Spotting the pests of tomorrow—Sampling designs for detection of species associations with woody plants

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Abstract

Aim: Early warning against potentially harmful organisms of woody plant species can be achieved by sampling sentinel plants in exporting countries. However, it is unclear where sentinel plants can best be located, and how many samples are required and when and how often sampling optimally should take place for the adequate assessment of the biodiversity associated with the target plant species. We aimed to review spatial and temporal factors affecting associate biodiversity of single woody plant species and to develop guidance for the design of global biodiversity sampling studies.

Location: Worldwide.

Taxon: Insects and Fungi.

Methods: Literature about factors affecting the diversity of insects and fungi in association with single plant species on global, regional, local and different temporal scales was reviewed. Case studies of insect and fungal diversity, primarily collected on single plant species, and the cost of collecting and analysing samples from locations around the world were analysed.

Results: The review of the literature illustrated various factors affecting diversity, and the case studies allowed quantification of the relative impact of some spatial, temporal and financial aspects on captured biodiversity and, thus, illustrate the need to consider all possible factors that may affect the result of the sampling when deciding on a sampling design.

Main conclusions: Our study illustrates the factors that should be considered when deciding on the location and timing of sampling for sentinel plants, which is important because of the trade-off between the number of samples and sampling locations needed to detect many of the species which may be potential pests, and the cost of (repeated) sampling in many locations. Decisions about the sampling design must be based on the objective of the sampling, but our recommendations apply irrespective of the targeted plant species or country.
1 | INTRODUCTION

Non-native pests (including pathogens; FAO, 2015) of woody plants are a serious threat to forest resources and have caused significant negative economic, biodiversity and livelihood impacts (e.g., Aukema et al., 2010; Kenis, Rabitsch, Auger-Rozenberg, & Roques, 2007). The increase in intercontinental trade volume coincides with an increase in the number of potentially serious pests in all parts of the world, and strong border biosecurity is needed to minimize the risk of additional pests being introduced (Wingfield, Brockerhoff, Wingfield, & Slippers, 2015). Most countries focus their efforts and measures on the number of potentially serious pests in all parts of the world, and strong border biosecurity is needed to minimize the risk of additional pests being introduced (Wingfield, Brockerhoff, Wingfield, & Slippers, 2015).

The associations between woody plants and invertebrates or microorganisms are relatively well-studied in comparatively rich countries (Beck, Böller, Erhardt, & Schwanghart, 2014) and countries with a tradition of natural history societies whose members have documented records of species in the environment (Silvertown, 2009). However, in many other countries, far less knowledge exists in publicly available databases or literature (Kier et al., 2005). When such information exists, it is commonly available as presence in a location or area, without any indication of the plant associations (Mitchell et al., 2016). There are few resources that provide records of associations between plants and other organisms, including scientific journals (e.g., the “Flora of the British Isles” series of the Journal of Ecology) or databases (e.g., www.cybertruffle.org.uk, https://nt.ars-grin.gov/fungaldata). However, such resources are likely to be biased towards certain taxa or regions. As a consequence, the information needed for the protection of forest resources against non-native pests is incomplete and additional data are required.

Collection of information about plant-pest associations across the distributional range of a plant species, including areas where the species has been introduced, requires sampling in a range of habitats. Multiple locations within and across countries are necessary, because differences among locations affect the identity and relative abundance of associated organisms. If the aim is to detect pest diversity it is necessary to sample in locations and at times that cover the widest range of relevant factors as possible. However, if the aim is to detect and identify the most damaging organisms it may be best to sample in locations where the host plant has been introduced and where it encounters pests and pathogens that it has not co-evolved with (Dickie et al., 2017; Lombardero, Alonso-Rodríguez, & Roca-Posada, 2012), or places where the host plant is growing in the suboptimal conditions, as this may increase the likelihood of disease symptoms developing (e.g., Agrios, 2005).

Sampling across a large geographic range requires considerable resources and, while it represents important opportunities, is challenging from both a financial and practical perspective. For example, when collections are made by collaborators in different countries, it is necessary to standardize sampling techniques (Eschen et al., 2018). Moreover, many factors affect biodiversity and it is difficult to take all into account. Careful planning is therefore needed to ensure that the entire environmental range of a plant species is covered to achieve appropriate spatial and temporal distribution of the samples. Finally, in order to decide on a sampling design, consideration of the aim of the sampling and the costs is needed.

The cost of sampling may be divided into fixed costs, such as the cost of processing a sample, and variable costs, such as travelling to a sampling location, and a balance needs to be sought to optimize the use of the resources depending on the aim of the sampling. For example, by reducing the number of sampling locations, resources...
may be allocated to increasing the number of samples taken at each location, which may lead to better assessment of within location diversity (i.e. alpha diversity), or sampling locations may be prioritized based on the presumed pest risk associated with trade volume originating from a region. It is also necessary to balance replication at the local and regional level, and within and between years, depending on the aim of the sampling, to avoid oversampling or undersampling (e.g. Hoban & Schlarbaum, 2014).

