

# 2024 review

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> The Research Agency of the Forestry Commission



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## Executive summary

Attacks on trees by invasive pests and pathogens, if left uncontrolled, can cause significant damage to the forestry industry across the UK, primarily the spruce and timber industries. However, values at risk extend beyond commercial woodland and forest industry, including natural forests and urban trees. These resources are essential to a healthy, biodiverse environment, which in turn provides key ecosystem services and benefits to human health. Infestation by some pests, such as the emerald ash borer, can be fatal to trees, and once established can cause significant harm to woodland biodiversity and ecosystems, as well as the timber industry.

This document is an Annex to the 'Welsh Plant Health Surveillance Network (WPHSN) 2022 Review document (March 2023)' <u>Welsh Plant Health Surveillance Network (WPHSN)</u> (forestresearch.gov.uk) and reports on the third year of the WPHSN activities undertaken on behalf of the Welsh Government. It describes a network of insect and spore traps placed at strategic woodland sites across Wales to monitor and record the presence/absence of invasive pests and pathogens which may negatively affect our trees, woodlands, and forests.

Data from the WPHSN are being used to inform the development of priority goals and policies relating to woodland management in Wales.

Details are given of the project objectives, the key biological threats being monitored, the trapping and analysis methodologies, and interim results from the 2024 trapping season, and recommendations are made for 2025 and beyond.



## Roles in the WPHSN team



Ariennir gan Lywodraeth Cymru Funded by Welsh Government

#### Welsh Government

Funder of the Welsh Plant Health Surveillance Network.







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# Glossary

Acute oak decline (AOD)	AOD is a disease caused by multiple agents	
	(especially bacteria) which can severely affect	
	native oak species.	
Agrilus biguttatus	Two-spotted oak buprestid beetle: a native bark	
	boring 'jewel' beetle which mostly lives in Quercus	
	species (oak trees).	
Agrilus convexicollis	A jewel beetle that has no common name: a non-	
	native pest of dying and dead Fraxinus species	
	(ash trees).	
Agrilus planipennis	Emerald ash borer beetle (EAB): an exotic beetle	
	pest of Fraxinus species (ash trees). Not currently	
	detected in the United Kingdom.	
Assay	An investigative procedure to quantitively and/or	
	qualitatively assess the composition of a sample.	
Biological sample	A 'living' specimen; that which contains cells of an	
	animal, plant, or other living organisms.	
Canker	Disease symptom, in which tree bark is killed and	
	appears discoloured and/or sunken and/or	
	cracked.	
Cryphonectria parasitica	Sweet chestnut blight: a fungal pathogen of the	
	Castanea genus (sweet chestnut trees).	
Curreya pithyophila	A fungal pathogen of <i>Pinus sylvestris</i> (Scots pine).	
Dendroctonus micans	Great spruce bark beetle: a non-native beetle pest	
	of Picea and Pinus species (spruce and pine trees).	
Dieback	Disease symptom, in which a shoot/stem begins to	
	die from the tip of its leaves backwards.	
DNA	Deoxyribonucleic acid: the chemical carrying the	
	genetic information enabling organisms to grow	
	and function. This information, and therefore the	
	DNA that carries it, is unique to every single living	
	species.	
Ecosystem services	The benefits that the natural environment provides	
	to human life. These include, for example, natural	
	pollination, clean air, extreme weather mitigation,	
	and human wellbeing.	
Forest Trapping Network	An UK wide insect surveillance programme	
(FTN)	targeting quarantine and priority species.	



GAPDC2 Project	Genomic for Animal and Plant Disease Consortium:
	a conglomerate of six different organisations with a
	primary focus to advance genomic surveillance
	approaches for terrestrial and aquatic animal and
	plant pathogens to improve disease management
	strategies.
Heterobasidion species	Conifer root and butt rot: a fungal pathogen of
	conifer trees.
Hylobius abietis	Large pine weevil: a beetle pest of conifer trees,
	mainly to newly planted plants.
Hymenoscyphus fraxineus	Ash dieback: a fungal pathogen of Fraxinus species
	(ash trees). Previously known as Chalara dieback
	of ash.
Invasive organism	A non-native organism the presence of which will
	cause, or is likely to cause, harm to an area into
	which it is introduced.
Ips amitinus	Small spruce bark beetle: a non-native beetle pest
	of Picea species (spruce trees).
Ips cembrae	Large larch bark beetle: a non-native beetle pest
	of Larix species (larch trees).
Ips typographus	Larger eight-toothed European spruce bark beetle:
	a non-native beetle pest primarily of <i>Picea abies</i>
	(Norway spruce trees).
Lymantria dispar	Gypsy moth: non-native moth pest of Quercus
	(oak trees) and <i>Populus</i> (poplar trees).
Metabarcoding	DNA-based method used to simultaneously identify
	many organisms within an individual biological
	sample.
Molecular assay	A DNA-based assay. Molecular assays can be used
	to detect a pathogen in a biological sample by
	detecting their specific DNA, providing a qualitative
	(presence/absence) or quantitative (amount)
	assessment of the target organism.
Monochamus alternatus	Japanese pine sawyer beetle: a non-native
	longhorn beelle which acts as a vector for the <u>pine</u>
	wood nematode – a pest of pine trees.
Multi-vial cyclone sampler	Air sampler for the collection of airborne particles.
iveonectria neomacrospora	Neonectria canker of fir: a fungal pathogen of
	Ables species (true fir trees). It causes severe
	cankers, crown dieback and eventually, tree death.
Pathogen	A microorganism that can cause disease.



Phloem	Transport tissue in plants. The innermost layer of
	tree bark, it transports the nutrients made during
	photosynthesis to the rest of the plant.
Phytophthora pluvialis	Phytophthora pluvialis is a fungus-like pathogen of
	softwood (coniferous) trees.
Phytophthora ramorum	Phytophthora ramorum is a fungus-like pathogen
	affecting many plants, including softwood
	(coniferous) and hardwood (broadleaved) trees.
Pine wood nematode (PWN)	A microscopic worm-like organism which poses a
	serious threat to the health of pine trees.
Pseudips mexicanus	Monterey pine engraver: a non-native beetle pest
	of Pinus species (pine trees).
Real-time PCR	Real-time polymerase chain reaction: a molecular
	assay enabling detection and quantification of a
	specific DNA in a biological sample. Used to assess
	presence/absence, and, in some cases, amount, of
	a specific organism.
Rhizoctonia butinii	A fungal pathogen of <i>Picea</i> species (spruce trees),
	Tsuga heterophylla (western hemlock) and
	Pseudotsuga menziesii (Douglas fir).
Sentinel site	An area of land, such as a botanic garden or
	arboretum, being monitored to inform the WPHSN
	programme of the presence/absence of organisms
	in that geographical area.
Spore	The reproductive structure of a fungus or fungal-
	like organism. Spores can be spread by water
	splashes, air currents or vectored by other
	organisms (e.g., insects).
Shotgun metagenomics	DNA-based method used to simultaneously identify
	many organisms within an individual biological
	sample; it differs from <b>metabarcoding</b> because it
	allows the analysis of all DNA present in a sample,
	rather than targeting a specific sequence ( <i>i.e.</i> , the
	barcode). Shotgun metagenomics requires
	considerably higher amount of starting DNA
	sample compared to metabarcoding.
Thaumetopoea processionea	Oak processionary moth (OPM): a non-native
	insect pest of Quercus species (oak trees).



