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Review

Research review

Silent invaders: the hidden threat of asymptomatic phytobiomes to forest biosecurity

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Summary

Populations of diverse, unknown, and potentially pathogenic fungi and fungus-like organisms are continuously introduced into new locations via asymptomatic infections (e.g. as endophytes or latent pathogens) within internationally traded live plants. Interactions between these asymptomatic fungi and novel recipient host trees can be unpredictable, and urban introductions may act as bridgeheads into natural and managed forests. Historical examples of novel, highly destructive forest tree diseases highlight the potential threat of this pathway. As the trade in live plants continues to expand, the likelihood of high-impact incursions increases. This has led to calls for more proactive management, including more stringent treatment and regulatory standards, and even the phasing out of trade in plants determined to be an untenable risk to forest ecosystems. In this review, we discuss how biosecurity systems should consider advances in understanding the diversity and ecology of phytobiomes associated with asymptomatic plants and what measures can be considered to reduce this threat to global forest health.

Introduction

The international movement of plant material, particularly plants for planting (P4P), is widely acknowledged as a principal driver of the global increase in invasive forest health epidemics (Brasier, 2008; Turbelin *et al.*, 2017). Defined as plants intended to remain planted, to be planted, or replanted (IPPC Secretariat, 2024), P4P have been implicated in the introduction and establishment of more than two-thirds of invasive forest pests and pathogens in well-studied systems (Liebhold *et al.*, 2012; Santini *et al.*, 2013). As the number of biological invasions continues to increase, it is an inevitable conclusion that the past and current mitigation efforts have been insufficient to keep pace with the accelerating speed of globalization (Seebens *et al.*, 2022). The increasing volume of trade in general, and P4P in particular, which overwhelm inspection services and introduce higher propagule pressures, is often held responsible for this increasing number of invasions (Eschen *et al.*, 2015; Wingfield *et al.*, 2015). However, we contend that another reason is the limited acknowledgment and integration of phytobiome-associated risks into regulatory frameworks, particularly fungal endophytes and latent pathogens with extended asymptomatic or presymptomatic phases.

Recent advances in sequencing technologies have expanded our understanding of phytobiomes, revealing a vast diversity of fungi causing asymptomatic infections. These fungi are often collectively referred to as endophytes, which are broadly defined as fungi that, for all or part of their life history, asymptomatically colonize plant tissue (Wilson, 1995). Some pathogens also have extended asymptomatic stages, and many invasive diseases are now recognized as avirulent endophytes in their native hosts (Slippers & Wingfield, 2007; Enderle *et al.*, 2019). Despite this knowledge, international phytosanitary regulations, such as International Standards for Phytosanitary Measures (ISPM) 36 (integrated measures for plants for planting), do not address asymptomatic phytobiomes, nor do any other regulatory frameworks (Box 1).

We examine the role of international trade of P4P in spreading phytobiomes that can infect naïve hosts and contribute to emerging

Box 1. Brief summary of the global phytosanitary framework

The Commission on Phytosanitary Measures (CPM), governing body of the International Plant Protection Convention (IPPC), aims to prevent plant pest spread and promote safe trade. Recognized by the World Trade Organization (WTO) under the Sanitary and Phytosanitary Measures Agreement, the IPPC sets global plant health standards for 184 contracting parties, which include most UN member states. Each country establishes a National Plant Protection Organization (NPPO) to implement IPPC functions.

The CPM adopts International Standards for Phytosanitary Measures (ISPMs) to protect biodiversity and facilitate trade through harmonized import guidelines. While ISPMs are not legally binding under the IPPC, WTO members are expected to base their phytosanitary measures on these standards in accordance with the WTO SPS Agreement. As of April 2024, 46 ISPMs have been adopted, including ISPM 27 (diagnostic protocols for regulated pests), ISPM 15 (phytosanitary measures for wood packaging), and ISPM 12 (phytosanitary certification).

Plants for planting (P4P) pose a high pest risk, prompting ISPM 36 to provide pest risk management guidance, for example outlining NPPO responsibilities for pest monitoring, production site approval, certifications, and pre-export inspections. Importing countries can conduct pest risk analyses to establish specific requirements, such as pest-free areas, surveillance, and quarantine. This framework relies on symptomatic hosts carrying named pathogens, regulated or otherwise, but existing mechanisms do allow for assessing and responding to unknown threats. For example, IPSM 2 (framework for pest risk analysis) notes that phytosanitary action may be justified for organisms not yet fully described or difficult to identify, based on a pest risk analysis using the information available, however limited.

forest tree diseases. We challenge the practical assumption that phytobiomes of traded plants are benign and argue that phytosanitary measures should focus more on phytobiomes as a biosecurity concern. We also discuss how emerging knowledge and technologies can address this issue and highlight the knowledge gaps that need to be filled.

Hidden passengers: the global trade of live plants – and their phytobiomes

Researchers world-wide have recovered endophytes from nearly all host plants studied, irrespective of plant species, habitat, or geography. Culture-based studies typically report variable isolation frequencies from surface-sterilized leaf tissues, but recovery rates ranging from 88% to 100% for leaves are reported across various tree hosts (Koukol *et al.*, 2012; Graham *et al.*, 2025). Endophytes are ubiquitous in plants and are routinely isolated from leaves, buds, inflorescences, fruit, seeds, xylem, phloem, cambium, and bark, across environments ranging from natural and managed ecosystems to glasshouses and even indoor settings (Mahnert *et al.*, 2018). Metabarcoding studies regularly recover hundreds or even thousands of phylogenetically diverse operational taxonomic units (OTUs) from leaf and root samples (Mishcherikova *et al.*, 2023; Chen *et al.*, 2024).

Endophytes are not only ubiquitous and diverse but also challenging to identify. Isolating endophytes from surface-disinfected leaves often results in sterile cultures that cannot be identified due to the absence of taxonomically informative features. Furthermore, sequencing informative loci or barcodes, such as the nuclear ribosomal internal transcribed spacer, may fail to identify taxa that lack related reference sequences in public databases (e.g. NCBI GenBank and UNITE). For instance, metabarcoding studies of fungal components of phytobiomes (i.e. mycobiomes) may fail to assign the majority of OTUs to even the family rank (Würth *et al.*, 2019). Given the phylogenetic diversity of endophytes and the immense taxonomic knowledge gap for fungi in general – only *c.* 150 000 species of an estimated 2.5 million (or more) have been described – identifying endophytes is understandably difficult (Niskanen *et al.*, 2023).

