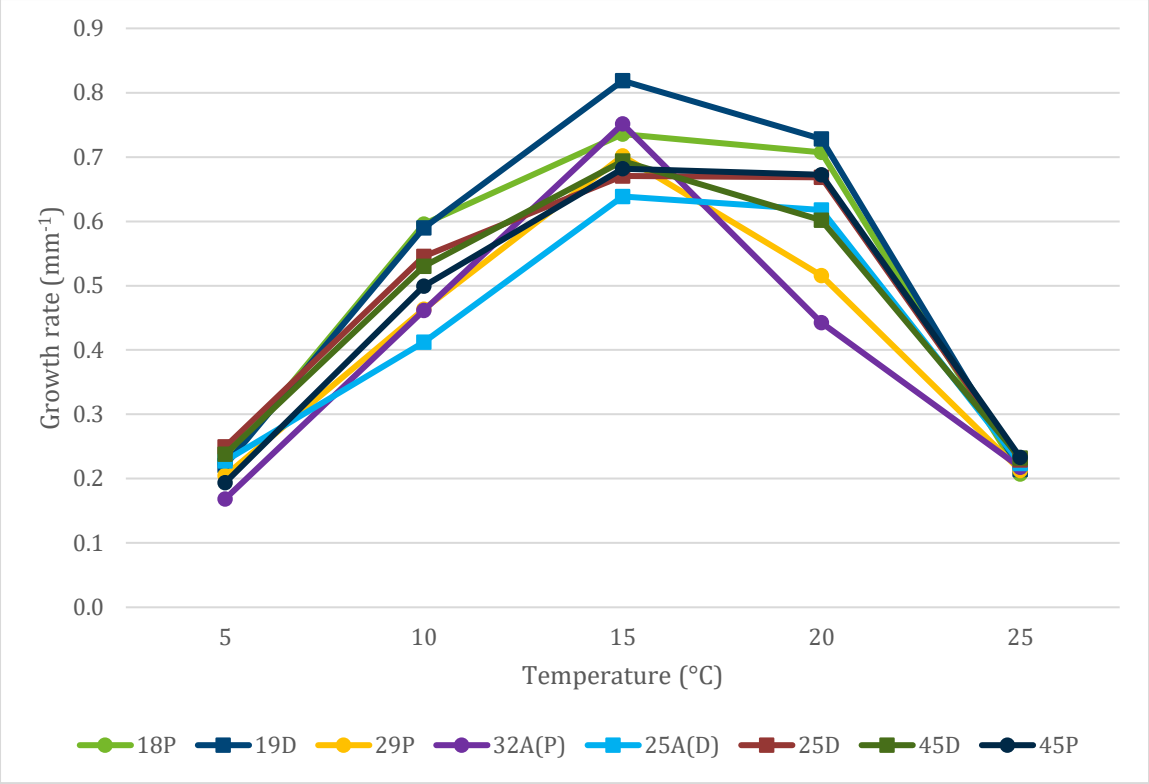


*Figure 6. Mean daily growth rate (mm) of* Curreya pithyophila *phragmospore (P) isolates, dictyospore (D) isolates and their anamorph (A) isolates after 8 weeks at different temperatures.*

# 5 Discussion

The main finding from this study was the identification of a previously rare (Cannon, 2011) stroma-forming fungus currently known as *Curreya pithyophila* as a primary causal agent of cankers on Scots pine. This is due to its fostering of damaging populations of the Scots pine woolly adelgid, *Pineus pini,* which initiates feeding wounds that are subsequently colonised by a common canker-causing pathogen, *Crumenulopsis sororia*. Given that *P. pini* and *C. sororia* are longstanding associates of Scots pine in the UK, it is *C. pithyophila* which appears to be driving this current outbreak of disease.

UK isolates of *C. pithyophila* had no close DNA sequence match in the GenBank NCBI database. The 94-95% match to *Alloleptosphaeria shangrilana,* a new species described from dead wood in China (Thiyagaraja et al., 2021), is not sufficiently close to be considered the same species. The fact that no DNA sequences exist in GenBank for this remarkably visible fungus illustrate just how obscure it has remained over the last two decades since the advent of routine DNA barcode sequencing.

