

2023 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 2023 'Welsh Plant Health Surveillance Network (WPHSN) 2022 Review document (March 2023)' <u>Welsh Plant Health Surveillance Network (WPHSN) (forestresearch.gov.uk)</u> and reports on the second 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, interim results from the 2023 trapping season, and recommendations are made for 2024 and beyond.



Roles in the WPHSN team



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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 beetle which mostly lives in Quercus species
	(oak trees).
Agrilus planipennis	Emerald ash borer beetle (EAB): an exotic beetle
	pest of Fraxinus species (ash trees). Not currently
	detected in the United Kingdom.
Airborne inoculum	Microorganisms suspended in, and carried through,
	the air, including microorganisms which can
	penetrate and infect another organism
	(pathogens).
АРНА	Animal and Plant Health Agency: a UK agency
	working to safeguard animal and plant health for
	the benefit of people, the environment, and the
	economy.
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 or plant.
Canker	Disease symptom, in which tree bark is killed and
	appears discoloured and/or sunken and/or
	cracked.
CEFAS	Centre for Environment, Fisheries and Aquaculture
	Science: marine and freshwater scientists
	providing data and advice to the UK government
	regarding safe and sustainable use of seas, oceans
	and rivers.
Cryphonectria parasitica	Sweet chestnut blight: a fungal pathogen of the
	Castanea genus (sweet chestnut trees).
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 well-being.
Fera Science	A leading provider of laboratory and field-based
	services in the agriculture, food, and environment
	sectors.
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	Conjfer root and butt rot: a fundal pathogen of
nerci obasiulon species	<u>oornior root and batt rot</u> r a ranger pathogon of
neter obasitation species	conifer trees.
Hylobius abietis	Conifer trees.Large pine weevil: a beetle pest of conifer trees,
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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 beetle which acts as a vector for the pine
	wood nematode – a pest of pine trees.
Neonectria neomacrospora	Neonectria canker of fir: a fungal pathogen of
	Abies 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.
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 case amount, of a
	specific organism.
RFS	Royal Forestry Society: a professional body for
	foresters and landowners where knowledge and
	information about is shared about woodland
	management.
RVC	Royal Veterinary College: a veterinary school
	leading in research and clinical services.
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).



The Pirbright Institute	A research organisation which studies and
	prevents viral diseases of farm animals and
	humans.
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, utilising the cones of Picea
	(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: 2023 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) programme 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 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.

The commercial element of the forest industry in Wales depends heavily on the healthy planting, growth and harvesting of one spruce species, namely Sitka spruce (*Picea sitchensis*). An outbreak affecting this one 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 compartments, 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 an invaluable support to human physical and mental well-being. 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 cause stress in trees, increasing their susceptibility to infection and disease.

The WPHSN programme 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	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
biguitatus	by carrying the causative bacteria from affected trees to
	healthy trees.
Agrilus	Fatal to infected trees as the larvae live and feed on internal
planipennis	tissues of trees preventing water and nutrient cycling.
Dondroctopus	Weakens tree through burrowing into the bark creating egg
micans	chambers in the cambium for breeding. Larvae feed on
	internal woody tissue. Fatality in prolonged colonisations.
	Destructive to conifer seedlings and newly planted young
Hylobius abietis	conifer trees by girdling stems through feeding on the bark
	tissue.
	Primarily attacks already compromised trees. Weakens
	trees further by boring tunnels through the bark to the
ips ceriibiae	phloem layer to create breeding chambers. Feeding larvae
	cause canopy dieback and needle defoliation. Vector of

Table 1.Summary of the WPHSN programme primary organisms and the key
threats they pose to tree health.



Organism	Threat to tree health
	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	A vector for the Pine wood nematode (PWN) which causes
alternatus	pine wilt disease (PWD) in pines, which can be fatal to trees.
Thaumetopoea processionea	Fatal to infected trees through vigorous feeding by the larvae (caterpillars) of the leaves, striping the canopy bare and leaving the tree open to secondary infection and more susceptible to drought stress.
Pathogens	
Cryphonectria parasitica	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.
Heterobasidion 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 the tree and causing dieback. Trees can die as a result of the infection, or due to increased susceptibility to secondary pathogens.
Neonectria neomacrospora	Infects fir (<i>Abies</i> spp.). Weakens trees by causing cankers, that kill off the phloem layer in branches, resulting in dieback. Excessive infection can lead to fatality.
Phytophthora pluvialis	Regulated pathogen. Weakens trees by causing needle loss, multiple resinous cankers and shoot dieback. Several aspects of its biology (<i>e.g.</i> , dispersal and host range in the UK) are not fully understood yet.