Internationally traded P4P can thus be expected to be colonized by endophytes and latent pathogens that are phylogenetically diverse, difficult to identify, and represent putatively unknown or novel species. We trade not just in plants but also in phytobiomes.

Endophytes have complex life histories primed for invasiveness

Endophytism is widespread across phylogenetically diverse species, likely because it enhances survival and reproduction in various ecological contexts. Many endophytes are latent saprotrophs, and researchers suggest that endophytism evolved to provide priority access to substrates upon host senescence, enabling rapid colonization and reproduction before more aggressive competitors invade (Carroll, 1988; Vaz *et al.*, 2020). Conceptually, endophytism is a life history strategy that supports fungal survival and dispersal, for example, allowing them to persist in living foliage, potentially avoiding challenging environmental conditions or the absence of primary hosts and even using leaves as vehicles for spread (Thomas *et al.*, 2016; Nelson *et al.*, 2020).

Host ranges of endophytes can span from narrow to exceptionally broad and may shift during their life cycle (Fig. 1). For example, some taxa have a wide host range as endophytes but exhibit more specific preferences (e.g. with primary hosts and substrates) for sexual reproduction. Other endophytes exhibit narrow host and substrate preferences, limited to specific plant species or genera and specific plant parts, such as foliage, branches, or stems (Wang *et al.*, 2023). Overall, the host ranges of endophytes are not well understood, as early studies relied on morphology to identify strains (often sterile in culture), leaving many taxa unidentified and contributing to a lack of comprehensive surveys (Carroll & Carroll, 1978).

While the transmission modes of many endophytes in woody plants remain poorly understood, most are believed to be horizontally transmitted (Fig. 2). Sexual or asexual reproductive propagules from senescent leaves or nonfoliar substrates can infect new living leaves via abiotic (e.g. wind, rain, and surface water movement) transmission and biotic (e.g. insect) vectors. Endophytes can also colonize woody substrates through direct physical contact and subsequent hyphal growth between colonized leaves and the receptive substrate (i.e. viaphytism; Thomas *et al.*, 2016). The potential for every colonized leaf to serve as a propagule, along with multiple transmission pathways, increases the likelihood of endophyte establishment and expansion.

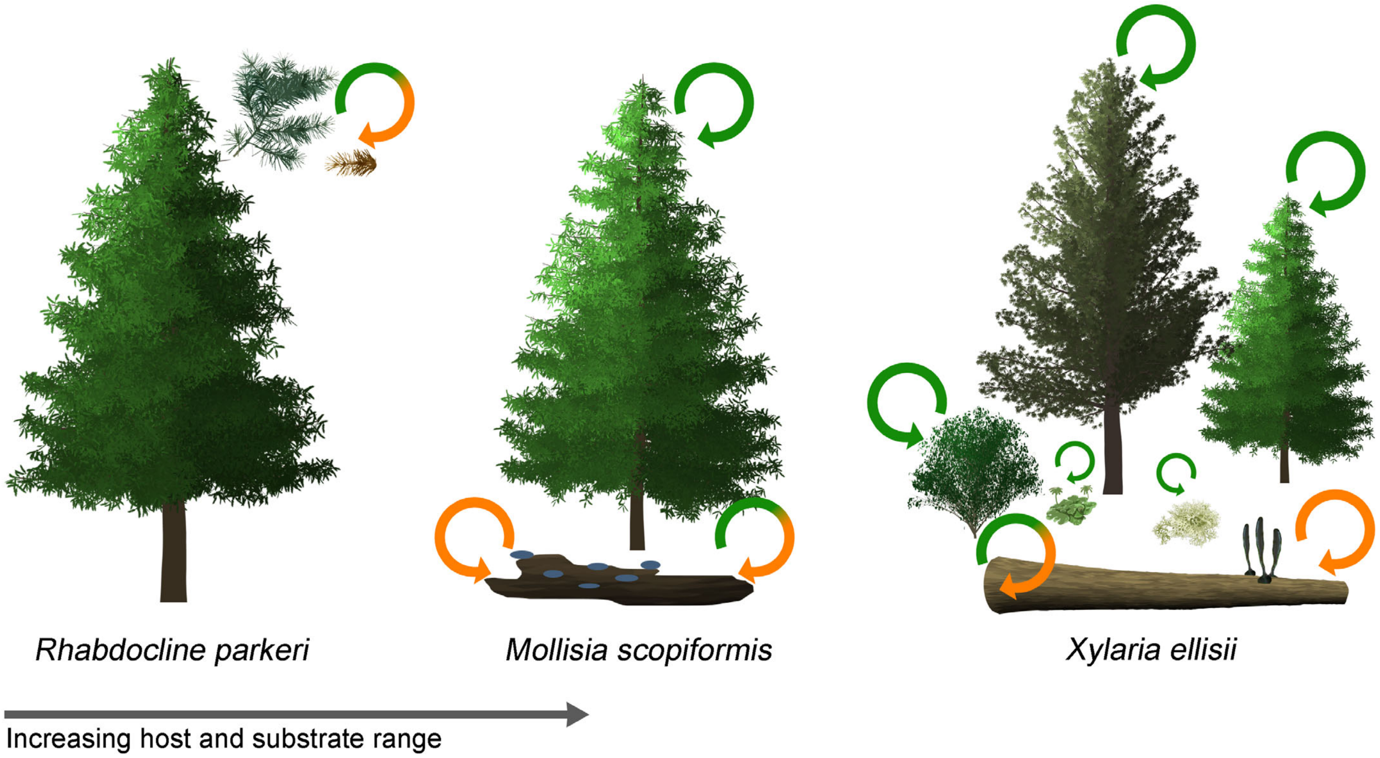

Fig. 1 Host and substrate range of three endophyte species along a gradient of ecological versatility. (1) *Rhabdocline parkeri* exhibits a narrow host and substrate range, restricted to needles of Douglas-fir (*Pseudotsuga menziesii*). It colonizes single host epidermal cells for the life of the needle (up to 8 yr or more), spreading rapidly as a saprotroph upon needle senescence. (2) *Mollisia scopiformis* has an intermediate range primarily on spruce (*Picea*), colonizing living needles, branches, and cambium as an endophyte and reproducing on decaying wood. (3) *Xylaria ellisii* demonstrates the broadest range, endophytically colonizing diverse hosts, including conifers, Ericaceae, *Vitis*, liverworts, and lichens, and saprotrophically colonizing angiosperm wood (*Acer*, *Betula*, and *Fagus*), where it undergoes sexual reproduction. These three examples are saprotrophs with endophytic phases that may act as mutualists by reducing pathogen or pest damage (Carroll, 1988; Richardson *et al*., 2015; Quiring *et al*., 2019). Circular arrows represent the life history transitions between endophytism (green) and saprotrophy (orange). The horizontal arrow indicates the progression toward increasing host and substrate range.