Thekopsora areolata	Cherry spruce rust: a rust fungus which conducts	
	its life cycle in stages over different host species,	
	infecting the cones of <i>Picea</i> (spruce) species and	
	the leaves of Prunus (cherry) species.	
THDAS	Tree Health Diagnostic and Advisory Service: part	
	of Forest Research which provides advice and	
	diagnosis of tree pests and pathogens.	
WEM	Wider Environment Monitoring: an insect	
	surveillance project spanning England, Scotland,	
	and Wales which monitors the presence of invasive	
	species, such as Ips typographus.	
WPHSN	Welsh Plant Health Surveillance Network: a Welsh	
	Government plant health initiative.	
Xylem	Transport tissue in plants. Situated internally to	
	the phloem in shoots and stems, it transports	
	water, as well as some nutrients, from roots to	
	leaves.	



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# Welsh Plant Health Sentinel Network: 2024 review

# 1. Project aims

- I. To assess presence/absence and abundance of invasive tree pests and pathogens across Wales.
- II. To build a 'real-time' map detailing where invasives are detected, which will act both as an early warning system and as a monitoring tool.
- III. To promote and facilitate collaborative working with external agencies to grow the Network, whereby information and advice can be shared.

# 2. Objective

The objective of the Welsh Plant Health Surveillance Network (WPHSN) project is to monitor and gather data – presence/absence – of native and invasive pests and pathogens that can pose a threat to the health of trees across Wales. A network of insect and spore traps has been deployed at sites deemed at high risk of invasion and colonisation. Biological samples are obtained and analysed by the Tree Health Diagnostic and Advisory Service (THDAS) laboratory staff based at Alice Holt in Surrey and at the Northern Research Station in Midlothian. The data gathered will be used to build distribution maps of pests and pathogens, inclusive of their abundance, and will inform the development of priority goals and policies relating to woodland management in Wales. To ensure adequate coverage of the geographical area and the sharing of information and advice within the sector, the surveillance network includes sites managed by Natural Resources Wales (NRW) as well as privately managed estates through collaboration with sentinel sites and private landowners.

The commercial element of the forest industry in Wales depends heavily on the healthy planting, growth, and harvesting of one spruce species, Sitka spruce (*Picea sitchensis*). An outbreak affecting this tree species has the potential to cause the loss of business



investments worth hundreds of millions of pounds, leading to negative consequences for nurseries, sawmills, timber transport companies, and other related industries dependent on the forestry sector. Other economically important, though relatively small, forest industry components, such as Christmas tree plantations (spruce and fir species), can also suffer serious damage. Trees in the countryside and urban areas, including the many old, veteran trees, are an essential part of the landscape and national heritage, and are an invaluable support to human physical and mental wellbeing. All these resources are directly exposed, and potentially vulnerable, to invasive pests and pathogens. These threats are increasing under the current pattern of climate change – where milder winters; wetter springs; and hotter, drier summers are likely to improve the survival rate of invasive pests and pathogens, as these more frequently-observed weather patterns simultaneously cause stress in trees, increasing their susceptibility to infection and disease.

The WPHSN project targets invasive organisms which have historically been detected in Wales, or which are likely to migrate to Wales with the warming climate. A summary of these organisms and the key threats they pose to tree health is provided in Table 1.

Organism	Threat to tree health
Insects	
Agrilus biguttatus	Weakens trees through the larvae's wood-boring activities and feeding from the vascular tissues on the bark. They possibly also contribute to the spread of acute oak decline by carrying the causative bacteria from affected trees to healthy trees.
Agrilus convexicollis	Feeds on dead and dying native ash trees ( <i>Fraxinus excelsior</i> ). Not detected in Wales to date. Further study required into the potential impact of this beetle in high numbers and its impact as a secondary pest on tree mortality.
Agrilus planipennis	Fatal to infected trees as the larvae live and feed on internal tissues of trees, preventing water and nutrient cycling.
Dendroctonus micans	Weakens tree through burrowing into the bark and creating egg chambers in the cambium for breeding. Larvae feed on internal woody tissue. Fatality in prolonged colonisations.

## Table 1Summary of the WPHSN programme primary organisms and the key<br/>threats they pose to tree health.



Organism	Threat to tree health
Hylobius abietis	Destructive to conifer seedlings and newly planted young conifer trees by girdling stems through feeding on the bark tissue.
Ips cembrae	Primarily attacks already compromised trees. Weakens trees further by boring tunnels through the bark to the phloem layer to create breeding chambers. Feeding larvae cause canopy dieback and needle defoliation. Vector of pathogenic fungi which can contribute to the death of the tree.
Ips typographus	Primarily attacks already compromised trees before colonising healthy trees. Tree death occurs as beetles bore into the tree creating 'galleries' to serve as nuptial and feeding chambers. This destroys the inner tissues of the tree, preventing water and nutrient flow.
Lymantria dispar	Destructive to broadleaf trees in dense populations by defoliating trees through feeding on the leaves. Repeated infestations can weaken a tree and leave it susceptible to disease.
Monochamus alternatus	A vector for the pine wood nematode (PWN) which causes pine wilt disease (PWD) in pines, which can be fatal to trees.
Thaumetopoea processionea	Can be fatal to infected trees through voracious feeding by the larvae (caterpillars) of the leaves, stripping the canopy bare and leaving the tree open to secondary infection and more susceptible to drought stress.
Pathogens	
Cryphonectria parasitica	A regulated pathogen of sweet chestnut ( <i>Castanea sativa</i> ). Fatal to infected trees by killing off cambium and woody tissue, inhibiting the flow of nutrients. Spores dispersed by wind and water. Not detected in Wales to date.
Curreya pithyophila	Infects Scots pine ( <i>Pinus sylvestris</i> ). Infests shoots and branches with high levels of infestation leading to crown thinning and dieback. Not detected in Wales to date.
<i>Heterobasidion</i> species	Infects conifers. Highly damaging to the timber industry. Causes decay in the lower part of the trunk and roots, weakening trees and killing them in some cases. Includes established ( <i>H. annosum</i> , <i>H. abietinum</i> ) as well as regulated species ( <i>H. irregulare</i> , not reported in the UK).
Hymenoscyphus fraxineus	Infects ash ( <i>Fraxinus</i> spp.). Spreads through the phloem and xylem layers, cutting off water and nutrient supplies to