Organism	Threat to tree health
Phytophthora ramorum	Regulated pathogen of larch (Larix spp.) and other tree
	species. Fatal to infected larches by causing shoot wilting
	and withering, premature needle cast and bleeding cankers
	on branches and stems. Spores are spread by wind, rain,
	and mechanical means: footfall, mammals, vehicles.
	Infects spruce (Picea spp.) and is a phytopathogenic fungi
Phizoctopia	which can cause needle death by parasitizing young shoots
butinii	and needles. Normally occurring in twigs at ground level,
	penetrates needles when air humidity is high, forming mats
	on the surface of the leaf tissue.
	A rust fungus that infects spruce and cherry (Picea spp. and
Thokonsora	Prunus spp.) trees. It can reduce seed yield in spruce as
areolate	infected cones produce infertile seeds and causes necrosis
	in leaf tissue in cherry trees. It relies on both these trees to
	complete its life cycle.



3. Detecting invasive organisms

3.1. Methodology

Site locations for the deployment of insect and spore traps in the pilot year (2022) of the WPHSN were selected using previous work led by Professor Hugh Evans. As a result of this, a 'J' formation was used to ensure zones that were at a higher risk of colonisation from migrating insects were selected (Figure 1).



Figure 1. Map of Wales illustrating the 'J' formation used to plot the trap locations in 2022 (purple spots).

In line with the forecast and targets for 2023 (as outlined in the <u>WPHSN 2022 Report</u>), the 'J' formation of the 2022 plan was maintained, with the inclusion of sites in a south to north direction to encompass mid-Wales in the surveillance plan (Figure 2).





Figure 2. Map of Wales illustrating the 'J' formation used to plot the trap locations in 2023 with inclusion of plots on the south to north mid-line (green spots).



4. Traps

4.1. Insect traps

Three types of insect trap were selected for the WPHSN programme: 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 at head height or in the tree canopy.

4.2. Spore traps

Two types of spore trap were selected for the WPHSN programme: Burkard volumetric air sampler and rotor arm spore traps. Both traps contain sticky surfaces (sticky rods for rotor arm traps and sticky tapes in Burkard traps), which airborne spores hit and become attached to. Unfortunately, due to a technical problem with the rotor arm traps at the time of deployment, only the Burkard spore traps were used for the 2022 surveillance programme. This problem has however since been resolved, allowing rotor arm spore traps to be deployed and trialled in 2023. Over the course of 2023, both Burkard samplers employed during 2022 were affected by electronic faults and were therefore collected and examined by the team. The cause to the problem was determined and the fault fixed, allowing Burkard samplers to be re-deployed in 2024.



5. Progress report

5.1. Insect monitoring

Targets in the <u>WPHSN 2022 review</u> (Lee & Olivieri, 2022, p. 15) outline a proposed expansion of the surveillance network by increasing the coverage across Wales and the number of traps deployed, as well as, building up the sentinel site network and continuing to collaborate with stakeholders to promote data sharing and information delivery into the public domain.

The addition of surveillance sites to the 'J' formation along the south/north axis of Wales was incorporated (Figure 2). Thus, the number of sites utilised for the WPHSN 2023 surveillance increased to 32, from 23 in 2022. Naturally, this enabled the targeted increase in the number of traps deployed – from 35 in 2022 to 73 in 2023. Of the 73 traps deployed, 67 were used to detect presence/absence of invasive insects and six for the detection of fungal pathogens. Additionally, a third canopy trap was deployed for the detection of *Monochamus alternatus*, a vector for a nematode which causes pine wilt disease in pine trees.

Of the sites selected for the 2022 surveillance, those which were deemed higher risk were selected again for surveillance in 2023. The data gathered each year will be compared to establish any patterns in populations and/or distribution of invasive organisms.

A breakdown of the numbers of each type of trap deployed in the 2023 season is presented in Table 2.

Тгар	Number
	deployed
X-vane to detect Ips typographus	16
X-vane to detect Ips cembrae	17
Total X-vane traps	33
Bucket to detect oak processionary moth	14
Multi-funnel to detect borer beetles	14
Multi-funnel to detect Monochamus alternatus	6
Total canopy traps	34
Burkard samplers to detect pathogenic spores	2
Rotor arm traps to detect pathogenic spores	4
Total spore traps	6
Total number of traps deployed	73

Table 2.Summary of traps deployed in the WPHSN 2023.



The biological samples taken during the WPHSN trapping programme show a presence of *Ips cembrae* at 14 of the 18 sites selected and absent in traps at 4 sites (Figure 3). *Ips cembrae* were detected in traps at Rheola Wood near Resolven, Llantrisant near Pontypridd, Cwmcarn near Cwmbran, Chepstow Park Wood in Chepstow, Manson's Grove near Monmouth, Cwmyoy in the Black Mountains, Irfon Forest near Llandrindod Wells, Radnor Forest near Knighton, Abbeycwmhir near Rhaeadr, Dyfnant Forest near Welshpool, Moel Famau near Mold, Clocaenog Forest near Ruthin, Beddgelert Forest near Blaenau Ffestiniog, and Abergwyngregn near Bangor. *Ips cembrae* were not detected in traps at Penllegare Valley Wood near Swansea, The Hendre near Monmouth, Sennybridge near Llandovery, and at Treborth Botanic Gardens near Bangor.