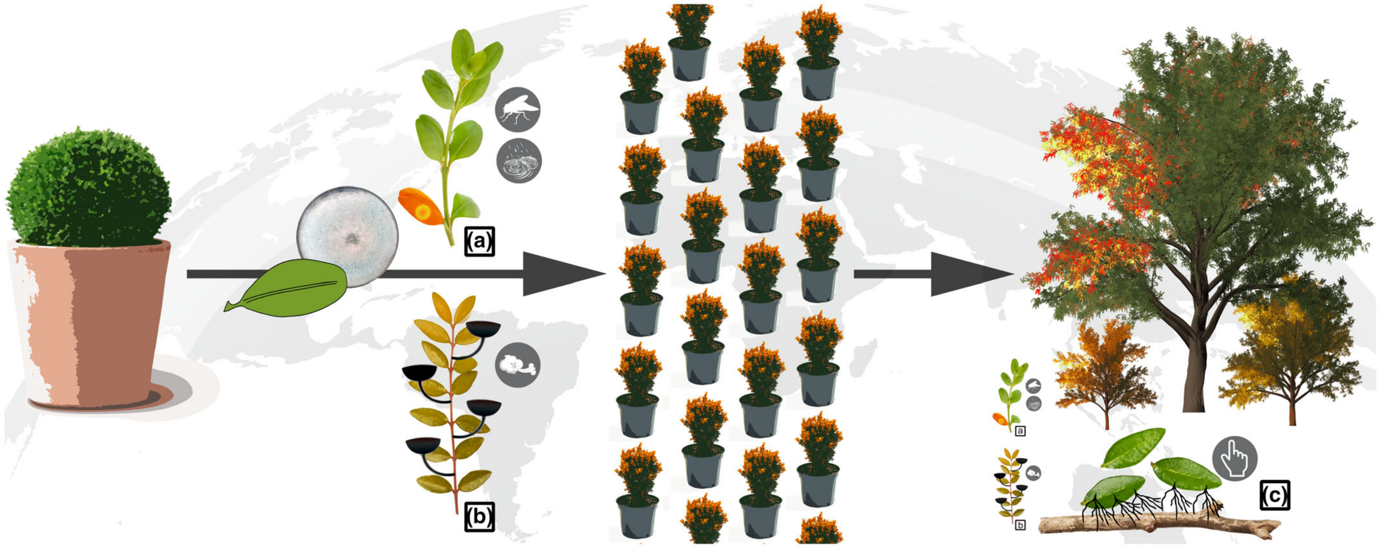

Fig. 2 Pathway illustrating the role of plants for planting (P4P) in the dispersal and establishment of a hypothetical endophyte, illustrating the varying life history traits that contribute to its invasiveness. An exported P4P colonized by an endophyte evading detection is introduced to a new location and established outdoors as an ornamental plant. The endophyte jumps to, and spreads among, a new related host species via (a) asexual spores that are produced from leaf lesions in slimy droplets and transmitted via insects and rain splash, and (b) sexual spores that develop on dead twigs and branches and are dispersed by air currents. Propagule pressure increases and a proximal native tree becomes a novel host, on which disease symptoms are produced, and the fungus spreads by asexual (as in a) and/or sexual spores (as in b), as well as to woody debris by (c) viaphytism.

Like many fungi, endophytes may reproduce both asexually and sexually, sometimes with different modes of propagule dispersal, such as insect- and water-dispersed asexual spores combined with air-dispersed sexual spores. While sexual reproduction benefits invasive pathogens, some species, such as *Ophiognomonia clavigignenti-juglandacearum* and *Melampsora larici-populina*, can also thrive clonally after losing sexual capability (e.g. due to absence of mating type or primary host; Barrès *et al*., 2008; Broders *et al*., 2015). Asexual reproduction paired with sexual reproduction enables rapid establishment, population growth, and adaptation (Gladieux *et al*., 2015).

Nutritional plasticity also enhances invasiveness, for example facultative pathogens switching between biotrophic and saprotrophic states. Pathogens, such as *Hymenoscyphus fraxineus* and *Diplodia sapinea*, can persist saprotrophically in environmental reservoirs (Laubray *et al*., 2024; Wingfield *et al*., 2025), making eradication virtually impossible. Persisting or even thriving without a living host may result in evading traditional virulence trade-offs (i.e. as sapronotic agents; Fisher *et al*., 2012; Kuris *et al*., 2014).

The complex life histories and mixed reproductive strategies of endophytes enhance their resilience, adaptability, and capacity for invasion.

Asymptomatic phytobiomes include pathogens – and this is not uncommon

Endophytes exist along a continuum from mutualists to pathogens, with their behavior influenced by the status of the host and environment (Schulz & Boyle, 2006). For example, endophytes can switch from being asymptomatic to pathogenic when hosts are subjected to temperature, drought, and light stress (Alvarez-Loayza *et al*., 2011; Tanney *et al*., 2024). Many examples can be found in Botryosphaeriaceae, which contain endophytic species that can cause disease in sufficiently stressed hosts (Slippers & Wingfield, 2007). Botryosphaeriaceae also offers an example of the extent and impact of the global movement of pathogens as asymptomatic infections in plant material. These fungi are normally dispersed over relatively short distances via rain splash and are unlikely to spread between continents naturally (Ahimera *et al*., 2004). Yet, a number of studies on global populations of the Botryosphaeriaceae suggest that these fungi have been introduced around the world multiple times, almost certainly in plant material, and have invaded and infected many native and non-native hosts in the new areas where they occur (Fig. 3). These include well-known pathogens, such as *Botryosphaeria dothidea* (Marsberg *et al*., 2017), *Lasiodiplodia theobromae* (Mehl *et al*., 2017), *Neofusicoccum parvum* (Batista *et al*., 2021), and *D. sapinea* (Wingfield *et al*., 2025; Table 1, Supporting Information Table S1).