While the identification of an optimal sampling design is a common problem in ecology, few studies have explored the effect of different sampling designs on the captured diversity on a large scale. Hoban and Schlarbaum (2014) studied how a set number of seed samples should be geographically distributed for ex-situ conservation of genetic diversity of a plant species. Their simulation results illustrated how sampling effort should be higher and in fewer populations if the expected alpha diversity is high, while it should be dispersed over a large geographic area if beta diversity is expected to be high. The specific study examined genetic diversity in populations of a single plant species and only considered geographic aspects of sampling. However, repeated sampling of a community is also needed to assess species diversity (e.g. McCain, 2004; O’Hanlon, 2012; Roques et al., 2015). Hence, the intensity of sampling should be adapted to the expected variation within and between years. Moreover, the expected spatial and temporal patterns may differ depending on the targeted taxa and detection and identification tools that are used.

The aim of this paper is to provide guidance for the design of studies for the assessment of species diversity associated with any single woody plant species on a large geographic scale, to create lists of (potentially harmful) organisms that may be used by plant protection organizations for PRA. We review factors that may affect species diversity and discuss how these may be included in the sampling design. Using case studies, we illustrate the effect of spatial and temporal aspects on the captured diversity and the cost of collecting and analysing the samples. We then explore the combination of relevant factors and costs and finish by providing guidelines for developing sampling designs on a global scale under resource constrains. Although we acknowledge that there are differences in the communities of organisms associated with different parts of a plant host, we consider individual trees as the basic experimental unit.

2 | FACTORS AFFECTING SPECIES OCCURRENCE AND DIVERSITY

2.1 | Spatial factors

2.1.1 | Global scale

In general, a negative relationship is found between latitude and biodiversity of different taxa (Hillebrand, 2004) The mechanisms affecting the latitudinal gradient in species diversity have their basis in climate, productivity, biotic interactions, historical perturbation, evolutionary rate and size of the area (Hodkinson, 2005). However, these mechanisms likely interact and depend on local conditions (Hodkinson, 2005) and significant variation in the pattern exists (Gaston, 2000).

Important aspects of climate are temperature and precipitation and these factors are likely to affect the species that can be found in a region. For example, global patterns in the diversity of soil fungi and terrestrial invertebrates are positively related to annual precipitation (Tedersoo et al., 2014), temperature and precipitation (Hawkins et al., 2013).

Many microorganism and invertebrate species are restricted to certain host plant species or genera (Branco, Brockerhoff, Castagneryrol, Orazio, & Jactel, 2015; Den Bakker, Zuccarello, Kuypers, & Noordeloos, 2014; May & Beverton, 1990; Nordén, Pentillí, Sitionen, Tomppo, & Ovaskainen, 2013). It seems therefore obvious that the sample locations should be within the natural distribution range of the host. The genetic diversity of the plant species may be related to the diversity of associated organisms (e.g. Belliotti, Braun, Arias, Castilho, & Guerrero, 1994), and if the center of origin of the plant species is known, focusing sampling locations in this region may increase the capture of associated species. However, locations outside the natural distribution range, such as areas where a plant species was introduced (e.g. plantations or botanic gardens), can also provide information about associations between woody plants and organisms in an area (Eschen et al., 2018). Moreover, species turnover is large among habitat types (Begon, Townsend, & Harper, 2009) and sampling multiple habitat types is likely to increase the number of associated organisms.

2.1.2 | Regional scale

Changes in elevation affect ambient temperature, UV radiation and composition of atmospheric gasses (Hodkinson, 2005), which changes the biochemical and physiological status of host plants (Witzell & Martin, 2008) and thus influences biodiversity, including the community composition of mycobiospheres (Cordier, Robin, Capdevielle, Fabreguettes, et al., 2012; Davey, Heegaard, Halvorsen, Kauserud, & Ohlson, 2013; Siddique & Unterseher, 2016; Zimmerman & Vitousek, 2012) or insects (Hodkinson, 2005). For example, altitude (i.e. ambient temperature) is among the predominant parameters shaping endophytic communities (Cordier, Capdevielle, Desprez-Loustau, & Vacher, 2012; Hashizume, Sahashi, & Fukuda, 2008; Osono & Hirose, 2009). In general, the species richness decreases with increasing altitude (Gaston, 2000). However, a consistent microbial (fungi, bacteria, Archaea) diversity pattern is lacking (Fierer & Jackson, 2006; Fierer, Strickland, Liptzin, Bradford, & Cleveland, 2009) and many studies of the impact of altitudinal gradients on microbial diversity have shown variable trends, mainly due to the complexity of underlying environmental changes and the diverse ecologies of microbial taxa (Peay et al., 2017). The species composition of insect communities also changes with altitude, but the nature of this relationship is not clear (Hodkinson, 2005).
While host plant identity is among the important determinants of fungal infection (i.e., Kato, Fukasawa, & Seiwa, 2017; Persøn, 2013; Sieber, 2007; Tedersoo et al., 2016; Unterseher, Reich, Finstermeier, Otto, & Morawetz, 2007) and herbivorous insect diversity (Novotny & Basset, 2005), the structure and diversity of the surrounding vegetation can also play a role (Helander, Ahiholm, Sieber, Hinneri, & Saikkonen, 2007). Local or regional tree diversity can be positively related to the number of fungi or invertebrates recorded on woody plants (Arnold, 2007; Arnold & Lutzoni, 2007; Vehviläinen, Koricheva, & Ruohomäki, 2007). Similarly, a high diversity of plants could promote greater microbial species richness, while low plant diversity may be associated with lower microbial diversity (Brodie, Edwards, & Clipson, 2003; Ishida, Nara, & Hogetsu, 2007; Pfenning & de Abreu, 2006). Several studies have found that the degree of relatedness of co-occurring introduced and native plants is correlated with the amount of damage the introduced plant suffers from native herbivorous insect species (i.e., Bush, 1969; Connor, Faeth, Simberloff, & Opler, 1980; Kiritchenko & Kenis, 2016; Pearse & Hipp, 2014; Strong, 1979).