Samples suggest an absence of *Ips typographus*, *Thaumetopoea processionea* (oak processionary moth), and *Agrilus planipennis* (emerald ash borer), and *Monochamus alternatus* (Japanese pine sawyer beetle).



Figure 3. Map illustrating the presence and absence of *Ips cembrae* in traps deployed in Welsh woodland locations selected for the WPHSN 2023 programme. Dark blue spots indicate traps which detected *Ips cembrae*. Light blue spots indicate traps which did not detect *Ips cembrae*. Green spots indicate other insect and spore traps in the network.



Other species of note present in the samples taken from the insect traps, but not of concern, were:

- Agrilus laticornis (jewel beetle)
- Hylesinus varius (ash bark beetle)
- Hylobius abietis (large pine weevil)
- Hylastes cunicularius (bark beetle)
- *Hylastes* species (crennulate bark beetle)
- *Pityogenes chalcographus* (six-toothed spruce bark beetle)
- Platypus cylindrus (oak pinhole borer)¹
- *Rhagium bifasciatum* (two-banded longhorn beetle)
- *Rhizophagus* species
- *Tetropium castaneum* (European spruce longhorn beetle)
- Thanasimus latreille (ant beetle)²
- Typodendron lineatum (striped ambrosia beetle)

¹Establishes in severely stressed trees, thus is a good indicator or poor tree health.

²An important predator of bark beetles.

A full list of insect species identified can be found in Appendix I.

The highest numbers of *Ips cembrae* were detected in traps at sites in mid north Wales where a total of 257 individuals were identified. Traps in southeast Wales also detected a high number of individuals – 163 - but fewer than in mid north Wales. Whilst traps in southwest Wales did not detect any individuals (Figure 4).

The sites in mid north Wales were \sim 5.4 miles west of the Wales/England border and those in the southeast between \sim 17.9 and 3.1 miles west of the border.

Sites in southwest Wales were ~57.0 miles west of the Wales/England border.

The data corroborate with the work led by Professor Evans (2021) whereby outbreaks and colonisation of bark beetles will most likely be from the east of England. As such, these data can be used for the site selection in the 2024 surveillance programme to ensure that priority areas, such as the Wales/England border and Southeast Wales are treated as 'higher risk' areas and have a steadfast trapping network established.





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

Although more traps to detect *Ips cembrae* were used in 2023 compared with 2022, insect numbers detected during routine servicing remained lower in 2023 than in 2022. In 2022, traps were routinely detecting >50 individuals each and in 2023 >20 individuals each (Figure 5).





Figure 5. Graphs illustrating a trend in detection of *Ips cembrae* in traps across Welsh woodlands in 2022 and 2023 (site abbreviations are indexed in Appendix 2).

Whilst *Ips cembrae* is not a primary pest, it is a secondary pest, it is prudent to continue with the surveillance of it to monitor the spread across Wales. This is particularly important since the detection of *Ips cembrae* in the pest free area in Scotland. It would indicate that the species is migrating and possibly increasing in population size, thus imperative that the WPHSN continues to monitor its ecology.



5.2. Spore monitoring

Over the course of 2023, surveys were carried out by the WPHSN team, in collaboration with FR THDAS and NRW, to inspect several forest sites for new and established pathogen threats and select areas suitable for spore traps deployment. The sites inspected included Radnor Forest (Powys), Mynydd Ddu (Powys), The Cot (Devauden, Monmouthshire) and Breidden Forest (Powys).



Figure 6. Setup of rotor arm trap and data logger for the monitoring of temperature and humidity at The Cott, Devauden; the data logger is enclosed in a white radiation screen to shield it from rain and direct solar radiation. The rotor arm trap is powered by a 12V battery.

After identification and mapping of high priority pathogen threats, rotor arm traps were deployed at The Cot and Mynydd Ddu, for the detection of *Neonectria neomacrospora* and *Heterobasidion* spp., respectively (see Glossary for details on the pathogens). At each of the two sites, two rotor arm traps were set up, one in close proximity (less than 0.5 m) from a *N. neomacrospora* or *Heterobasidion* spp. fruit body, and the other



approx. 150 m away from the inoculum source. Additionally, at each site a data logger was deployed to monitor for temperature and relative humidity (Figure 6). This setup was devised to detect and quantify spore release in response to environmental and seasonal factors, and help identify the optimal conditions for sporulation, which could potentially inform management procedures.

Burkard seven-day volumetric samplers remained at the same locations as during the previous year, namely the FR field station in Talybont-on-Usk, Brecon, Powys, and the National Botanical Garden of Wales, Carmarthen, Carmarthenshire. A map with all spore trap locations is provided in Figure 7.



Figure 7. Location of Burkard seven-day volumetric samplers and rotor arm traps, updated to March 2024.