Many other notorious tree pathogens, often not considered endophytes, have in recent years been shown to have endophytic phases (Table 1). For example, *Fusarium circinatum*, which causes pine pitch canker in susceptible *Pinus* species, can persist as a biotroph for at least 1 yr in asymptomatic seedlings (Swett *et al*., 2016), as well as survive saprotrophically in needles, branches, and wood chips (Serrano *et al*., 2017). Additionally, *F. circinatum* has been found to form endophytic associations with various Poaceae (Swett & Gordon, 2012; Swett *et al*., 2016) and dicots in the Asteraceae, Lamiaceae, and Rosaceae families (Hernandez-Escribano *et al*., 2018). These alternative hosts not only serve as a reservoir for field infections but could also serve as vehicles for dispersal when such plants are traded.

Phytophthora species are among the most serious pathogens of woody plants. Apart from their potential to spread in soils of live traded plants (Jung *et al*., 2016; Rooney-Latham *et al*., 2019), *Phytophthora* species have also been reported on asymptomatic plants (Puertolas *et al*., 2021). For example, Migliorini *et al*. (2015) found that 70% of asymptomatic plants sampled from European nurseries harbored *Phytophthora* species. Similarly, Denman *et al*. (2009) reported that *c*. 30% of *Phytophthora ramorum* and *Phytophthora kernoviae* infections in *Rhododendron* trap plants were asymptomatic. While not typically recognized as endophytes, their presence in asymptomatic plant tissue and infected roots highlights the threat of only relying on symptoms to identify infection. Interestingly, Beaulieu *et al*. (2021) reported significantly greater relative growth rates of isolates obtained from asymptomatic vs symptomatic hosts. *Phytophthora* can have impressively broad host ranges and varying saprotrophic abilities in aquatic environments or soil, which may contribute to their persistence and spread on the landscape once introduced to a new region (Aram & Rizzo, 2018; Hardham & Blackman, 2018; Dunstan *et al*., 2020).

Cryphonectriaceae are necrotrophs that have caused some of the most serious tree disease outbreaks historically. The most famous of these is *Cryphonectria parasitica*, causal agent of the destructive chestnut blight that killed billions of American and European chestnuts, which causes perennial cankers on branches and stems, eventually killing the aboveground parts (EFSA Panel on Plant Health, 2014). The fungus, however, can also infect the shoots of its primary and other hosts endophytically (Nicoletti *et al*., 2021). Similarly, other pathogens in the Cryphonectriaceae have been shown to have an endophytic phase, including *Chrysoporthe austroafricana* and *Celoporthe woodiana* (Mausse-Sitoe *et al*., 2016) and *Chrysoporthe cubensis*, *Chrysoporthe inopina*, and *Aurapex penicillata* (Granados *et al*., 2020).

In recent years, various studies, particularly those focusing on phytobiomes of asymptomatic plants, have revealed that the previously mentioned pathogens are not isolated occurrences. Instead, the phytobiomes of healthy plants frequently harbor organisms with pathogenic potential (Table 1). For example, Kemler *et al*. (2013) have shown that Mycosphaerellaceae and Teratosphaeriaceae make up the majority of leaf endophytes in some tree species, and these are common leaf and stem pathogens or are related to well-known pathogens of *Eucalyptus*. Lin *et al*. (2023) showed that genera, such as *Cercospora*, *Pseudocercospora*, and *Elsinoë*, are common in healthy *Populus* tissue and that this varies with drought stress and between male and female plants. The commonality of this finding among such phytobiome studies suggests that this phenomenon might be near universal.

Novel endophyte–host interactions are unpredictable

Some biological invasions are caused by endophytes that may be avirulent or weakly pathogenic on their endemic hosts but cause