Results from a detailed taxonomic study with investigations into the behaviour of the fungus in culture at different temperatures were compared with the findings of previous research (Franz, 1955; Holm, 1967; Casagrande, 1969; Murray & Parry, 1969). Our isolates grew similarly to those of Casagrande (1969) who undertook growth studies with dictyospore isolates indicating the optimal temperature for growth is 18-21 °C with no growth after 27 °C. We also found the two ascospore types to differ not only in the spore morphology, but also in asci and ascospore dimensions and culture characteristics. Our work supports the designation of the dictyospore and phragmospore types as two species, rather than just varieties (Holm, 1967) or a single species with variable spore morphology and culture characteristics (Casagrande, 1969).

The known geographical distribution of specimens assigned to *C. pithyophila* has been extended to include many new sites in the south-west, north-west, east and central belt of Scotland (e.g. Fig. 1). Although this fungus has apparently been observed previously on spruce and fir in the UK (Peace, 1962; Holm, 1967), all our findings to date have been on Scots pine. In the UK outside of Scotland this fungus, or a fungus similar in morphology, has previously been reported in Wales (on *Piceae* see Holm, 1967), north-east England (Copley, FRDBI Record No.: 1221008; ABFGID 383090, on *Picea*) and in Devon (the present study, on *Pinus*). For such a distinctive fungus, all previous reports are very sparse with records only from the years 1879 (see Holm, 1967), 1907 (McIntosh, 1915), 1969 (Murray & Parry, 1969) and then subsequently just 10 records from the BMS Fungal Records database and Fungus Conservation Trust CATE database (1977, 1980, 1986, 1996 and 2004). Anecdotally we have noticed that the fungus is more likely to occur in sites that experience high moisture levels, which also promote lichen growth. The effect of site conditions and climate on prevalence of *C. pithyophila* is an avenue for further investigation.

DNA sequencing of single spore isolates from UK phragmospore and dictyospore specimens of *C. pithyophila* revealed significant genetic differences between spore types. The ITS region is regarded as the standard DNA barcode for ascomycetous fungi due to its high level of variability between species and its multicopy nature within the genome which makes it easy to amplify (Tekpinar & Kalmer, 2019). The extent of genetic differentiation between phragmospore and dictyospore types across the ITS region was backed up by further substantial differences across the three protein coding genes (TEF1-α, B-tub and actin). Although there is no standard sequence divergence for delineating fungal species (Xu, 2020), the genetic differences encountered here, together with observed differences in spore shape and colony morphology, provide strong evidence to support the taxonomic reclassification of phragmospore and dictyospore types of *C. pithyophila* as two different species, as was proposed by Holm (1967). A formal taxonomic treatment, including phylogenetic analysis, will be the subject of a future publication.

There was less genetic variation among isolate populations within each spore type, which is unsurprising as the four loci are known for providing interspecific rather than intraspecific resolution (Tekpinar & Kalmer, 2019). Although intraspecific ITS sequence variation does exist within fungi, its estimated rate in ascomycete fungi (based on a search of 2509 species within the GenBank NCBI nucleotide database) was less than 2% (Nilsson et al., 2008). In our study, the ITS region was the most informative locus, revealing one dominant sequence and two variant sequences for dictyospore isolates and one dominant sequence with a single variant for phragmospore isolates.

Most intriguing was the ITS sequence variation observed within dictyospore isolates from a single ascoma. This requires further investigation but may indicate outcrossing (heterothallism) requiring the presence of two mating types. If different mating types of the same fungal species have different ITS sequences, then this might suggest separate evolutionary histories (Berbee et al., 2003) and in this case, the presence of introduced genotypes of *C. pithyophila*. The mating system in our populations of *C. pithyophila* should be investigated further by identifying and sequencing the mating type genes that control sexual reproduction in ascomycete fungi (Santos et al., 2010). More traditional approaches such as isolate crossing and vegetative compatibility tests (Glass et al., 2000; Brasier & Webber, 2013) could also provide useful information on the speciation, ecology and genetic structure of *C. pithyophila*.