Plants in regions with different land use types, such as forests, urban or agricultural areas, can differ in their diversity and richness of associated organisms, which is in part related to land use intensity (Newbold et al., 2015). This is exemplified by differences between two sentinel plantings in China. The first was located in an intensively managed agricultural area and the insects found on the trees were mainly insects found in agricultural land (Roques et al., 2015). Application of fertilizers including macronutrients, primarily nitrogen, generally decrease fungal species diversity and may cause shifts in community structures (i.e., Allison, Hansen, & Treseder, 2007; Avis, Mueller, & Lussenhop, 2008; Treseder, 2008). The negative impact of pesticides on microbial and insect community structure as well as the ecosystem services provided by them is known from several studies (Griffiths et al., 2006; Rose & Dively, 2007). The second sentinel planting was located in a landscape with smaller-scale agriculture and a larger fraction of nearby forests, and the overall diversity of insects was higher (Roques et al., 2015). Hence, the number of sampling locations should be higher in regions including a large diversity of land use types, and lower in regions with homogeneity of land use types. By contrast, botanic gardens typically harbour taxonomically diverse collections of woody plants from various origins, which may enable detection of a wide range of associated organisms and represent a good choice for sampling location in urban areas.

A further factor which should be considered for the position of the sampling locations is the trade connections and the transportation infrastructure, because they may be associated with high risk of unintentional transport of potential invasive non-native species to other countries (Hulme, 2009). For wood products, these could be the locations where untreated wood or wood products are stored for transport (Hulme, 2009; Schrader & Unger, 2003). Regardless transport of plants for planting, plant nurseries are high risk locations for transporting pests (Hulme, 2009; Schrader & Unger, 2003). However, new pathways can emerge, or the location where production takes place may shift from one region to another (Eschen et al., 2017). In addition, many pathogens can be transported on footwear, camping gear or car tires, although the extent to which this occurs is poorly understood (Anderson, Rocliffe, Haddaway, & Dunn, 2015). Hence, locations close to transportation infrastructure or with high numbers of visitors or tourists should be considered for sampling.

### 2.1.3 Local scale

Soil characteristics are a key factor determining the occurrence and diversity of soil borne organisms (Johnson & Rasmann, 2015; Tedersoo et al., 2014; Willsley, Chatterton, & Cárcamo, 2017). Local variation may be affected by topography and the immediate and legacy effects of plant–soil feedback, which in turn may lead to differences in microorganisms and invertebrates associated with roots or the rhizosphere (Bardgett, 2002; Dickie et al., 2019; Ehrenfeld, Ravit, & Elgersma, 2005; Ettema & Wardle, 2002; Fierer & Jackson, 2006; Fierer et al., 2009; Tedersoo et al., 2014).

Host genotype can influence biodiversity and the composition of insect or fungal communities (e.g. Johnson & Agrawal, 2005; Peacock, Hunter, Turner, & Brain, 2002). For example, previous studies have shown that the composition of aboveground (e.g. phyllosphere endophytic fungi: Bálint et al., 2013; Cordier, Robin, Capdevielle, Fabreguettes, et al., 2012; Elamo, Helander, Saloniemi, & Neuvonen, 1999; Todd, 1988) and belowground (e.g. arbuscular mycorrhizal fungi: Lindeman, 2004) fungal communities is partly determined by host genotype.

Diversity and frequency of insect herbivores vary with host plant age; some insect guilds are more diverse on saplings and others on mature plants (Basset, 2001; Lowman, 1992). Additionally, studies from gallery forest insects have shown frequency of gall causing insects to be related to the age of their host plants (i.e., Craig, Itami, & Price, 1989; Price, 1989), and that younger plants are more susceptible to attack. Similarly, the foliar endophyte community of *Sequoia sempervirens* changed with leaf age and some species are associated with young and others with older leaves (Espinoza-Garcia & Langenheim, 1990).