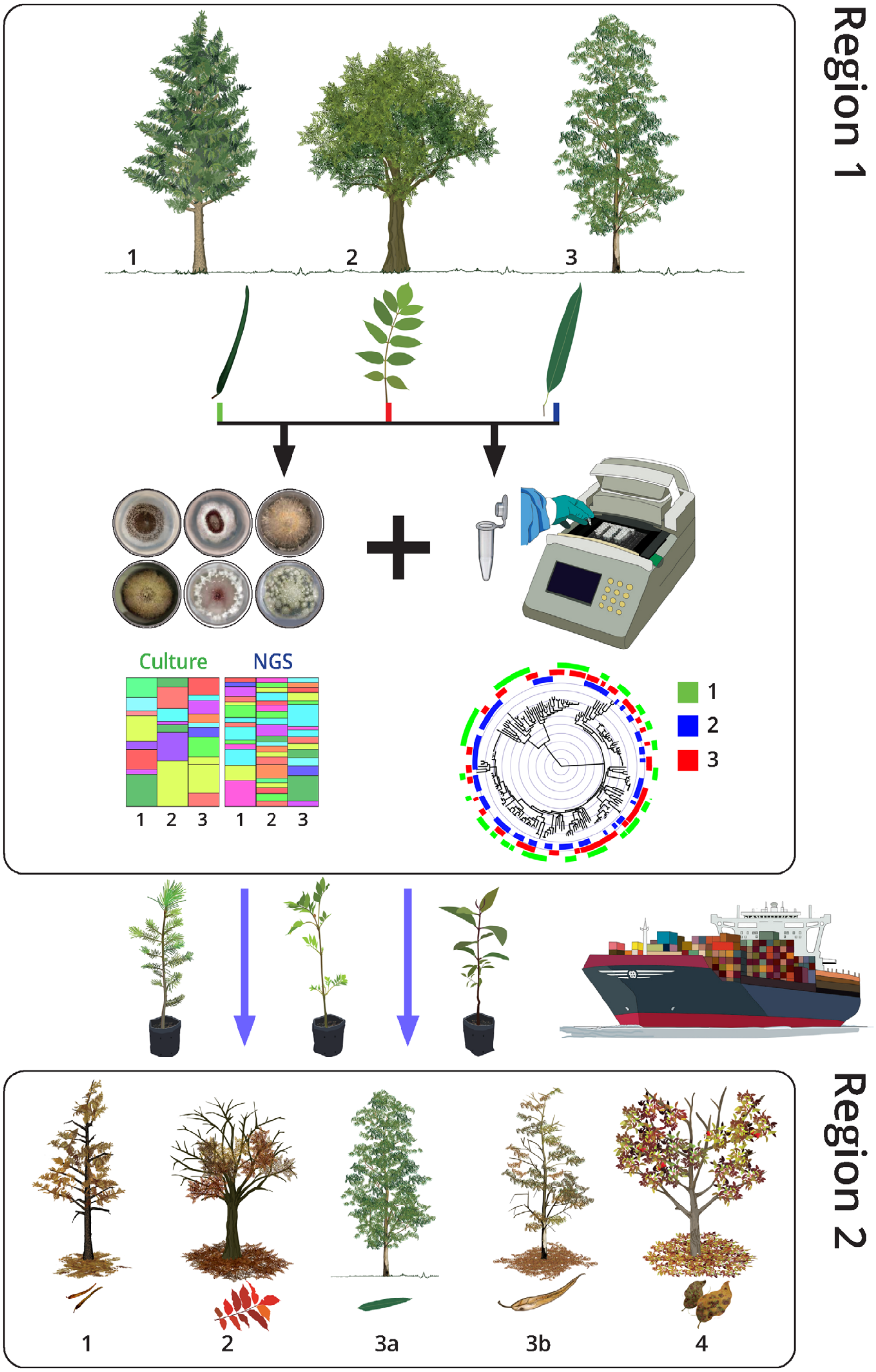

Fig. 3 Consequences of unintended phytobiome trade via healthy plant material. Healthy plants host diverse phytobiomes, including asymptomatic pathogens and unknown fungi. Trade regulations focus on visual inspections for disease symptoms in limited material, overlooking the risks posed by diverse microbial communities. This becomes evident when exploring the microbiota associated with healthy plant material by culture and culture-independent methods. Here, we highlight examples linked to (1) *Pseudotsuga menziesii*; (2) *Fraxinus* spp.; (3) *Eucalyptus* spp.; and (4) *Malus domestica*. Upon introduction to receptive environments (blue arrows), potential outcomes on these hosts may include the following: (1) co-evolved endophytes accompanying their host to new regions, where they may cause severe disease due to conducive environmental conditions (e.g. Swiss needle cast); (2) host jumps to exotic species, leading to the emergence of new diseases (e.g. ash dieback); (3a) cryptic invasions remaining unnoticed and (3b) becoming virulent under specific conditions (e.g. Botryosphaeriaceae or sooty bark disease); and (4) introduction of pathogens with broad host ranges infecting new phylogenetically diverse hosts (e.g. Botryosphaeriaceae; Table 1).

unpredictable and destructive diseases in their new hosts (Fig. 3). *Hymenoscyphus fraxineus* is a contemporary example of a devastating disease (ash dieback) in Europe, caused by a fungus that is typically a harmless endophyte–saprotroph on its native host (Enderle *et al*., 2019; Hill *et al*., 2019). In Japan, where it is native, *H. fraxineus* endophytically colonizes Manchurian ash (*Fraxinus mandshurica*) leaves until senescence and abscission, after which it transitions to a saprotrophic phase involving both asexual and sexual reproduction (Inoue *et al*., 2019). *Hymenoscyphus fraxineus* was likely introduced into Europe through the import of endophytically colonized Manchurian ash trees (Enderle *et al*., 2019). On novel hosts, such as European ash (*Fraxinus excelsior*), *H. fraxineus* shifted from a biotrophic endophyte to an aggressive necrotrophic pathogen, causing symptoms, such as leaf lesions, wilting, twig and branch dieback, and cankers, leading to widespread mortality.

Before the recognition of the outbreak, and assuming its unlikely detection in asymptomatic imported trees, detecting *H. fraxineus* would not have triggered a pest risk analysis or any phytosanitary measures or quarantine. None of the *c.* 200 species of

Table 1 Examples of invasive pathogens with latent/endophytic life histories that were introduced outside of their native range via plants for planting (P4P) pathway.

Disease and causal agent	Major hosts	Probable pathways and origin	Species known preinvasion?
Eastern filbert blight (*Anisogramma anomala*)	*Corylus* spp.	Imported from eastern United States on infected nursery stock. Endemic to eastern North America.	Yes
Botryosphaeria canker, dieback and fruit rot (*Botryosphaeria dothidea*, *Neofusicoccum parvum*, *Lasiodiplodia theobromae*, etc.)	Broad host range in agriculture, urban environment, native and planted forests	Live plants, seedlings, and plant parts (including fruit).	Yes
Cryphonectria canker of *Eucalyptus* (*Chrysoporthe cubensis*/*deuterocubensis*)	*Eucalyptus* spp.	Propagative material of various Myrtaceae. Possibly endemic to Asia.	No
White pine blister rust (*Cronartium ribicola*)	*Pinus* subg. *Strobus*, *Ribes* spp.	Multiple introductions via infected nursery stock (e.g. from France to Vancouver, BC, 1910). Endemic to Asia.	No
Chestnut blight (*Cryphonectria parasitica*)	*Castanea* spp.	Propagative material of *Castanea crenata* from Asia into North America, possibly introduced into Europe via North America. Endemic to Asia.	No
Cryptostroma corticale	*Acer* spp., *Fraxinus excelsior*, *Aesculus hippocastanum*	Infected wood and/or asymptomatically infected plants. Endemic to eastern North America.	Yes
Diplodia sapinea	*Pinus* spp.	Multiple introductions into the Southern Hemisphere and invading Northern Europe. Infected seedlings and possibly seed. Unknown origin.	Yes
Dogwood anthracnose (*Discula destructiva*)	*Cornus* spp.	Multiple introductions probably from asymptomatic *Cornus kousa* plants imported from Asia. Endemic to Asia.	No
Dothistroma needle blight (*Dothistroma septosporum* and *D. pini*)	*Pinus* spp.	Infected seedlings and other plant material.	Yes
Scab and shoot malformation (*Elsinoë* species)	*Citrus*, *Populus*, *Eucalyptus*, and other woody species	Disease on various woody hosts. Shown to be endophytes on *Populus* and *Corylus*. Unknown origin.	No
Eutypella canker (*Eutypella parasitica*)	*Acer* spp.	Infected saplings and logs. Endemic to North America.	Yes
Pitch canker (*Fusarium circinatum*)	*Pinus* spp., Poaceae, and Asteraceae	*Pinus* seed and seedlings, possibly other hosts. Believed to be endemic to Mexico.	No
Scleroderris canker (*Gremmeniella abietina*, European race)	*Pinus* spp.	Asymptomatic exotic pine seedlings. Possibly endemic to central Europe.	Yes
European ash dieback (*Hymenoscyphus fraxineus*)	*Fraxinus* spp.	Live *Fraxinus* plants. Endemic to East Asia.	No
European larch canker (*Lachnellula willkommii*)	*Larix* spp.	Live plants (nursery stock). Endemic to Asia.	Yes
Brown spot needle blight (*Lecanosticta acicola*)	*Pinus* spp., *Cedrus libani*	Multiple introductions into Europe from infected North American *Pinus* P4P. Likely endemic to North America.	Yes
Beech bark disease (*Nectria ditissima*[a] and *N. faginata*[b]) and *Cryptococcus fagisuga*	*Fagus grandifolia*	Ornamental *Fagus sylvatica* trees from Europe. Scale vector endemic to Europe.	Yes[a]/No[b]
Swiss needle cast (*Nothophaeocryptopus gaeumannii*)	*Pseudotsuga menziesii*	Live plants. Endemic to the natural range of its host.	No
Butternut canker (*Ophiognomonia clavigignenti-juglandacearum*)	*Juglans* spp.	Live plants imported from Asia (e.g. Japanese walnut *Juglans ailantifolia*). Endemic to Asia.	No
Phyllosticta citricarpa	*Citrus*	Citrus fruit and latently infected plants. Probably endemic to Southeast Asia.	Yes
Alder Phytophthora (*Phytophthora* × *alni*)	*Alnus glutinosa*, *A. incana*, *A. cordata*, *A. viridis*, and *A. rubra*	Planting materials. Origin of parental species and hybridization unknown.	No
Jarrah root rot (*Phytophthora cinnamomi*)	*c.* 5000 spp.	Multiple introductions via infected nursery stock. Possibly endemic to Southeast Asia.	Yes
Phytophthora lateralis	*Chamaecyparis lawsoniana*	Imported *Chamaecyparis* from Asia.	No
Phytophthora pluvialis	*Pseudotsuga menziesii*, *Notholithocarpus densiflorus*, *Pinus radiata*, *P. patula*, *P. pinea*, *P. strobus*, *T. heterophylla*, *Larix kaempferi*, others	Moved with infected plant material. Possibly endemic to American Pacific Northwest.	Yes