A frequent observation in this study was the presence of dictyospore and phragmospore specimens on Scots pine at the same sites, on the same trees and even on the same branches. This raises questions as to whether two apparently distinct species, occupying the same unique ecological niche, would have both evolved undetected on Caledonian pine in the UK. For two separate species to evolve from an ancestral population requires an ecological or geographical

barrier to gene flow between the two sub-populations (Xu, 2020). Murray & Parry (1969) observed only the dictyospore type in the 1960s outbreak on Scots pine in north-east Scotland. The report from the Perthshire outbreak in 1907 does not mention spore type but suggests that the affected Scots pine trees were of foreign origin (McIntosh, 1915). A phragmospore type of this fungus was reported in Wales in 1876 (Holm, 1967) but not on Scots pine. Based on the evidence gained so far, we infer that one, or both, of these fungi may represent recent introductions into the UK. Studies of the genetic variation within UK phragmospore and dictyospore populations using genomics tools are proposed, since whole-genome sequencing sets a much better standard than individual loci for defining species boundaries (Matute & Sepúlveda, 2019; Xu, 2020). Whole genome sequencing will also help to unravel the population structure and dynamics of *C. pithyophila* and should indicate how long each population has been evolving for in the UK. Additionally, genetic analyses of global herbarium specimens of *C. pithyophila* on different conifer hosts could provide significant insights into where our specimens are likely to have evolved.

Of obvious concern is the impact that this new health threat is having on Scots pine, how long has it been going on for, and what can be done to minimise the effects? Our study has focused on *C. pithyophila* and its association with *P. pini* as the primary drivers of disease. However, *C. sororia,* described by Ennos & Swales (1987) as a facultative wound pathogen of Scots pine, is ultimately responsible for the perennating cankers that cause branches to die and the apparently progressive dieback from the lower crown upwards. McIntosh (1915) reported that the infestations resulted in branch killing, as did Murray & Parry (1969) who also concluded that the infestations had no economic significance being limited to the lower branches of Scots pine. Neither of these previous reports described the secondary role of *C. sororia* in the observed symptoms.

The current, widespread *C. pithyophila*/*P. pini* infestations on Scots pine in Scotland is providing an extensive resource of wound sites for the development of *C. sororia* cankers. We know that *C. pithyophila* was present at Assynt in 2014 but have no further evidence to indicate when or where this wider outbreak might have started in Scotland. Dating cankers through growth ring analysis at a range of sites might provide some insight into this. Examination of recent Scottish climate records and site factors linked to high disease prevalence might help us to understand if the outbreak is also linked to climatic factors.

Given the vast potential for inoculum production by all three main agents, and the knowledge gaps that still exist in terms of understanding the drivers of this outbreak, it is difficult to give a prognosis for the future development of the disease in Scotland or provide clearly informed management guidance. However, this study has shown that the presence of the adelgid is required for colonisation by *C. pithyophila*, that there is almost certainly the potential to introduce the problem to new sites via infested planting material as young trees are susceptible, that weak or stressed trees appear most susceptible, and infestations typically start on the lower branches. Therefore, we offer the following interim recommendations for the management of Scots pine until more information is available pending further research.

- Plant only healthy stock which is visibly free of adelgids or fungal infection (as illustrated in Figure 7).
- Source stock that has been propagated and grown in the UK by a reputable nursery, preferably one that is Plant Healthy certified Welcome to Plant Healthy - Plant Healthy.
- Only plant Scots pine on sites that are deemed suitable for this species. The Ecological Site Classification (ESC) tool can be used to assess site suitability Ecological Site Classification (ESC) - Forest Research.
- Match Scots pine provenance to site to ensure maximum vigour.
- Early brashing to remove susceptible lower branches may help reduce disease establishment.