Organism	Threat to tree health
	the tree and causing dieback. Trees can die as a result of
	the infection, or due to increased susceptibility to
	secondary pathogens.
Neonectria	Infects fir (Abies spp.). Weakens trees by causing cankers
neomacrospora	that kill off the phloem layer in branches, resulting in
	dieback. Excessive infection can lead to mortality.
	A regulated pathogen. Weakens trees by causing needle
Phytophthora	loss, multiple resinous cankers, and shoot dieback. Several
pluvialis	aspects of its biology (e.g., dispersal and host range in the
	UK) are not fully understood yet.
	A regulated pathogen of larch (Larix spp.) and other tree
	species. Induces mortality in infected larches by causing
Phytophthora	shoot wilting and withering, premature needle cast, and
ramorum	bleeding cankers on branches and stems. Spores are
	spread by wind, rain, and mechanical means: footfall,
	mammals, vehicles.
	A relatively unknown and poorly investigated tree
	pathogenic fungus. It is known to infect spruces (Picea
	spp.), western hemlock (Tsuga heterophylla) and Douglas
Rhizoctonia	fir (Pseudotsuga menziesii), and can cause needle death
butinii	by parasitising young shoots and needles. Normally
	occurring in twigs at ground level, penetrates needles
	when air humidity is high, forming mycelial mats on the
	surface of the leaf tissue.
	A rust fungus that infects spruce and cherry ( <i>Picea</i> spp.
Thekopsora	and <i>Prunus</i> spp.) trees. It can reduce seed yield in spruce
areolata	as infected cones produce infertile seeds, and causes
	necrosis in leaf tissue in cherry trees. It relies on both tree
	species to complete its life cycle.



# 3. Detecting invasive organisms

### 3.1. Methodology

As in the first two years, locations for the deployment of insect and spore traps were selected using previous work led by Professor Hugh Evans (2021). Sites deemed at higher risk of colonisation from migrating organisms were selected, including: at the Wales/England border; along major transport corridors, such as the M4 in South Wales and the A55 in North Wales; sites closely situated to industrial areas, *e.g.*, ports and sawmills; and parks and gardens containing a wide variety of potential host tree species ('sentinel sites') (Figure 1).



Figure 1 Welsh Plant Health Surveillance Network 2024 surveillance sites.



## 4. Traps

### 4.1. Insect traps

Three types of insect trap are used in the WPHSN project: X-vane traps, bucket traps, and multi-funnel traps. Each is deployed with a chemical lure comprising a synthetic cocktail mimicking the sexual pheromone emitted by females to attract a male for breeding. Depending on the target insect, traps are deployed either at head height or in the tree canopy.

## 4.2. Spore traps

Burkard volumetric air samplers and rotor arm spore traps, the latter constructed inhouse, were used; both operate with adhesive surfaces (sticky tapes and sticky rods respectively) to which airborne spores adhere upon impact. It is these sticky surfaces that are analysed to establish what fungal organisms are present in an area.

Unfortunately, due to technical problems with some rotor arm traps and one Burkard air sampler, the spore trapping network was not as extensive as initially planned in 2024. Alternative options are being investigated for future spore trapping surveillance so that a wider monitoring programme can be established.



## 5. Progress report

## 5.1. Insect monitoring

Targets in the <u>Welsh Plant Health Surveillance Network</u> 2023 review (Lee and Olivieri, 2023, p. 31) outline a proposed expansion of the surveillance network for 2024 by widening the surveillance to include other tree pests, such as *Agrilus biguttatus* (two-spotted oak buprestid), which is associated with acute oak decline, and *Lymantria dispar* (gypsy moth). Additionally, collaboration with the Wider Environment Monitoring (WEM) surveillance programme (an EU monitoring programme for the detection of *Ips typographus* and *Ips amitinus*) was forecast.

Other targets included a revision to the spore trapping network to focus surveillance efforts on specific fungal pathogens, such as *Neonectria neomacrospora* (canker of fir), and a revision to the site selection process to cover a higher percentage of priority regions in Wales through the inclusion of surveillance along major road corridors and in industrial areas.

A total of 46 sites were used for surveillance in 2024 (Appendix 3), an increase from 32 sites in 2023, and 23 sites in 2022. In total, 86 insect traps were deployed across these sites (Table 2) - an increase over the total of 35 traps deployed in 2022, and 73 traps in 2023. Of the 86 insect traps deployed, 24 were allocated for use in the WEM programme as well as the WPHSN survey. Two spore traps were deployed – one at Talybont-on-Usk (Brecon) and one at The Cott (Monmouthshire).

Тгар	Number deployed
X-vane to detect Ips typographus	19
X-vane to detect Ips amitinus	5
X-vane to detect Ips cembrae	5
Bucket to detect Thaumetopoea processionea	20
Bucket to detect Lymantria dispar	8
Multi-funnel to detect Agrilus planipennis	3
Multi-funnel to detect Agrilus biguttatus	16
Multi-funnel to detect Monochamus alternatus	10
Total insect traps	86
Burkard samplers to detect pathogenic spores	1
Rotor arm traps to detect Neonectria neomacrospora spores	1
Total spore traps	2
Total number of traps deployed	88

#### Table 2Summary of traps deployed in the WPHSN 2024.



In May 2024, three adult *Ips typographus* beetles were detected in one insect trap deployed in a stand of *Picea abies* (Norway spruce) managed by NRW in Slade Wood, Monmouthshire, Southeast Wales (grid reference: ST 45506 89292) (Figure 2).

Throughout the 2024 survey season, this was the only detection of *Ips typographus* in Wales. Action was immediately taken to prevent the possible establishment of a breeding colony and further spread. The extent of the interception was investigated by deploying an additional three X-vane traps with lures, undertaking detailed visual surveys of any dead and dying *Picea* trees in the stand, and felling of 10 randomly-selected *Picea abies* for inspection. No signs or symptoms of colonisation by *Ips typographus* were observed from these additional investigations, and further detailed studies suggested that the three beetles intercepted had arrived as a result of a rare blow over event from Continental Europe.