Table 1 (Continued)

Disease and causal agent	Major hosts	Probable pathways and origin	Species known preinvasion?
Sudden oak death (*Phytophthora ramorum*)	Very broad range including *Larix*, *Notholithocarpus*, *Quercus*, *Rhododendron*	Multiple introductions via nursery pathway: ornamental shrubs, such as *Rhododendron*, *Viburnum*, *Pieris*, and *Camellia*. Evidence suggests endemic to Indochina/Japan.	No
Tar spot (*Rhytisma acerinum*)	*Acer platanoides*	Introduced at some point with European *A. platanoides* trees, which were first imported in the 1750s. Endemic to Europe.	Yes
Cypress canker (*Seiridium cardinale*)	*Cupressus* and other Cupressaceae spp.	Imported *Cupressus* ornamental trees. Possibly endemic to California.	No
Septoria canker (*Sphaerulina musiva*)	*Populus* spp. (e.g. *P. deltoides*, *P. trichocarpa*, and *P.* hybrids)	Moved with infected *Populus* used in forestry plantations, affecting susceptible hybrids and Asian species. Endemic to eastern North America.	Yes

Expanded table with references found in Supporting Information Table S1.

Hymenoscyphus have ever been reported as pathogens; before *H. fraxineus*, they were all considered benign saprotrophs and benign endophytes. Ash dieback therefore provides an alarming example of an endophyte belonging to a genus previously of no phytosanitary concern being introduced into a new environment, jumping hosts, and leading to the destruction of a dominant tree species and billions of dollars' worth of damage (Hill *et al.*, 2019). This is the destructive potential of a 'benign' endophytic litter decomposer being accidentally introduced into a naïve host. Interestingly, *Hymenoscyphus albidus*, a close relative of *H. fraxineus*, is endemic to Europe, where it exhibits a similar endophyte–saprotroph life cycle on European ash without causing disease, apparently due to its low infection pressure and timing of colonization (Hietala *et al.*, 2022).

Similar to the *H. fraxineus* example, much of the impact of pathogens described in earlier sections (e.g. *Phytophthora*, *Cryphonectria*, and *Chrysoporthe* species) has likely been the outcome of novel interactions with naïve hosts that lack resistance (Table 1). The changes in behavior of fungi in these novel host interactions are not always well understood, but it is notable that some of the most devastating impacts are on what is presumed to be novel hosts when they are under stress, a phenomenon that is increasing under climate change (Sturrock *et al.*, 2011).

Trading in phytobiomes: is this a lost cause?

Our growing understanding of fungal life histories involving endophytic phases highlights an important introduction pathway that challenges the efficacy of visual-based inspections and certification systems reliant on the apparent absence of known pests. Considering the growing trade in plants and plant products and its substantial economic impact, it is imperative that more informed measures are implemented to mitigate pathway risks while promoting sustainable industry growth. Some potential treatments and strategies to reduce the risks of P4P trade are discussed below, with approaches to reduce knowledge gaps highlighted in Box 2.

P4P treatments

The phytosanitary risks associated with moving wood and wood packaging are well recognized, and the adoption of ISPM 15 was a crucial step in mitigating these risks through standardized heat treatment and fumigation (Ormsby, 2022). By contrast, fewer advances have been made in treating P4P due to the challenges of sterilizing living plants without harming their health. For instance, while ionizing radiation is commonly used for fresh fruit and vegetable treatments, its application to P4P remains unexplored and raises concerns about potential devitalization and mutagenesis.

Fungicide treatments are unlikely to eradicate endophytes and may instead select for resistant strains (Mohandoss & Suryanarayanan, 2009; Batzer & Mueller, 2020; Win *et al.*, 2021). The rising use of fungistatics and fungicides also poses risks, including masking pathogen symptoms that may otherwise be detected and contributing to resistance in both plant and animal pathogens (Linderman & Davis, 2008; Al-Hatmi *et al.*, 2019; Toda *et al.*, 2021). Heat treatment (thermotherapy), including methods like aerated steam, hot water, and ambient heat exposure, has been demonstrated to reduce or eliminate the occurrence of pathogenic viruses, bacteria, oomycetes, and fungi in live plants and seeds (Pisetta *et al.*, 2016; Wang *et al.*, 2018; Baggio *et al.*, 2021; Kranjec Orlović *et al.*, 2021). Combining thermotherapy with treatments, such as cryotherapy, chemotherapy, and micrografting, improves pathogen eradication, although these are better suited for propagative material than for treating whole plants (Müller & Hamberg, 2021; Swiecki & Bernhardt, 2022). Heat treatment for eradicating fungal endophytes is limited by insufficient research on effective lethal doses and host tolerances, which, given the nature of the commodity, must consider not only the risk of devitalizing plants but also minimizing aesthetic damage and recovery time. Evidence of thermotolerance in some plant-associated taxa (e.g. Ramsfield *et al.*, 2010; Noseworthy *et al.*, 2024) suggests that thermotherapy may be more effective against nonfungal and non-oomycete pathogens, particularly when treatment temperatures must be limited to avoid damaging a living host. Heat tolerance also varies among plant hosts, making a universal approach to apply to all live plants unrealistic. More

Box 2. Approaches to closing knowledge gaps and addressing biosecurity challenges associated with phytobiomes

Predicting invasiveness *a priori*
(1) Sentinel planting networks to identify potential invasive pathogens.
(2) Elucidate endophyte–plant interactions between endemic and non-native hosts with inoculation studies.
(3) Explore phylogenetic signals to predict host ranges, pathogenicity, and invasiveness.
(4) Genomic approaches to identify traits or genetic markers that cause or predict invasiveness (e.g. genomic signatures of pathogenicity and invasiveness).