### 2.2 Temporal factors

The abundance of a species can vary across both long and short time-scales. Long-term variation can be due to climate change, with some species increasing in abundance in association with warming temperatures (Bebber, 2015; Gange, Gange, Sparks, & Boddy, 2007; Kauslerud et al., 2008). Continued monitoring and sampling over years may also reveal emerging genotypes of a pest. The Sudden larch death epidemic caused by the oomycete *Phytophthora ramorum* in the UK and Ireland is an example of a genotype of a pest causing unexpectedly high damage on a new host. Until 2009 *P. ramorum* was known in Europe mainly as a pest of horticultural and amenity plants and trees, but then it was found causing extensive damage to Japanese larch (*Larix kaempferi*) in plantations, which was related to lineage and genotype diversity changes in 2009–2014 (Harris, Mullett, & Webber, 2018; King, Harris, & Webber, 2015).
Variation at shorter time-scales is often in line with the normal biological responses of the pest to environmental or seasonal variation, such as temperature, day length or rainfall, or in response to seasonal variation in host quality (Jumpponen & Jones, 2010; O’Hanlon, 2012; Rudolph, Marcíá-Vincente, Lotz-Winter, Schleuning, & Piepenbring, 2018; Vorišková, Brabcová, Cajthami, & Baldrian, 2014; Wolda, 1988). For instance, insects that feed on the leaves of deciduous trees will be less abundant in autumn, when their main food source is scarce. Some pathogens may be present in the host year-round, but cause the most noticeable symptoms early in the growing season, and thus are easier to detect during this period (Steele, Laue, MacAskill, Hendry, & Green, 2010). Seasonality of abundance varies among taxa and capturing a large fraction of the taxa that are associated with a plant host in a sampling location is likely to require repeated sampling over the course of one or multiple years. The length of time the plants have been present in the environment, which may be related to the age of the plants, may also affect the abundance and identity of associated organisms.

3 | CASE STUDIES

Here, we explore some of the aspects of sampling design that were mentioned in the previous section. We do this using four original datasets that enable us to appraise the effects of local, regional and temporal factors. In addition, we assess the costs associated with sampling and identifying detected organisms.

3.1 | Inter-annual and spatial patterns in insect diversity

The assessment of the inter-annual and spatial variation in insect diversity was done using a dataset of insects reared from dormant twigs of the pedunculate oak (Quercus robur) from continental Croatia. Samples (seven 50 cm long twigs of each tree) were collected every January during 2001–2018 in Croatian oak (Quercus) forests and put in jars with water at room temperature (22°C). Emerged insects were assigned to taxonomic groups that ranged from species to family level; scale insects were identified to superfamily level only. The dataset was subsampled in order to get the data for the plots that were sampled continuously during all 18 years. This resulted in a total of 2,196 samples collected from 42 plots within eight forest locations. Additionally, data on various factors were collated for each of these plots, including precipitation, temperature, elevation, average tree age and coordinates.

Species accumulation curves were calculated for each plot, forestry location and for all plots together. Twelve taxa were detected during 18 years of sampling and the curve for the combined forestry units did not reach an asymptote during this period. On average, $8.1 \pm 0.6$ taxa were collected in each forestry location and $5.4 \pm 0.2$ in each plot. In none of the eight forestry locations were all of the insect taxa detected during the 18 years of sampling (Figure 1).

Species accumulation curves calculated for plots were log-transformed to assess the rate of increase in insect taxon richness during the study (slope). The effect of various environmental factors and plot characteristics on total insect taxon richness of a plot and the rate of increase in richness were analysed. The average age of the trees in a plot was significantly positively related to the number of taxa detected in a plot ($p = 0.005$, $R^2 = 0.18$). No significant relationships with annual precipitation, temperature, plot size or altitude were found. This result suggests that more samples should be collected in older trees in this case (Dickie et al., 2019).

3.2 | Intra-annual patterns in insect diversity

The effect of repeated sampling throughout a year on detected biodiversity associated with woody plants was assessed in a nursery in western Serbia, close to the city Šabac. The nursery of deciduous trees belonging to the genera Betula, Acer, Fraxinus, Prunus, Tilia, Ulmus and their cultivars was established in 2011 in an area of ca. 2 ha.

Four yellow sticky traps (10 × 25 cm, “Horiver”, Koppert Biological Systems) were hung on branches in the crown of randomly selected trees from mid-March until the beginning of November in 2013. The traps were changed every second week (17 sampling periods in total). The insects stuck to the traps were identified to 21 different taxa, ranging from order to family, and counted. The numbers for each sampling period were pooled, as the results of individual traps were nearly identical (Appendix S1). We calculated how many of the identified groups were detected by the repeated sampling using an ordered accumulation curve.

Seasonal patterns in insect abundance were clear, with the lowest monthly insect numbers found in July (Figure 2). Homoptera, Diptera, Hymenoptera and Coleoptera were the most numerous orders. Individuals of the remaining six orders were found sporadically. The number of detected groups increased from April until
September, when all 21 insect taxa had been recorded. The results revealed that just sampling any one of the 17 fortnightly sampling periods would on average miss more than half of the total diversity (mean 12.3 ± 0.8 taxa missed). Sampling in June and August, the two months with the highest number of orders, would have resulted in capturing all orders in the dataset. Any other combination of two months would have missed at least one order, even if the numbers of insects may be higher.