All 70 spore samples collected from Burkard samplers during the financial year 2022-2023 were processed in the pathology laboratories at Alice Holt. Total DNA was extracted from 48 mm-long segments (corresponding to 24 hours air samples) of Melinex tape from the Burkard traps, using a protocol adapted from Zajc *et al.* (2022). DNA quality and suitability for downstream analysis (*i.e.*, qPCR and metabarcoding) was then checked by running the extracted DNA in a PCR and subsequently analysing the PCR products on agarose gel. DNA was successfully extracted and amplified from 58 samples. Twelve samples, all collected at Talybont-on-Usk, yielded no amplicon band on agarose gel; of these, 11 had been collected during the months of December 2022 and January 2023. All 12 samples that failed amplification were excluded from downstream analysis.

The individual 24 hours-DNA samples were pooled by week to obtain 10 samples, of which five from Talybont and five from the National Botanical Garden. These 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.

Briefly, the raw data (sequence reads) obtained by sequencing were filtered to obtain clean data, then the Divisive Amplicon Denoising Algorithm 2 (DADA2) was used to obtain Amplified Sequence Variants (ASVs, Callahan *et al.*, 2017) as detailed in in Li *et al.* (2020). ASVs were taxonomically annotated to obtain the corresponding fungal organism information. Based on the choice of the sequenced DNA barcode (Internal Transcribed Spacer 1 – ITS1), the taxonomical information was assumed reliable up to the genus level, but not suitable for fungal species identification. Relative abundance of fungal taxa was determined for each sample, and FunGuild was used to obtain the corresponding ecological functions of fungal taxa based on their species classification.

After filtering data, samples included between 40,000 and 60,000 clean sequence reads. These figures suggest that amount and quality of the pooled DNA samples was suitable for metabarcoding analysis, and further support the suitability of spore samples collected with Burkard samplers for metabarcoding analysis. The number of ASVs per sample ranged from 51 to 773 (Table 3), with samples collected during late summer-early autumn showing a higher number of ASVs compared to those collected later in the season, at each site. This might depend on DNA amount and quality of the individual pooled samples but could be also partially explained by seasonal effects (for example, a higher diversity of airborne fungal inoculum during warmer months).



Table 3.Alpha diversity: observed features, *i.e.*, ASVS per sample. Sample
name: B1 = Talybont-on-Usk; B2 = The National Botanical Gardens
of Wales. The six-digit number refers to the spore sample collected
(ddmmyy).

Sample	Observed features (ASVs)
B1.150922	363
B1.041022	773
B1.131222	185
B1.060123	79
B1.180123	79
B2.150822	431
B2.230822	516
B2.200922	379
B2.270922	533
B2.051022	51

The relative abundance of fungal taxa across the ten samples analysed is illustrated in Figure 8. Basidiomycota showed higher relative abundance in most samples, followed by Ascomycota (Figure 8.a).





Figure 8. Relative abundance of (a) fungal phyla, and (b) ten most abundant fungal genera across samples. Sample name: B1 = Talybont-on-Usk; B2 = The National Botanical Gardens of Wales; the six-digit number = data spore sample collected (ddmmyy).



Overall, among the most abundant fungal genera (Figure 8.b) were common and widespread taxa of temperate woodland, such as *Cladosporium*, *Hypholoma*, *Stereum* and *Coprinellus*, which include endophytic or soil/wood saprotrophic species. Genera including weed and grass pathogens (such as *Cladosporium* and *Phaeosphaeria*), as well as grass and tree pathogens (such as *Fusarium*) were also present. Other genera that include common forest pathogens were also detected in lower amount, including among the others *Armillaria*, *Heterobasidion*, *Ganoderma*, *Rigidoporus*, *Botryosphaeria*, *Hymenoscyphus* and *Diaporthe*.

These initial findings provide a general overview of the composition of fungal airborne inoculum at FR Talybont field station and the National Botanical Garden. The data could be used to inform and guide further, more in-depth investigations, for example using the available real time qPCR assays to identify specific forest pathogens within the genera detected. The collection of additional spore samples during 2024-25 will also allow comparison of the profile (taxa identity and relative amount) of airborne fungal spores at different locations, at different times of the season and between different years.

During 2023, work was also carried out to set up the real time qPCR assay for the detection of *Neonectria neomacrospora* (Nielsen *et al.*, 2019). The assay now allows relative quantification of *N. neomacrospora* airborne inoculum and comparison between samples collected at different times and locations. The assay will be used to detect spore release from infected stands at The Cott, Devauden, and can be further utilised to screen samples from rotor arm traps at Mynydd Du and/or Burkard samplers. Further optimisation steps will allow to determine the limit of detection (*i.e.*, minimum amount of pathogen spores that the real time qPCR can detect) and the limit of quantification (*i.e.*, the minimum number of spores at which reliable quantification of the airborne inoculum is possible) of the real time qPCR technique.

