Improving knowledge of *Xylella fastidiosa* vector ecology: modelling vector occurrence and abundance in the wider landscape in Scotland

PHC2020/04 - Project Final Report

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Content

1 Summary

The insect-vectored, bacterial plant pathogen *Xylella fastidiosa* is currently not known to be present in Scotland or the UK, but if introduced could present a serious threat to a wide range of tree and other plant species. Previous research on *X. fastidiosa* for the Plant Health Centre made progress on spatial risk mapping, vector abundance in woodlands and spatial epidemiological modelling of potential outbreaks. However, uncertainty about the wider distributions of insect vectors was a common factor across these projects.

All known vectors of *X. fastidiosa* are xylem-feeding members of the sharpshooter (Cicadellidae) and spittlebug (Aphrophoridae) families, which are in the suborder Auchenorrhyncha of order Hemiptera (the true bugs). In European outbreaks, the dominant vector is the meadow spittlebug *Philaenus spumarius*. This project aimed to improve knowledge of the ecology and distribution of these vectors in Scotland and use this to improve outbreak modelling by addressing four related objectives.

Objective 1: *To sample potential X. fastidiosa vectors in Scottish landscapes over time and space to gain a better understanding of species composition and habitat preferences.*

Regular surveys for potential vectors at Loch Leven, and a wider survey across 16 sites in central Scotland, showed that the major vector *P. spumarius* was the numerically dominant species in Scotland (77-92% of individuals sampled). Nymphs were present between mid-May and mid-July and the adult flight period (where disease transmission would occur) lasted approximately three months between mid-July and mid-September. This is a similar length to the flight period on olive trees in Italy, suggesting phenological constraints (i.e. too short duration of the flight period) may not limit any *X. fastidiosa* transmission in Scotland.

Unexpectedly, *P. spumarius* showed a clear habitat preference for heather-dominated habitats (referred to as heathland throughout), with numbers of nymphs and adults being an order of magnitude higher than in the woodland understorey or grassland. Adult densities in the latter two habitats were comparable to those recorded in outbreak areas of Italy suggesting vector densities are sufficient to drive transmission. The high densities in Scottish heathland, combined with recent demonstration that heather is a host plant of *X. fastidiosa,* also raises concern that heathlands might be at previously unanticipated risk.

However we sampled very low densities of *P. spumarius* in tree canopies. This contrasts to the situation in Italy where a seasonal migration of vectors from the understorey to the canopy drives transmission between olive trees. This might limit potential *X. fastidiosa* outbreaks in Scottish woodlands, although in tree canopies we did record reasonable numbers of another potential but as yet unconfirmed vector species (alder spittlebug *Aphrophora alni*).

Objective 2: *To compare sampling techniques for potential X. fastidiosa vectors to identify the most suitable methods for surveying.*

Sweep netting is the standard method for sampling potential vectors and was used in the surveys above. We compared this method to vacuum sampling (G-vac) and malaise trapping,and showed that sweep netting captured the greatest number of individuals, including *P. spumarius*. However, malaise trapping yielded more individuals of other rarer

species and so might complement sweep netting in future surveillance and surveys, especially if knowledge of the full range of species present is needed.

We also compared field identification of nymphs with identification using molecular methods from DNA barcoding. Both identification methods showed extremely high levels of agreement (>99%) suggesting both are suitable for future surveys.

Objective 3: *To use the new information on vector densities and the X. fastidiosa risk map for Scotland to update an existing X. fastidiosa spread model to identify locations of potential high spread risk.*

A spatially-explicit epidemiological model developed for the outbreak in Italy was adapted to simulate outbreaks in Scotland, while attempting to plausibly account for effects of lower temperature and hydric stress on disease transmission. A range of epidemiological scenarios were considered, factoring in elevated vector densities in heathlands and emerging evidence that there may be little long-distance vector dispersal in Great Britain.

In all scenarios, simulated outbreaks in Scotland grew and spread much more slowly than the outbreak in Italy (due to lower temperatures) and were dominated by asymptomatic trees (due to lower hydric stress). This suggests a lower potential impact of the disease in Scotland than in current outbreak areas, but also greater difficulty in detecting outbreaks. Modelled disease spread was greatest in areas with higher summer temperature and host plant connectivity. For epidemiological scenarios with high vector densities in heathlands, spread was greatest in heathland-dominated areas suggesting these habitats might be at highest risk of a large outbreak in Scotland.

These results are subject to considerable uncertainty about transferring the model from Italy to Scotland. Important caveats include uncertainty about host plant distribution and density, uncertainty about the *X. fastidiosa* strain that might arrive in Scotland, and uncertainty about the interaction between lower temperatures and higher vector densities on transmission in heathlands.

Objective 4: *To model the efficacy of eradication strategies applied to potential Scottish X. fastidiosa outbreaks, using the new UK contingency plan (DEFRA 2021) (a modification of latest EU strategy - Commission Implementing Regulation (EU) 2020/1201) as a reference point.*

The model was used to simulate UK and EU eradication strategies in Scotland, based on felling in the vicinity of known infections and demarcation of buffer zones around them for further surveillance. Both strategies performed similarly and achieved modest reductions in outbreak growth, suggesting that control was hampered by the high proportion of asymptomatic infections reducing the effectiveness of visual inspections. Indeed, greater testing of asymptomatic host plants also led to better control of the disease spread, as more infections were detected and removed. For the worst-case epidemiology scenarios, control effectiveness was also increased by early detection and larger buffer zone radii. It may be especially important to use a large buffer zone immediately after initial detection, even if subsequently reducing it in size, to increase the chance that the initial demarcation encloses the invasion front.

Overall, the modelling suggests that refining current control strategies for Scotland should account for epidemiological parameters of the outbreaks in Scotland, with factors such as lack of symptom prevalence and long-distance dispersal compared to Italy identified as key to the effectiveness of control. Better understanding of *X. fastidiosa* epidemiology in a Scottish outbreak will therefore be key to effective management of the disease.In conclusion, this project has revealed new information for understanding the risk of *X. fastidiosa* spread in Scotland, should the disease be introduced. It provides better understanding of vector phenology, species composition and habitat preferences as well as guidance for sampling and identification methods. Our unexpected findings of very high vector densities in heathland and very low densities in tree canopies are significant. When factored into the modelling, these suggest transmission risk of *X. fastidiosa* in Scotland may be greater in heathlands than between forest trees. Fortunately, the modelling suggested that *X. fastidiosa* may spread more slowly and cause less impact in Scotland than in Italy. However, the effectiveness of eradication strategies is likely to depend on how *X. fastidiosa* manifests in Scotland. Better understanding of factors such as the extent of symptom prevalence and long distance dispersal will be needed to refine surveillance and eradication efforts.

2 Introduction

The insect-vectored plant pathogen *Xylella fastidiosa* poses a significant threat to Scottish forestry, horticulture and the wider environment due to its ability to disperse across borders in plant trade and cause severe disease in a wide range of host plants. *X. fastidiosa* was once restricted to the Americas, where it caused long-standing losses to production (e.g. Pierce's disease of grapevines), emergence of new economically significant plant diseases (e.g. citrus variegated chlorosis) and damage to wild plants and forest trees (e.g. leaf scorch in oak, elm and sycamore) (Hopkins and Purcell 2002). The past decade has seen detection and spread of multiple subspecies and strains of *X. fastidiosa* into Europe (Sicard et al. 2018), with major outbreaks in Italy, France, Portugal and Spain and smaller transient outbreaks or interceptions reported in several other countries (EPPO 2021). These have raised the profile of *X. fastidiosa* such that it is now regarded as one of Europe's most important plant health threats (Pautasso et al. 2015).

In Scotland, *X. fastidiosa* remains absent but concern over its introduction and potential outbreaks has also been a focus of previous research carried out for the Plant Health Centre (Broadmeadow et al. 2019, Kenyon 2019, Park et al. 2019, White et al. 2019). Previous projects have made progress towards:

- Developing a spatial risk map for *X. fastidiosa* in Scotland in PHC2018/04 (Broadmeadow et al. 2019). This was done through overlay of factors including relatively suitable climate conditions (mild winter temperatures) and likely suitable land cover for vector insects.
- Understanding abundance of potential *X. fastidiosa* vector spittlebugs in woodlands utilising existing malaise trap samples and new molecular tools for identification in PHC2018/06 (Park et al. 2019). Analyses of spittlebug abundance suggested that landscape-level attributes were more important than site characteristics, with more spittlebugs in woodlands where the surrounding landscape has little woodland, other semi-natural habitats or hedgerows.
- Modelling potential outbreaks of *X. fastidiosa* in Scotland and detection by different surveillance strategies, using an existing model developed for the Italian outbreak in PHC2018/05 (White et al. 2019). This suggested a mixture of national random sampling and risk-based surveillance was most likely to pick up an outbreak.

However, following these previous Plant Health Centre projects, remaining uncertainty about potential insect vectors of *X. fastidiosa* was highlighted as a common factor that hampers risk assessment and preparedness for an outbreak in Scotland. *X. fastidiosa* is obligately insect-vectored, requiring xylem-feeding insects to acquire the bacterium in their foregut when feeding on infected plants and then to transmit the bacterium to new host plants when moving around and feeding. All known vectors of *X. fastidiosa* are members of the suborder Auchenorrhyncha of order Hemiptera (the true bugs). Specifically, confirmed vectors are members of the sharpshooter (Cicadellidae) and spittlebug (Aphrophoridae) families. In Italy, the meadow spittlebug *Philaenus spumarius* (Aphrophoridae) is considered the most important vector transmitting *X. fastidiosa* among olive trees, while *P. italosignus* and *Neophilaenus campestris* are also documented vectors under laboratory conditions (Cornara et al. 2017, 2018, Cavalieri et al. 2018). Vector transmission has been less studied in the other European outbreak areas, but *P. spumarius* individuals carrying *X. fastidiosa* have been detected in those regions, suggesting it may also be an important vector elsewhere (Cornara et al. 2019). In the rest of Europe beyond the current outbreak areas, *P. spumarius* is common and widely distributed, reaching as far north as the Arctic Circle. Furthermore, all other xylem-feeding insects have an unknown potential to act as vectors for *X. fastidiosa*, especially

abundant species with wide geographical distribution and a polyphagous diet featuring susceptible host plants (EFSA Panel on Plant Health (PLH) et al. 2019).

In Scotland, several potential *X. fastidiosa* vector species are present, including the major vector *P. spumarius* and other species such as *Cicadella viridis, Neophilaenus lineatus*, *Aphrophora alni* and *Evacanthus interruptus*. The previous Plant Health Centre project PHC2018/06 (Park et al. 2019) provided useful information about vector abundance in woodlands, but significant knowledge gaps remain about their wider distributions, abundances in different habitats, phenology and host plants. Better understanding of these would improve risk assessment for *X. fastidiosa* outbreaks in Scotland. For example, it would indicate the habitats most at risk of disease spread, the timing and length of the potential *X. fastidiosa* transmission season, and the seasonal activity of vector insects at ground level and in tree canopies. As such, a first aim of this project was to improve understanding of vector ecology and sampling methods by sampling nymph and adult vectors in multiple habitats over time, across multiple sites and using multiple sampling methods.

Additionally, this project aimed to use better information on potential vectors to refine the existing contingency modelling of *X. fastidiosa* outbreaks in Scotland from PHC2018/05 (White et al. 2019). The previous project investigated surveillance strategies, and specifically whether surveillance should be concentrated around potential "at risk" introduction sites or spread more widely across Scotland. In this project, we aimed to improve existing modelling by using the new vector sampling data alongside equivalent sampling from Italy to estimate potential transmission rates in different habitats in Scotland. In addition, the new modelling attempted to account for other factors likely to alter the trajectory of *X. fastidiosa* outbreaks in a Scottish environment as compared to Italy (Occhibove et al. 2020), including the distributions of potential host plant species in the Scottish landscape and climatic differences between Italy and Scotland. The improved model was used to simulate outbreaks and eradication strategies based on current EU measures and UK contingency plans. These measures are based on demarcation of buffer zones around detected infections in which surveillance is focused, and the felling or removal of infected plants. Here, we aimed to use modelling to explore the efficacy of these eradication strategies in a Scottish setting, taking into account the localised epidemiological biotic and abiotic drivers (e.g. temperature, host species and distribution etc).

2.1 Objectives

To meet the project aims of improving knowledge of *X. fastidiosa* vector ecology and using this to improve outbreak modelling, the project has four related objectives:

1. To sample potential *X. fastidiosa* vectors in Scottish landscapes over time and space to gain better understanding of species composition and habitat preferences.
2. To compare sampling techniques for potential *X. fastidiosa* vectors to identify the most suitable methods for surveying.
3. To use the new information on vector densities and the *X. fastidiosa* risk map for Scotland to update an existing *X. fastidiosa* spread model to identify locations of potential high spread risk.
4. To model the efficacy of eradication strategies applied to potential Scottish *X. fastidiosa* outbreaks, using the new UK contingency plan (DEFRA 2021) (a modification of latest EU strategy - Commission Implementing Regulation (EU) 2020/1201) as a reference point.

3 Spatio-temporal variation in abundance of potential *Xylella fastidiosa* vectors in central Scotland

3.1 Introduction

Vectors of *X. fastidiosa* such as the spittlebug *P. spumarius* play an important role in the transmission of disease in Mediterranean areas of Europe where seasonal movement of the insects, from the herbaceous undergrowth up into the olive trees in the drier summer months, drives transmission of the disease (Cornara et al. 2017, Bodino et al. 2019). Improving our understanding of the behaviour of vectors in different climates is vital in preventing the introduction and spread of the disease. Our previous work in PHC2018/06 (Park *et al*, 2019) demonstrated that the presence and abundance of xylem-feeding insects such as *P. spumarius* in woodlands is related to attributes of the surrounding landscape. For example, there was an increased likelihood of finding the vectors in woodlands located in landscapes with relatively low percentages of broadleaved woodland and other semi-natural habitats. However, little is known about how the abundance of these vectors varies seasonally or spatially (between and within habitats) or how these patterns are affected by choice of sampling methods. Here, we addressed this by sampling nymphs and adults of potential vector species to characterise the seasonal and spatial variation in abundance of vectors within contrasting habitats in central Scotland.

3.2 Methods

3.2.1 Seasonal variation in nymph and adult abundance

To examine how spittlebug abundance varies seasonally, repeated field surveys for vectors were carried out in three habitats (heathland, grassland and woodland) at the RSPB reserve located at Loch Leven, Kinross.

Nymphal populations of Aphrophoridae (*Aphrophora* sp, *Neophilaenus* spp and *Philaenus* sp*)* were monitored every two weeks from May to July using quadrats (a total of 40 quadrats of 0.25m^2 in each habitat). Quadrats were randomly positioned on the ground and the vegetation was inspected for nymphs: the number of nymphs, their genus, instar stage and plant host were recorded. Nymphal spittlebugs were collected at one sampling timepoint only (17th July) to confirm visual identifications via DNA barcoding (see below).

Adult populations of Aphrophoridae and other potential vectors (*Cicadella viridis and Evacanthus* spp. of Cicadellidae, subfamily Cicadellinae) were monitored weekly from July to October by sweep netting. Sampling consisted of a total of 120 sweeps along a transect, the total number of vectors caught were recorded and the insects released. This was converted to insects per sweep and per m^2. Five sweeps sampled approximately 1 m^2 (approx. 0.7 m step x 0.29 m net diameter x 5 sweeps = 1.012 m^2). Within the woodland habitat a further 120 sweeps were carried out on the lower canopies of 30 trees to monitor presence and abundance of adult vectors in trees over the summer.

3.2.2 Landscape-scale spatial variation

To determine fine-scale habitat preferences of vectors more widely across the landscape, 16 sites across the central belt of Scotland were selected for adult spittlebug sampling, including

the Loch Leven site used for seasonal sampling (Figure 1). Site choice targeted regions identified as having a higher risk of disease spread in PHC2018/04 and PHC2018/05 (Broadmeadow et al. 2019, White et al. 2019), while also including all three habitat types (grassland, woodland and heathland). Sites were separated from their nearest neighbouring site by a mean of 10.6 km (min. 4.3 km, max. 20 km). Sampling locations for each habitat type within each site were located as close as possible together (mean separation = 565 m, min. 79 m, max. 2.2 km).

Adult populations of Aphrophoridae and other potential vectors (Cicadellinae) were sampled from 26th July to 26th August. Sampling consisted of a total of 120 sweeps of ground-level vegetation cover along a transect in each habitat. Vectors caught were collected and preserved for enumeration and identification in the lab. This was converted to insects per sweep and per m^2 as above. Within the woodland habitat a further 60 sweeps were carried out on the lower canopies of 15 trees to assess abundance and presence, if any, of adult vectors in trees.