- Monitor Scots pine plantings for signs of *C. pithyophila* infestations (e.g. Figure 7) and report any findings to the Tree Health Diagnostic and Advisory Service of Forest Research via Tree Alert [Tree Alert (forestresearch.gov.uk)](Tree Alert (forestresearch.gov.uk)).

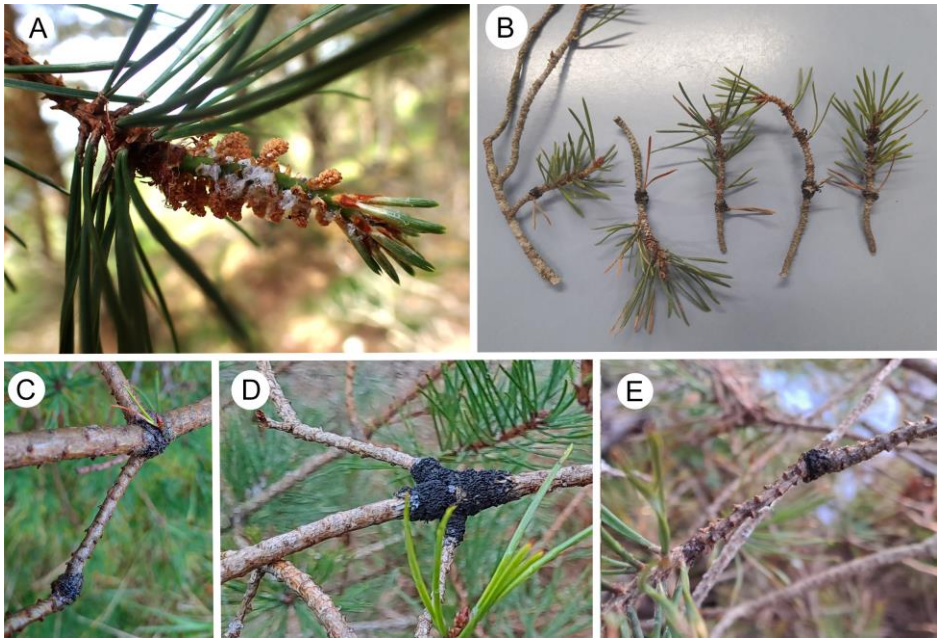


*Figure 7. A. Free-living Pineus pini on Scots pine indicated by white 'woolly' wax deposits often visible at bases of buds/flowers and needles; (B-E) Curreya pithyophila infections on young shoots and branches of Scots pine where the stromata can be small and quite inconspicuous.*

# 6 Conclusions

The primary agents responsible for the symptoms of cankering and crown dieback on Scots pine now prevalent across Scotland are a previously obscure fungus called *C. pithyophila* which associates with the Scots pine woolly adelgid, *P. pini*. It is *P. pini* which directly damages the tree through feeding, with feeding sites subsequently colonised by the wound pathogen *C. sororia,* which in turn causes the blackened cankers that are so disfiguring on Scots pine. *Curreya pithyophila* populations in the UK comprise two genetically and morphologically distinct forms which should be renamed as distinct species. Historical reports suggest that previous *C. pithyophila* outbreaks occurred on plantation Scots pine in Perthshire in the 1900s and in north-east Scotland in the 1960s, with sporadic reports since the late 1970s. The relatedness of our specimens of *C. pithyophila* to the fungal specimens causing the previous outbreaks is unknown. Further investigations are required to understand the drivers behind this current widespread outbreak of *C. pithyophila* and *P. pini* on Scots pine in Scotland. Management recommendations based on our current understanding focus on the planting of 100% healthy and locally adapted stock on sites deemed suitable for Scots pine.

# 7 References


Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*, 403-410.

Aveskamp, M. M., Verkley, G. J., de Gruyter, J., Murace, M. A., Perello, A., Woudenberg, J. H., Groenewald, J. Z. & Crous, P. W. (2009). DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. *Mycologia, 101*, 363-382.

