

Welsh Plant Health Surveillance Network Programme:

2022 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 sector across the UK, primarily the spruce and timber industries. However, values at risk can extend well beyond the commercial woodland and forest industries to include all types of rural woodland as well as 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 to the timber industry.

This document describes and reports on the first year of activities undertaken for the Welsh Plant Health Surveillance Network (WPHSN), a ground-breaking Welsh Government funded project to monitor native and invasive pests and pathogens that may pose a threat to health of plants and trees across Wales.

A network of insect and spore traps placed at strategic woodland sites across Wales, coupled with state-of-the-art laboratory analysis by specialist Forest Research personnel, allows for the early detection, monitoring, and recording of the presence and/or abundance of insect and fungal species which may negatively affect our trees, woodlands, and forests.

Data from the WPHSN will be 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 2022 trapping season, and recommendations are made for 2023 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.



Tom Jenkins BSc, BSc, FICFor

Head of Forest Research Wales / Pennaeth Ymchwil Coedwigaeth yng Nghymru

Leads the FR unit in Wales and is responsible for the management and growth of the Unit, and the assessment of research opportunities within Wales.



Dr Leone Olivieri PhD

Forest Pathologist and Technical Advisor

Plant pathologist with a background in molecular biology. Carries out the laboratory-based work for the WPHSN programme and is based at the southern research station in Alice Holt, Surrey.



Racheal Lee BSc (Hons)

Research Worker – Tree Health

Delivers field-based support to the Tree Health Diagnostic and Advisory Service (THDAS) team in their work to detect and monitor tree health, pests and diseases.



Glossary

Agarose gel	A lab tool employed to visually assess a sample of
	DNA. It can be used to visualise DNA which has been
	run in a PCR (or real time PCR) <i>i.e.</i> , PCR products.
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).
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.
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.
Heterobasidion species	Conifer root and butt rot: a fungal pathogen of
· · · · · · · · · · · · · · · · · · ·	coniter 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 <i>Fraxinus</i> 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 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 Picea abies
	(Norway spruce trees).
KingFisher Apex	A laboratory instrument used in the automated,
	high-throughput processing of biological samples to
	isolate DNA.
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.
Neonectria neomacrospora	Neonectria canker of fir: a fungal pathogen of Abies
	species (true fir trees).
Pathogen	A microorganism that can causes disease.
PCR	Polymerase chain reaction: a molecular assay
	enabling detection of a specific DNA in a biological
	sample. Can be used to assess presence/absence of
	a specific organism (species) or group of organisms
	(genus, family, or a higher taxonomical level).
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	A fungus-like pathogen of softwood (coniferous)
	trees.
Phytophthora ramorum	A fungus-like pathogen of softwood (coniferous)
	and hardwood (broadleaved) 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.



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).					
Thaumetopoea processionea	Oak processionary moth (OPM): a non-native insect					
	pest of Quercus species (oak trees).					
Thermal cycler	A laboratory instrument to carry out real-time PCR.					
THDAS	Tree Health Diagnostic and Advisory Service: part					
	of Forest Research which provides advice and					
	diagnosis of tree pests and pathogens.					
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 Programme: 2022 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, although comparatively small, parts of the forestry sector, 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 and veteran trees, are an essential part of the landscape and national heritage, and provide 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					
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 living tissues of the tree, preventing water and nutrient flow.				
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.				
Dendroctonus micans	Weakens tree through burrowing into the bark creating egg chambers in the cambium for breeding. Larvae feed on internal woody tissue. Fatality in prolonged colonisations.				

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



Organism	Threat to tree health					
Insects						
Hylobius abietis	Destructive to conifer seedlings and newly planted young conifer trees by girdling stems through feeding on the bark tissue.					
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.					
Agrilus planipennis	Fatal to infected trees as the larvae live and feed on internal tissues of trees preventing water and nutrient cycling.					
Pathogens						
Hymenoscyphus fraxineus	Infects ash (<i>Fraxinus</i> sp.). 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> sp.). Weakens trees by causing cankers, that kill off the phloem layer in branches, resulting in dieback. Excessive infection can lead to fatality.					
<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).					
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.					
Phytophthora ramorum	Regulated pathogen of larch (<i>Larix</i> sp.) 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.					
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.					