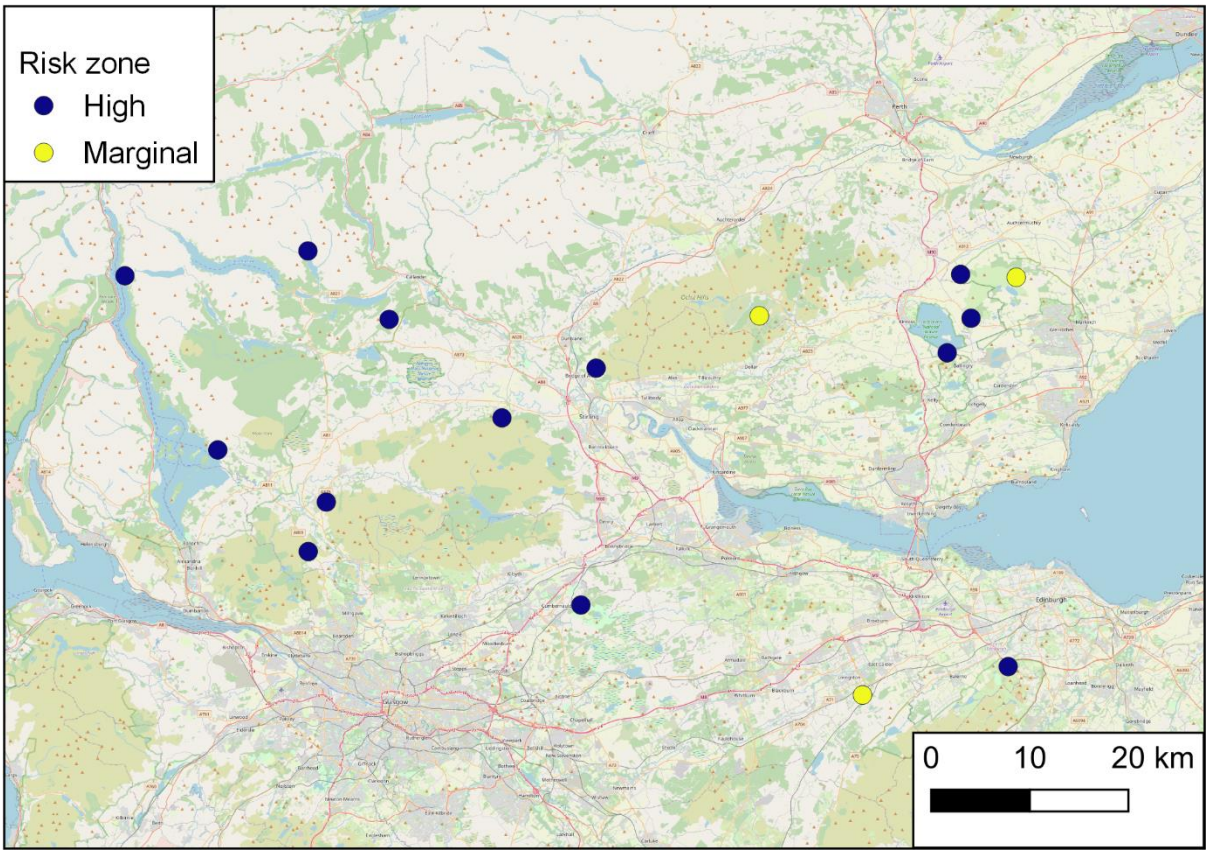


Figure 1. Sampling site locations in central Scotland and associated X. fastidiosa risk zones, taken from the previous Plant Health Centre risk mapping project PHC2018/04 (Broadmeadow et al. 2019).

As explanatory variables for vector abundance, altitude and coordinate data were taken at the beginning of each transect. An estimate of the maximum temperature at each location on its sampling day was obtained from the nearest region in an online database (www.worldweatheronline.com). To characterise the landscape surrounding the sampled locations, ArcGIS software was used to create circular buffers (1km radius) around each sampling location (n=64). Land Cover Map data v1.5.1 was used to calculate the proportion of the following land cover classes within each buffer: broadleaved woodland (LCM class 1), agricultural land (LCM classes 3 & 4), heathland (LCM classes 9 & 10), unimproved grassland (LCM classes 5, 6 & 7) and urban areas (LCM classes 20 & 21).

The influence of these factors on vector abundance was analysed using Generalised Linear Mixed Models (GLMMs) on the number of *P. spumarius*. Models used a negative binomial error distribution appropriate for count data and specified a random effect of the site (random intercept). Habitat type was specified as a fixed effect categorical factor. The proportions of broadleaf woodland, agricultural, land and heathland within 1km of each sampling location were included as continuous fixed effects, as surrounding land use was previously associated with spittlebug abundance in our earlier analyses (Park et al. 2019). In addition, geographical, sampling and meteorological variables that may influence spittlebug abundance and were included in the model were: latitude and longitude, altitude (m), date (day of year), and maximum temperature (°C) on the day of sampling. To aid model convergence, all continuous fixed effects were centred on zero and scaled to unit variance. For date, both linear and quadratic terms (i.e. terms for date and date squared) were included in the starting model to

represent a unimodal seasonal trend. From this wide range of fixed effects, we used all subsets model selection to find the combination of fixed effects with the best fit to the data. This involved fitting GLMMs with every possible combination of fixed effects and selecting the one with the lowest Akaike Information Criteria (AICc). All analyses were performed in R (R Core Team 2021).

3.2.3 Comparison of adult vector trapping techniques

To determine the most suitable method for collecting large numbers of adult *P. spumarius* and other potential vectors to detect the presence of *X. fastidiosa*, three sampling techniques were compared: sweep netting, G-vac (suction) and malaise traps. Each technique was compared in the grassland, woodland and heathland habitats at the RSPB reserve at Loch Leven, Kinross. Sweep netting and G-vac sampling were carried out once per week from 12th August to 2nd September 2021.

The sweep netting procedure was the same as previously described. Briefly 120 sweeps were carried out once per week for a total of four weeks. The total number of vectors caught was recorded and the insects released. From these data, the number caught per m^2 sampled was calculated as described previously.

Suction sampling was performed using the G-vac or 'bug vac' to collect insects from the vegetation. On each sampling, an area within a 0.25m^2 quadrat was sampled for approx. 10 seconds. A total of 30 quadrats were sampled, resulting in a total sample area of 10m^2. This was carried out once per week for four weeks. The total number of vectors caught was recorded and the insects released.

Finally, nine malaise traps were set-up, distributing three in each habitat. Malaise traps are tent-like structures that intercept flying insects and direct them towards a collection pot in which the insects are preserved in ethanol. The traps were set out for the same 3 week period as above and the pots were collected every 7 days.

3.2.4 Species identification using DNA barcoding

To test the accuracy of species identification of the Aphrophoridae samples collected in the field during this study, DNA barcoding was carried out on a sub-sample of specimens collected at Loch Leven, Fife, on 17th June 2021. Each specimen was collected by hand, a record made of its putative species and then placed in a labelled tube with the habitat type and quadrat number also noted.

DNA barcoding involves the generation of a short sequence of DNA from a sample that can then be used to interrogate a database of sequences generated from the same region in other species. The mitochondrial COI gene contains sufficient polymorphism to enable species-specific identification, whilst also containing regions of conserved sequence that enable PCR primers to bind across a range of species.

A total of 109 samples, comprising a selection of different species and nymph stages were included in the DNA barcoding, with representative samples included from each of the three habitats (woodland, grassland and heathland) in the study.

DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. A non-destructive DNA extraction approach was taken i.e., samples were not ground

up prior to DNA extraction; rather whole specimens were incubated overnight in the lysis buffer. This would allow future phenotypic examination should the results merit revisiting a particular specimen (although the integrity of the smaller nymph stages may not survive this approach (K. Lester, pers comm.)).

DNA was eluted in 100 ul elution buffer and a 5 ul aliquot of the elution run on a 1.2 % agarose gel to check the extraction was successful (high molecular weight band).

A PCR reaction was then carried out using the mitochondrial COI (cytochrome oxidase) "Folmer" primer pair, which generates a ~700 bp fragment and has been used extensively for metazoan species identification. This is often referred to a Universal COI primer as it is known to work on a wide range of species.

Forward Primer: LCO1490 GGTCAACAAATCATAAAGATATTGG

Reverse Primer: HCO2198 TAAACTTCAGGGTGACCAAAAAATCA

PCR amplification was carried out in a Techne PCR machine as follows: the total reaction volume of 20 µl consisted of: 1.2 µl template DNA, 2 µl 10x PCR buffer (NH_4, pH 8.8, 0.1% Tween 20, 2.5 mM $MgCl_2$) (Bioron, Germany), 2 pmol of each primer, 0.2 mM of each dNTP, and 0.5 U Superhot *Taq* DNA polymerase (Bioron, Germany). The PCR profile was as follows: 3 mins at 94 ^oC for initial denaturation, then 5 cycles of 30s 94 ^oC, 30s 45 ^oC, 1 min 72 ^oC followed by 30 cycles of 30 s 94 ^oC, 1 min 51 ^oC and 1 min 72 ^oC. The run was concluded with a final extension step of 72 ^oC for 10 mins. Negative controls replacing DNA template with water were run with PCR reactions to monitor potential contamination.

A 5 ul aliquot of each PCR product was electrophoresed on a 1.4% agarose gel to check PCR and samples which produced a clear single-ban, amplicons were cleaned up with EXO-SAP IT (Affymetrix, UK) as per manufacturer's instructions prior to sequencing. Sequencing was carried out at the James Hutton Institute, Dundee using the forward LCO1490 primer. Sequence data were edited and aligned using Sequencher v. 5.4 (Gene Codes Corporation, USA). All sequence chromatograms were visually checked to ensure reliability of calls e.g., bases masked by dye peaks and corrected manually where necessary.

The sequences were BLASTED against the Genbank database to confirm species ID based on percentage sequence match.

3.3 Results

3.3.1 Seasonal variation in nymph and adult abundance

A total of 1011 nymphs were recorded during the study, of which 932 (92%) were identified as the confirmed *X. fastidiosa* vector *P. spumarius*. There were also 68 *Neophilaenus spp.* and 11 *Aphrophora spp.* nymphs recorded, which could not be identified to species level in the field. As the numerically dominant species, it was only possible to construct phenology curves for *P. spumarius* (Figure 2). *Philaenus spumarius* nymphs were first sighted in woodland and grassland habitats at the beginning of May 2021, with nymphs in the heathland observed at the next sampling timepoint (mid-May). These were first sighted at a later instar stage, suggesting the small difference was due to difficulties in surveying heather for early nymphal stages. Peak nymphal abundance was at week 24 (mid-June) within all three habitats, and then showed a similar decline in abundance with nymphs largely absent after week 28 (mid-July) (Figure 2).

The number of sampled adults per species and habitat are shown in Table 1, again showing the numerical dominance of *P. spumarius*. Adults of *P. spumarius* were first recorded in heathland and grassland habitats at week 26 (the start of July) (Figure 2), with adults in the woodland understorey first recorded about two weeks later in mid-July. The abundance of *P. spumarius* adults in the grassland and woodland stayed relatively low and consistent throughout mid-July to mid-September (often <1 per m²). For unknown reasons, the numbers of *P. spumarius* in the heathland fluctuated widely throughout July to September, with the highest peak recorded at week 33 (mid-late August). Adult *P. spumarius* numbers dropped to <1 per m² by October within all habitats, with grassland abundances being the lowest.

Table 1 Numbers of adults of potential X. fastidiosa vectors recorded at Loch Leven in repeat sampling between May-October 2021 using sweep netting.

Species	Woodland (understorey)	Woodland (canopy)	Heathland	Grassland	Total
Philaenus spumarius	176	9	1288	87	1560
Aphrophora alni	0	34	0	0	34
Neophilaenus lineatus	0	0	23	2	25
Cicadella viridis	0	0	0	13	13
Evacanthus interruptus	5	0	0	0	5
Total	181	43	1311	102	1637

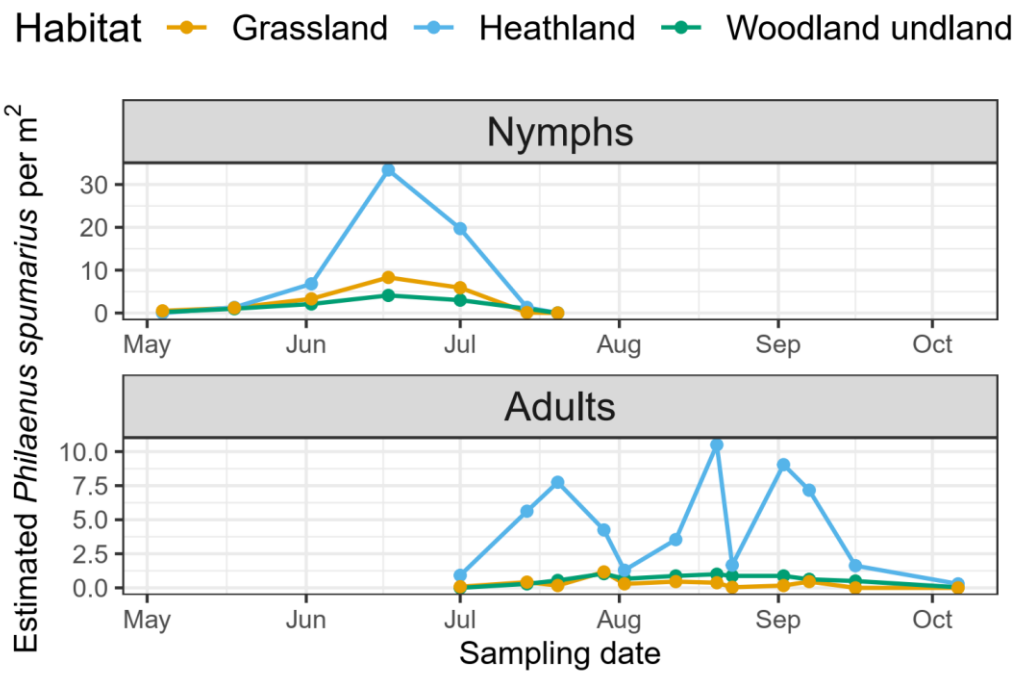


Figure 2. Phenology of P. spumarius nymphs and adults in three habitats at Loch Leven in 2021, shown as their sampled densities over time. This figure excludes woodland canopy data.

Table 1 also suggests differences in local habitat preference between adults of the different potential vector species. *Philaeneus spumarius* was the most common vector within all three habitats (excluding woodland canopies) but was most abundant by far in heathland. *Neophilaenus lineatus* adults were only present in the heathland and (much more rarely) in grassland. In the woodland tree canopies *P. spumarius* and *A. alni* adults were both recorded, however the abundance of *A. alni* was much greater than *P. spumarius*. *Philaneus spumarius* were more abundant in woodland understorey compared to the tree canopies. Two other potential leafhopper vectors, *E. interruptus* and *C. viridis* were recorded at lower densities than the other species and restricted to woodland and grassland habitats, respectively.

3.3.2 Landscape-scale spatial variation

The numbers and species composition of adult potential *X. fastidiosa* vectors (Aphrophoridae and Cicadellinae) sampled by sweep netting across four habitat types at 16 sites in central Scotland (64 unique sampling locations) are shown in Table 2. As at the single sampling site in Loch Leven presented above, *P. spumarius* was the dominant species, comprising 77% of individuals. As such it formed the focus of the statistical analysis. However, because only one *P. spumarius* individual was sampled in the woodland canopy, this habitat type was excluded from the subsequent analysis.

Table 2 Numbers of adults of potential X. fastidiosa vectors recorded in four habitat types in one-off sampling of 16 sites in central Scotland (Figure 1) from May-October 2021 using sweep netting. Note that Loch Leven was one of the sampled sites.

Species	Woodland (understorey)	Woodland (canopy)	Heathland	Grassland	Total
Philaenus spumarius	237	1	1610	231	2079
Neophilaenus lineatus	113	0	46	273	432
Cicadella viridis	42	0	4	57	103
Aphrophora alni	4	53	0	3	60
Evacanthus interruptus	8	0	0	0	8
Neophilaenus exclamationis	0	0	3	0	3
Total	404	54	1663	564	2685

The model selection procedure found that the best fitting GLMM for *P. spumarius* abundance included effects of only the habitat type and date squared (i.e. its quadratic term) (Table 3). Together these explained approximately 40% of the variation in *P. spumarius* abundances (marginal $R^2 = 0.397$). A further approximately 20% was explained by the random effect of site (conditional $R^2 = 0.596$), i.e. variation in abundance between sites regardless of the habitat type. Other potential fixed effects including land cover in the surrounding landscape, spatial coordinates, elevation and temperature were omitted from the best fitting model.