Work is currently ongoing at Alice Holt laboratories to setup a real time qPCR assay for the detection of different species of *Heterobasidion* (loos *et al.*, 2019). This will allow detection of four different species of the pathogen, including *H. annosum* and *H. abietinum* (both present in Wales), and will be utilised to detect spores released from infected stands at Mynydd Du. The assay could be also used to test samples from Burkard traps where *Heterobasidion* spp. were detected, allowing identification at the species level.

5.3. Sentinel site network collaboration

Collaboration with the sentinel site network has continued, with a total of nine sites onboard with the WPHSN in 2023, from five in 2022. The first sites on board in 2022 have continued to work with the WPHSN in 2023 and have expressed an interest in continuing into 2024 and beyond. At the time of writing, there are 14 sentinel sites that



have agreed to host WPHSN traps in 2024 and two private landowners, giving a total of 16 sentinel sites working with the WPHSN project (Figure 9).



Figure 9. An infographic illustrating the different entities working in collaboration with the WPHSN Project.

Attendance at networking events, such as those organised by the Animal and Plant Health Agency (APHA) and NRW, and interactions at public engagement events, such as the Royal Welsh Show, have led to an increase in interest from senior team members within sentinel site organisations. Furthermore, interactions with Royal Forestry Society (RFS) members at quarterly field meetings has led to the inclusion of private landowners into the Project. Below, Table 4 shows the development of the sentinel site network with the WPHSN project since its inception in 2022.



Table 4.Sentinel sites and private landowners collaborating with the WPHSN
project: 2022 – 2024.

Sentinel Site	2022	2023	2024
Forest plantations across Wales (NRW)	•	•	•
National Botanical Gardens of Wales, Carmarthen	•	•	•
Cardiff Golf Club, Cardiff	•	•	•
Powis Castle (National Trust), Welshpool	•	•	•
Loggerheads Country Park, Llanferres	•	•	•
Plas Newydd, Anglesey (National Trust)	•	•	•
Penllegare Valley Wood, Swansea		•	•
Bute Park, Cardiff		•	•
Landmarc, Brecon		•	
Treborth Botanic Gardens, Bangor		•	•
Pengelli Forest (Wildlife Trust of South & West Wales)			•
Celtic Manor, Newport			•
Leighton Estate, Welshpool (RFS)			•
Chirk Castle (National Trust)			•
Private landowners			
Dingestow Court, Monmouthshire			•
Taliaris Forest, Llandeilo			•

Working relationships with senior project leads and research scientists at FE and FC aided the planning phase of the WPHSN 2023 and will continue to do so into 2024 and onwards. The information obtained, such as flight patterns of beetles and moths, informs the best deployment methods for the trapping network in Welsh woodlands. Additionally, support from FR entomologists provides verification of insect identification from samples obtained during the survey season.

The WPHSN is proving to be of interest to the wider audience in 2023 with external stakeholders, *i.e.*, conservation organisations, displaying an interest in how the WPHSN project is operated and the results it is getting. They are interested to learn how they



can support the Project and are willing to offer their woodlands as host sites for traps. Other similar projects, such as the Forest Trapping Network (FTN) led by the FR entomology team based at the Holt Laboratory, have sought to collaborate with the WPHSN and seek advice and information to aid the planning of their work. For example, their project requires them to utilise five Welsh woodland sites and to avoid duplication of host sites and obtain reliable local information, they have liaised with the Project Lead (Racheal Lee) as part of a collaborative approach to their work.

Furthermore, for 2024 the WPHSN has collaborated with the Wider Environment Monitoring (WEM) surveillance programme overseen by FR's Head of Entomology, Dr Max Blake. This will provide the WPHSN with an opportunity to align its surveillance network with that of the WE and provide returnable data to the EU regarding the presence/absence of *Ips typographus* specifically.

Another collaboration, and more recent for 2024, is that with the Genomic for Animal and Plant Disease Consortium (GAPDC2 project). This is a collaborative programme involving leading organisations in the fields of terrestrial and aquatic animal health, such as APHA, RVC, Cefas, The Pirbright Institute, Fera Science, and Forest Research. The aim is to implement surveillance approaches based on both targeted and pathogen agnostic genomics, to safeguard the health of animals, plants, and ecosystems. The role of the WPHSN in this collaboration will be the provision of spore samples to the GAPDC2 project for analysis via the development of the spore surveillance network across Wales.



WPHSN 2024 – Forecast and Targets

1 Expand surveillance network.



¹AOD – Acute oak decline

²WEM – Wider Environment Monitoring surveillance for *Ips typographus*



In their study, Blake *et al.*, (2014) state *Ips typographus* capable of transport of >100 km (62 miles) in optimal flight conditions. Thus, the WPHSN 2024 site selection should ensure that traps used to detect presence/absence of *Ips typographus* are within this distance in a westerly direction from the Wales/England border (Figure 10). This method can also be applied for the surveillance of other invasive insect species to ensure a rigorous surveillance programme is operated.