Forest Research

4.1. Detecting invasive organisms

Site locations for the deployment of insect traps were selected using previous work led by Professor Hugh Evans. In his report, Evans (2021) illustrates, with zones, the likelihood of an establishment of a colony of *Ips typographus* in the UK in relation to climate suitability and noted this to be suitable for estimating establishment of other species. Trap locations were consequently plotted using a 'J' formation which started on Anglesey, tracking east across the north coast of Wales to the Wales/England border, then south from Mold, along the border to Monmouth, and then into Cardiff heading west along the south coast towards the Gower (Figure 1).



Figure 1. Map of Wales illustrating the 'J' formation used to plot proposed trap locations.

Following advice from the Forest Research (FR) entomology team at Alice Holt, forest stands at the chosen locations were assessed for their risk of potential colonisation. For



example, sites that contain compromised trees, such as windthrown or damaged trees, are of high risk and were therefore chosen to host a trap. Other stands of interest were those that had been recently felled, or were about to be felled, and those hosting tree species of concern, such as spruce, larch, oak, and ash. Additionally, specific time scales were determined for the deployment of the insect traps (as different insects emerge at different times of the year) and for the routine servicing of traps and sample collection.

Immediate screening of all insect samples was planned to detect presence/absence of invasives in real time. Samples were sent securely to the THDAS team where a secondary screening was carried out to confirm absence of invasive species and identify the presence of other insects to build a picture of the insect biodiversity in Welsh woodlands.

Woodland sites for the deployment of spore traps were selected according to different criteria. Some locations were chosen because of the historical presence of pathogens in the stands, such as *Heterobasidion* species, *Neonectria neomacrospora* and *Hymenoscyphus fraxineus*. Other sites were chosen because they were considered to be high-risk areas. These are defined as generally dense woodland sites, with high relevance to forest industry or conservation, and exposed to extreme weather conditions (high humidity, temperature, rainfall, and wind).

On sites with established pathogens, spore release and spread from infected stands is assessed over time, for example under different environmental conditions, or throughout the season. Conversely, on high-risk areas where no specific pathogens have been reported, an overview of the spore population is first obtained, and this information is subsequently used to inform visual surveys targeted to specific threats.

Samples collected with spore traps are analysed with DNA-based methods, which allow accurate identification of fungal and fungal-like pathogens present in the air. Different methods are used to assess presence/absence, and in some cases quantities, of a specific pathogen (real time PCR), or to analyse the overall composition of the airborne spore population (metabarcoding). Since DNA-based methods require laboratory infrastructures and equipment, spore samples cannot be immediately analysed on site. Instead, once collected they are sent directly and securely, following established biosecurity procedures, to the Forest Pathologist at Alice Holt for analysis.

4.2. External agency collaboration

Site selection visits, knowledge sharing, and collaboration with our external colleagues at sentinel sites are invaluable tools for the success of the WPHSN programme. Following a networking meeting with the Animal and Plant Health Agency at the beginning of the Programme, connections to external agencies were shared and developed to facilitate collaboration. The sentinel site organisations which came on board are Natural Resources



Wales, National Botanical Gardens of Wales, the National Trust, the Woodland Trust, Loggerheads Country Park, and Cardiff Golf Club. Together they help deliver the project through hosting site specific traps and conducting the routine servicing of them. This enables greater spread of trap deployment, thus widening the network, providing inclusion and education to our professional colleagues, and distributing the workload.

5. Traps

5.1. 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.

See Appendix 1 for the information slides outlining the specifics of the insect traps used and the target species.

5.2. Spore traps

Two types of spore trap were selected for the WPHSN programme: Burkhard 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 Burkhard spore traps were used for the 2022 surveillance programme. This problem has however since been resolved, allowing all spore traps – rotor arm and Burkhard – to be used from 2023.

See Appendix 2 for the information slides outlining the specifics of the spore traps and the target pathogens.



6. Processing biological samples

6.1. Insects

Primary screening is conducted on site at the time of collection to determine the presence/absence of invasive species. Samples are then processed at base to preserve the specimens for full examination at the end of the surveillance year. At the end of the year (autumn through to winter) a secondary screening is carried out by the entomology team at Forest Research's Holt laboratory in Farnham to verify the findings from the primary screening and to determine other species of note. This will help to build a picture of the insect biodiversity in Welsh woodlands. These data are fed back to the Welsh Government, and in due course, the sentinel site network (Figure 2).