The final GLMM revealed highly significant and strong effects of habitat on *P. spumarius* abundance across sites (Table 3). Consistent with the results from Loch Leven, *P. spumarius* abundance was substantially higher in heathland than in any other habitat sampled, as revealed by significant negative coefficients for grassland and woodland understorey habitats (Table 3, Figure 3). Indeed, there were approximately 5 times as many individuals in heathland compared to grassland and woodland understorey (95% confidence range approximately 2 to 9 times as many). Based on a negative coefficient for the date squared term (Table 3), the model also suggested that abundance declined over the sampling period, although this did not quite reach statistical significance.

Table 3 Results of the final negative binomial GLMM for P. spumarius abundance as explained by habitat and date squared (i.e. its quadratic term in the model). For the two habitat effects heathland is the reference habitat to which other habitats are compared. As such, the intercept term is the log of the expected mean abundance in heathland at the central date. Effects of surrounding land cover, temperature, spatial location and elevation were omitted from the model.

Variable	Standardised coefficient	Std. Error	Z value	P value
Intercept	4.717	0.305	15.492	< 0.0001***
Habitat: Grassland	-1.5130	0.3627	-4.171	< 0.0001***
Habitat: Woodland understorey	-1.4287	0.3410	-4.189	< 0.0001***
Date squared	-0.4594	0.2424	-1.895	0.0581

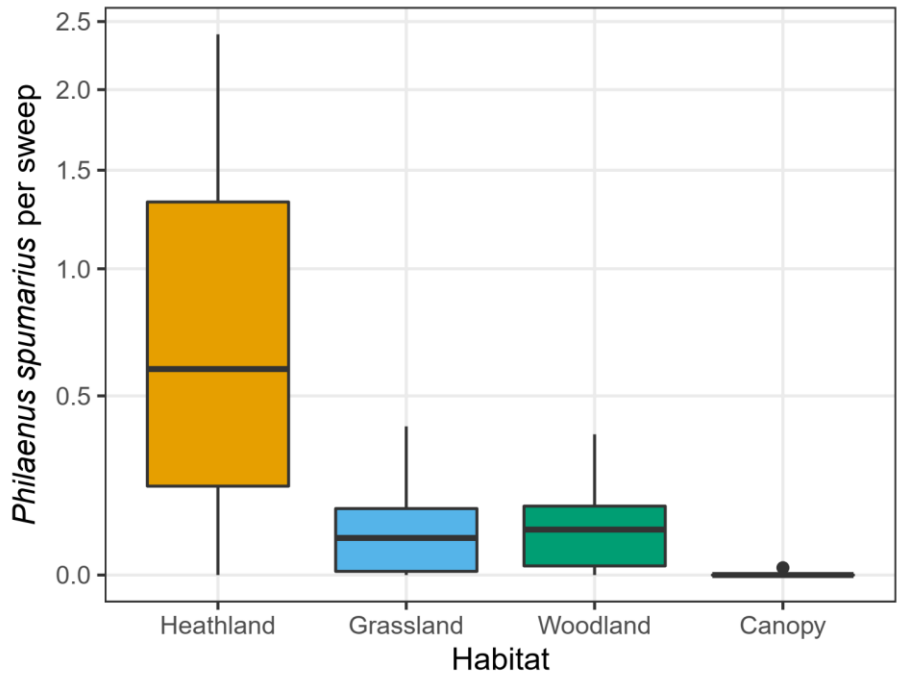


Figure 3 Boxplot showing variation in adult P. spumarius abundance (number of individuals per sweep) between habitats sampled in 16 sites across central Scotland (including Loch Leven). Note the logarithmic scaling of the y-axis.

3.3.3 Comparison of vector trapping techniques

Overall, sweep netting caught by far the most potential vector insects and the most *P. spumarius*, compared to suction sampling with the G-vac or use of malaise traps (Figure 4). However, malaise trapping appeared similarly or more effective than sweep netting for capturing some of the less common species, such as *C. viridis* and *A. alni* (Figure 4). We may also have slightly underestimated the numbers sampled in the malaise trapping since two traps were damaged by high winds in the heathland habitat.

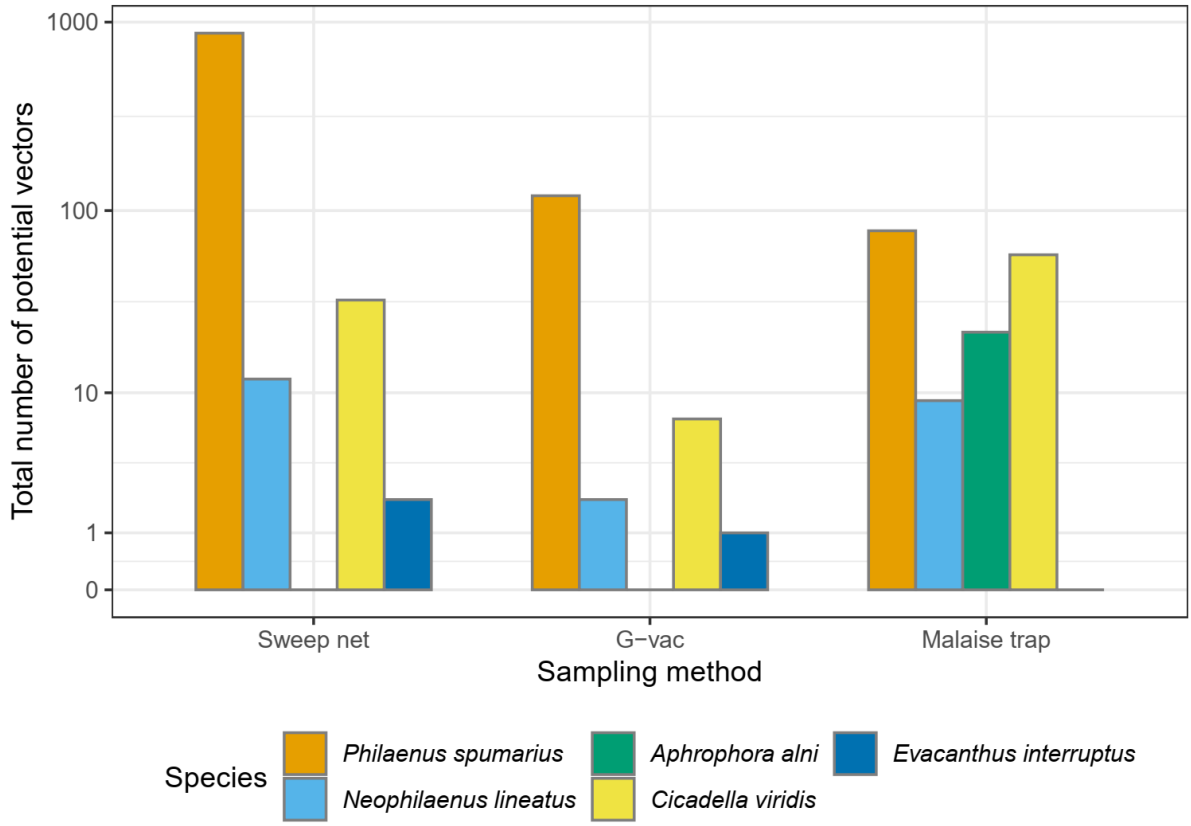


Figure 4 Sampling method comparison at Loch Leven. This figure shows the total number of potential vectors collected across all three habitats (woodland, heathland and grassland) by three different sampling methods. Vector numbers are shown on a logarithmic scale, to aid comparison. Sweep netting caught the most P. spumarius compared to the G-vac and the malaise traps (although two malaise traps were damaged by high winds in the heathland habitat).

3.3.4 DNA barcoding

Preliminary results from the DNA barcoding confirmed the accuracy and reliability of the nymph identification. Of the 109 samples taken for barcoding, DNA sequences were successfully obtained for 101 (93%). Manual identification in the field and DNA barcoding gave the same identification for 100 of the 101 samples, meaning there was approximately 99% agreement (Table 4). The single mismatch was a stage 4 nymph sampled in grassland. In addition, the barcoding provided species-specific identifications for *Aphrophora* and *Neophilaenus*, which it was not possible to do manually in the field.

Table 4 Confusion matrix comparing identification of spittlebug nymphs made manually in the field and in the laboratory through DNA barcoding.

	DNA barcoding ID		
Field ID	***Aphrophora alni***	***Neophilaenus lineatus***	***Philaenus spumarius***
Aphrophora spp.	2	0	0
Neophilaenus spp.	0	20	0
Philaenus spumarius	0	1	78

3.4 Discussion

3.4.1 What affects the abundance of potential X. fastidiosa vectors in Scotland?

Potential *X. fastidiosa* vectors were widespread across all our sites. The confirmed vector species *P. spumarius* (the meadow spittlebug) was the most abundant in our samples and reached especially high abundances as both nymphs and adults in heathland habitat. Whilst it has previously been documented that spittlebugs are typically associated with grasslands and that *P. spumarius* is found in most terrestrial habitats (Weaver and King 1954, Yurtsever 2000), this is the first study we know of to demonstrate a clear habitat preference for heathland, with average densities approximately five times higher than in grassland or woodland. This pattern was revealed seasonally in the repeat sampling at Loch Leven and more widely in the survey across central Scotland (including Loch Leven and a further 15 sites).

Furthermore, comparison of vector densities estimated in this project with those recorded by sweep netting in Italian olive groves (Xf-ACTORS vector working group 2020) suggests they reach comparable densities to Italy in woodland and grassland, but that the densities observed on heathland in Scotland are much higher. A concerning aspect of this finding is that dominant Scottish heathland genera such as *Calluna* and *Vaccinium* are known to be naturally-infected host species of *X. fastidiosa* in Portugal and USA respectively (EFSA et al. 2021). If they were to be infected in Scotland, those host species are widely and distributed in relatively contiguous heathland areas that cover approximately one third of Scotland, and could provide connectivity for disease spread. As well as the risk of spread to other habitat types, potential damage to heathlands from *X. fastidiosa* would be extremely concerning as they are a globally rare habitat, especially lowland heaths, and provide important benefits including wildlife habitat, grazing and recreation. As such the very high vector density we recorded in those habitats adds to concern about potential outbreaks in heathland in Scotland and more widely in northern Europe.

At the Loch Leven site, *P. spumarius* showed similar seasonal variation across habitats, with nymphs present between May and July, peaking sharply in mid-May. Adults were present in high numbers for about 3 months from July to September, without a clear peak in abundance. This phenological pattern is similar to that documented in the Italian outbreak region in inland Puglia, though there the phenology is shifted earlier in the season, with nymphs peaking in mid-April and high adult abundances between May and August (Bodino et al.

2019). Despite this phenological shift, our results suggest a similar potential length of the *X. fastidiosa* transmission season in Scotland and in Italy. Phenological constraints therefore seem unlikely to prevent an outbreak in Scotland.

However, a point of difference between Scotland and Italy is that in Italy adults of *P. spumarius* undergo a clear vertical migration during the dry summer period from the herbaceous understorey up to the olive tree canopy and that of other trees and bushes (Bodino et al. 2019). Our data suggest this seasonal vertical migration does not happen in Scotland. At Loch Leven, only nine *P. spumarius* adults were sampled in woodland canopies, which was approximately 5% of the total sampled in the woodland understorey vegetation. Likewise in the multi-site study, only one adult *P. spumarius* was reported in the woodland canopy (<1% of the woodland total). It is possible that sweep netting was not an effective method for sampling *P. spumarius*' use of trees, for example if they preferentially use the tops of the trees, which would be hard to sample from ground level. However, it also suggests that an apparent lack of vertical migration might strongly limit the potential transmission of *X. fastidiosa* among susceptible trees in Scotland, limiting the scale of an outbreak. A caveat to this is that the alder spittlebug, *A. alni*, was sampled at relatively high densities in tree canopies in both surveys, and its potential role as a vector of *X. fastidiosa* remains unknown.

The seasonal sampling at Loch Leven also revealed very high fluctuations in numbers of adults sampled in heathland habitat during their flight season. One possible explanation for this is temporal variation in weather conditions, since the effectiveness of sweep netting is strongly affected by weather (Hughes 1955). Furthermore, the position of the 100m transect varied from week to week and even at local scales insect numbers can be very patchily distributed. Previous studies (including our own work over the last three years and work in olive monocultures) also show similar variability. This emphasises the importance of repeat sampling throughout a season and across multiple locations and favours the use of more than one sampling methodology (see below).

Interestingly, the multi-site survey did not reproduce our previous findings from PHC2018/06 (Park et al. 2019) where the analysis of malaise trap data found an effect of the wider landscape context on the local abundance of all spittlebugs. Specifically there were fewer spittlebugs when the surrounding landscape contained more woodland and other semi-natural habitats. By contrast, we found no significant influence of the surrounding landscape in our analysis. This may be because this study considered a wider range of habitat types and was based on a relatively small number of sites, giving a relatively low power to detect weaker effects. However, given the very high numbers of *P. spumarius* in heathland habitat and a known ability to move over hundreds of metres (Bodino et al. 2021), it is surprising that we did not detect a spill-over effect from adjacent heathland causing a positive influence on abundance. This may reflect the relatively low power of our analysis, but also raises the possibility that there is less movement by Scottish *P. spumarius* populations than has been observed in Italy, mirroring preliminary field monitoring of movements and genetic results from Great Britain conducted in the BRIGIT project (S. Hogenhout pers. comm.).

3.4.2 Optimal sampling methodologies

The comparison of sweep netting, suction sampling (G-vac) and malaise traps found that sweep netting caught the greatest numbers of adult *P. spumarius,* and is therefore recommended as a sampling methodology for catching large numbers of vectors to test whether they are carrying *X. fastidiosa*. It is also the method used most widely for vector

surveys in other parts of Europe (Cornara et al. 2019, Di Serio et al. 2019, Bodino et al. 2020, Avosani et al. 2022), allowing comparison of sampled vector densities in Scotland with those in outbreak regions, as was discussed above.

Sweep netting may perform better than the suction sampling because during the fieldwork, it was observed that spittlebugs were frequently able to jump out of the way before being captured by G-vacs, due to the disturbance this technique involves, whilst this was not the case with sweep netting. Similar results have been found in previous studies (Doxon et al. 2011).

Malaise traps captured the lowest number of *P. spumarius*, so would not be well suited to surveillance should that species be the main vector of an *X. fastidiosa* outbreak in Scotland. However, malaise trapping collected comparable or better numbers of other potential vector species than both sweep netting and G-vac, including the canopy-dwelling *A. alni* and the woodland and grassland species *C. viridis*. It also samples over a long time period and so may be less sensitive than sweep netting to short term fluctuations associated with weather. As such, malaise trapping could be useful for identifying the full range of vector species present within a landscape with minimal effort and complement sweep netting in future surveillance and surveys. A caveat to this is that malaise traps may suffer damage in high winds in open habitats, as happened in this study while sampling heathlands. As such, they may be best suited to sheltered habitat such as woodland.

Comparison of nymph identification conducted manually in the field with that made by DNA barcoding showed excellent levels of agreement. Therefore, both are considered reliable methods for identifying species from survey data, providing experienced entomologists or expertise in molecular methods are available. DNA barcoding has an advantage over field identification in that it gives better ability to identify nymphs to species level but has the disadvantage of additional costs. Therefore the decision to use barcoding versus field identification may come down to the resources available and the need for species-level nymph identification. With greater resources it would be desirable to extend the comparison to nymphs of the rarer species, and potentially also the adults.

4 Modelling *Xylella fastidiosa* outbreaks and eradication efforts in Scotland

4.1 Introduction

In PHC2018/05 (White et al. 2019) we used a spread model based on Italian data to simulate *X. fastidiosa* outbreaks in woodland and urban areas in Scotland. This showed that the detection of *X. fastidiosa* is highly sensitive to the model's epidemiological parameters, which might vary from Italy due to environmental differences, and whether the introduction is in an area under surveillance due to its perceived risk of importing the disease. Here, we updated the model with the new data regarding Scottish vector distribution and density from this project, as well as new information on *X. fastidiosa* epidemiology and vector dispersal from the BRIGIT project. We used this more realistic Scottish-focused model to simulate outbreaks under eradication, based on the new UK *X. fastidiosa* contingency plan management strategy (DEFRA 2021), which differs from the current EU regulation (Commission Implementing Regulation (EU) 2020/1201) (DEFRA 2021) only with regards to the survey intensity in the outer section of the demarcated area. These simulations aimed to understand the relative efficacy of current disease management strategies under different epidemiological and introduction conditions and how variations of these might impact pathogen spread and management effectiveness in each condition.