Berbee, M. L., Payne, B. P., Zhang, G., Roberts, R. G. & Turgeon, B. G. (2003). Shared ITS DNA substitutions in isolates of opposite mating type reveal a recombining history for three presumed asexual species in the filamentous ascomycete genus *Alternaria*. *Mycological research, 107*, 169-182.

Boyce, J. S. (1952). *Cucurbitaria pithyophila*, an entomogenous fungus. *Plant Disease Reporter, 36*, 62-63.

Brasier, C. & Webber, J. (2013). Vegetative incompatibility in the ash dieback pathogen *Hymenoscyphus pseudoalbidus* and its ecological implications. *Fungal Ecology, 6*, 501-512.

CABI. (2009). Compendium datasheet on *Crumenulopsis sororia*. https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.16278 (accessed December 2023).

Cannon, P. F. (2011). *Cucurbidothis pithyophila* var. *pithyophila*. Fungi of Great Britain and Ireland. https://fungi.myspecies.info/taxonomy/term/4692/descriptions (accessed 12th December 2023).

Carbone, I. & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia, 91*, 553-556.

Casagrande, F. (1969). Ricerche biologiche e sistematische su particolari ascomiceti pseudosferiali. *Phytopathologische Zeitschrift, 66*, 97–136.

Cavara, F. (1897). Über eine neue Pilzkrankheit der Weisstanne. *Cucurbitaria pithyophila* (Kunze) De N. *Zeitschrift für Pflanzenkrankheiten, 7*, 321-325.

De Notaris, G. (1863). *Comm. Soc. crittog. Ital. , 1(fasc. 4)*, 214.

Ellis, M. B. & Ellis, J. P. (1997). *Microfungi on Land Plants. An Identification Handbook*. Slough, UK: The Richmond Publishing Co. Ltd.

Ennos, R. & Swales, K. (1987). Estimation of the mating system in a fungal pathogen *Crumenulopsis sororia* (Karst.) Groves using isozyme markers. *Heredity, 59*, 423-430.

Franz, J. (1955). Tannenstammläuse (Adelges piceae Ratz.) unter einer Pilzdecke von Cucurbitaria pithyophila (Kze. et Schm.) De Not., nebst Beobachtungen an Aphidoletes thompsoni Möhn (Dipt, Itonididae) und Rabocerus mutilatus Beck (Col.. Pythidae) als Tannenlausfeinde. *Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz*, 49-61.

Fries, E. (1823). *Systema Mycologicum II*: Grypswalddeae. 620 pp.

Fuckel, K. W. G. L. (1863). Fungi rhenani exsiccati. *fasc. 6*, no. 538.

Glass, N. L., Jacobson, D. J. & Shiu, P. K. (2000). The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. *Annual Review of Genetics, 34*, 165-186.

Havelka, J., Danilov, J. & Rakauskas, R. (2021). Relationships between aphid species of the family *Adelgidae* (*Hemiptera, Adelgoidea*) and their endosymbiotic bacteria: a case study in Lithuania. *Bulletin of Insectology, 74*, 1-10.

Holm, L. (1967). Taxonomical notes on ascomycetes. V. On *Sphaeria parmeliarum* Phill. & Plowr. and the genus *Cucurbidothis* Petr. *Svensk Botanisk Tidskrift, 61*, 449-456.

Kinloch, B. B., Westfall, R. & Forrest, G. (1986). Caledonian Scots pine: origins and genetic structure. *New phytologist, 104*, 703-729.

Matute, D. R. & Sepúlveda, V. E. (2019). Fungal species boundaries in the genomics era. *Fungal Genetics and Biology, 131*, 103249.