Figure 10. Map plotting target sites for the WPHSN 2024 programme. Orange spots denote proposed sites for insect traps and green spots for spore traps.



From an invasive insect species perspective, to date, only *Ips cembrae* has been detected in Wales. With this data, and the data from the forthcoming 2024 surveillance programme, results will be analysed to establish a pattern in the distribution and population of *Ips cembrae*.

Similarly, other insect species of note (those not classed as invasive species) that have been repeatedly detected in WPHSN traps over the course of this pilot project (2022 – 2025), *e.g., Rhizophagus* species, *Platypus cylindrus* and *Thanasimus latrielle*, can be monitored for their positive impact on Welsh woodlands. To note, *Rhizophagus grandis* are a natural predator of *Dendroctonus micans* (a non-native pest of spruce trees); presence of *Platypus cylindrus* in oak is an indicator that the tree is stressed and potentially suffering with disease; *Thanasimus latrielle* is a natural predator of a range of bark beetles and brings ecological value to protecting woodlands.

If we were to take this project forward from 2025, the WPHSN data can be compared with historic data gathered by FR and FC colleagues over the last 20 years and used to predict the impact of insects on Welsh woodlands in the future.

Additionally, further spore surveillance can be carried out via a general survey over a longer window, with experimentation of different spore collection instruments to optimise spore capture. It would also be possible to consider designing a hybrid collection instrument to maximise the results we are looking for and increase the number of spore traps deployed.

2 Evolve/advance stakeholder engagement

Continue to partake Nurture working Continue to in networking and relationships with collaborate with, and promotional external colleagues in grow, the sentinnel opportinties at furtherance of data site network, bringing regional events, sharing and project on board private industry related collaboration to widen landowners to host shows, such as the the scope of the traps in addition to Royal Welsh Show, WPHSN public parks and and at local places. knowledge sharing seminars to promote and educate stakeholders



3 Expand testing capacity



Recommendations

To assist with the 2024 trap servicing workload, a member of the FR Technical Services Unit (TSU) can be subcontracted to the Project for the period April – October. Should the WPHSN receive funding in consecutive years from 2025, a seasonal appointment can be considered. This could be a student with a research interest in tree health, entomology, and/or forest pathology, and who would gain from the field experience.

To improve the sample analysis element of the system, a Wales based FR laboratory should be considered. In the longer term, this would enhance the research and development component of the Wales Plant Health Surveillance Network and develop the collaboration with our sentinel sites. In the interim, basic laboratory equipment, such as a microscope, can be sourced for the field station office in Talybont-on-Usk.



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Appendices

Appendix 1

Results from biological samples collected from all insect traps deployed in Welsh Woodlands in 2023.

Organism	Order		
Adonis ladybird (Coccinelidae)	Coleoptera		
Ambrosia beetle	Coleoptera		
Ant beetle (Thanasimus latreille)	Coleoptera		
Ant species (Formicidae)	Hymenoptera		
Aphid species (Aphidoidea)	Hemiptera		
Ash bark beetle (Hylesinus varius)	Coleoptera		
Bark beetle (Hylastes cunicularius)	Coleoptera		
Black arches butterfly (Lymantria monacha)	Lepidoptera		
Black-clouded longhorn beetle (Leiopus nebulosus)	Coleoptera		
Black-spotted longhorn beetle (Rhagium mordax)	Coleoptera		
Click beetles (Elateridae)	Coleoptera		
Common green lacewing (Chrysoperla carnea)	Neuroptera		
Common sexton beetle (Nicrophorus vspilloides)	Coleoptera		
Common wasp (Vespula vulgaris)	Hymenoptera		
Common woodlouse (Oniscus asellus)	Isopoda		
Crane Fly (Tipulidae)	Diptera		
Crenatus torania	Coleoptera		
Crenulate bark beetle (Hylastes species)	Coleoptera		
Damsel fly species	Odonata		
Darkling beetle (Tenebrionidae)	Coleoptera		
Earwig species	Dermaptera		
European spruce longhorn beetle (Tetropium castaneum)	Coleoptera		
False flower beetle (Scraptidae)	Coleoptera		
Fan-bearing wood-borer beetle (Ptilinus pectinicornis)	Coleoptera		
Feather-winged beetles (Ptiliidae)	Coleoptera		
Fly species	Diptera		
Fungus beetle (Endomychidae)	Coleoptera		
Garden chafer beetle (Phyllopertha horticola)	Coleoptera		
Giant ichneumon wasp (Megarhyssa macrurus)	Hymenoptera		
Ground beetle (Carabidae)	Coleoptera		
Harvestman spider (Leiobynum rotundum)	Opiliones		
Horntail wasp (Sirex spp.)	Hymenoptera		
Hoverflies (Syrphidae)	Diptera		