Figure 2 Map illustrating the location of the *Ips typographus* interception in May 2024 - red spot.



Other insect samples taken during the 2024 survey season show a presence of *Ips cembrae* (large larch bark beetle) at Llantrisant Forest near Pontypridd, Cwmcarn Forest near Cwmbran, Chepstow Park Wood in Chepstow, Radnor Forest near Knighton, and Clwyd Forest at Moel Famau near Ruthin (Figure 3).

Samples suggest an absence of *Thaumetopoea processionea* (oak processionary moth), *Lymantria dispar* (Gypsy moth), *Agrilus planipennis* (emerald ash borer), *Agrilus biguttatus* (two-spotted oak buprestid), and *Monochamus alternatus* (Japanese pine sawyer beetle).



Figure 3 Map illustrating the presence of *Ips cembrae* in traps deployed in Welsh woodlands selected for the WPHSN 2024 programme – blue spots.



Insect traps to detect *Ips cembrae* were deployed at five locations in Wales where the beetle has previously been detected. Data gathered in 2024 will be compared with data from 2023 and 2022, allowing population density and distribution patterns to be analysed and monitored for use in future surveillance planning.

The highest numbers of *Ips cembrae* (120 beetles) were caught in traps in Radnor Forest in mid-north Wales. Traps in southwest Wales, south-central Wales, southeast Wales, and northeast Wales caught fewer than 50 individuals at each location over the 2024 survey season (Figure 4).

Region	Site	# Ips cembrae	
Southwest	Llantrisant Forest	45	
South- central	Cwmcarn Forest	21	
Southeast	Chepstow Park Wood	16	
Mid-north	Radnor Forest	120	
Northeast	Clwyd Forest	29	
			PEMBRON Stewart Stewart Stewart Stewart



## Figure 4 Map illustrating the population density of *Ips cembrae* detected in traps across Wales in 2024.

Higher numbers of *Ips cembrae* were caught in Radnor Forest in 2024 than in 2022, but fewer than in 2023. The numbers of *Ips cembrae* detected in Cwmcarn Forest in 2024 were fewer than were detected in both 2022 and 2023. Overall, the number of *Ips cembrae* caught in 2024 were the lowest since the project commenced in 2022 (Figure 5).





Figure 5 Charts illustrating the numbers of *Ips cembrae* detected in traps across Welsh woodlands 2022–2024 (site abbreviations are given in Appendix 2).



While *Ips cembrae* is not a primary pest, it is prudent to continue with surveillance to monitor changes in its population density and distribution across Wales. This is particularly important following the detection of *Ips cembrae* in the pest free area in Scotland. The latter finding suggests that the species is migrating and possibly increasing in population size. This insect species will therefore continue to be included in the WPHSN.

Other insect species found in the samples taken from the traps, but not of concern, included:

- Agrilus laticornis (jewel beetle)
- Hylobius abietis (large pine weevil)
- Hylastes cunicularius (bark beetle)
- *Hylastes* species (crennulate bark beetle)
- Pityogenes chalcographus (six-toothed spruce bark beetle)
- Rhagium bifasciatum (two-banded longhorn beetle)
- Rhizophagus species
- Thanasimus latreille (ant beetle)<sup>1</sup>
- *Trypodendon domesticum* (European hardwood ambrosia beetle)
- Trypodendron lineatum (striped ambrosia beetle)

<sup>1</sup>An important predator of bark beetles and ambrosia beetles.

A full list of insect species identified is given in Appendix I.

### 5.2. Spore monitoring

In collaboration with the Genomic for Animal and Plant Disease Consortium (GAPDC2 project), surveys were carried out at two sites in 2024: The Cot (Monmouthshire) using a single rotor rod spore trap for the detection of *Neonectria neomacrospora*; and Talybont-on-Usk (Brecon) using a Burkard volumetric air sampler for general surveillance of fungal pathogens (Figure 6).





## Figure 6 Locations of the Burkard seven-day volumetric sampler and rotor rod traps for 2024 spore surveillance.

The intention for the 2024 surveillance programme was to have widened the spore trapping network through the deployment of an additional Burkard sampler in North Wales and an additional three rotor rod spore traps at locations in south, southwest, and mid-Wales. The length of the survey window was also to have increased, to maximise the number of samples collected across the season. A new technique for collecting airborne particles was also to be tested through the deployment of a multi-vial cyclone sampler in mid-Wales.

A number of unanticipated mechanical and technical issues with the sampling equipment meant it was not possible to undertake the spore trapping as intended during the 2024 season. Details of the issues, and the proposed solutions, are presented in Table 3.



## Table 3Summary of equipment failures in the 2024 spore trapping<br/>programme.

2024 plan	Failure	Proposed solution for the 2025 season
Deploy a second Burkard sampler in North Wales.	Mechanical malfunction of sampler motor in cooler temperatures.	Insulate sampler motor.
Deploy three additional rotor rod spore traps across Wales.	Rotor rod connection points corroded, causing motor malfunction.	Use alternative design – Rotorod Spore Sampler by Agri Samplers Ltd.
Deploy multi-vial cyclone sampler in mid-Wales.	Installation issues – returned to manufacturer for investigation and repair.	Installation by manufacturer.

#### 5.2.1. 2024 Burkard spore traps

From August to October 2024, a total of 18 daily Burkard samples were collected at the Forest Research (FR) field station in Talybont-on-Usk. Each sample comprised a 48 mmlong Vaseline-coated Melinex tape segment, corresponding to a 24-hour sampling window. All Burkard samples were processed in the pathology laboratories at Alice Holt:

- (1) to compare two different methods currently employed at FR for total DNA extraction from Burkard samplers, with the aim of harmonising DNA extraction methods for spore traps across FR, and
- (2) to obtain total DNA from spore trap samples for subsequent real-time qPCR and metabarcoding analysis.

The sample tapes were each divided longitudinally to obtain two subsamples. Total DNA was then extracted using (A) a hybrid protocol (based on CTAB and Macherey-Nagel's NucleoSpin Plant II kit) adapted from Zajc *et al.* (2022) and (B) a commercial kit (Macherey-Nagel's NucleoSpin Food Kit). The first of these protocols had previously been used to extract DNA from WPHSN Burkard samples collected in 2022-23. DNA quality and suitability for downstream analysis (*i.e.*, real-time qPCR and metabarcoding) was then checked by:

- a. measuring DNA concentration with Nanodrop spectrophotometer and Qubit Fluorometer (ThermoFisher Scientific)
- b. measuring DNA quality using Nanodrop
- c. running the extracted DNA in an end-point fungal ITS PCR and subsequently analysing the PCR products on agarose gel.