McIntosh, G. (1915). *Cucurbitaria pithyophila* Fries. *Transactions of the Royal Scottish Arboricultural Society, 29-30*, 209-210.
Murray, J. & Parry, W. (1969). The association of *Pineus pini* and *Cucurbitaria pithyophila* on Scots Pine in northern Scotland. *Scottish Forestry, 23*, 8-13.
Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K. H. (2008). Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary bioinformatics, 4*.
Peace, T. R. (1962). *Pathology of Trees and Shrubs with Special Reference to Britain*. Oxford: Clarendon Press. 753 pp.
Petrak, F. (1921). Mykologische Notizen. III. *Annales Mycologici 19(3-4)*, 176-223.
Piotrowska, M. J., Riddell, C., Hoebe, P. N. & Ennos, R. A. (2018). Planting exotic relatives has increased the threat posed by *Dothistroma septosporum* to the Caledonian pine populations of Scotland. *Evolutionary applications, 11*, 350-363.
Rehm, H. (1873). Ascomyceten: no. 147.
Santos, J. M., Correia, V. G. & Phillips, A. J. (2010). Primers for mating-type diagnosis in *Diaporthe* and *Phomopsis*: their use in teleomorph induction in vitro and biological species definition. *Fungal Biology, 114*, 255-270.
Takahashi, I. & Saho, H. (1972). *Cucurbitaria pithyophila* on *Pinus* spp found at Tokyo University Forest Institute, Hokkaido, Japan. *Journal of the Japanese Forestry Society, 54*, 282-284.
Tekpinar, A. D. & Kalmer, A. (2019). Utility of various molecular markers in fungal identification and phylogeny. *Nova Hedwigia, 109*, 187-224.
Thiyagaraja, V., Wanasinghe, D. N., Karunarathna, S. C., Tennakoon, D. S., Hyde, K. D., To-Anun, C. & Cheewangkoon, R. (2021). *Alloleptosphaeria shangrilana* sp. nov. and first report of the genus (*Leptosphaeriaceae*, *Dothideomycetes*) from China. *Phytotaxa, 491*, 12–22.
White, T. J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In A. Xufre & F. Simões (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315-322). London, UK: Academic Press.
Xu, J. (2020). Fungal species concepts in the genomics era. *Genome, 63*, 459-468.

# 8 Appendix 1: Specimens of *Curreya pithyophila* used for the temperature growth rate experiment and analyses of ITS, TEF1- α, B tub and actin loci.

Specimens of *Curreya pithyophila* used for the temperature growth rate experiment (indicated by *) and analyses of ITS, TEF1- α, B tub and actin loci. All specimens were collected in Scotland except for SP23-29. Specimens of the dictyospore type are indicated by shaded rows whereas non-shaded rows indicate specimens of the phragmospore type. The number of isolates per specimen for which sequences were obtained are indicated by + for each locus. The number of isolates per specimen that differed from the most common sequence for each spore type, as held by 'type' specimens SP23-18 (phragmospore) and SP23-19 (dictyospore), are indicated with the number of single nucleotide polymorphisms (snp) in parentheses.

| **Specimen number** | **Location of collection** | **Date of collection** | **Spore type** | **ITS sequence** | **TEF1- α sequence** | **B-tub sequence** | **actin sequence** |
|---|---|---|---|---|---|---|---|
| SP23-11 | Insh, Highland | October 2022 | Dictyospore | +++ | | | |
| SP23-13 | Whitewell, Highland | October 2022 | Phragmospore | +++ | | | |
| SP23-18* | Assynt tree 7, Highland | January 2023 | Phragmospore | +++ | + | + | + |
| SP23-19* | Assynt tree 8, Highland | January 2023 | Dictyospore | +++ | + | ++ | |
| SP23-20 | Leetown, Highland | January 2023 | Dictyospore | +++ | | | |
| SP23-20a | Leetown, Highland | January 2023 | Anamorph of phragmospore type | +++ | | | |
| SP23-21 | Bush Estate, Midlothian | January 2023 | Phragmospore | +++ | | | |
| SP23-22 | Cumnock, East Ayrshire | January 2023 | Phragmospore | +++ | | | |
| | | | | | | | |