Figure 2. Primary and secondary screening of insect samples.

6.2. Spores

Spore trap samples are collected and immediately sent to Alice Holt for analysis. Once received, the first step consists in extracting the DNA from spores. Due to the nature of the sticky substrate employed to capture spores, a highly efficient DNA extraction protocol is required. Notably, at Alice Holt two different protocols are utilised for sticky tapes (from Burkard traps) and sticky rods (from rotor arm traps). DNA extraction from rotor arm sticky rods involves the use of the KingFisher Apex robot (Figure 3) that carries out automated extraction from multiple samples at once. Once extracted, the DNA can be analysed to obtain information on specific pathogens, or on the overall composition of the sample. Real-time PCR can be used to determine presence/absence,



and in some cases amount, of spores of a specific pathogen, whereas metabarcoding provides an overview of the spore population (*i.e.*, the fungal genera and/or families) present in the air sample. Real-time PCR can be carried out in the laboratory facilities at Alice Holt, whereas metabarcoding requires the DNA to be sent to an external contractor for analysis.



Figure 3. Laboratory equipment for DNA extraction (KingFisher Apex) and real-time PCR (Roche LightCycler) for the detection of pathogens.



7. Progress report

The administrative phase of the WPHSN programme, *i.e.*, planning and organising trap deployment, began in January 2022. The Senior Tree Health Officer at Natural Resources Wales (NRW) assisted with site selection and the timing of trap deployment was optimised following discussions with the entomology and forest pathology teams in Forest Research.

Collaboration with sentinel sites was initiated via a networking meeting organised by the Animal and Plant Health Agency (APHA) in May 2022 and was further developed through networking with external colleagues at Tree Health update meetings organised by NRW. Knowledge sharing takes place during site visits with external colleagues, such as the NRW forestry team; at public events, such as the Royal Welsh Show, the Association of Professional Foresters (APF) Exhibition, and the International Plant Health Conference; and at regional seminars, such as those organised by the Institute of Chartered Foresters (ICF) and Focus on Forestry First (FFF) (Figure 4).

The operational phase of the WPHSN programme, *i.e.*, the deployment and servicing of traps, and the collection of biological samples and sample analysis, ran from 4th April 2022 until 31st January 2023; insect traps were decommissioned week commencing 24th October 2022 and spore traps on 25th January 2023. During this stage a total of 35 traps were deployed in woodlands across Wales (Figure 5). An index of exact trap locations and their grid reference is detailed in Appendix 3.



Figure 4. Promotion of the WPHSN programme and knowledge exchange taking place at the Royal Welsh Show, 2022 (left) and during a site visit to an NRW managed plantation in 2023 (right).





Figure 5. Map of Wales illustrating locations of the 35 insect and spore traps deployed in 2022. N.B. where a site hosts more than one trap, only one circle is illustrated.

A breakdown of the numbers of each type of trap deployed in the 2022 season is presented in Table 2.

Table 2.Summary of deployed traps during the active stage of the WPHSN
programme 2022.

Тгар	Number deployed
X-vane to detect Ips typographus	10
X-vane to detect Ips cembrae	10
Total X-vane traps	20
Multi-funnel to detect borer beetles	5
Bucket to detect oak processionary moth	8
Total canopy traps	13
Burkard to detect pathogenic spores	2
Rotor arm to detect pathogenic spores	0
Total spore traps	2
Total number of traps deployed	35



The biological samples collected during the WPHSN trapping programme show a presence of *Ips cembrae* at nine of the 10 sites selected and absent in traps at one site. The sites where *Ips cembrae* were present in traps were Cwmcarn Forest, Fedw Wood, Manor Wood, Talybont-on-Usk, Coed Mawr, Radnor Fishpools, Mynydd Ddu, Fforest Fawr, and Mynydd Margam. *Ips cembrae* were not detected in traps at the Tair Onen Forest Nursery (Figure 6).

Samples suggest an absence of *Ips typographus*, *Thaumetopoea processionea* (oak processionary moth), and *Agrilus planipennis* (emerald ash borer). Results from the biological samples collected from the *Ips cembrae* traps can be found in Appendix 4.