The results of DNA concentration measurements are shown in Table 4.



# Table 4Concentration of total DNA extracted from Burkard samples using<br/>CTAB + NucleoSpin Plant II kit (method A) vs Nucleospin Food kit<br/>(method B), as measured by Qubit fluorometer.

Note: the sample name is coded to indicate the location of trap (B1 = Talybont-on-Usk), date of deployment (DDMMYY), and progressive tape segment (*i.e.*, day of sampling, starting from deployment); a dash in the table indicates no DNA was detected; NEC = negative extraction control.

Sample number	Sample name	Method A: CTAB + NS Plant II kit (ng/µl)	Method B: NS Food kit (ng/µl)
1	B1.130824-1	0.14	-
2	B1.130824-2	0.46	0.29
3	B1.130824-3	0.13	-
4	B1.130824-4	0.13	-
5	B1.130824-5	0.14	-
6	B1.130824-6	0.13	-
7	B1.130824-7	0.16	-
8	B1.260924-1	0.43	0.16
9	B1.260924-2	0.12	-
10	B1.260924-3	0.12	-
11	B1.260924-4	0.12	-
12	B1.260924-5	0.11	-
13	B1.291024-1	0.24	0.05
14	B1.291024-2	0.12	-
15	B1.291024-3	0.12	-
16	B1.291024-4	0.13	-
17	B1.291024-5	0.14	-
18	B1.291024-6	0.15	0.02
	NEC	0.07	-



DNA concentration as measured by Qubit fluorometer was higher for subsamples extracted with the hybrid method (method A) employing CTAB + NucleoSpin Plant II kit compared to the method using only the NucleoSpin Food kit (method B). However, this apparent difference should be treated with caution, as only four of the subsamples extracted using method B contained DNA detectable by the Qubit fluorimeter. Because of the very low DNA concentration over both sets of subsamples, it was not possible to use the Nanodrop spectrophotometer (data not shown). Following the end-point fungal ITS PCR, 14 out of 18 Burkard subsamples extracted with method A successfully yielded an ITS amplicon band on agarose gel, whereas this was observed for only 8 out of 18 subsamples extracted using method B (Figure 7).

To confirm these results, accurate DNA quantification using a general real-time qPCR assay for fungal organism will be required. However, the combined Qubit and end-point PCR results suggest that the hybrid method employing CTAB and the NucleoSpin Plant II kit could achieve higher concentration and quality of total DNA extracted from Burkard trap samples. Therefore, our partial findings support the use of the hybrid method based on Zajc *et al.* (2022) for DNA extraction from the sampling matrix employed in Burkard traps, or similar (*e.g.*, Vaseline on Melinex/plastic support).



Figure 7 Results of end-point PCR amplification of total DNA from Burkard traps using primers ITS1F and ITS4; (A) subsamples extracted using CTAB + NucleoSpin Plant II kit and (B) subsamples extracted using NucleoSpin Food kit; samples are numbered according to Table 4. "+" = positive control; "-" = negative control. Presence of a horizontal DNA band (or multiple bands) indicates successful amplification of total DNA. A faint band(s) can be observed for subsamples 4, 5, 12 and 14 in (A), and for subsamples 3, 14 and 16 in (B).

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#### 5.2.2. 2024 rotor rod spore traps

From July to December 2024, a total of six rotor rod samples, each consisting of two plastic rods covered in sticky tape ('sticky rods') were collected at The Cot, Devauden. Each spore trap was run continuously for 24 hours. All samples were processed in the pathology laboratories at Alice Holt for the DNA-based detection of *Neonectria neomacrospora* spores. Total DNA was extracted by using Applied Biosystem's MagMAX DNA isolation kit for automated KingFisher extraction. DNA quality and suitability for downstream analysis (*i.e.*, real-time qPCR and metabarcoding) was then checked by running the extracted DNA in an end-point fungal ITS PCR and subsequently analysing the PCR products on agarose gel. Samples are listed in Table 5 and the results of end-point PCR analysis on agarose gel are shown in Figure 8.

# Table 5List of rotor rod samples. The sample name comprises the location<br/>of trap (1A = The Cot, Devauden) and date of deployment<br/>(DDMMYY).

Sample number	Sample name
1	1A.170724
2	1A.130824
3	1A.240924
4	1A.250924
5	1A.291024
6	1A.041224





# Figure 8 Results of end-point PCR amplification of total DNA from rotor rod traps using primers ITS1F and ITS4; samples are numbered according to Table 5. "+" = positive control; "-" = negative control. Presence of a horizontal DNA band (or multiple bands) indicates successful amplification of total DNA.

Of the six samples, only one (1A.041224) successfully amplified in the fungal ITS PCR. No fungal DNA was detected in the other five samples. This result is unexpected, and unlikely to have been caused by low numbers of fungal spores in the air at the time of sampling. A more likely explanation is that spores were not captured due to a malfunction in the rotor spinning mechanism. This has highlighted the need for more reliable and efficient rotor rod samplers, which have now been acquired for deployment in spring 2025.

Ten pooled DNA samples that had been processed during 2022-23 for ITS metabarcoding (see previous WPHSN report) were further subjected to 16S metabarcoding (for the detection of bacterial pathogens) and shotgun metagenomics (for the detection of any DNA species present in the sample, including fungi and bacteria). Samples were sent to Novogene, Cambridge, UK, for library preparation, Illumina sequencing, and subsequent bioinformatic and statistical analyses of sequencing results using QIIME2. Details of the library preparation and analysis steps were provided in the Novogene ITS Amplicon QIIME2 Analysis Report, and further information can be obtained from the authors of this project report upon request.

The 16S barcodes were successfully amplified and sequenced in all samples, and data analysis is ongoing. Conversely, only seven out of ten samples were successfully sequenced by shotgun metagenomics. The lower success rate of shotgun metagenomics compared to metabarcoding is not surprising, given the low concentration of DNA measured in extracted Burkard samples (see Table 4). These results suggest that metabarcoding may be a more effective approach for the analysis of air samples collected with spore traps.



## 5.3. Collaboration

Collaboration with the sentinel site network has continued, with an additional five sites included in the 2025 WPHSN programme (Table 6). Feedback from the sentinel site network has been positive with many expressing an interest in continuing their collaboration with the WPHSN for the foreseeable future.

# Table 6Table illustrating the development of the sentinel site network and<br/>private landowners collaborating with the WPHSN project: 2022–<br/>2025.