Jewel beetle (Agrilus laticornis)	Coleoptera
Jumping plant lice (Psyllidae)	Hemiptera
Large pine weevil (Hylobius abietis)	Coleoptera
Leaf beetle species (Chrysomelidae)	Coleoptera
Leaf weevil (Phyllobius glaucus)	Coleoptera
Lesser thorn-tipped longhorn beetle (Pogomocherus hispidus)	Coleoptera
Longhorn beetle (Cerambycid)	Coleoptera
Longhorn beetle species (Cerambycidae)	Coleoptera
Meadow brown butterfly (Maniola jurtina)	Lepidoptera
Millipede species	
Mite species	
Moth species	Lepidoptera
Narrow-waited bark beetle (Salpingus ruficolis)	Coleoptera
Oak pinhole borer (Platypus cylindrus)	Coleoptera
Orange ladybirds (Coccinelidae)	Coleoptera
Parasite wasp species	Hymenoptera
Rhinoceros beetle (Sinodendron cylindricum)	Coleoptera
Ridged violet ground beetle (Carabus problematicus)	Coleoptera
Root-eating beetles (Rhizophagus species)	Coleoptera
Round fungus beetle (Leoididae)	Coleoptera
Rove beetles (Staphylinids)	Coleoptera
Saw fly species	Hymenoptera
Scarab beetle (Aphodius species)	Coleoptera
Scarab beetle (Scarabaeidae)	Coleoptera
Scorpionfly (Panorpa communis)	Mecoptera
Shield bugs (Pentatomidae)	Hemiptera
Sixtoothed spruce bark beetle (Pityogenes chalcographus)	Coleoptera
Slugs	Soleolifera
Soldier beetles (Cantharidae)	Coleoptera
Speckled longhorn beetle (Pachitodes cerambycidformis)	Coleoptera
Spider species (Araneae)	Araneae
Spiderweb beetles (Ptinidae)	Coleoptera
Spot ladybird (Coccinelidae)	Coleoptera
Spot ladybirds (Tytthaspis species)	Coleoptera
Striped ambosia beetle (Typodendron lineatum)	Coleoptera
Swollen fungus beetle (Aridius nodifer)	Coleoptera
Two banded longhorn beetle (Rhagium bifasciatum)	Coleoptera
Wasp beetle (Clytus arietis)	Coleoptera
Wood boring beetle species	Coleoptera



Appendix 2

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

Date	Site																
	PV W	RH	CN	CP W	MG	СҮ	LT	SB	IF	RF	АН	DF	CF	CL	BF	AG	TB G
22 May			2	1													
24 May		0					1										
30 May									?		0						
01 Jun																0	
05 Jun		2					27										
13 Jun											10	2	0	2			
14 Jun									2							1	
19 Jun			13				72										
20 Jun								0									
21 Jun				13		35											
03 Jul									2								
04 Jul												6					
05 Jul														3			
06 Jul															2		
07 Jul										73	4						
11 Jul			3	11		10											
12 Jul							13										
17 Jul													0				
18 Jul										78	13						
20 Jul																0	
26 Jul																	0
28 Jul								0									
01 Aug											6						
02 Aug													0		0		
04 Aug										27							
09 Aug				3	7												
14 Aug											18	0		0			
15 Aug										10							
21 Aug	0																
22 Aug				1	50	2											
23 Aug									0								
30 Aug														0			



31 Aug										1			
05 Sep								0	9				
06 Sep						2							
07 Sep	0		1		0		0						
20 Sep								1		0			
21 Sep												0	
03 Oct			2	9									
05 Oct	0												



Site abbreviations (2023)

- PVW Penllegare Valley Wood
- RH Rheola
- CN Cwmcarn
- CPW Chepstow Park Wood
- MG Manson's Grove
- CY Cwmyoy Wood
- LT Llantrisant Forest
- SB Sennybridge
- IF Irfon Forest
- RF Radnor Forest
- AH Abbeycwmhir DF Dyfnant Forest
- CF Clwyd Forest
- CL Clocaenog Forest
- BF Beddgelert Forest
- AG Abergwyngregn
- TBG Treborth Botanic Garden

Site abbreviations (2022)

- 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).



Ips typographus trap

Ips cembrae trap

Borer beetle trap (e.g., emerald ash borer and two-spotted oak buprestid).