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

- Hylesinus varius (ash bark beetle)
- Hylasiuns toranio (olive bark beetle)
- Taphrorychus species
- Hylobius abietis (large pine weevil)
- Rhizophagus depressus
- Thanasimus formicarius (ant beetle)
- Nicrophorus vespilloides (burying beetle)
- *Elateridae* species (click beetles)
- Chrysomelidae (leaf beetles)
- Neuroptera (lacewings)
- *Rhagium bifasciatum* (two-banded longhorn beetle)
- Hylurgops palliates (lesser spruce bark beetle)
- *Trypodendron lineatum* (striped ambrosia beetle)
- Hylastes cunicularis (bark beetle)
- Hylastes ater/brunneus (black pine bark beetle)
- Pityogenes trepanatus
- Pityogenes chalcographus
- Curculionoidea (weevils)
- Anobium species (wood boring beetles)
- Carabidae (ground beetles)
- Pentaforma rufipes (forest bug)
- Halyzia sedecimguttata (orange ladybird)





Figure 6. Map illustrating the presence and absence of *Ips cembrae* in traps deployed in Welsh woodland locations selected for the WPHSN programme.

The core process phases of the WPHSN programme, *i.e.*, planning and preparation of site visits for the 2023 trapping season, the analysis of spore samples in the laboratory, the optimisation of molecular assays, such as that for *Neonectria neomacrospora*, and data recording, are ongoing.

Burkard trap samples were analysed microscopically, and spores were observed on the sticky tape (Figure 7). This confirmed the correct setup and performance of spore traps. DNA extraction methods for spore samples were acquired, set up and validated. DNA extraction was carried out with a subset of the samples collected at the National Botanical Garden of Wales. DNA amount and quality was checked by running the extracted DNA in a PCR and subsequently analysing the PCR products on agarose gel. The analysis showed that all Burkard trap samples yielded fungal DNA suitable for real time PCR and metabarcoding. Additionally, agarose gel analysis showed that the spore trap preparation, DNA extraction and PCR methods were reliable and ensured no contamination of samples at any stage of the workflow. In fact, DNA wasn't present in



any of the negative controls, whilst it was present in the positive control (Figure 8). Work is currently ongoing to finish extracting DNA from Burkard trap samples, which will then be sent for metabarcoding analysis.

Work is also currently ongoing to calibrate the real time qPCR assay for the detection of *Neonectria ditissima* (*Neonectria* canker of fir), and notably to determine the limit of detection and the limit of quantification of this technique. These results will allow us to establish the minimum amount of pathogen spores that the real time qPCR can detect, and the minimum number of spores at which reliable quantification of the airborne inoculum is possible. These values are essential for the correct interpretation of presence/absence and quantity data for spores. Work has also been done at Alice Holt to ensure that the rotor arm traps are both fully functional and properly calibrated for use throughout 2023. Traps will be delivered and will be ready for deployment at the beginning of the new sampling season (April 2023).



Figure 7. Microscope picture of spores collected on a Burkard sampler sticky tape.





Figure 8. Agarose gel analysis of PCR products. DNA was extracted from Burkard trap samples, run in a PCR to obtain a PCR product and then analysed with agarose gel. Every column corresponds to a different PCR product (1-19). PCR products appear on the agarose gel as a bright horizontal band (yellow arrow), whereas absence of band indicates absence of PCR product, and therefore of DNA. PCR products correspond to samples from the National Botanical Garden (NBG); negative controls prepared at Alice Holt when traps were set up prior to field deployment (AH control 01 and 02); negative DNA extraction control (NEC), non-target (*i.e.*, negative) control for the PCR (NTC) and positive control for the PCR (POS).



WPHSN 2023 – Forecast and Targets

1 Expand surveillance network



2 Progress public engagement activities





3 Expand testing capacity



Recommendations

To help manage the trap deployment and servicing workload, an additional field worker could be considered for the period April – October. This could be in the form of a seasonal employment, or a student with a research interest in tree health, entomology, 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.

References

Evans, H. (2021). *The threat to UK conifer forests posed by* Ips *bark beetles*. Research Report. Forest Research, Edinburgh. pp. 22.



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Appendices

Appendix 1

Slides outlining the specifics of the insect traps used and the target insect species.