### 3.3 Spatial patterns in diversity of seed-borne fungi

The effect of sampling of multiple individuals at a location, as well as of repeated spatial sampling within a region was assessed using fungal isolates obtained from ponderosa pine (*Pinus ponderosa*) seeds in western North America. The fungal community was assessed for 100 surface sterilized seeds each from six locations by growing fungi on non-selective agar media (1.5% water agar (PPA, Pronadisa Lab. Conda) with streptomycin (100 mg/L) to inhibit bacterial growth) and grouping the obtained isolates based on their morphology (Franić et al., 2019). The diversity of fungal morphotypes in each sample was assessed by calculating rarefaction (interpolation) and extrapolation (prediction) of sampling curves. A total of 73 morphotypes was found in samples from the six locations and 16.3 ± 3.2 in each location. The curves of only two locations reached the asymptote (Figure 3). At all other locations more seeds would need to be sampled to obtain a complete estimate of the species richness.

![Figure 2](image-url) Monthly numbers of insects belonging to ten Orders, found on yellow sticky traps in a nursery in Serbia in 2013. Insects were identified into 21 taxa, which were pooled into Orders. The size of the circles is relative to the number of insects found (1–343 individuals). Each circle is the mean of four sticky traps [Colour figure can be viewed at wileyonlinelibrary.com]

![Figure 3](image-url) Estimated diversity of fungal morphotypes associated with seeds of *Pinus ponderosa* from six locations in North America. The regional diversity ("Total") was estimated using seeds from all six samples [Colour figure can be viewed at wileyonlinelibrary.com]

Seed borne fungi were cultured as above from seed lots of eight species from China, Europe and the north western USA. Each seed lot consisted of 100 seeds obtained from commercial suppliers. To explore whether the diversity can be best assessed by taking few samples each from many locations or many samples from a few locations, we sampled the fungal diversity in 100 random seeds, taken from 1 to 5 seed lots per tree species. We repeated the sampling 100 times per tree species and seeds-to-seed lot ratio and calculated the average fraction of the total diversity in the species that was captured during
the sampling. The detected fraction of the fungal diversity in each species increased significantly with the seeds-to-seed lot ratio, indicating that distributing a fixed number of samples over more locations leads to a better estimate of the total diversity associated with each tree species (mixed effects model with seeds-to-seed lots ratio as fixed and tree species as random effect: $\chi^2 = 257, p < 0.001$; Appendix S2).

3.4 | Spatial and temporal patterns across and within regions, locations and years

To determine which of the spatial and temporal factors may be most influential for the assessment of diversity, we used a dataset of ladybirds associated with forest edges and hedgerows in North-western Switzerland (Roy et al., 2012). The data were collected from 15 sites in three topographically and climatically distinct regions, up to seven times a year over 8 years (2006–2013). At each sampling location and occasion, ladybirds were collected by beating all branches up to 3m height over a distance of 50m. All collected specimens were identified to species level and counted prior to being released. This dataset allows assessment of all design aspects described above, apart from the effect of replicate samples at each sampling location and occasion. We therefore split the data from each sampling location and occasion into five artificial subsamples by subsampling without replacement. The first artificial subsample was generated by removing a number (calculated as a random number in Microsoft Excel) of the counted individuals of each detected species in the dataset. This was repeated five times, whereby the number of individuals of each species in a subsample was reduced by the number taken in the previous subsample, so that the species composition in the combined five subsamples did not differ from the actual sample. In order to minimize potential effects of the subsampling, we repeated the subsampling procedure ten times and took means of the results of analysis of the ten generated datasets. We assessed the effect of repeated sampling at each of the spatial and temporal levels by calculating species accumulation curves using each of the factors as base unit and comparing the slope of the curves for the first three sampling units.

The number of species detected at these 15 sites in north-western Switzerland was approximately half of the species known from Switzerland (Lucht, 1987). The slopes of the curves varied, with the steepest slope in months and sites (4.90 ± 0.16 and 4.24 ± 0.06 species per additional sample [mean ± SE]) and the flattest slopes for replicates (within site and year) and among regions and years (3.66 ± 0.08, 3.53 ± 0.03 and 3.88 ± 0.07 species per additional sample, respectively). This indicates that there was strong seasonality in the occurrence of ladybird species and large differences among sites.

3.5 | Cost versus sampling effort

We assessed these fixed, such as the cost of processing a sample, and variable costs, such as travelling to a sampling location (Fagan, Bithell, & Dick, 2008) through a short questionnaire sent to participants in a global study of insects and fungi associated with trees belonging to eight genera (I. Franić et al., in prep). Samples were collected in 32 counties, on all continents, with a proportionally higher number of samples taken in European countries. Sampling was similar to that in the Croatian case study above. Each participant was asked to indicate the number of tree species that were sampled (i.e. the number of samples taken) and to estimate the distance travelled to the sampling location, the time and number of people involved in the sampling and extraction of DNA for assessment of the fungal community or rearing of insects from the samples. We also asked for the cost of field and laboratory materials and shipping of the extracted DNA and insects to Switzerland, where the samples were analysed. Responses were obtained for 25 out of the 32 countries, including five from non-European countries. The cost estimates were separated into fixed and variable costs and the average values were taken for each (Table 1).