Improving interception via inspections
(1) High-throughput sequencing/metabarcoding to detect known and potential pathogens.
(2) Develop appropriate sampling methods to improve detection in large consignments.
(3) Explore use of detection methods, such as biosensors, volatile markers, and hyperspectral sensing.

Updating best practices to meet risks
(1) Metagenomics tools to elucidate imported phytobiomes and characterize risk assessments based on commodity type and origin.
(2) Shift toward trade in plants for planting (P4P) commodities that are less risky (e.g. meristematic tissue cultures and treated seeds) and bulking within countries for domestic usage vs focus on importing retail-ready plants.
(3) Advancing treatments of P4P to reduce phytosanitary risk, for example thermotherapy.
(4) Curtail international movement of ornamental P4P commodities that are deemed unacceptable risks, for example plants that are known pathways for harmful organisms, especially if they are phylogenetically related to economically/ecologically important trees endemic to the importing country.
(5) Consider the adoption of combined blacklist and whitelist approaches to phytosanitary regulations.

Advancing fungal biodiversity and phytobiome research
(1) Concerted global effort to accelerate fungal biodiversity research.
(2) Integrating fungal taxonomic research (e.g. collect-and-sequence approach) into next-generation sequencing (NGS)-based biodiversity studies.
(3) Adopting a voucherless, sequence-based nomenclature or formal designation of unidentified eDNA sequences.
(4) Omics approaches to characterize phytobiomes of hosts in endemic and non-native locations.
(5) Characterize endemic phytobiomes pre- and postinvasion.

research is needed to assess the feasibility, efficacy, optimal dosage, and potential risks of devitalizing plants in high-throughput treatments to reduce phytosanitary risks in P4P.

Risk reduction practices

A potential approach to circumvent unknown risks is to shift the P4P trade to a system that favors trading in propagation material with subsequent bulking up in the importing countries for domestic consumption. Propagative materials, such as tissue cultures, are less risky than rooted plants in pots, as are plants grown in growth chambers and glasshouses vs field-grown plants (IPPC Secretariat, 2020). Seeds generally show a diversity bottleneck, for example one or zero fungus per seed, and this diversity may exclude common pathogens of vegetative tissues; however, they are still considered a potentially high risk (Newcombe *et al.*, 2018; Hill *et al.*, 2021; Franić *et al.*, 2024). Overall, this approach would significantly disrupt the current P4P trade paradigm and is unlikely to be implemented; however, its adoption may be advisable on an individual basis for high-risk commodities, such as *Rhododendron* and its risk in transferring *P. ramorum* (Denman *et al.*, 2009). Banning the importation of high-risk commodities may also be considered if they pose an excessive risk and lack effective mitigation measures.

Evolutionary- and value-based risk assessments

Host jumps within the same host genus are likely for many invasive pathogens, though many remain cryptogenic, with 25% of forest pathogens in Europe of unknown origin (Santini *et al.*, 2013). While broad host ranges are normal for many species of oomycetes, Botryosphaeriales, Diaporthales, and other fungi, others exhibit more restricted host preferences that may be used to infer risks. Santini *et al.* (2013) noted that the majority (77%) of invasive forest pathogens in Europe are specialists, while a smaller number (16%) are polyphagous.

The likelihood of a pathogen infecting two plant species decreases with phylogenetic distance (Gilbert & Webb, 2007), and this pattern is evident in pathogens, such as *O. clavigignenti-juglandacearum* on *Juglans* spp., *Bretziella fagacearum* on *Quercus* spp., and *Lachnellula willkommii* on *Larix* spp. Phylogenetic distance can thus serve as a proxy for pathogen risk and impact. Gilbert *et al.* (2015) suggested that pathogens affect plant species within the same genus at 30–50% of the severity observed in the most susceptible species of that genus. By contrast, their impact is lower (10–30%) on species within the same family but belonging to a different genus. Phylogenetic distance models are valuable for pest risk analysis, helping to assess the risk of pathogen introduction and its economic and environmental consequences (Lynch *et al.*, 2021).

Invasion risk and disease severity increase when endemic plants in the receiving ecosystem are phylogenetically closely related to imported P4P species. Importing P4P into areas with congeneric endemic plants poses a higher phytosanitary risk, requiring a careful assessment of the reward ratio – balancing the economic and social benefits of trade with the potential ecological, economic, and social consequences of invasion. Shifting from pest risk analyses focused on specific pests to commodity-based risk assessments that consider unknown but plausible threats offers a more proactive approach. For example, the Not Authorized Pending Pest Risk Analysis category prohibits specified P4P taxa from importation until a pest risk analysis determines whether they should be authorized for entry (CFIA, 2015; USDA-APHIS, 2024). A phylogenetic-based approach, incorporating the value of at-risk endemic biodiversity, would help mitigate risks while supporting P4P trade and guiding inspection and regulatory efforts more effectively.

Improved detection and surveillance methods

Molecular methods targeting viable cells, such as quantitative reverse transcription polymerase chain reaction (RT-PCR) and viability PCR, may be more practical for phytosanitary applications by reducing false alarms from detection of nonviable cells. Pathogen-specific simplex and multiplex tests (e.g. loop-mediated isothermal amplication (LAMP) and qPCR) can also be deployed in the field to rapidly detect pathogens with high sensitivity and accuracy (Luchi *et al.*, 2020), especially for high-risk groups, such as *Phytophthora* (Leal *et al.*, 2024). While promising, more work must be performed to address considerations, such as detecting DNA from nonviable cells, and to develop international standards. Current capabilities may not yet support evidence from such tools as definitive proof of infection or the presence of viable organisms in commodities, but these results can still inform decision-making, such as sample triaging.