| Specimen number | Location of collection | Date of collection | Spore type | ITS sequence | TEF1- α sequence | B-tub sequence | actin sequence |
|---|---|---|---|---|---|---|---|
| SP23-25* | Rosehall, Highland | February 2023 | Dictyospore | +++ (1 snp in all isolates) | +++ | + | |
| SP23-26 | Abernethy, Highland | February 2023 | Phragmospore | +++ | | | |
| SP23-29* | Haldon, Devon, England | February 2023 | Phragmospore | +++ | ++ | ++ | |
| SP23-31 | Flowerdale, Highland | February 2023 | Dictyospore | +++ | | | |
| SP23-32* | Loch Maree, Highland | February 2023 | Anamorph of phragmospore | +++ | +++ | ++ | |
| SP23-34 | Loch Cluanie, Highland | February 2023 | Dictyospore | +++ | | | |
| SP23-35 | Whitewell, Highland | February 2023 | Phragmospore | +++ | | | |
| SP23-36 | Black Loch, Dumfries and Galloway | March 2023 | Phragmospore | +++ | | | |
| SP23-36 | Black Loch, Dumfries and Galloway | March 2023 | Dictyospore | +++ | | | |
| SP23-37 | Tentsmuir, Fife | April 2023 | Phragmospore | +++ | | | |
| SP23-39P | Glensaugh, Aberdeenshire | April 2023 | Phragmospore | +++ | | + | |

| Specimen number | Location of collection | Date of collection | Spore type | ITS sequence | TEF1- α sequence | B-tub sequence | actin sequence |
|---|---|---|---|---|---|---|---|
| SP23-40 | Nethybridge, Highland | December 2022 | Phragmospore | +++ | | | |
| SP23-41 | Glen Affric, Highland | January 2023 | Anamorph of dictyospore type | + | | | |
| SP23-44 | Pitmedden Forest, Fife | May 2023 | Phragmospore | +++ | | | |
| SP23-45P* | Muir of Dinnet, Aberdeenshire | March 2023 | Phragmospore | +++ | + (1 snp) | +++ | |
| SP23-45D* | Muir of Dinnet, Aberdeenshire | March 2023 | Dictyospore | +++ | + | + | |
| SP23-47 | Glentress site 1, Scottish Borders | May 2023 | Anamorph of phragmospore type | +++ | | | |
| SP23-48 | Glentress site 2, Scottish Borders | June 2023 | Phragmospore | +++ (1 snp in all isolates) | +++ (1 snp in all isolates) | +++ | ++ |
| SP23-52P | Glen Affric Dog Falls, Highland | June 2023 | Phragmospore | +++ | | + | |
| SP23-52D | Glen Affric Dog Falls, Highland | June 2023 | Dictyospore | +++ | ++ | ++ | ++ |
| SP23-53D | Glen Tarbert site 1, Highland | June 2023 | Dictyospore | +++ (2 snp in 1 isolate) | ++ | ++ | ++ |

| Specimen number | Location of collection | Date of collection | Spore type | ITS sequence | TEF1- α sequence | B-tub sequence | actin sequence |
|---|---|---|---|---|---|---|---|
| SP23-53A | Glen Tarbert site 2, Highland | June 2023 | Anamorph of dictyospore type | +++ (2 snp in 1 isolate) | + | + | + |
| SP23-59 | Loch Cluanie, Highland | June 2023 | Anamorph of dictyospore type | +++ | + | +++ | |
| SP23-71 | Tairlaw Forest, South Ayrshire | October 2023 | Dictyospore | +++ (2 snp in all isolates) | | | |
| SP23-72 | Black Loch, Dumfries and Galloway | October 2023 | Anamorph of the phragmospore type | + | | | |
| SP23-74 | Changue Forest, South Ayrshire | October 2023 | Phragmospore | +++ | | | |
| SP23-75 | Devilla, Fife | November 2023 | Anamorph of the phragmospore type | + | | | |
| SP23-76 | Devilla, Fife | November 2023 | Phragmospore | +++ | | | |

Plant Health Centre
c/o The James Hutton Institute
Invergowrie,
Dundee, DD2 5DA

Tel: +44 (0)1382 568905

Email: Info@PlantHealthCentre.scot
Website: www.planthealthcentre.scot
Twitter: @PlantHealthScot