29 March 2023

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Appendix 2

Slides outlining the specifics of the spore traps and the targeted pathogens.



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Spore pathogens







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Appendix 3:

Trap index with site names and grid references (GR). Red boxes indicate an *Ips typographus* trap and blue boxes indicate an *Ips cembrae* trap. Green funnel and bucket traps are collectively known as 'canopy traps'.

Trap	Trap #	Site name	Site GR		
X-Vane	1	Limekiln Wood, Monmouth	SO 4665013033		
X-Vane	2	Raglan	SO 5137705795		
X-Vane	3	Radnor Fishpools	SO 1814166313		
X-Vane	4	Radnor Fishpools	SO 1837565539		
X-Vane	5	Wentwood, Chepstow	ST 4349494166		
X-Vane	6	Fedw Wood, Chepstow	ST 5061798408		
X-Vane	7	Fourteen Lochs, Caerphilly	ST 2824189232		
X-Vane	8	Coed Mawr, Caerphilly	ST 2478588749		
X-Vane	9	Tair Onen Forest Nursery, Vale of Glamorgan	ST 0386274389		
X-Vane	10	Tair Onen Forest Nursery, Vale of Glamorgan	ST 0383774342		
X-Vane	11	Mynydd Margam, Port Talbot	SS 8119788703		
X-Vane	12	Mynydd Margam, Port Talbot	SS 8118888581		



Тгар	Trap #	Site name	Site GR
X-Vane	13	Afan Argoed, Port Talbot	SS 8150094207
X-Vane	14	Mill Wood, Gower	SS 48328746
X-Vane	15	Black Mountains, Brecon Beacons	SO 2502626112
X-Vane	16	Black Mountains, Brecon Beacons	SO 2505326033
X-Vane	17	Manor Wood, The Narth	SO 5255506089
X-Vane	18	Talybont-on-Usk	SO 1041318855
X-Vane	19	Fforest Fawr, Caerphilly	ST 1335183309
X-Vane	20	Cwmcarn Forest, Cwmbran	ST 2569395350
Multi-funnel	21	National Botanical Gardens	SN 5289818706
Multi-funnel	22	Loggerheads Country Park	SJ 1999062884
Multi-funnel	23	Nash Wood, Presteigne	SO 3084563302
Multi-funnel	24	Briton Ferry, Port Talbot	SS 7564294223
Multi-funnel	25	Powis Castle	SJ 21680652
Bucket	31	National Botanical Gardens	SN 5284018712
Bucket	32	Loggerheads Country Park	SJ 1997362851
Bucket	33	Nash Wood, Presteigne	SO 3085563300
Bucket	34	Briton Ferry, Port Talbot	SS 75319363
Bucket	36	Powis Castle	SJ 21680652
Bucket	37	Plas Newydd	SH 5198869474
Bucket	38	Manor Wood, The Narth	SO 51370582
Bucket	39	Cardiff Gold Club	SO 3084363314
Burkard 1	52	FR Field Station, Talybont-on-Usk	SO 10482338
Burkard 2	53	National Botanical Gardens	SN 52761873



Appendix 4

Results from the biological samples collected from the *Ips cembrae* insect traps giving the dates the biological sample were taken and the numbers of individual *Ips cembrae* beetles present in each trap.

Date	Site									
	СВ	сн	мw	тв	СА	RF	BB	FF	РТ	FN
25-Apr						0				
05-May	27							0	0	0
10-May				0			0			
17-May		1						204	0	0
30-May			3	10		0	0			
31-May	88	0			83					0
14-Jun						1				
15-Jun	36				10				0	0
16-Jun			0				11			
24-Jun				2						
27-Jun		0	2					0		
28-Jun									0	
11-Jul						2	45			
12-Jul	25				42					
14-Jul			5	25						
26-Jul	1	0		3	0		18	0		
27-Jul			2						0	
03-Aug										0
08-Aug				0		7				
09-Aug		0	1				1			
10-Aug	23				26					
11-Aug									5	
17-Aug						0				
30-Aug									3	
02 Sep										0
06-Sep				1		0				
07-Sep		3					1			
08-Sep	5				46			142		
14-Sep										0
20 Sep				0		0	0			
04-Oct		0	0				0			
06-Oct	0				0			0		0
07-Oct				0					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







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