Using this Scotland-specific mechanistic spread modelling approach allows us to address questions relevant for plant health policy:

- How might the severity of an outbreak be affected by Scottish eco-epidemiological conditions?
- How might *X. fastidiosa* spread risk vary in space across the Scottish landscape in light of the new data on vector dispersal and abundance in different habitats?
- What is the relative effectiveness of the new UK contingency plan for *X. fastidiosa* eradication compared to other options for controlling a potential outbreak in Scotland under different epidemiological and introduction assumptions? How sensitive is this to assumptions about pathogen transmission and vector ecology?

4.2 Methods

4.2.1 Model overview

We developed a mechanistic spatially-explicit spread model to simulate potential spread of *X. fastidiosa* in Scotland, for different scenarios reflecting the uncertainty about possible disease dynamics in a Scottish environment. This was done by considering plausible modifications of the Italian epidemiological parameters in the model to reflect the effect of climate, new understanding of vector distribution in Scotland and differences in dispersal behaviour between Italy and Great Britain. To identify areas where introduction might lead to risk of larger outbreaks, we model infection introduction in different locations. Results from the spread in absence of disease management measures informed the scenarios simulating outbreaks under eradication, based on current policy.

The Scottish model runs over a 200 x 200 m gridded spatial landscape of the whole of Scotland. Within each grid cell, host plant density varies according to land cover type. Transmission dynamics are simulated at a yearly temporal scale to correspond with vector seasonality (Figure 5). Modelled transmission involves deterministic compartmental local

transmission within grid cells (White et al. 2020) and stochastic vector dispersal that spreads *X. fastidiosa* to new grid cells (Figure 5).

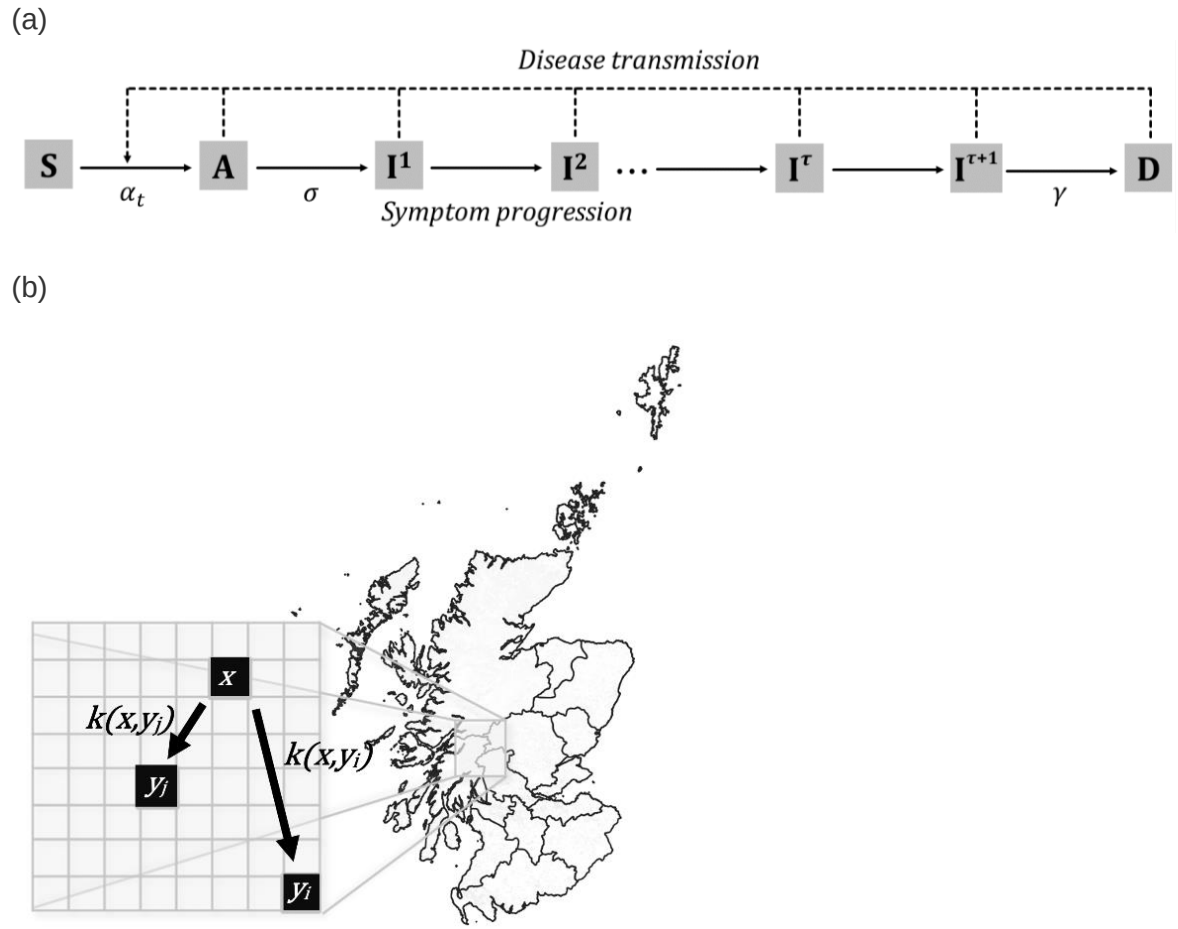


Figure 5 Model schematic overview. (a) The compartmental epidemiological model for within-grid cell transmission. Solid arrows indicate the direction and rates of movement of host plant individuals between compartments: S = *susceptible;* A = *infected asymptomatic;* I = *infected symptomatic;* D = *desiccated. (b) Stratified short- and long-distance dispersal, driving stochastic transmission between grid cells according to the kernel* k(x,y).

4.2.2 Definition of the susceptible host population

Information on the exact location and abundance of potential *X. fastidiosa* hosts across Scotland is lacking, so we adopted a "worst-case scenario" approach to estimate the distribution and density of the overall potential susceptible population over the entire Scottish landscape. Areas where potential *X. fastidiosa* hosts might occur were selected by cross referencing, at the lowest taxonomic level available, the updated database of *X. fastidiosa* susceptible hosts (EFSA et al. 2021) with the PLANTATT database of British native and non-native plant attributes (Hill et al. 2004) and their main habitats in the UK land cover map (Rowland et al. 2017). This allowed us to exclude habitats where *X. fastidiosa* hosts did not occur and map host distribution. As there is most concern about *X. fastidiosa* tree disease, only woody and semi-woody hosts were considered.

The main land cover classes harbouring host species were broadleaved woodland, arable and horticulture, heather/heathland, urban and suburban (Figure 6), consistent with previous risk mapping in PHC2018/04 (Broadmeadow et al. 2019). According to this report, these habitats harboured vector presence allowing pathogen transmission. Arable land with non-susceptible crops and urban areas with no green spaces were excluded using UK CEH Crop Map (Jarvis et al. 2019) and OS Open Greenspace Map (© Crown copyright and database right 2020) respectively.

The susceptible population density of the identified susceptible areas was estimated according to a global tree density model (Crowther et al. 2015). This density was downscaled to each 200 x 200 m grid cell of the model by the percentage vegetation cover, which was estimated using a satellite vegetation index (2008/2018 average NDVI from MOD13Q1 16-day 250 m resolution resampled at 200 m). The resulting landscape comprised susceptible grid cells arranged in patches of variable size and connectivity, due to the exclusion of the areas where susceptible host species were assumed not to occur (Figure 6).

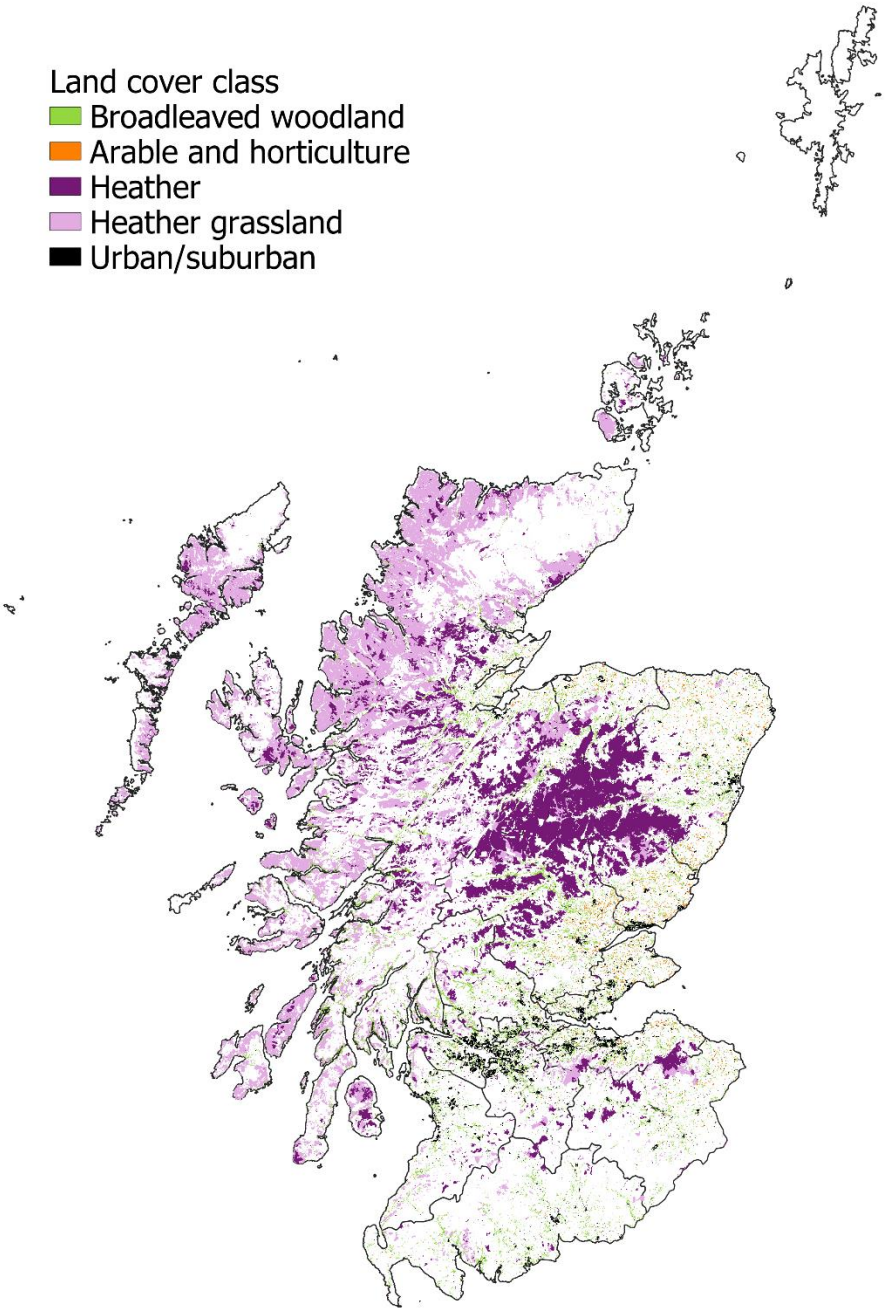


Figure 6 Scottish susceptible areas with potentially high densities of host plants for X. fastidiosa, classified by land cover class. Note that only those arable and horticultural areas known to be growing potentially susceptible crops are included.

4.2.3 Local transmission

The local infection growth within a grid cell, was an adaptation from the UK-model developed in the BRIGIT project (Occhibove et al. in prep.). The deterministic compartmental model (Figure 5) included four host plant stages, susceptible (*S*), infected asymtomatic (*A*), infected symptomatic (*I*), and desiccated (*D*). In this model, density of symptomatics (*I*) is the main drivers of transmission, with asymptomatic (*A*) and desiccated (*D*) individuals being less infectious (White et al. 2020). After infection, individuals go through an asymptomatic incubation phase (*A*) before developing symptoms and entering the symptomatic (*I*)

compartment. The transition from infected to desiccated classes only starts τ years after symptom appearance (White et al. 2020). These dynamics are described by the equations below (Eq. 1 to 7; see Table 5 for full notation):

$$S_{t+1} = (1 - \alpha_t)S_t \quad \text{(Eq. 1)}$$

$$A_{t+1} = \alpha_t S_t + (1 - \sigma)A_t \quad \text{(Eq. 2)}$$

$$I^1_{t+1} = \sigma A_t \quad \text{(Eq. 3)}$$

$$I^2_{t+1} = I^1_t \quad \text{(Eq. 4)}$$

$$\vdots$$

$$I^\tau_{t+1} = I^{\tau-1}_t \quad \text{(Eq. 5)}$$

$$I^{\tau+1}_{t+1} = I^\tau_t + (1 - \gamma)I^{\tau+1}_t \quad \text{(Eq. 6)}$$

$$D_{t+1} = D_t + \gamma I_t \quad \text{(Eq. 7)}$$

Above, we denote τ^{th} the compartment of symptomatic sojournment by I^τ_t . The total proportion of infected symptomatic trees is given by

$$I_t = \sum_{j=1}^{\tau+1} I^j_t$$

The transition rate (Eq. 8), and the transition probabilities from infected asymptomatic to symptomatic (Eq. 9), and from symptomatic to desiccated (Eq. 10) are (see Table 5 for full notation):

$$\alpha_t = e^{-\beta \frac{b_A A_t + I_t + b_D D_t}{N_t}} \quad \text{(Eq. 8)}$$

$$\sigma = 1 - e^{-\frac{1}{T_A}} \quad \text{(Eq. 9)}$$

$$\gamma = 1 - e^{-\frac{1}{T_D}} \quad \text{(Eq. 10)}$$

Epidemiological parameters were adapted from Occhibove et al. (in prep.) to represent *X. fastidiosa* dynamics in the cooler and wetter Scottish climate compared to the current outbreak area of Puglia in Italy. The infection rate α_t (Eq. 8), accounted for the likely effect of average summer temperature in each grid cell (Eq. 11) through the temperature scaling parameter C which causes reduced *X. fastidiosa* transmission at lower temperatures. C was estimated by fitting a curve to *in vitro* growth data for several *X. fastidiosa* strains (Occhibove et al., in prep.). The temperature-growth curve was then normalised to have a value of 1 at the average summer temperature in Puglia (which was also the optimal growth temperature) to give the scaling factor C as a function of temperature. Gridded average summer temperature

from the CHESS climate data (Robinson et al. 2020) were then used to determine local C value in each grid cell across the Scottish landscape.

We also assumed that due to cooler and wetter conditions in Scotland, the disease progression would be slower than is observed in olive trees in Italy. To capture this, mean incubation period (T_a) and desiccation time (T_d) were dependent upon scaling factors, w_I and w_D respectively (Eq. 12 and 13) estimated based on the relative climatic water deficit in Scotland compared to Puglia (Table 5). Climatic water deficit is linked to plant hydric stress (Vicente-Serrano et al. 2013, Dilts et al. 2015), which has been observed to influence *X. fastidiosa* symptom expression and time to desiccation (McElrone et al. 2001, Choi et al. 2013, Saponari et al. 2019). Our assumptions on the links between plant hydric stress and climatic variables are also confirmed by (Rivington et al. 2018) in a Scottish focused study on agrometeorological indicators.

The model variables scaling transmission and disease progression, including by local environmental factors (see Table 5) are:

$$\beta = Cv\beta_P \quad \text{(Eq. 11)}$$

$$T_A = w_I T_a \quad \text{(Eq. 12)}$$

$$T_D = w_D T_d \quad \text{(Eq. 13)}$$

Table 5 Eco-epidemiological parameters used in the model with associated meaning and estimation method.

Parameter	Parameter name/meaning	Value
β_p	Effective contact rate for infected individuals	17.88[a]
b_A	Infectivity of asymptomatic individuals, relative to fully infectious individuals	0.01[a]
b_D	Infectivity of desiccated individuals, relative to fully infectious individuals	0.5[a]
T_a	Mean duration of the symptomless period (years)	1.19[a]
T_d	Mean time to desiccation (years)	1.36[a]
τ	Desiccation delay parameter (years)	3[a]
C	Temperature scaling factor for transmission, based on *in vitro X. fastidiosa* growth data and gridded summer temperature in Scotland	0-0.16[b]
v	Vector density scaling factor affecting transmission rate, based on data from this report	10 (heathland)[c] 1 (other habitats)[b]
w_I	Scaling factor affecting incubation period, based on the ratio between the maximum climatic deficit of the infected area in Puglia and UK	3[b]
w_D	Scaling factor affecting time to desiccation, estimated as above	3[b]
θ	Scale parameter of dispersal kernel	10^{-6}[d]
k_l	Long-distance dispersal (km)	5.92[d]
k_s	Short-distance dispersal (km)	0.32[d]

[a]from White et al. (2020)
[b]from Occhibove et al. (in prep.)
[c]data from this project
[d]from Chapman et al. (in prep.)