We calculated the cost of sampling, including travel, and preparing samples for sequencing and assessed differences in costs between sampling insects and fungi, and the different costs of classical plating of fungi and next-generation sequencing (NGS) based identification (Figure 4). Furthermore, we investigated the effect of the number of samples per location on total cost and the effect of the fraction of sampling locations on other continents. The result illustrates that NGS was the least expensive of the three methods for identification of diversity in these samples and reveals the high labour cost associated with rearing live organisms for identification. The results further indicate that, although sampling was a small part of the total cost, this becomes rapidly larger if the fraction of sampling locations on other continents increases. Finally, it is unsurprising that the required resources are positively related to the total number of collected samples, but the results reveal that a larger fraction of the budget will be spent on analysing samples if more samples are collected at each location.

4 | DISCUSSION

Detection of potentially harmful organisms in exporting countries allows timely PRA and proactive implementation of risk mitigation measures by importing countries prior to introduction, but it is unclear where and when samples for this purpose should be collected. Our case studies reveal important effects of many spatial and temporal aspects that may affect the captured diversity and, thus, should be considered when deciding on a sampling design. Although here we discuss the tree as the sampling unit, the number of samples within the individual tree should be enough to record a large proportion of the richness of the selected organism group Morales-Rodríguez et al. (2019). The sampling effort for the selected organism group could focus on the tree organ (e.g. stem, roots and crown), where that particular species group is most abundant. Sampling of non-host substrates associated with trees, such as soil or water, may allow detection of additional pests and pathogens that may be transported with the plants, in particular those that have dormant survival structures and opportunistic pathogens that may become pathogenic when they come into contact with naïve hosts.
4.1 Spatial aspects of global sampling designs

The global distribution and species richness of many organism groups are affected by large-scale patterns, such as climate, latitude (Gaston, 2000) and trade connections (Meurisse, Rassati, Hurley, Brockerhoff, & Haack, 2018; Van Kleunen et al., 2018) and more regionally by elevation, habitat type and soil (e.g. Hodkinson, 2005; Tedersoo et al., 2014). The locations for the sampling of diversity associated with the chosen tree species on a global scale should therefore be selected in such a way that these factors are considered across the geographic extent of occurrence of the targeted tree species. If the distribution of species richness in the targeted taxon or the factors driving species richness are unknown, the spatial distribution of sampling locations could be random or based on climatic parameters. When diversity patterns appear in the collected data, this information may be used to improve the spatial distribution of sampling locations (Albert et al., 2010).

A wide spatial distribution of sampling locations is necessary to capture differences in species occurrence as a result of environmental variation, coinciding with the above-mentioned factors. However, it is equally important to consider comparatively small-scale variation in species occurrence, such as those that result from differences in soil type, elevation, intraspecific differences within the tree species and landscape complexity. The effects of such intermediate and small-scale spatial replication in sampling were illustrated by our case studies when samples were taken from multiple individuals (case studies about seeds and ladybirds), as well as multiple sampling locations (case studies about seeds and ladybirds) and regions (case studies about ladybirds and forestry locations). We therefore propose that a sampling design should be spatially nested, with multiple trees per location and multiple locations within regions or continents. When it is too expensive, or practically difficult to have multiple locations per region, multiple trees should be sampled at each location, taking care to select trees that are as far apart within the sampling location as possible and, where possible, considering also age and other characteristics of the trees (diverse tree phenotypes). Sampling spatially separated trees may increase the likelihood of capturing a larger species richness of organisms than when a single tree, or multiple trees growing close together are sampled, because of potential variety in the surrounding habitat and the larger species pool generally associated with larger habitat fragments (Roques et al., 2015; Tscharntke & Brandl, 2004).

Selection of sampling locations and collection of samples using a nested design have rarely been done on a global scale, with most studies of global biodiversity patterns using samples from a relatively large number of locations that appear to have been selected based on availability of collaborators that are able or willing to collect samples. In other cases, the selection of locations was based on the presence of the target species in botanic collections outside the natural distributional range of the species (Fagan et al., 2008). In one global study of soil microorganisms, 40 samples were taken at each location (Tedersoo et al., 2014). These samples were pooled for analysis of a small subsample of each, effectively reducing the number of samples to one per location, but this still is representative of more local diversity than most studies have achieved. Such an approach may be an effective surrogate for assessing total diversity if the ratio between diversity found in each sample and the diversity in the pooled sample is constant.

4.2 Temporal aspects of sampling designs

Sampling should be repeated to capture the temporal aspects of species occurrence within and between years. Kenis et al. (2018) suggest that sampling over at least 2 years is needed in sentinel nurseries to ensure that potential pests have time to invade the site. Temporal replication may be more important for insect than for microorganisms (e.g. fungi), as many microorganisms may be present inside the host throughout the year, whereas adult insects leave the host after emergence. The need for temporal replication for the detection of microbial diversity may depend on the applied detection techniques and sampled tree organ. Repeated sampling within or between years is valuable and likely to yield a much larger species richness, mainly because of the seasonality of the occurrence of different insect taxa, as illustrated by the Serbian, Croatian and Swiss case studies. The ability to detect insect species, as opposed to the orders and families studied in the Serbian case study, will increase with the frequency of sampling, i.e. the chance of detecting all of the species increases with more samples. In sentinel nurseries, another important temporal factor relevant to sampling is the time of the year at which the target plants are exported (Kenis et al., 2018). This is especially relevant for insect pests, as if they have left the plants then they are no longer at risk of being transported with the plants (e.g. Frančić et al., 2019).