Sentinel sites	2022	2023	2024	2025
Forest plantations across Wales (NRW)	•	•	•	•
National Botanical Gardens of Wales, Carmarthen	•	•	•	٠
Cardiff Golf Club, Cardiff	•	•	•	٠
Powis Castle, Welshpool (National Trust)	•	•	•	•
Plas Newydd, Anglesey (National Trust)	•	•	•	•
Loggerheads Country Park, Llanferres	•	•	•	
Treborth Botanic Gardens, Bangor		•	•	•
Penllegare Valley Wood, Swansea		•	•	
Bute Park, Cardiff		•	•	
Landmarc, Brecon		•		
Llyn Parc Mawr, Anglesey			•	•
Pengelli Forest, Pembrokeshire (Wildlife Trust of South & West Wales)			•	•
Celtic Manor, Newport			•	٠
Leighton Estate, Welshpool (Royal Forestry Society)			•	•
Chirk Castle, Chirk (National Trust)			•	•
Roath Park, Cardiff				•
St Pierre Golf Club, Chepstow				•
Tredegar House, Newport (National Trust)				•



Sentinel sites	2022	2023	2024	2025
Tintern Abbey, Tintern (Cadw)				•
Gregynog Hall, Newtown				•
Total number of sites per year	6	10	14	16
Private landowners				
Dingestow Court, Monmouthshire			•	•
Taliaris Forest, Llandeilo			•	٠

Working relationships with senior project leads and research scientists at FR and Forestry Commission (FC) Forest Services have continued into 2025 since the inception of the project in 2022. Shared data, such as site information, trapping locations, sporulation patterns, and emergence and flight patterns of beetles and moths, all help to optimise the trapping network in Welsh woodlands. Additionally, support from FR entomologists provides verification of insect identification from samples obtained during the survey season.

Collaboration with the Genomic for Animal and Plant Disease Consortium (GAPDC2 project) in 2024 provided the WPHSN an opportunity to be involved in a multidisciplinary programme to help develop methods for an agnostic genomics project looking at ways to safeguard the health of animals, plants, and ecosystems through spore surveillance. Spore samples and data were collected by the WPHSN and shared with GAPDC2 for analysis. GAPDC2 is a research project and is managed by a separate service level agreement (SLA), as such, it is not possible for the WPHSN to continue to collaborate with this project in 2025, primarily due to technical difficulties experienced with the spore traps and differing methodologies in spore surveillance. The WPHSN report authors are exploring alternative avenues for spore analysis to support that part of this project. Discussions are ongoing at the time of writing.

Multiple collaborations with insect surveillance projects will continue into 2025. The Forest Trapping Network (a national and FR-led insect surveillance project) will work in tandem again with the WPHSN to ensure adequate coverage across Wales and to prevent duplication of sites used for monitoring. The WPHSN will continue to support the Wider Environment Monitoring programme for the presence/absence of *Ips typographus*, providing both surveillance for Wales and data returnable to the EU.



## WPHSN 2025 – Forecast and targets

The WPHSN has received funding from the Welsh Government for a further two years. This funding will enable the project to continue the surveillance network for invasive pests and pathogens until 31 March 2027, for ongoing collaboration with the sentinel site network and private landowners, and for essential fieldwork support through the appointment of a dedicated Fieldwork Technician.

#### 1 Refine surveillance network



<sup>1</sup>AOD – Acute oak decline

- <sup>2</sup>SCB Sweet chestnut blight
- <sup>3</sup>WEM Wider Environment Monitoring surveillance for *Ips typographus*

<sup>4</sup>Inward *et al.* (2024).



During the pilot years of the project (2022–2024), surveillance sites were selected based on research by Professor Evans (2021) and his forecast of potential establishment of *Ips typographus* from Continental Europe. Subsequently, sites deemed high risk were those in SE Wales and along the Wales/England border. Site selection for surveillance in 2025 (Figure 9) have been further informed by historical wind patterns (Inward *et al.*, 2024), in conjunction with sites that have been wind damaged as a result of Storms Darragh and Éowyn. Once again, sites in SE Wales will be prioritised because of the dispersal projections and following the interception of *Ips typographus* in Monmouthshire in 2024. Similar methods can also be applied for the surveillance of other invasive insect species to ensure the surveillance programme remains as effective as possible.

A total of 35 pheromone traps are planned for deployment in Welsh woodlands for the monitoring and detection of *Ips typographus* between 01 April 2025 and 30 September 2025, serviced fortnightly. Primary sample analysis for *Ips typographus* will take place in the field at the time of sample collection to ensure early detection.

Additionally, the WPHSN will again carry out surveillance on behalf of the Wider Environment Monitoring (WEM) scheme for the early detection of *Ips typographus*.

Monitoring absence/presence of other invasive pests and pathogens across Wales will take place between April and September and will include insect/spore trap deployment and routine servicing for:

- *Ips cembrae* (large larch bark beetle)
- The jewel beetles *Agrilus planipennis* (emerald ash borer), *A. biguttatus* (two-spotted oak buprestid), and *A. convexicollis*
- Thaumetopoea processionea (oak processionary moth)
- Monochamus alternatus (Japanese pine sawyer beetle)
- Pseudips mexicanus (Monterey pine engraver)
- *Heterobasidion* species (conifer root and butt rot fungus)
- Neonectria neomacrospora (canker of fir)
- Cryphonectria parastica (sweet chestnut blight)





## Figure 9 Map illustrating planned surveillance sites for the WPHSN 2025 programme.

Visual surveys will also be carried out (in areas of insect surveillance) for the detection and monitoring of acute oak decline disease, *Diplodia corticola* (Bot canker of oak), the fungus *Curreya pithyophila*, sooty bark disease, and sweet chestnut blight.

A wider network of spore traps is planned for the 2025 and 2026 monitoring seasons through the deployment of an additional five traps across Wales. Additionally, the performance of different spore collection instruments, such as the Cyclone sampler, will be evaluated, with a view to optimising spore capture for DNA extraction. Sampling is scheduled to take place monthly between May 2025 and December 2025 at six strategically chosen sites in Wales (Figure 10).





Figure 10 Map illustrating planned locations for spore traps. Orange rhombi indicate sites which will host a single Rotorod trap; blue circle with orange rhombus indicate sites which will host a Rotorod trap and a Burkard air sampler; yellow circle with orange rhombus indicate sites which will host a Rotorod trap and a Cyclone sampler.