4.2.4 Between-cell transmission

The model spatial component was based on presumed *X. fastidiosa* vector dispersal mechanisms, i.e. short-distance flights possibly aided by wind, or long-distance dispersal through flight and/or unintentional hitchhiking on human vehicles (Cornara et al. 2019). To reflect this, stochastic dispersal was represented as a distance-decay in transmission using a mixture of a Gaussian short-distance kernel and an exponential long-distance kernel (Figure 5):

$$k(x,y) = (1-\theta)e^{\frac{(x-y)^2}{-2k_s^2}} + \theta e^{\frac{|x-y|}{-k_l}} \quad \text{(Eq. 14)}$$

of which parameters are defined in Table 5. A weighted probability of infection was assigned to each uninfected grid cell according to the kernel distribution (i.e. the distance from all infected cells), so that the long-distance spread events depended upon the distribution of *X. fastidiosa* over the landscape (Chapman et al., in prep.).

The parameter θ determines the proportion of long-distance and short-distance events. We used the value estimated for Puglia by Chapman et al. (in prep.) to represent a dispersal type mainly involving short-distance events with some rare random long-distance jumps. However, vector dispersal is still under investigation and in Great Britain there is preliminary genetic evidence for very little long-distance dispersal (BRIGIT project; S. Hogenhout pers. comm.) (see next section).

4.2.5 Eco-epidemiological scenarios for a Scottish outbreak

Xylella fastidiosa has not been detected or intercepted in Scotland, so we hypothesised a range of plausible dynamics to represent uncertainties on vector abundance effect on transmission (see Section 2.3) and vector dispersal abilities, which could not be estimated directly. We simulated four relevant scenarios to investigate the potential impact of vector habitat preference (based on high abundance of vectors in heathland found in this project) and dispersal patterns (based on new data from BRIGIT project) on *X. fastidiosa* spread. All simulations were performed in R (R Core Team 2021) over a 16 year timeframe to give sufficient opportunity for disease spread. Since the model is stochastic, each scenario was simulated 62406 times with a single introduction of *X. fastidiosa* at a random susceptible grid cell, selected using a stratified random sampling by county. With this level of replication, introductions were simulated at 10% of the susceptible grid cells.

To explore different scenarios about vector behaviour in the model, all four combinations of the following scenarios were run (Table 5):

- **Scenarios for habitat variation in vector densities.** Based on the findings in this report, maximum densities of potential vectors in woodland and grassland in Scotland are comparable to those previously reported from olive groves in the outbreak area of Italy (Xf-ACTORS vector working group 2020). Xf-ACTORS vector data (Xf-ACTORS vector working group 2020) reports maximum vector abundances instead of averages, as the most relevant metric for transmission, and the maximum densities found in this study in heathland habitats were an order of magnitude greater than those in grassland or the woodland understorey (Figure 3). Therefore, to investigate the potential effect of this, we ran both a baseline scenario (vector densities are the same as Italy in all habitats) and a scenario in which vector densities in heathland drive higher rates of transmission in that habitat;
- **Scenarios for long-distance vector dispersal.** The baseline scenario assumed dispersal was equivalent to Italy where frequent long-distance dispersal is driving the spread. However, recent population genetic investigation of *P. spumarius* in the BRIGIT project suggested little long-distance dispersal in Great Britain (S. Hogenhout pers. comm.). Therefore, as well as the scenario where long-distance dispersal is similar to that in Italy, we also ran a scenario in which there is no long-distance dispersal (θ is set to 0 in Eq. 14).

To check the effects of our modifications compared to original parameter values estimated for Puglia, we also ran a limited set of simulations a Puglia-like scenario (i.e. including both long and short-distance vector dispersal, and setting temperature, incubation/desiccation, and vector scaling factors to 1).

Table 6. Vector scenarios used in the simulation experiment. Values of parameters not specified here are from Table 5.

Scenario	Description	Parameter values
Baseline densities and dispersal	Vector densities in all habitats are equivalent to Italy, as is the long-distance dispersal	$v = 1$ (all habitats)
Heathland densities	As a result of higher vector densities recorded in this project, transmission is 10 times higher than Italy in heathland habitat. Long-distance dispersal is equivalent.	$v = 10$ (heathland); $v = 1$ (other habitats)
Short dispersal	Vector densities in all habitats are equivalent to Italy but there is no long-distance dispersal	$\theta = 0$ $v = 1$ (all habitats)
Heathland densities and short dispersal	Transmission is 10 times higher than Italy in heathland habitat and there is no long-distance dispersal	$\theta = 0$ $v = 10$ (heathland); $v = 1$ (other habitats)

4.2.6 Scenarios for Scottish control measures

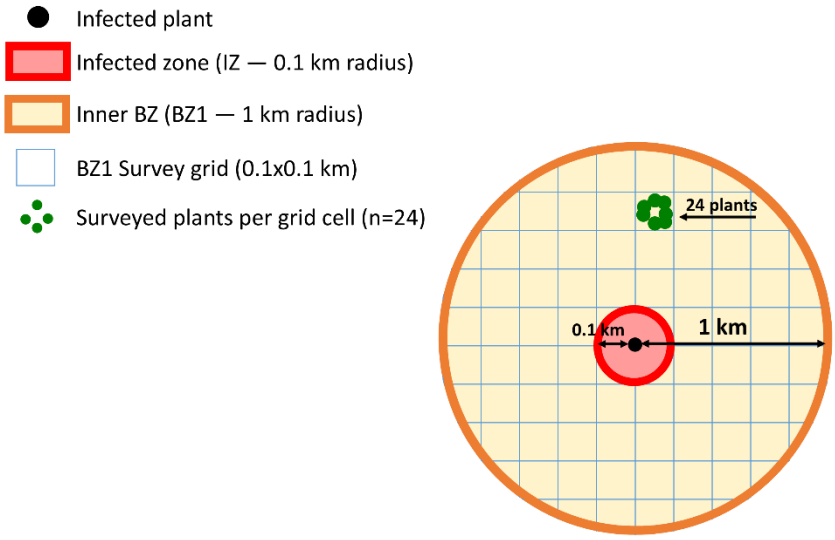
The current *X. fastidiosa* management strategy in the UK is described in the new contingency plan (DEFRA 2021), which is derived from the latest EU relevant policy (Commission Implementing Regulation (EU) 2020/1201). The simulated measures are based on an eradication approach (as opposed as containment, which is implemented only in larger outbreaks where eradication is not possible), and include:

- Demarcation of a buffer zone (BZ) with a radius of 2.5 km around the positive detections (Figure 7).
- Survey based on visual detection of symptoms for *X. fastidiosa* with a 100 x 100 m grid in the first 1 km radius of the BZ (inner BZ or BZ1) and a 0.5 x 0.5 km grid in the subsequent 1.5 km, which represents the outer BZ (BZ2) (Figure 7). In each grid cell 24 plants are surveyed, and samples are taken from all symptomatic and a random proportion of asymptomatic plants to be tested in the lab.
- When new positives are confirmed by PCR, fell/remove all host plants within a 100 m radius infected zone (IZ), and all symptomatic plants in the whole BZ.
- The demarcated areas are updated every year using the new positives uncovered in the surveys.

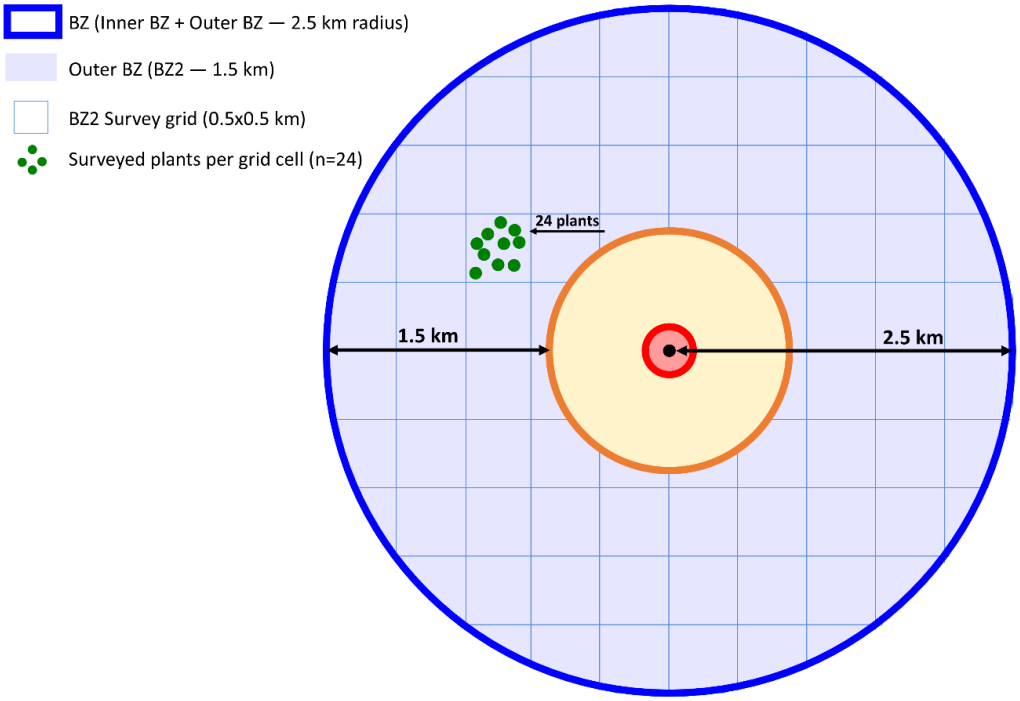
These UK measures differ from current EU regulation with regards to the survey grid in the outer BZ (BZ2), which is set as 1 x 1 km in EU (Figure 7), determining a lower survey intensity in the outer BZ compared to the UK. The BZ size, in both EU and UK plans, was only recently reduced from 5 km to the current 2.5 km with the aim of reducing the potentially negative

effects of control measures on the landscape and the horticultural industry. However, previous studies indicate that greater BZs are more effective in controlling *X. fastidiosa* potential spread, although they might require higher surveillance costs (White et al. 2017, EFSA Panel on Plant Health (PLH) et al. 2019).

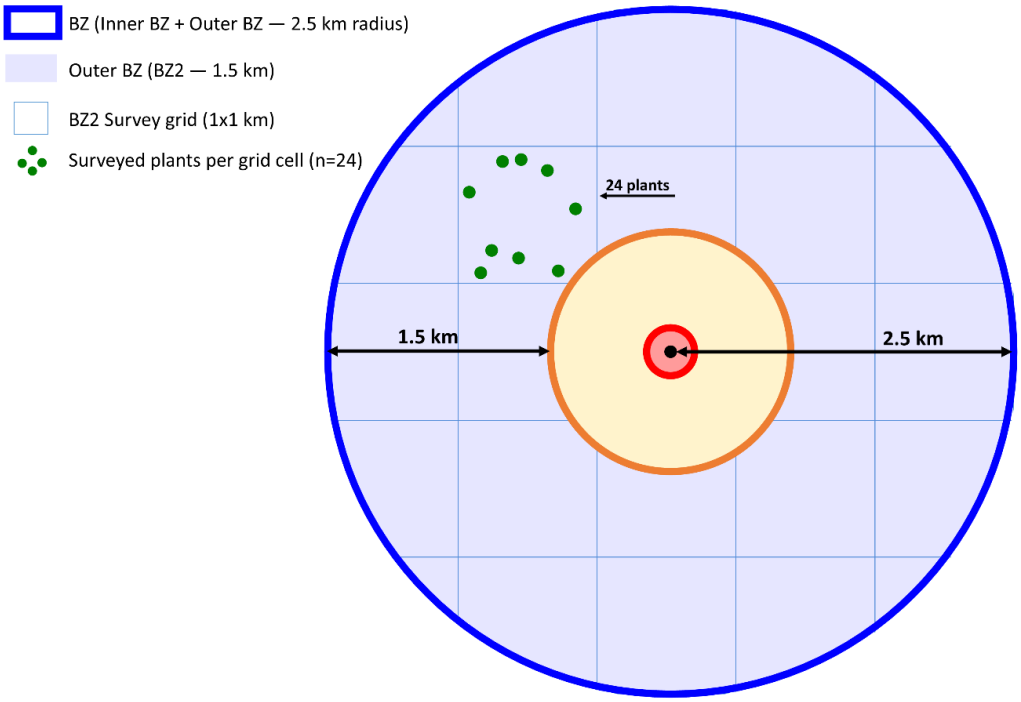
a) Infected area and inner Buffer Zone



b) Buffer zone from UK contingency plan



c) Buffer zone from EU measures



d) Alternative extended Buffer Zone

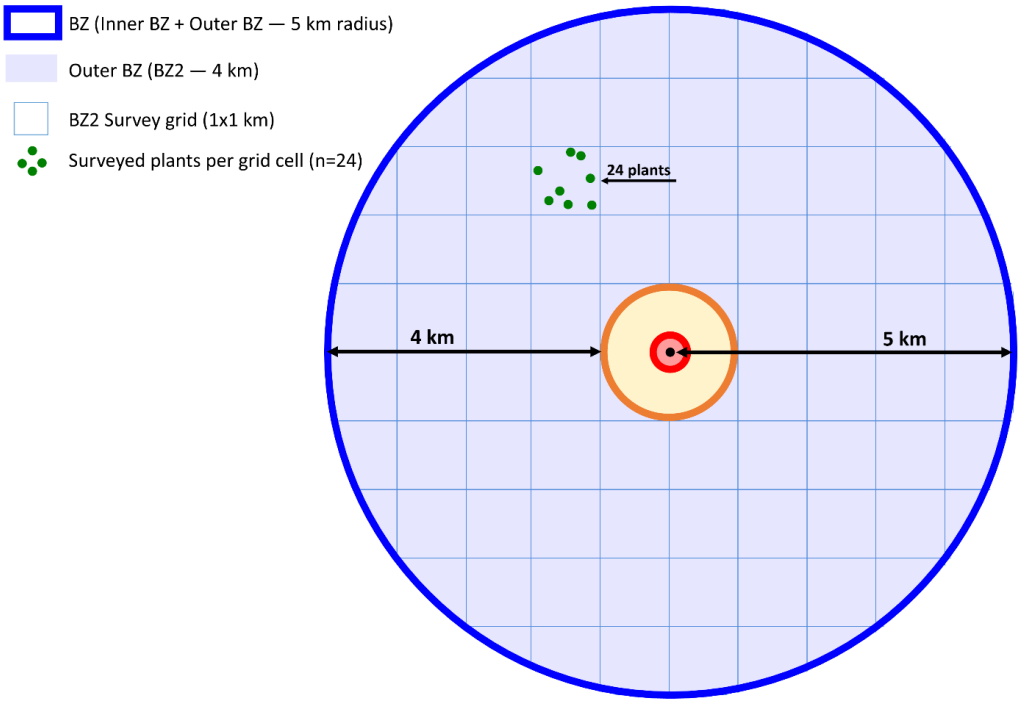


Figure 7. Schematics of demarcated infected (IZ) and buffer zones – inner buffer zone (BZ1) and outer buffer zone (BZ2) – for an outbreak of one infected plant. a) IZ and inner buffer zone (BZ1): 0.1x0.1 km grid for surveys within 1 km of IZ (these characterise both UK and EU regulations and they will not be varied in our scenarios). b) Outer BZ (BZ2) survey grid specified in the new UK contingency plan for surveys between 1km and 2.5 km from infected plant. c) Outer BZ (BZ2) survey grid specified in the EU regulation (Commission Implementing Regulation 2020/1201) for surveys between 1km and 2.5 km from infected plant. d) Example of alternative scenario: outer BZ (BZ2) survey grid of 1x1 km with a total BZ of 5 km.

The main aim of our Scottish surveillance and control scenario simulations was to test the relative effectiveness of the UK and EU eradication strategies, and variations of these motivated by our epidemiological assumptions. As a result, we highlight which combination of measures might be relatively more effective to control an outbreak, while reducing felling, under Scottish specific hypothesised epidemiological and introduction conditions based on the spread model results. To do this we chose to simulate variations in outer BZ (BZ2) survey intensity, BZ radius, and asymptomatic testing rate during surveys – due to the high proportion of asymptomatic individuals resulting from our spread simulations – in combinations with variations in epidemiological scenario, introduction habitat, and time to detection (all combinations listed in Table 7).