Oak processionary moth trap

Monochamus alternatus trap

Trap	Trap #	Site name	Site GR
X-Vane	1	Abergwyngregyn	SH 66863 71662
X-Vane	2	Penllegare Valley Woods	SS 62550 99232
X-Vane	3	Beddgelert	SH 56932 48812
X-Vane	4	Clwyd Forest, Moel Famau	SJ 18183 62235
X-Vane	5	Chepstow Park Wood	ST 48750 98315
X-Vane	6	Irfon Forest, Abergwesyn	SN 85562 50716
X-Vane	7	Cwmyoy, Llanthony	SO 28905 23197
X-Vane	8	Cwmyoy, Llanthony	SO 29427 21784
X-Vane	9	The Hendre, Monmouth	SO 46658 13766
X-Vane	10	Abbeycwmhir	SO 05016 71177
X-Vane	11	Clwyd Forest, Moel Famau	SJ 18155 62159
X-Vane	12	Clocaenog Forest	SJ 03308 51474
X-Vane	13	Chepstow Park Wood	ST 48790 97934
X-Vane	14	Not deployed	-
X-Vane	15	Clocaenog Forest	SJ 03337 51573
X-Vane	16	Battle Hill	SO 01412 34578
X-Vane	17	Abergwyngregyn	SH 66863 71662
X-Vane	18	Irfon Forest, Abergwesyn	SN 85560 50744
X-Vane	19	Beddgelert	SH 56932 48812
X-Vane	20	Radnor Forest	SO 22068 66700
X-Vane	22	Cwmcarn	ST 24946 94883
X-Vane	23	Radnor Forest	SO 23144 67403
X-Vane	24	Abbeycwmhir	SO 04391 71568
X-Vane	25	Resolven	SN 84192 04809
X-Vane	26	Resolven	SN 84215 04793
X-Vane	27	Llantrisant	ST 02552 84407
X-Vane	28	Llantrisant	ST 02490 84422
X-Vane	29	Sennybridge	SN 86424 35982
X-Vane	30	Ceri Forest	SO 14360 86532



Trap	Trap #	Site name	Site GR		
X-Vane	31	Dyfnant Forest	SJ 01085 13097		
X-Vane	33	Dyfnant Forest	SJ 02425 15740		
X-Vane	34	Treborth Botanic Gardens, Bangor	SH 54945 71138		
X-Vane	43	Sennybridge	SN 86692 38367		
X-vane	PL	Manson's Grove	SO 49791514		
Green Funnel	21	The Hendre, Monmouth	SO 46662 13809		
Green Funnel	36	Abergwyngregyn	SH 67026 71607		
Green Funnel	32	Penllegare Valley Woods	SS 62530 99299		
Green Funnel	35	Nash Wood, Presteigne	SO 30420 63453		
Green Funnel	38	Resolven	SN 84445 04728		
Green Funnel	40	Britton Ferry	SS 75585 94249		
Green Funnel	42	Sennybridge	SN 85755 36547		
Green Funnel	47	Cardiff Golf Club	ST 19507 81489		
Green Funnel	48	Bute Park, Cardiff	ST 17811 77014		
Green Funnel	51	Cwmyoy, Llanthony	SO 30613 22159		
Green Funnel	53	National Botanical Gardens of Wales	SN 5250 1877		
Green Funnel	56	Powis Castle	SJ 21592 06225		
Green Funnel	59	Loggerheads Country Park	SJ 19957 62858		
Green Funnel	61	Treborth Botanic Gardens, Bangor	SH 54980 71097		
Bucket	41	Sennybridge	SN 85772 36539		
Bucket	45	Nash Wood, Presteigne	SO 30420 63453		
Bucket	46	Cardiff Golf Club	ST 19507 81461		
Bucket	50	Bute Park	ST 17811 77014		
Bucket	52	National Botanical Gardens of Wales	SN 5253 1871		
Bucket	55	Penllegare Valley Woods	SS 6253 9927		
Bucket	57	Powis Castle	SJ 21592 06225		
Bucket	58	Loggerheads Country Park	SJ 19957 62858		
Bucket	60	Treborth Botanic Gardens, Bangor	SH 54804 71063		
Bucket	63	Abergwyngregyn, Gwynedd	SH 66864 71641		
Bucket	64	Plas Newydd, Anglesey	SH 51983 69472		
Bucket	66	The Hendre, Monmouth	SO 46578 13831		
Bucket	67	Britton Ferry	SS 75550 94230		
Bucket	68	Resolven	SN 84445 04728		
Black funnel	37	Cwmcarn	ST 24946 94883		
Black funnel	39	Brechfa Forest - Arboretum	SN 5341 35624		
Black funnel	44	Sennybridge	SN 86717 38345		
Black funnel	49	Chepstow Park Wood	ST 48472 98249		
Black funnel	54	Penllegare Valley Woods	SS 6294 9873		
Black funnel	62	Treborth Botanic Gardens, Bangor	SH 54945 71196		



Trap	Trap #	Site name	Site GR		
Spore traps					
Rotor Arm 1	71(a)	The Cot, Devauden	ST 50726 99243		
Rotor Arm 2	71(b)	The Cot, Devauden	ST 50771 99474		
Rotor Arm 4	72 (a)	Mynydd Ddu	SO 269241		
Rotor Arm 5	72 (b)	Mynydd Ddu	SO 269243		
Burkard 1	B1	Talybont-on-Usk	SO10482338		
Burkard 2	B2	National Botanical Gardens of Wales	SN 5269 1869		



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