Some of the differences in occurrence of organisms on a host species may be related to host age and population dynamics of the

### Table 1

Average costs of sampling in Europe and on other continents (Frančić et al., in prep.)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Euros</th>
<th>Other continent</th>
<th>Europe</th>
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Variable Sampling per location and sample

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organisms. As a consequence, it is very unlikely that the entire diversity will be captured in a single sample, or a single year of sampling, and it may be necessary to continue sampling over many years. This is clearly illustrated by our case study from Croatia, where even after 18 years of sampling not all defoliating taxa in the country have been captured and the total diversity in the dataset has not been captured in any single site. A similar picture arose from the Swiss case study of ladybirds. New, exotic species may become established during the sampling period, which may be captured. However, given the unpredictable nature of invasions of non-native organisms into new regions, it is very difficult to design a sampling scheme to ensure that these invasive organisms are recorded. Some new non-native species may appear very rapidly, as illustrated by A. leucopoda in our Serbian case study, a species that was first recorded in Serbia only in the year prior to the study (Glavendekić, Petrović, & Petaković, 2013). Other species may take longer, for example in the case of species with strong population fluctuations. The non-native Asian longhorn beetle (Anoplophora glabripennis) was estimated to be present in England at least 10 years before it was recorded (Straw, Fielding, Tilbury, Williams, & Cull, 2016). Similarly, it can be several years before non-native pathogens are recorded, such as in the case of the ash dieback fungal pathogen Hymenoscyphus fraxineus, which was probably present in England for at least 8 years before it caused a level of symptoms high enough to facilitate detection (Wylder, Biddle, King, Baden, & Webber, 2018). It is very difficult to define the number of years that sampling should continue because of differences in taxa, climatic and site conditions and periodic inspection of the data, for example using species accumulation curves, could provide guidance. In our Croatian case study, we found significant correlations between the diversity captured in each plot after eighteen years and the diversity captured after less than 10 years, indicating that early results can provide an indication of the total diversity at a location. Such indication of total diversity may be more useful, and more cost-effective, than compiling a complete list of associated organisms for every plant species and it would allow plant health authorities to prioritize commodities for risk assessment and inspection at the border.

4.3 | Costs and required sampling effort

Repeated sampling may be difficult or expensive, in particular without local collaborators. Our assessment of real costs associated with sampling and identification of organisms at sites in 32 countries provides a more accurate estimate than in previous studies (e.g. Fagan et al., 2008) and allows for planning of sampling efforts based on realistic budget estimates. Unsurprisingly, the results illustrate the high cost of sampling and identification, but also reveal that a considerable amount of resources is needed for travelling to the sampling locations. This highlights the need for effective international collaboration to achieve repeated sampling at individual locations, not only for data collection according to common protocols, but also for more cost-efficient use of resources. The countries in which samples are collected will have an interest in knowledge about harmful organisms found in the other countries, which may be a strong incentive for collaboration.

Our cost versus sampling effort case study confirms that a scenario where specialists from one country visit all sampling locations is not necessarily the most cost-efficient (Fagan et al., 2008). We calculated costs related to the collection of individual samples, or samples per location, from the cost of processing...
the samples until they were shipped to a central location for final preparation for high throughput sequencing. The cost and investment in time in the field on other continents, as well as shipping of the samples, was consistently ca. twice that of sampling in European countries. Equal or higher costs were incurred in the laboratory. Large scale collaboration can be challenging to establish, and this effort was largely successful because of large-scale international collaboration that was coordinated through an EU-funded COST Action (FP1401). There was also a large amount of effort dedicated to developing the common protocols for sampling and sample processing and explaining these to the collaborators, which we feel increased the efficiency of the sampling process. Hence the true costs are higher than those we estimated, although probably not as much as the estimates of Fagan et al. (2008).

4.4 Conclusions and recommendations

Assessment of the biodiversity associated with a single tree species requires careful planning and significant resources. Our review and case studies provide indications for the factors that should be considered when deciding on the location and timing of sampling for sentinel plants, which is important because of the trade-off between the number of samples and sampling locations needed to detect many of the species which may be potential pests, and the cost of (repeated) sampling in many locations. Decisions about the sampling design must be based on the objective of the sampling. In sentinel nurseries the objective is to identify as many of the associated organisms on the plants as possible (Kenis et al., 2018). This is because the risk of any of the associated organisms being transferred to another region by trade of plants for planting is high. In sentinel plantations, with

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**FIGURE 5** Decision support scheme for the design of global sampling designs [Colour figure can be viewed at wileyonlinelibrary.com]

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1Factors on the global, continental or local scale affecting species occurrence and diversity described in the manuscript

2Number of locations is dependent on the logistical and financial circumstances, distribution weighted by expected diversity patterns

3With the NGS method for endophytic fungi. For foliar fungi or fungi that respond to certain weather conditions two sampling occasions may be better.
plants native to an importing country, sampling should focus on the damage causing organisms, as these are the most likely to also cause damage to the plants in the native range of the plants, i.e. to become invasive pests (Roques et al., 2015). If it is sufficient to identify higher taxonomic groups, for example to inform the development of systems approaches to manage a range of species associated with imported plant species, fewer samples may suffice.