Since the high vector abundance found in heathlands might have a significant impact on transmission (this report), we simulated the two contrasting epidemiological scenarios with and without the increased transmission rate in heathlands: Heathland vector density versus baseline vector density (Table 6). We accounted for uncertainty in potential infection location by simulating introduction in two different habitats: urban/suburban areas (representing introduction through horticultural trade of ornamentals used in private gardens/urban planting) or heathland (representing worst case scenario of introduction into a potentially high-risk habitat). In addition, two time-to-detection scenarios were simulated representing early detection (5 years post introduction) versus late detection (10 years, comparable to Italy).

All combinations of the above conditions were simulated for all combinations of the following surveillance measures (Table 7):

1. **Outer BZ (BZ2) survey intensity.** We simulated high survey intensity, as in the UK *X. fastidiosa* contingency plan management strategy (0.5 x 0.5 km survey grid) (DEFRA 2021), as well as medium intensity represented by the EU regulations (1 x 1 km survey grid), and low intensity (2 x 2 km survey grid), representing lower effort compared to both strategies. In each grid cell 24 plants are surveyed for symptoms, and samples are taken to be tested via PCR (Figure 9).
2. **BZ radius.** The BZ radius simulated varied from 1 km, representing smaller BZ size compared to UK and EU measures, to 2.5 km the current BZ size for UK and EU, and 5 km, representing the greater BZ size in the previous EU regulation (Figure 9d).
3. **Asymptomatic testing during surveys.** As mentioned above, during survey activities within the BZ all symptomatic trees inspected are sampled and sent to the laboratory to confirm positivity through PCR testing. Currently, a random number of asymptomatic trees, among those inspected, are sampled and tested. Based on the modelling results in this report we predict a high percentage of asymptomatic individuals over the total infected. Therefore, we simulated the current random survey method and alternatively a targeted approach to detect asymptomatic infections, setting the asymptomatic testing rate to the 30% or 60% of the inspected trees. In this way, a greater number of asymptomatic individuals is overall tested, among those may be included infected and healthy plants.

In addition, we considered two contrasting introduction areas: central Scotland, previously identified as having the highest introduction risk in PHC2018/04 (Broadmeadow et al. 2019) (Figure 8a); and northern Scotland due to the abundance of heathland, which has been observed to be a potentially high-risk habitat for *X. fastidiosa* transmission due to the combination of great vector abundance and high spatial connectivity (this report) (Figure 8b).

However, in the second case we only introduced the infection in heathland, being urban and suburban land cover virtually absent in the selected portion of landscape.

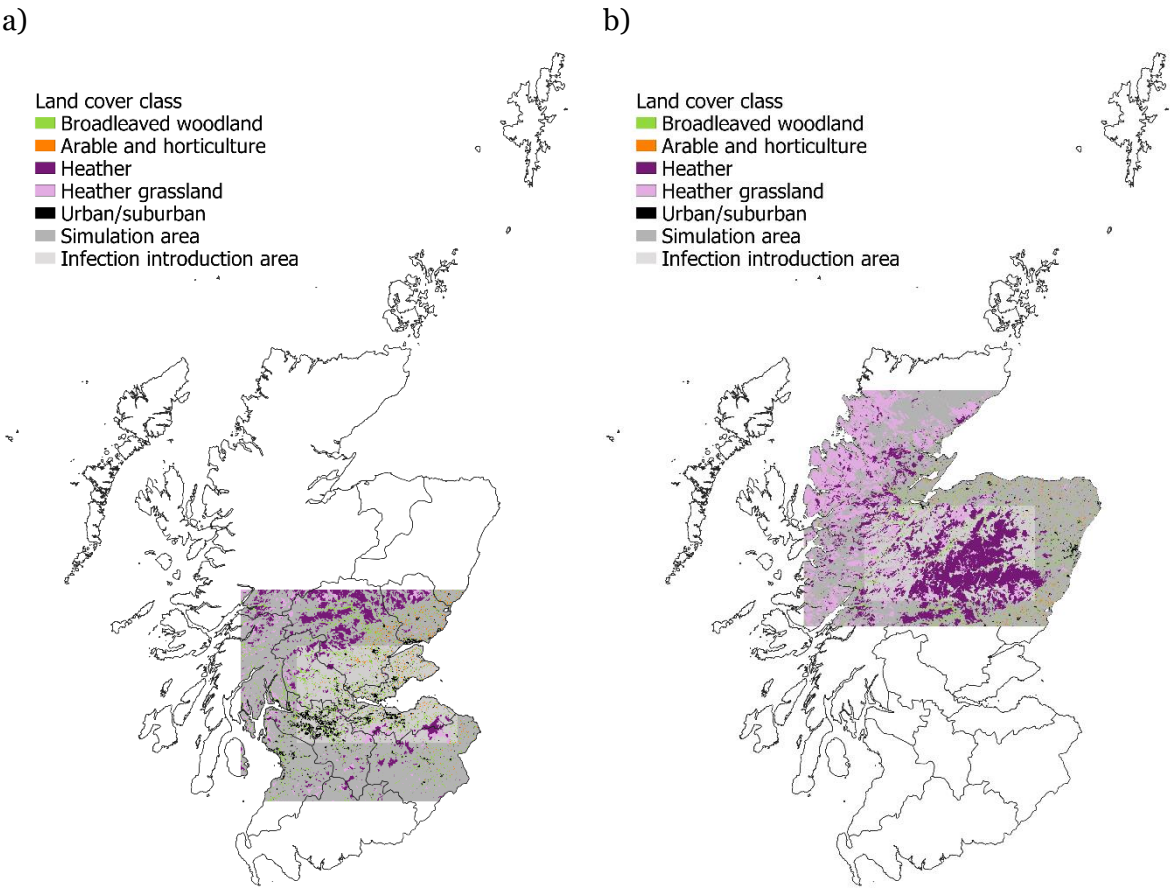


Figure 8. Maps of simulation areas (dark grey) for surveillance and control scenarios with relative land cover classes and infection introduction area (light grey). a) Introduction in central Scotland; b) introduction in northern Scotland.

Table 7. Summary table of the different settings for the surveillance and control scenarios. All combinations were simulated.

Model setting	Options simulated
Epidemiological scenario	Baseline or heathland vector density
Introduction region	Central or Northern Scotland
Introduction habitat	Heathland or Urban/suburban*
Time to detection	Early (5 years) or Late (10 years)
BZ radius	1 km, 2.5 km[a, b], or 5 km
Survey intensity in outer BZ	Low (2x2 km grid), Medium[a] (1x1 km grid) or High[b] (0.5x0.5 km grid)
Testing of asymptomatic trees	UK/EU (random) [a, b], Targeted (30%) or Targeted (60%)
Felling in IZ	All host plants[a, b]
Felling in BZ	All symptomatic plants

*[a] EU strategy (EU 2020/1201); [b] UK strategy (DEFRA 2021); * not simulated when the introduction was in northern Scotland.*

Due to the model being stochastic, every combination of variables in Table 7 was simulated 1000 times. To evaluate the effectiveness of the control, we also simulated equivalent epidemiological and introduction scenarios but without implementation of any surveillance or control. From these results, we calculated a relative "averted infections ratio" (AIR):

$$AIR = \frac{N_{\text{inf}\_NC} - N_{\text{inf}\_E}}{N_{\text{inf}\_E}} \quad \text{(Eq. 15)}$$

where N_{inf_NC} is the total infections in a scenario without control, and N_{inf_E} is the total infections with control. This ratio increased with the greater proportional number of infections averted by the simulated surveillance and control measures, and equated to 0 when there was no difference between the uncontrolled and controlled scenario.

4.3 Results

4.3.1 Effect of eco-epidemiological scenarios on disease spread

Single simulations of the four eco-epidemiological scenarios (Table 6) suggested that the increased transmission in heathlands and inclusion of long-distance dispersal markedly increased the growth rate of *X. fastidiosa* outbreaks (Figure 9). Figure 9, however, also shows that the model produced an order of magnitude less potential spread in Scotland than would be expected if the epidemiological parameters matched those from Puglia, Italy.

The generality of these results from single simulations was confirmed in the multiple replicate simulations (Figure 10 and Figure 11). For scenarios where transmission was the same in heathlands as in other habitats, greater epidemics were produced by introduction in areas with higher summer temperature and larger and more continuous patches of susceptible grid cells, such as urbanised areas in the central belt and other counties with high proportion of broadleaved forest and heathlands. When the simulations accounted for the greater abundance of vectors on heathland by increasing transmission in heathlands, larger epidemics were produced, especially when introduced in counties where heathland habitat was more abundant and less fragmented, such as northern and eastern counties. In this case lower temperatures in the north were more than compensated for, in terms of *X. fastidiosa* spread, by the effect of vector abundance in heathland. Additionally, long distance dispersal markedly increased outbreak size for any county of introduction. Nevertheless, due to lower temperatures the spread in Scottish conditions remained orders of magnitude lower than is expected from the dynamics in Puglia (considering both the spread and in the landscape and the prevalence of symptoms) (Figure 10).

Finally, due to slower disease progression the modelled Scottish dynamics in all scenarios resulted in outbreaks with a very high percentage of asymptomatic hosts, ranging from 40% to 70% and being higher in areas of greater transmission (i.e. higher proportion of asymptomatics where the epidemic spread faster due to higher temperature, greater vector abundance, and lower host fragmentation).

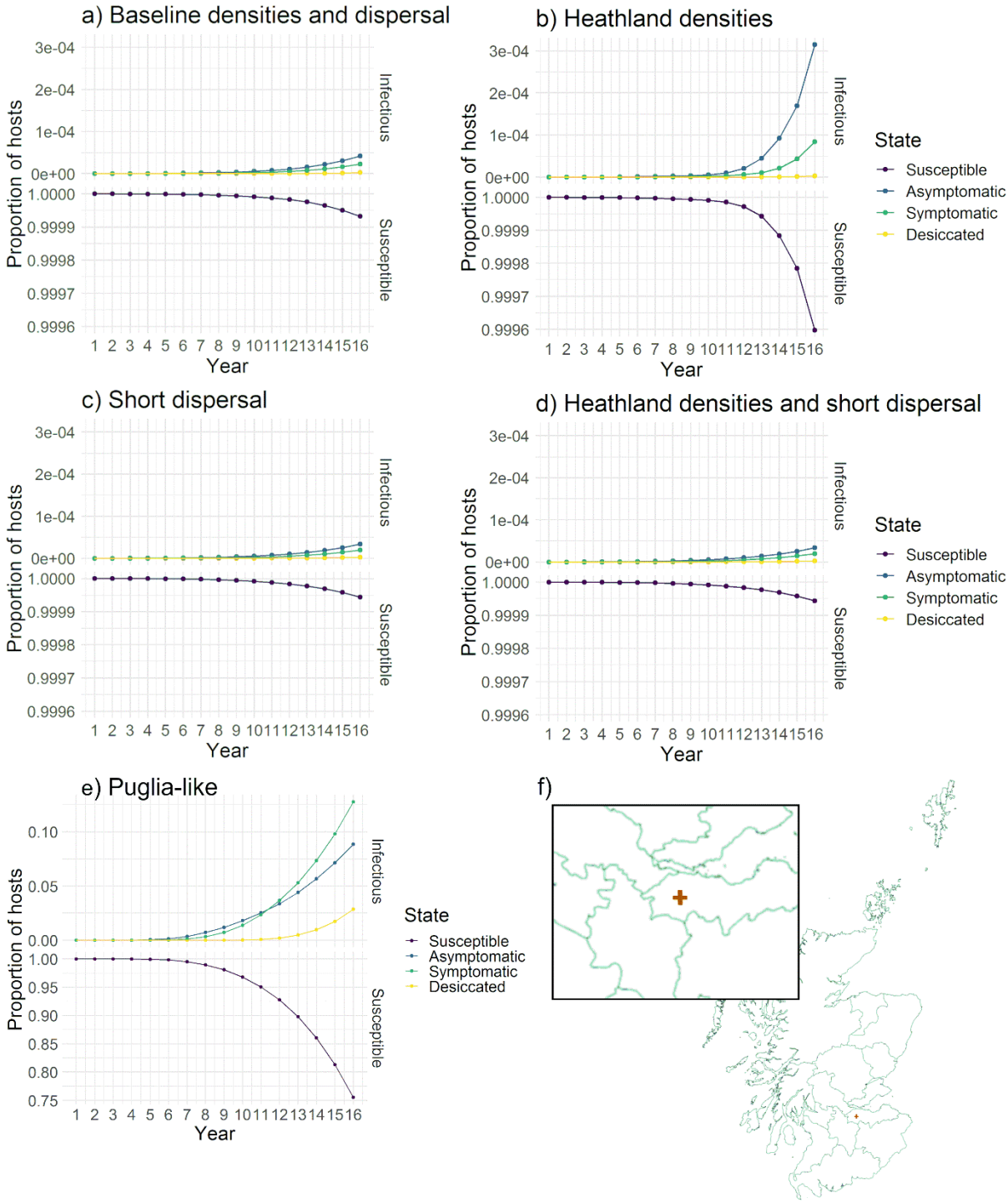
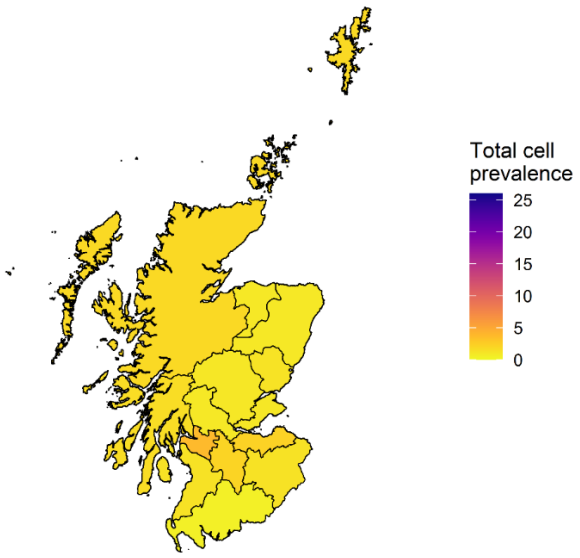
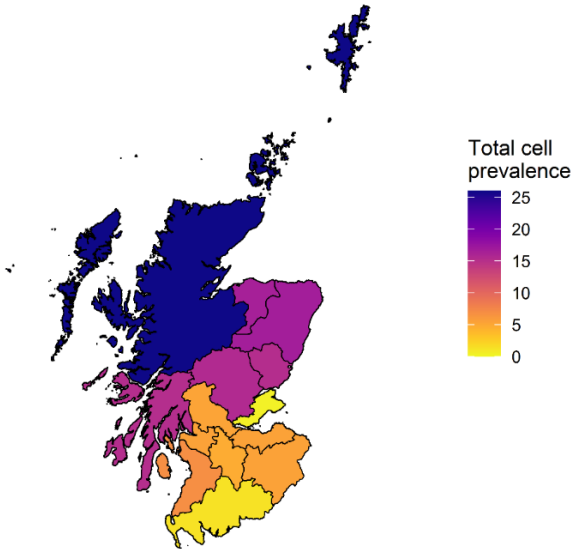


Figure 9 Examples of single simulations showing the proportion of hosts in each model compartment over 16 years after a single infection introduction for the scenarios in Table 6. (a) Baseline densities and dispersal; (b) Heathland densities; (c) Short dispersal; (d) Heathland densities and short dispersal; (e) Puglia-like. In (f) the red cross shows the introduction location used in the simulations (coordinates: x 306425; y 666450). Note from the y-axis scales that simulations using the parameters from Puglia produce much larger epidemics than those expected for Scottish scenarios.