#### 2 Cultivate stakeholder engagement

Continue to partake in networking and public engagement activities, and knowledge sharing events, to raise the profile of the work of the WPHSN project and raise awareness of plant health issues, pests, and pathogens

Nurture working relationships with external colleagues in furtherance of data sharing and project collaboration to widen the scope of the WPHSN Cultivate collaborations with sentinel sites and private landowners to ensure needs of both parties are met and support the work of the WPHSN project

#### 3 Develop the programme with an expanded WPHSN team





## Recommendations

Site selection for the detection and monitoring for *Ips typographus* in 2025 and 2026 should be determined based on assessments of wind damage on the Welsh Government Woodland Estate as a result of Storms Darragh and Éowyn, as well as on those sites deemed at high risk of an outbreak based on their geographical location in conjunction with the pattern of likely wind plume events from Continental Europe.

Additionally, ports and processing facilities, together with woodland sites adjacent to these, are high-risk sites and will be considered for inclusion in future monitoring activities.

*Heterobasidion* monitoring using spore traps and air samplers can be incorporated into the spore trapping network to assist researchers with an agenda into the concerns over the increasing distribution of this pathogen and its impact on spruce, *e.g.*, it's resilience to wind damage in extreme weather events.

## References

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# Appendices

## Appendix 1

Results from biological samples collected from all insect traps deployed in Welsh Woodlands in 2024.

Organism	Organism	Organism
Agrilus laticornis	Halyzia sedecimguttata	Rhagium bifasciatum
Anisandrus dispar	Hydrophilidae species	Rhagonycha fulva
Anobium species	Hylastes attenatus	Rhipalapion longirostre
Aphantopus hyperantus	Hylastes cunicularius	Rhizophagus depressus
Aphid species	Hylergops palliatus	Rhizophagus nitidulus
Aphidecta obliterata	Hylobius abietis	Rutpela maculata
Apis mellifera	Hylurgini xylechinus	Salpingus planirostris
Archips xylosteana	Hymenoptera species	Salpingus ruficolis
Aromia moschata	Ichneumonid species	Sawfly species
Autographa gamma	Lymantria monacha	Scolytus intricatus
Bombus lapidarius	Malthodes species	Scolytus rugulosus
Bombus terrestris	Millipede	Scraptiidae species
Broscus cephalotes	Monotoma angusticollis	Sevica brunnea
Cantharis pellucida	Monotoma conicollis	Staphylinidae species
Carabus problematicus	Nalassus laevioctostriatus	Stenocorus meridianus
Catops grandicollis	Nemopjora degreella	Syrphus ribesii
Chrysoperla carnea	Nicrophorus vespilloides	Thanasimus latreille
Click beetles	Oligia latruncula	Tomicus minor
Cnephasia incertana	Pachytodes cerambyciformis	Tomicus piniperda
Crypturgus subcribrosus	Pammene albuginana	Trixagus species
Curculia glandium (acorn weevil)	Pammene fasciana	Trypodendron domesticum
Ditula angustiorana	Panorpa communis	Trypodendron lineatum
Dryocetes villosus	Parasitoid hymenoptera	Vespula vulgaris
Ernoporicus fagi	Pentatoma species	Vincenzellus ruficollis
Exochomus quadripustulatus	Phalangium opilio	Weevil species
Formica rufa	Pityogenes calcographus	Xyleborini saxesenii
Grammoptera abdominalis	Platydema violaceum	Xylita laevigata
Grammoptera ruficornis	Podabrus alpinus	Zabrus tenebrioides
Grammoptera ustulata	Polygraphus polygraphus	
	Ptilinus pectinicornis	1



### Appendix 2

Results from biological samples collected from *Ips cembrae* insect traps in 2024, inclusive of dates biological samples were taken and the number of individual *Ips cembrae* beetles present in each trap.

Date	RF	MF	LT	CPW	CN	Site abbreviations (2024)
13-May	95	20				RF Radnor Forest Presteigne
15-May			37		0	MF Clwvd Forest, Moel Famau
16-May				10		LT Llantrisant Forest, Llantrisant
28-May	7					CPW Chepstow Park Wood, Devauden
30-May			0		1	CN Cwmcarn Forest, Cwmbran
10-Jun	1	0				1
13-Jun			0	1	0	Site abbreviations (2023)
24-Jun		1				PVW Penllegare Valley Wood
25-Jun	1					RH Rheola
27-Jun			1	0	1	CPW Chepstow Park Wood
08-Jul	5	0				MG Manson's Grove
09-Jul			1	1	0	CY Cwmyoy Wood
22-Jul	9	1				LT Llantrisant Forest
24-Jul			4	2	9	SB Sennybridge
05-Aua	2	3				RF Radpor Forest
07-Aua				2		AH Abbeycwmhir
08-Aua			2		10	DF Dyfnant Forest
19-Aua		4				CF Clwyd Forest
20-Aug				0		BF Beddgelert Forest
21-Aua			0		0	AG Abergwyngregn
02-Sep		0				
05-Sep				0		1
06-Sep					0	
17-Sep				0		CB Cwmcarn Forest Cwmbran
						CH Fedw Wood, Chepstow
						MW Manor Wood, The Narth
						TB Talybont-on-Usk
						CA Coed Mawr, Caerphilly

- RF Radnor Fishpools, near Presteigne
- BB Mynydd Ddu, Black Mountains
- FF Fforest Fawr, Caerphilly
- PT Mynydd Margam, Port Talbot
- FN Tair Onen Forest Nursery, near Cowbridge