That more organisms are found when more samples are taken is unsurprising, but we are unaware of experimental or observational studies that show the relative importance of these factors. Most studies focus on one or two of the factors, possibly for budget reasons. In recent sentinel studies of insect pests on European and Chinese tree species planted in China, the temporal replication was very intense: the plants were inspected every second week or every month, while the number of locations was limited to two. At each location, there were over a hundred plants of each study species, in an area of less than one hectare, arranged in a randomized block design with five blocks (Roques et al., 2015). At the other extreme, studies of global patterns in diversity of different taxa had no temporal replication but samples were taken in many countries (e.g. Tedersoo et al., 2014). Most studies will have a similar design, because of limited resources for travelling that limit the ability to sample repeatedly, or the presence of the studied host plant species in few botanic gardens. These examples and our case study show the cost of sampling on a global scale indicate the need to consider which factors are the most important for the assessment of biodiversity on the chosen tree species.

Our review and case studies indicate that the sampling design (where, when, how often and how much to sample) may differ depending on the targeted taxa (e.g. fungi vs. insects), sampled part of the tree (e.g. perennial vs. annual parts) or identification method (e.g. isolation or rearing vs. high throughput sequencing). Such differences are due to the ecology of the targeted hosts and pests and to the specific detection or identification techniques that are used (Morales-Rodríguez et al., 2019). In order to decide on the appropriate sampling for insects and fungi in the same locations it is necessary to understand the ecology of the sampled trees and targeted pests, as well as the purpose of the sampling. However, to save travel costs it would be meaningful to, whenever possible, combine sampling insects and fungi at one or two occasions per year.

In order to facilitate the design of global studies of diversity associated with single woody plant species, we developed a decision support scheme based on the factors that were discussed above (Figure 5). We recommend starting the design of a sampling study by deciding on the target tree species, the purpose of the sampling and taxa of interest. The tree species affects the regions of the world where sampling may take place, the purpose of the sampling may define whether all or only damaging organisms are targeted, and the sampled taxa could have an influence on the need for repeated sampling within years. Following selection of the target tree species and identification of the distributional range (natural and introduced) of the species, we recommend assessing the availability of potential and established collaborations with institutions or individuals in those regions. If no suitable contacts exist, some sampling may be carried out by the initiator of the sampling. This is obviously a more expensive option and if financial limitations exist this may affect the number of large-scale spatial factors that can be considered when selecting sampling locations. When the factors of interest have been identified and corresponding data have been collated, the sampling locations may be distributed over the gradients of these factors.

When the locations have been identified and the costs of sampling in those locations may be estimated, decisions must be taken regarding the number of trees per location and the number of samples per year. These decisions may not have a large impact on the fixed costs, in particular if sampling is done by a local collaborator, but may allow assessment of local or seasonal variation in species occurrence. In many cases a multi-level design with global or regional arrangement of sampling locations and then sampling within locations (local variation, or among trees) will yield more information than taking a single (pooled) sample per location. It is therefore important that this local variation is captured by analysing samples and documenting the results separately for each sample.

Detection and identification of potential pests of woody plants before their introduction is an important component of successful biosecurity, because it allows PRA to be carried out and phytosanitary measures to be developed and implemented (Eschen et al., 2018). In this manuscript we reviewed and illustrated some aspects of survey design that affect the captured biodiversity. Every sentinel study will be different and the data and decision scheme presented here may provide guidance for the design of such studies, resulting in more cost-effective surveys for pests and pathogens associated with single woody plant species.

ACKNOWLEDGEMENTS

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DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository (www.datadryad.org) https://doi.org/10.5061/dryad.95752p7

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King, K. M., Harris, A. R., & Webber, J. F. (2015). In planta detection used to define the distribution of the European lineages of Phytophthora ramorum on larch (Larix) in the UK. Plant Pathology, 64, 1168–1175.


**BIOSKETCH**

This manuscript was conceived and developed as part of COST Action Global Warning, an EU-funded networking project to, among other things, develop standardized monitoring protocols to collect data required for preparing appropriate and reliable risk assessments. The projects brought together entomologists, pathologists and PRA specialists from many countries and has worked on harmonized sentinel planting design, data collection and identification for a range of organisms based on direct observation and symptoms, as well as morphological and molecular techniques.

Author contributions: All authors designed the study. MG, IF, RE, NL and DM provided datasets for the case studies. IF, MdG and RE analysed the data. RE wrote the manuscript with input from all authors.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.