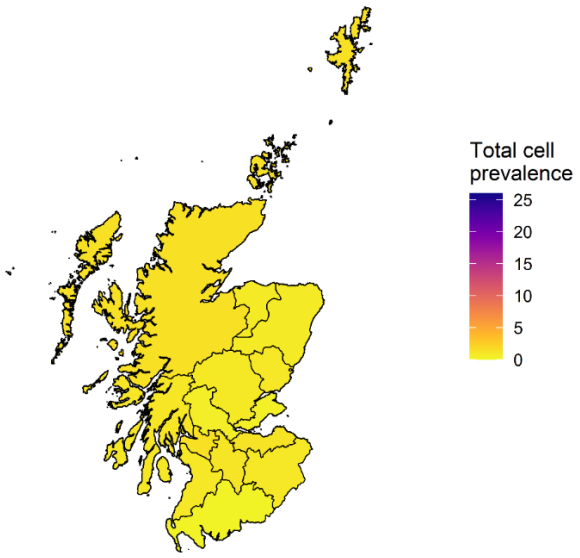
a) Baseline densities and dispersal



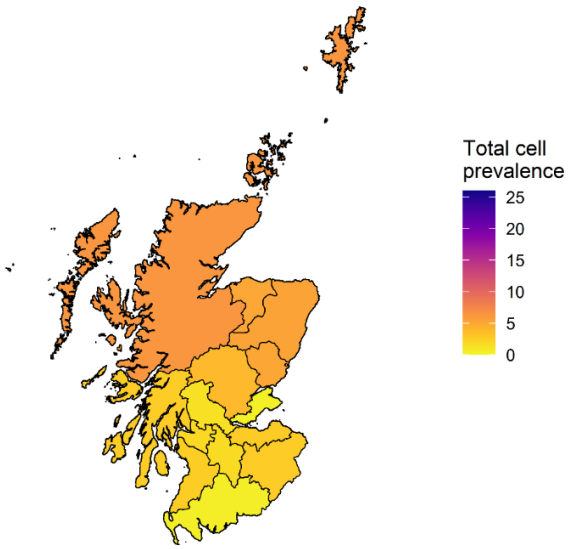
b) Heathland densities



c) Short dispersal



d) Heathland densities and short dispersal



e) Puglia-like

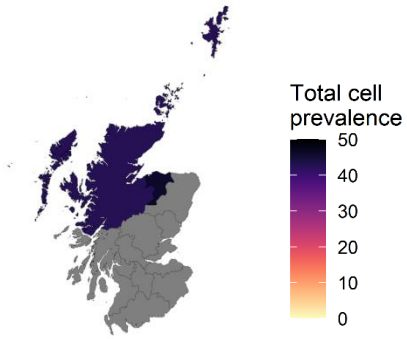


Figure 10 Regional variation in spread risk at county level. Shading shows the average cell prevalence (percentage of susceptible cells infected) 16 years after introductions into each county. (a-d) Maps show the different epidemiological scenarios (Table 6) and (e) for comparison a simulation in one county with parameters from Puglia, Italy.

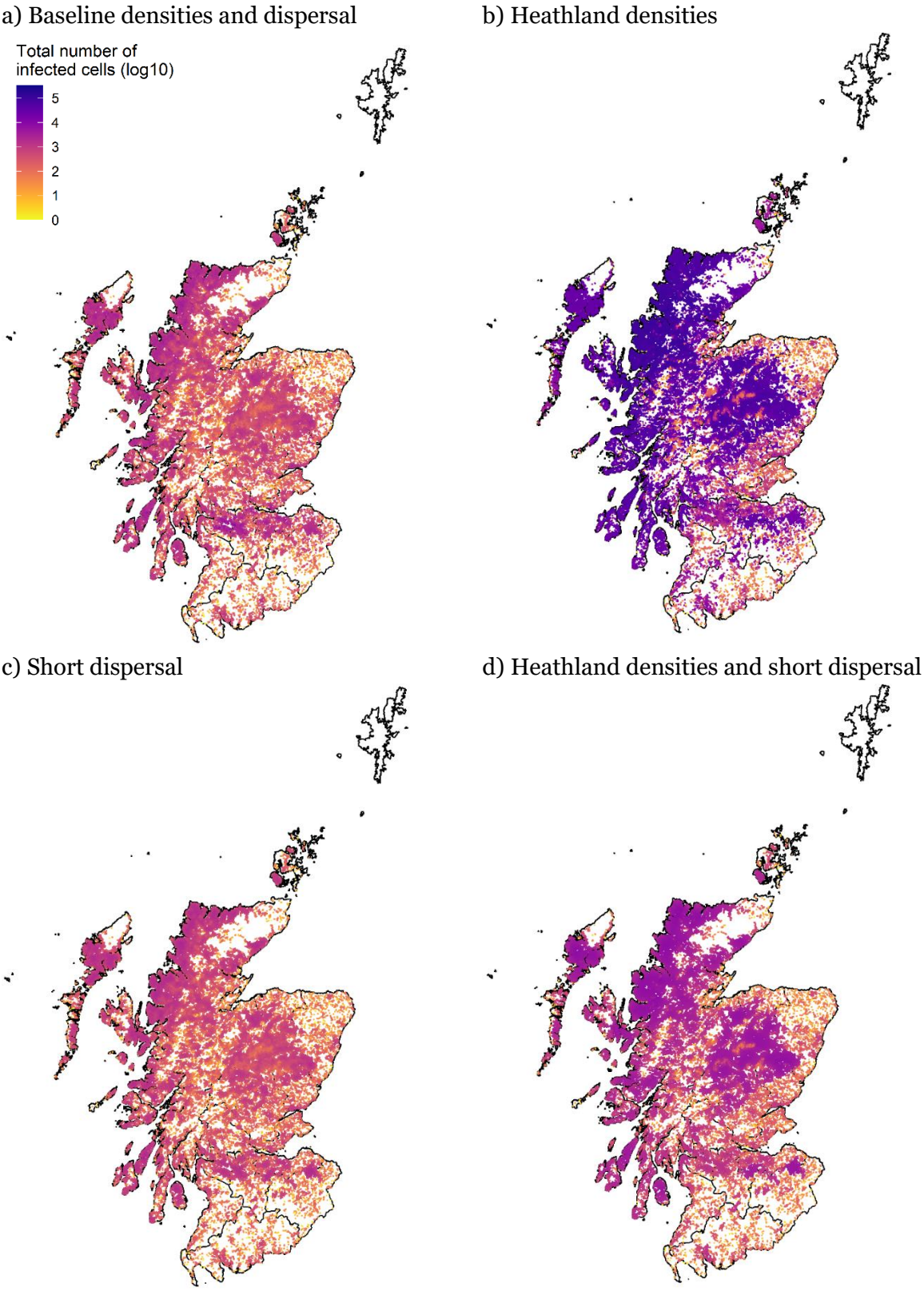


Figure 11 Effect of introduction location on spread risk. Infection introduction points are shaded by the number of infected cells after 16 years. Maps show the different epidemiological scenarios (Table 6).

4.3.2 Surveillance and control scenarios

4.3.2.1 Introduction in central Scotland

Figure 12 shows the averted infections ratio (AIR – a measure of control effectiveness) with the baseline vector density epidemiological scenario and introduction in central Scotland in heathland, followed by early detection (5 years after introduction). In terms of averting infections, the simulations only achieved modest reductions in outbreak size and the UK measures proposed in the new contingency plan (DEFRA 2021) performed similarly to the EU strategy (Figure 12 first panel). In general, control effectiveness increased with greater BZ size and greater testing of asymptomatic trees but was little affected by survey intensity in the outer BZ (Figure 12). Our proposed targeted asymptomatic testing strategy increased the number of averted infections in each scenario, probably because the percentage of positive inspections (a proxy of monitoring effectiveness) also increased when asymptomatics were targeted for testing. This suggests that the current UK/EU random testing might not be the best approach when an outbreak is characterised by a high proportion of asymptomatic infections.

Early detection enhanced control effectiveness, but most markedly when we assumed higher transmission in heathland and larger buffer zone sizes (Figure 13). Thus, early detection seemed crucial to reduce spread in the worst-case epidemiological scenarios simulated.

Fewer trees were felled with the smallest buffer zone sizes (Figure 14), which may partly explain the lower effectiveness of this strategy (Figure 12). Interestingly, when there was early detection of the outbreak relatively few trees were felled with the largest buffer zones (Figure 14) and this also had the greatest effectiveness (Figure 12). This was likely because the 5 km buffer zone encompassed the invasion front of the disease when it was detected early, while the 2.5 km buffer zone did not. As such more infections were cleared earlier, preventing a higher number of infections with less need for subsequent felling. Therefore, if the outbreak was detected early then the largest buffer zones gave the most effective control with the lowest felling.

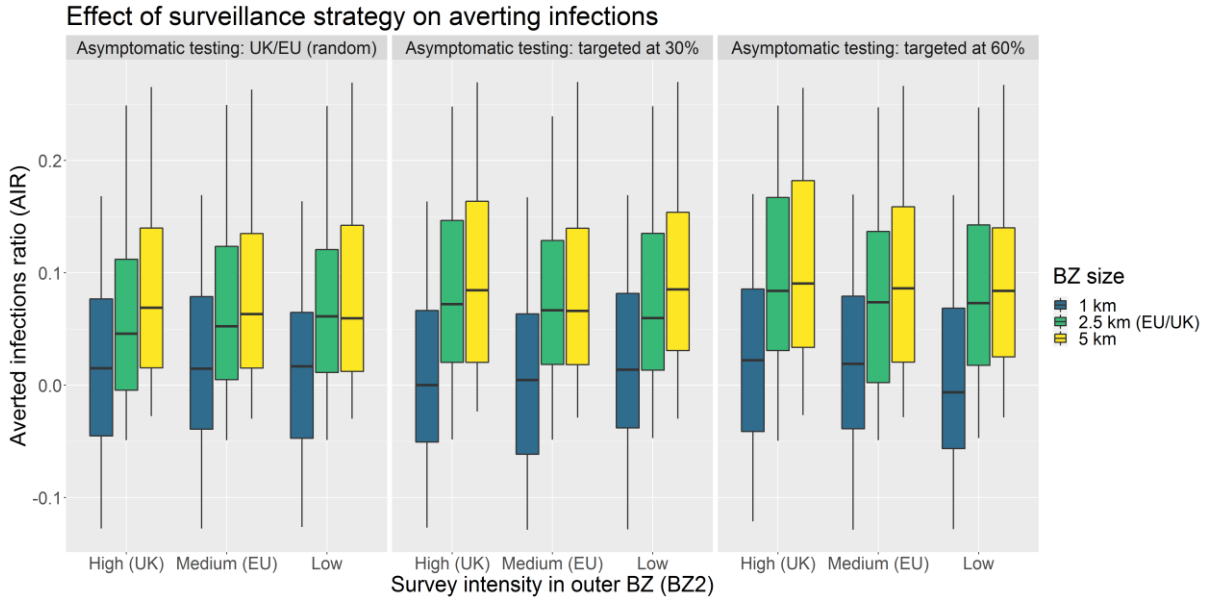


Figure 12. Averted infections ratio (AIR) for an outbreak in central Scotland showing the relative effectiveness of control for variation in buffer zone size, survey intensity, and asymptomatic testing rate. Simulations assumed the baseline vector density epidemiological scenario, introduction in heathland and early detection.

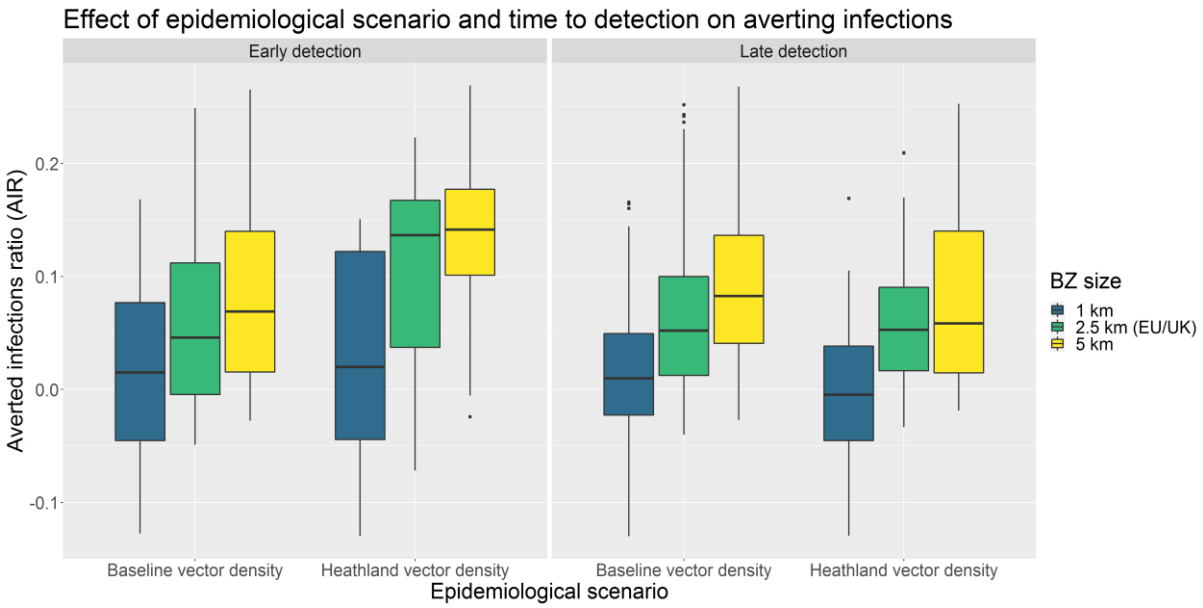


Figure 13. Averted infections ratio (AIR) for an outbreak in central Scotland showing the relative effectiveness of control for variation in detection time, epidemiological scenario and buffer zone size. Simulations assumed introduction in heathland and high survey intensity (UK strategy), and random asymptomatic testing (UK/EU strategy).

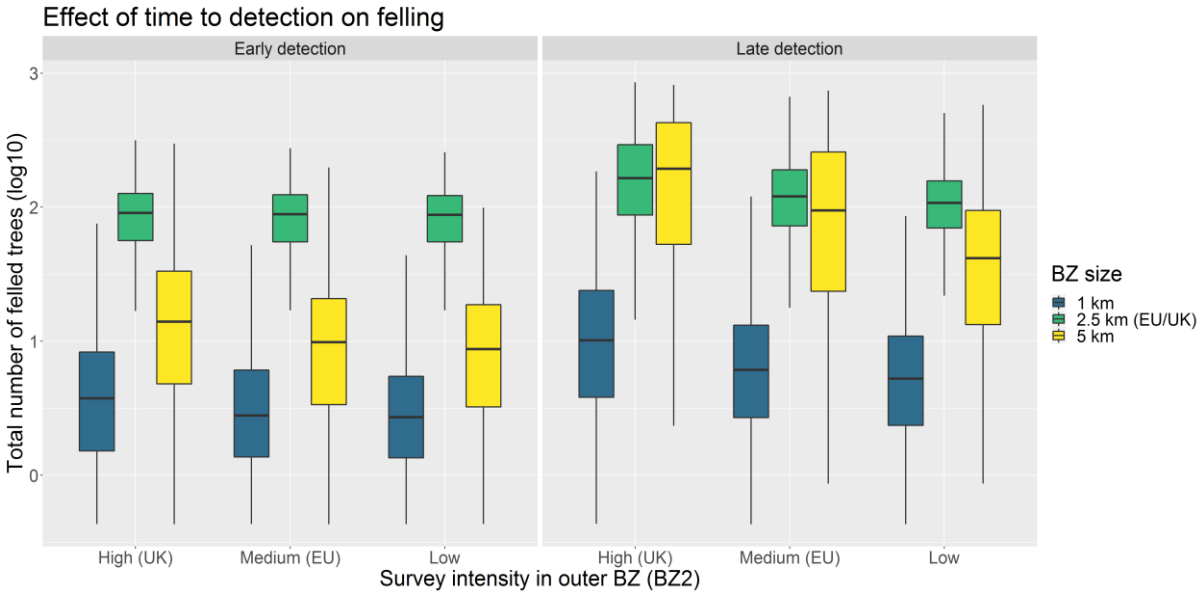


Figure 14. Number of felled trees for an outbreak in central Scotland with different detection times and buffer zone survey intensities and sizes. Simulations assumed the baseline vector density scenario, introduction in heathland and random asymptomatic testing (UK/EU strategy).

4.3.2.2 Introduction in northern Scotland

Results for outbreaks in northern Scotland were qualitatively similar to those for central Scotland, but it was noticeable that the variability of the results was much lower, and the effectiveness of control was greater (Figure 15). This may reflect the more contiguous distribution of habitat and the impact of lower temperatures on spread rates.