## Appendix 3

#### Trap index with site names and grid references (GR).

Тгар	Site name	Site GR	Pest/pathogen
X-Vane	Mynydd Emroch	SS 78317 90053	Ips amitinus
X-Vane	Coed Naviges, Stanner Hill	SO 26764 58854	Ips amitinus
X-Vane	Slade Wood, Highmore Hill	ST 45449 89304	Ips amitinus
X-Vane	Craig y Llan, Rudry	ST 19854 86486	Ips amitinus
X-Vane	Moel Famau	SJ 16982 61409	Ips amitinus
X-Vane	Radnor Forest	SO 23144 67403	Ips cembrae
X-Vane	Coed Smaelog, Llantrisant	ST 02509 84414	Ips cembrae
X-Vane	Chepstow Park Wood	ST 4880 9825	Ips cembrae
X-Vane	Moel Famau	SJ 18224 62199	Ips cembrae
X-Vane	Cwmcarn, Risca	ST 23857 97314	Ips cembrae
X-Vane	Wentwood	ST 42748 95445	Ips typographus
X-Vane	Mynydd Margam	SS 81458 91448	Ips typographus
X-Vane	Coed Smaelog, Llantrisant	ST 02561 84401	Ips typographus
X-Vane	Coed y Gedrys, Taff Vale	ST 11203 85259	Ips typographus
X-Vane	Wentwood	ST 42287 95588	Ips typographus
X-Vane	Chepstow Park Wood	ST 48739 97923	Ips typographus
X-Vane	Newborough Forest	SH 41433 67051	Ips typographus
X-Vane	Wentwood	ST 42257 95675	Ips typographus
X-Vane	Craig yr Aber Wood	SS 84571 87057	Ips typographus
X-Vane	Chirk Castle	SJ 27448 37459	Ips typographus
X-Vane	Radnor (Fforest Fach)	SO 21707 66393	Ips typographus
X-Vane	Slade Wood, Highmore Hill	ST 45506 89292	Ips typographus
X-Vane	Wentwood	ST 42833 95452	Ips typographus
X-Vane	Radnor (mid)	SO 20062 66105	Ips typographus
X-Vane	Cwmcarn, Risca	ST 23856 97231	Ips typographus
X-Vane	The Hendre	SO 46707 13851	Ips typographus
X-Vane	Moel Famau	SJ 18153 62187	Ips typographus
X-Vane	Coed Cwmgolog	SO 18663 87471	Ips typographus
X-Vane	Taliaris Park	SN 63634 28453	Ips typographus
Green Funnel	The Hendre	SO 46663 13838	Agrilus biguttatus
Green Funnel	Loggerheads Country Park	SJ 19957 62858	Agrilus biguttatus
Green Funnel	Colonel's Park, Pwllplythin	SO 52077 07302	Agrilus biguttatus
Green Funnel	Stanner Hill	SO 26780 58910	Agrilus biguttatus
Green Funnel	Bute Park	ST 17783 76967	Agrilus biguttatus
Green Funnel	Dingestow Court	SO 45395 09296	Agrilus biguttatus
Green Funnel	Powis Castle	SJ 21628 06234	Agrilus biguttatus



Green Funnel	Dingestow Court	SO 45227 09663	Agrilus biguttatus
Green Funnel	Penllegare Valley Wood	SS 62471 99327	Agrilus biguttatus
Green Funnel	Glyn Wood, Tintern	ST 52492 99924	Agrilus biguttatus
Green Funnel	Briton Ferry	SS 74958 94481	Agrilus biguttatus
Green Funnel	Chirk Castle	SJ 27662 37927	Agrilus biguttatus
Green Funnel	Chepstow Park Wood	ST 48525 98067	Agrilus biguttatus
Green Funnel	Pengelli Forest	SN 13028 39268	Agrilus biguttatus
Green Funnel	Canaston Wood, Narberth	SN07580 14082	Agrilus biguttatus
Green Funnel	Cardiff Golf Club	ST 19526 81494	Agrilus biguttatus
Green Funnel	Craig yr Llan, Rudry	ST 19854 86486	Agrilus planipennis
Green Funnel	Stanner Hill	SO 26780 58910	Agrilus planipennis
Green Funnel	Forest Fawr, Tongwynlais	ST 14071 83850	Agrilus planipennis
	•		
Bucket (OPM)	Plas Newydd	SH 5197 6465	Thaumetopoea processionea
Bucket (OPM)	Loggerheads Country Park	SJ 19957 62858	Thaumetopoea processionea
Bucket (OPM)	Stanner Hill	SO 26780 58910	Thaumetopoea processionea
Bucket (OPM)	Dingestow Court	SO 45378 09296	Thaumetopoea processionea
Bucket (OPM)	Bute Park	ST 17783 76967	Thaumetopoea processionea
Bucket (OPM)	The Hendre	SO 46666 13862	Thaumetopoea processionea
Bucket (OPM)	Chepstow Park Wood	ST 48505 98066	Thaumetopoea processionea
Bucket (OPM)	Briton Ferry	SS 75415 94265	Thaumetopoea processionea
Bucket (OPM)	Chirk Castle	SJ 27662 37927	Thaumetopoea processionea
Bucket (OPM)	Glyn Wood, Tintern	ST 52515 99907	Thaumetopoea processionea
Bucket (OPM)	Colonel's Park, Pwllplythin	SO 51949 07424	Thaumetopoea processionea
Bucket (OPM)	Treborth Botanic Gardens	SH 54804 71063	Thaumetopoea processionea
Bucket (OPM)	Powis Castle	SJ 21688 06292	Thaumetopoea processionea
Bucket (OPM)	Canaston Wood, Narberth	SN 07577 14043	Thaumetopoea processionea
Bucket (OPM)	Pengelli Forest	SN 1302 3926	Thaumetopoea processionea
Bucket (OPM)	Taliaris Park, Llandeilo	SN 63569 28444	Thaumetopoea processionea
Bucket (OPM)	Penllegare Valley Wood	SS 62471 99327	Thaumetopoea processionea
Bucket (OPM)	Cardiff Golf Club	ST 19481 81451	Thaumetopoea processionea
Bucket (OPM)	Celtic Manor	ST 35668 91217	Thaumetopoea processionea
Bucket (OPM)	Celtic Manor	ST 35791 89822	Thaumetopoea processionea
Bucket (GM)	The Hendre	SO 46611 13811	Lymantria dispar
Bucket (GM)	Loggerheads Country Park	SJ 199 628	Lymantria dispar
Bucket (GM)	Canaston Wood, Narberth	SN 07639 14122	Lymantria dispar
Bucket (GM)	Dingestow Court	SO 45392 09376	Lymantria dispar
Bucket (GM)	Colonel's Park, Pwllplythin	SO 52014 07378	Lymantria dispar
Bucket (GM)	Briton Ferry	SS 75343 94247	Lymantria dispar
Bucket (GM)	Treborth Botanic Gardens	SH 54950 71042	Lymantria dispar
Bucket (GM)	Powis Castle	SJ 21529 06170	Lymantria dispar



Black funnel	Chepstow Park Wood	ST 48408 98356	Monochamus alternatus
Black funnel	Slade Wood	ST 45509 89340	Monochamus alternatus
Black funnel	Stanner Hill	SO 26764 58854	Monochamus alternatus
Black funnel	Newborough Forest	SH 41395 67113	Monochamus alternatus
Black funnel	Smaelog Wood, Llantrisant	ST 02653 84395	Monochamus alternatus
Black funnel	Craig yr Llyn	ST 19849 86515	Monochamus alternatus
Black funnel	Moel Famau	SJ 16982 61409	Monochamus alternatus
Black funnel	Treborth Botanic Gardens	SH 54778 71067	Monochamus alternatus
Black funnel	Mynydd Emroch	SS 78310 90048	Monochamus alternatus
Black funnel	Taliaris Park	SN 63447 28429	Monochamus alternatus
Rotor Arm 1	The Cot, Devauden	ST 50726 99243	Neonectria neomacrospora
Burkard 1	Talybont-on-Usk field station	SO 10496 23386	Random sampling



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