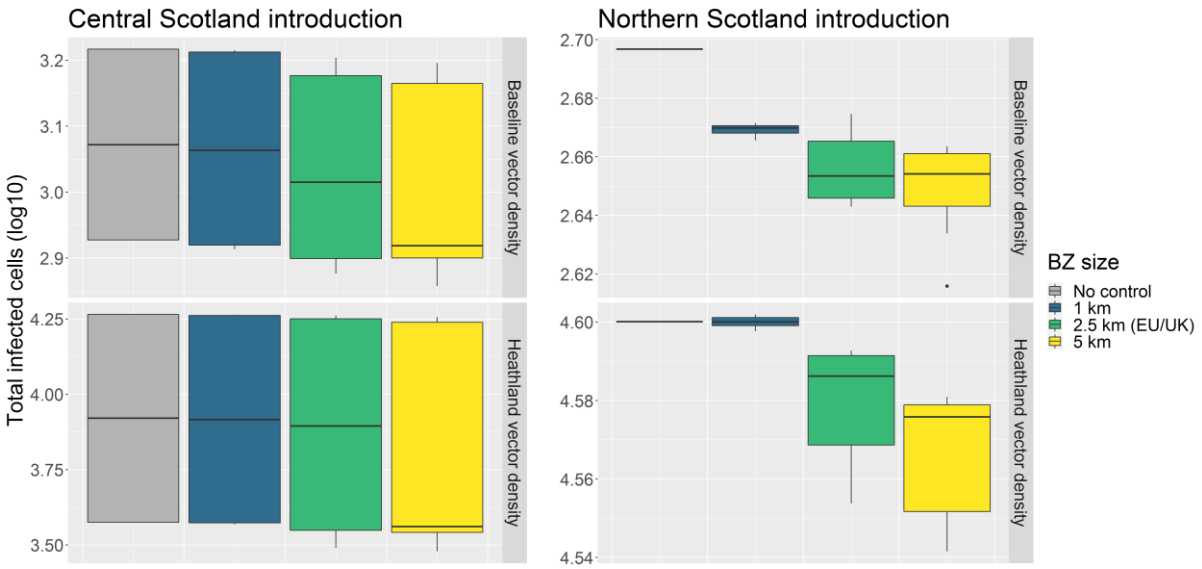


Figure 15. Outbreak sizes for introductions in central and northern Scotland for different buffer zone sizes and for different epidemiological scenarios. Note the logarithmic scaling of the y-axes. Simulations assumed introduction in heathland and random asymptomatic testing (UK/EU strategy).

4.4 Discussion

4.4.1 Xylella fastidiosa spread potential in Scotland

The modelling suggested that *X. fastidiosa* transmission in Scotland might be possible in all hypothesised scenarios. However, it would lead to far smaller outbreaks than in the outbreak region of Italy due to the assumption of reduced transmission and prolonged disease progression in the cooler and wetter Scottish environment. This also implies that Scottish outbreaks may be characterised by a high percentage of asymptomatic infected hosts, which would likely hinder detection efforts. Large populations of asymptomatic but infected host plants may also represent a potential risk of future disease emergence under climate change, as symptom development and pathogen growth might be favoured by predicted increases in temperatures and plant hydric stress (McElrone et al. 2001). In Corsica, the apparently long-undetected *X. fastidiosa* outbreak in a diverse range of symptomless host species led to the hypothesis of the existence of a hidden reservoir of asymptomatic infection (Soubeyrand et al. 2018). This hidden reservoir might not cause a severe outbreak but might represent a risk of future disease emergence due to climate and land-use change. Given that *X. fastidiosa* symptom appearance is related to hydric stress (McElrone et al. 2001), the possibility of significant asymptomatic-but-infected host populations in the humid environment of Scotland seems plausible.

Modelled disease spread was greater in areas with higher summer temperature and host plant connectivity, i.e. where potentially susceptible grid cells are arranged in large and continuous patches. This was observed not only in the central belt, characterised by urban/suburban areas assumed to harbour potential *X. fastidiosa* hosts, but especially in regions with a high proportion of heathland, and to a lesser degree broadleaved woodland. In addition to its high connectivity, heathland might represent a further source of risk due to the greater vector

abundance documented earlier in this report, enhancing spread and counteracting the effect of generally lower temperatures found where this habitat is particularly abundant and dominant (e.g. northern Scotland).

Comparing our results with the previous risk map from PHC2018/04 (Broadmeadow et al. 2019), areas of relatively higher modelled spread only partially overlap those identified in the previous study. This is due to differences in methodology and assumptions about risk based on availability of newly generated data on *X. fastidiosa* in this project and beyond (e.g. data in Occhibove et al., in prep. from Brigit project). Previous risk mapping placed more emphasis on limitation by low winter temperature, while here only summer temperatures were considered. Furthermore, Broadmeadow et al. (2019) considered heathlands as medium risk, while the model here revised this upwards using new information on heathland host plants (EFSA et al. 2021) and vector densities (this project). Additionally, Broadmeadow et al. (2019) assumed vectors would be absent in cold, wet and windy locations such as at high elevation, while the model here assumed vectors would be present in all grid cells with host plants regardless of climate. We sampled vectors at sites up to nearly 400 m in elevation and the species has been recorded up to 639 m in Scotland (NBN Atlas 2022). The presence of *P. spumarius* is confirmed in the Italian Alps from 1000 m to 2210 m (Di Serio et al. 2019), as well as at high altitudes in Scandinavia (Boucelham and Raatikainen 1987) and at lower elevations in the Arctic circle (Halkka et al. 2006). Thus, it seems possible that mountainous areas in Scotland might well sustain vector populations, although not necessarily at the high densities found in the survey from this report. Further studies on vector abundance on heathland across elevational gradients are needed to further investigate this.

The simulations modified the parameters of a model calibrated for the Italian outbreak on olive trees to try to capture changes in eco-epidemiology in a novel Scottish environment. This inevitably leads to uncertainty and strong caveats on the model outputs (Occhibove et al. 2020), discussed below.

1. Host density estimation was based on a global tree density product (Crowther et al. 2015) whose accuracy for Scotland and ability to represent host density in heathlands is not clear. Even in woodlands, lack of knowledge about specific host tree species meant we had to assume a worst-case scenario where every tree was susceptible.
2. We did not model a particular *X. fastidiosa* subspecies or strain or particular host species, but instead considered an "average" *X. fastidiosa* outbreak, assuming that Scottish abiotic conditions would generally prolong asymptomatic and desiccation periods compared to those of olive trees in Puglia. These effects might vary depending on the specific pathogen strain-host interaction, which are virtually impossible to predict in novel locations (Occhibove et al. 2020).
3. In the model, higher vector densities in heathlands counteracted low transmission due to low temperatures, leading to substantial spread on heathlands, though still at much lower rates than if transmission was at Italian levels. Empirical data on the scaling of transmission rates with both vector densities and temperature are needed to confirm this interaction and improve its modelling.

4.4.2 Modelling of surveillance and control strategies

Simulations of UK and EU eradication strategies (differing only in the survey intensity in the outer buffer zone) for outbreaks in Scotland performed similarly and achieved modest

reductions in outbreak growth. This was likely because lower temperatures and hydric stress in Scotland, compared to Italy, meant the model simulated a high proportion of asymptomatic infected plants in potential Scottish outbreaks which are difficult to detect and remove. Currently, inspectors perform visual surveys in the demarcated area to identify symptomatic individuals that are later confirmed by lab testing, while only a small percentage of asymptomatic individuals are tested. In our simulations, setting a fixed proportion of asymptomatic plants to be sampled per inspection benefitted control efforts considerably. If these assumptions are correct, current UK/EU asymptomatic testing might not be the most appropriate approach when epidemiological dynamics are not characterised by high symptom severity.

Control effectiveness was also increased by increasing the buffer zone (BZ) size and (especially in the worst-case epidemiological scenarios) through early detection, consistent with previous modelling for Italy (White et al. 2017, EFSA Panel on Plant Health (PLH) et al. 2019). However, increasing BZ sizes may not always be possible due to limited resources, the complexity of a heterogeneous landscape or public acceptability of control measures. Public and stakeholder engagement are key to successful and cost-effective disease management (Gottwald 2010, Marzano et al. 2017). This is likely to be especially important at the early stage of an outbreak where reporting of new cases by the public and land managers facilitates epidemiological understanding and initial demarcation of the infected area. Stakeholder engagement could also play a role in early detection, though this may be hampered by a high proportion of asymptomatic infections. To increase the chance that outbreaks are detected early, measures such as surveillance for infection of vector insects and of asymptomatic host plants might also be considered.

In our simulations, UK and EU strategies also led to a very similar amount of felling, which is a crucial metric to take into account as it is ecologically and economically costly and might lead to additional undesirable consequences when an excess of uninfected plants needs to be felled to control the infection. Simulations suggest that at the time of detection the current 2.5 km BZ (UK/EU) may not effectively circumscribe the infection front, especially in the scenarios of greater spread. As a consequence, a higher level of felling was needed over a longer period of time. This suggests a larger buffer might be considered upon first detection of the disease, even if it is then reduced in size.

Our simulations suggested that different introduction locations (and to a lesser extent habitat) affected the characteristics of the resulting outbreaks. When the simulated outbreak was initiated in central Scotland outbreaks were more variable in size and severity, depending on the interaction between the more fragmented distribution of susceptible habitats (a mix of broadleaved woodland, urban/suburban areas, and some heathland) and the stochastic disease dispersal. Simulated outbreaks introduced in northern Scotland spread through a more homogenous and continuous area of heathland. This led to large outbreaks when the effects of increased vector densities on transmission were included in the model.

In summary, the modelling suggests improvements to current control strategies might be made by increasing efforts for early detection, increasing buffer zone sizes at least in the immediate aftermath of detection and greater testing of asymptomatic host plants. However, refining the control strategy should account for epidemiological parameters of the outbreaks in Scotland, with factors such as symptom prevalence and long-distance dispersal identified as key to control effectiveness. Better understanding of *X. fastidiosa* epidemiology in a Scottish outbreak will therefore be key to effective management of the disease.

5 Conclusions

This project has revealed new information for assessing the risk of *X. fastidiosa* spread in Scotland, should the disease be introduced. At Loch Leven the phenology of the confirmed vector species *P. spumarius* was shifted approximately two months later than is observed in Italy. However, the period of adult activity was of a similar length, which might provide opportunity for potential *X. fastidiosa* transmission. Unlike in Italy, however, there was no evidence of a summer vertical migration from the ground level herbaceous vegetation into tree canopies, probably due to the ground vegetation remaining green and being available to feeding insects in Scotland. This might limit vector transmission of *X. fastidiosa* among trees. However, our sampling was confined to lower branches of the trees and so this lack of vertical migration should be confirmed with dedicated sampling higher in the tree canopies.

Across central Scotland, *P. spumarius,* was the numerically dominant species in the surveys of woodland understorey, grassland and heathland habitats. However, it showed a clear preference for heathland, occurring at a density approximately five times greater than in the other habitats. To our knowledge this finding is novel and made more significant because of the recent confirmation of common heather *Calluna vulgaris* as a naturally-infected host of *X. fastidiosa multiplex* in Portugal (EFSA et al. 2021) and the high proportion of Scottish land represented by upper heathland (21-31% - NatureScot) . In addition, five species of *Vaccinium* are known to be naturally or experimentally susceptible hosts of two subspecies *X. fastidiosa multiplex* and *X. fastidiosa fastidiosa* in USA, causing leaf scorch symptoms, and species of *Erica* and *Arctostaphylos* are also confirmed hosts (EFSA et al. 2021). This raises the possibility that common Scottish heathland species such as bilberry *Vaccinium myrtillus,* bell heather *Erica cinerea*, cross-leaved heath *Erica tetralix* and bearberry *Arctostaphylos uva-ursi* might also be susceptible, although to our knowledge this remains untested.

If Scottish heathland does support high densities of susceptible hosts and vectors then heathlands might pose a significant *X. fastidiosa* outbreak risk in Scotland, which has until now not been the focus of attention. Indeed, the modelling suggested elevated vector densities in heathland combined with its high landscape connectivity might compensate for low temperature limitation of transmission and bacterial growth and allow relatively large outbreaks – although they would still be much smaller, less symptomatic and slower growing than those observed in Italy. Much uncertainty remains, however, as this result is derived by adjusting a model calibrated to the Italian outbreak for eco-epidemiological conditions in Scotland (Occhibove et al. 2020). In particular, we remain uncertain about the interaction between lower temperatures and vector densities in driving transmission, disease progression and transmission for *Calluna vulgaris* and other heathland species, and the relative scaling of host densities compared to trees for which the model was developed. Despite these important caveats, the empirical finding of very high vector densities in a known European host species that is a dominant species in much of Scotland raises a significant concern and suggests that heathlands should be part of ongoing surveillance for *X. fastidiosa.*

Other potential vector species were at much lower abundance than *P. spumarius* except in woodland tree canopies where *A. alni* was dominant. This species has not previously been identified as a vector of *X. fastidiosa*, but there remains the possibility that as a canopy dwelling xylem-feeder it could play a role in transmitting the disease between trees in Scotland. As such, we suggest that monitoring of vector insects to detect presence of *X. fastidiosa* should target both *A. alni* and *P. spumarius*. In this way surveillance would cover the tree canopy specialist and the numerically dominant confirmed vector species. Based on

our comparison of sampling techniques, vector surveillance might combine both sweep netting to actively sample *P. spumarius* from ground level vegetation and malaise traps for passive sampling of other vector species including *A. alni*. Malaise trapping is probably most effective in relatively sheltered environments such as woodland and for flying insects. We found that it was the most effective method for sampling *A. alni*, although relatively small numbers were detected overall. We also found that for nymphs, both manual field identification and molecular methods using DNA barcoding were accurate and effective.

Regarding surveillance and control scenarios, simulations suggested that current UK eradication strategy proposed in the new contingency plan (DEFRA, 2021) might mitigate a potential outbreak. Simulations also highlighted the importance of early detection, larger buffer zones and targeted asymptomatic testing during surveys to improve control and minimise felling of uninfected trees. In addition, the effectiveness of control measures might depend on the location and habitat of introduction, and the actual epidemiological dynamics in Scotland. In the worst-case epidemiological scenario, or in the case of an outbreak in a particularly valuable host, an increase in BZ might be desirable to reduce both spread and felling. In case of limited resources, increased asymptomatic testing rate might be preferable instead of an increased survey intensity. The implementation of these measures needs to be evaluated in light of landscape configuration and type of hosts involved in the relative outbreak.

Overall, this project has provided important new information on the species composition, density, distribution, habitat preferences and phenology of potential *X. fastidiosa* vectors in Scotland, as well as their sampling and identification methods. It has used this information to update previous epidemiological modelling for the disease in Scotland and extended the modelling to simulate plausible eco-epidemiological scenarios and control strategies for an outbreak. Together the empirical and modelling results highlight heathland habitats as potentially being at relatively high risk of *X. fastidiosa* spread, should introduction occur, and provide guidance for improving outbreak control strategies. Should an outbreak occur in Scotland, this evidence will support management decisions and help minimise impacts.

6 References

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7 Appendix 1. List of 3rd party datasets used

Data and reference	Link
EFSA Update of the Xylella spp. host plant database (EFSA et al. 2021)	https://zenodo.org/record/5004966#.YdjFR_6nxPZ
Survey data of xylem-sap feeding Auchenorrhyncha (Xf-ACTORS vector working group 2020)	https://zenodo.org/record/3775537#.YdjGG_6nxPZ
Land Cover Map 2015 (25m raster, GB) (Rowland et al. 2017)	https://catalogue.ceh.ac.uk/documents/bb15e200-9349-403c-bda9-b4300938o7c7
CEH Land Cover plus: Pesticides 2012-2016 (England and Wales) (Jarvis et al. 2019)	https://www.ceh.ac.uk/services/ceh-land-cover-plus-crops-2015
OS Open Greenspace Map	https://www.ordnancesurvey.co.uk/business-government/products/open-map-greenspace
Global tree density map (Crowther et al. 2015)	https://elischolar.library.yale.edu/yale_fes_data/1/
Climate hydrology and ecology research support system meteorology dataset for Great Britain (1961-2017) [CHESS-met] (Robinson et al. 2020)	https://catalogue.ceh.ac.uk/documents/2ab15bf0-ad08-415c-ba64-831168be7293
MODIS MOD13Q1 16-day NDVI (Didan 2015)	https://lpdaac.usgs.gov/products/mod13q1v006/
PLANTATT-attributes of British and Irish plants: status, size, life history, geography and habitats (Hill et al. 2004)	https://www.brc.ac.uk/biblio/plantatt-attributes-british-and-irish-plants-spreadsheet

8 Appendix 2. Species glossary

Focal bacterial pathogen

Xylella fastidiosa (family: Lysobacteraceae; order: Lysobacterales, class: Gammaproteobacteria; phylum: Proteobacteria)

Potential insect vectors of *X. fastidiosa* recorded in the surveys

The potential vector species covered in this report are all members of suborder Auchenorrhyncha in order Hemiptera. Within this suborder, the following vector species that we sampled in Scotland came from two families.

Family Aphrophoridae:

Philaenus spumarius

Aphrophora alni

Neophilaenus lineatus

Neophilaenus exclamationis

Family Cicadellidae:

Cicadella viridis

Evacanthus interruptus

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