Metabarcoding and metagenomics approaches are widely used to characterize phytobiomes, including endophytes, from specific plant tissues. Unfortunately, few such analyses are currently carried out to study phytobiomes associated with P4P or for surveillance of microbes in such pathways. Systematic studies of regularly traded P4P throughout their lifetimes from areas of origin (or sites of production) to eventual destinations, captured in structured open-access databases, will go a long way to providing a foundation for assessing the risk of traded phytobiomes. This approach can also help to determine the effectiveness of different management approaches to reduce risk aligned with ISPM 36 (see Box 1) and the approaches proposed in this article. Development of such approaches for human health (Ko *et al.*, 2022) and other fields related to biosecurity (Bell *et al.*, 2024) demonstrate opportunities for broader adoption of this technology in plant health biosecurity.

Machine learning operations have been used to develop advanced real-time identification systems for insects (Chiranjeevi *et al.*, 2024) and, for example, classifying anthracnose disease, including presymptomatic infections, in mango fruit (Siripatrawan & Makino, 2024). Phytosanitary applications could eventually involve real-time monitoring of plant health within glasshouses and high-risk forests (e.g. near ports), and detecting infected plants (including asymptomatic) in glasshouses and consignments based on signatures from hyperspectral imaging or molecules like volatile organic compounds (Brilli *et al.*, 2020; Sherwood *et al.*, 2024). Following transmission to symptomatic hosts, AI-driven scanning tools for identifying key signals for novel pests and species of interest from text analysis pipelines (e.g. from sources like news articles and publications; Antoniou *et al.*, 2024) and images from citizen science and social media may also be deployed for early detection of invasive species.

Horizon scanning – predicting the next invasion

To prevent further biological invasions, it is crucial not only to detect invading pathogens before they become widespread but also to identify potential invasive pathogens and host ranges. Sentinel-based surveillance, which involves planting and monitoring susceptible hosts in urban areas or near ports and other high-risk sites, can facilitate earlier pathogen detection and uncover new pathogen–host interactions, in turn enhancing databases that help classify the risks of unknowns in phytobiomes (Eschen *et al.*, 2019; Lovell-Read *et al.*, 2023; Chang *et al.*, 2024).

Omics-based approaches to predict invasiveness, virulence, and host interactions are becoming increasingly feasible and should be considered in coming years as an additional tool for biosecurity management. McTaggart *et al.* (2016) suggest a paradigm shift from name-based biosecurity to a biologically meaningful gene-based risk assessment. Advances in omics approaches to understanding genetic signatures of lifestyle, pathogenicity factors, and elements linked to pathogen genome evolution all offer targets to focus on in such an approach. While such an approach is not currently feasible for most plant biosecurity systems due to costs, capacity, and gaps in scientific knowledge, it is technically feasible and should be a priority in biosecurity-related research.

One major caveat is that the fungus alone may not inform potential host interactions, as the gene repertoire is the same for an endophyte that is benign on its endemic host and pathogenic on a naïve host. Therefore, host response must also be considered, which adds complexities to predicting interaction outcomes. Combining omics-based approaches, sentinel plantings, and modeling approaches will become especially interesting in this respect. Large-scale, high-throughput identification using omics approaches (e.g. metabarcoding) of endophytic species captured in sentinel plantings can guide efforts to isolate the specific species on artificial media to identify life-history traits of these species. Subsequent modeling and machine learning approaches that use species information, as well as morphological and molecular trait data, can then help in prioritizing those P4P species posing severe threats to plants and ecosystems in the importing country (Paini *et al.*, 2011; Philibert *et al.*, 2011).

Conclusions

In this review, we argue that phytobiomes – particularly endophytes and latent pathogens with prolonged asymptomatic phases – are ubiquitous, diverse, and pose a significant challenge to the future health of planted forests and natural woody ecosystems. These concerns are amplified when considering the significant knowledge gaps relating to the detection, identification, and overall biology of endophytes. The functional diversity of endophytes, which span the mutualist–pathogen continuum within the same or across different hosts, contributes to an overall uncertainty in predicting their interactions with novel hosts, especially when overlaid with a changing climate (Herpin-Saunier *et al.*, 2022). Deliberate introductions of P4P permeated with endophytes unintentionally provide unique opportunities to observe unexplored interactions – with potentially disastrous outcomes.

Some of the most devastating biological invasions can be traced to the spread of phytobiomes along the P4P pathway (Table 1). Yet, despite the widely recognized risks, P4P trade continues to accelerate with inadequate mitigation efforts. These invasions often go undetected, either as dead ends or cryptic threats, until a catastrophic spillover draws attention. The cryptic nature of endophyte-driven biological invasions demands the development of methods and policies that address the common pathway represented by trade in living plant material, especially P4P. Such an approach must better account for unnamed and unknown organisms, considering factors like life history and pathogenic potential within the phytosanitary paradigm.

It has been nearly a decade since the first call to shift from a name-based to a gene-based biosecurity risk mitigation approach (McTaggart *et al*., 2016). The growing use of genomics and other molecular tools in biosecurity (Bell *et al*., 2024), along with lessons from the SARS-CoV-2 pandemic (Ko *et al*., 2022), demonstrates the technical feasibility of such approaches. Developing effective tools is critical for improving detection, surveillance, and management strategies to mitigate risks and enhance biosecurity measures. As climate change accelerates and amplifies the impact of biological invasions, research supporting science-based biosecurity decisions, ensuring global biodiversity and ecosystem integrity while maintaining trade, has never been more critical, and we acknowledge the efforts of organizations, such as the International Forestry Quarantine Research Group, the North American Plant Protection Organization, and the European and Mediterranean Plant Protection Organization.

The global trade in live plants is not just a pathway for economic growth but also a Trojan horse for the silent spread of invasive fungi and fungus-like organisms, many of which persist asymptomatically as endophytes or latent pathogens. Traditional phytosanitary measures, focused on visible symptoms, overlook these hidden threats and leave forests vulnerable to catastrophic outbreaks. To safeguard forest biodiversity, biosecurity must evolve beyond outdated pathogen-centric approaches and strive to integrate genomic surveillance, sentinel networks, and risk-based trade policies. Without urgent action, accelerating global trade will continue to outpace our ability to mitigate biological threats, reshaping forests in unpredictable ways.

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None declared.

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References

Ahimera N, Gisler S, Morgan DP, Michailides TJ. 2004. Effects of single-drop impactions and natural and simulated rains on the dispersal of *Botryosphaeria dothidea* conidia. *Phytopathology* **94**: 1189–1197.

Al-Hatmi AMS, de Hoog GS, Meis JF. 2019. Multiresistant *Fusarium* pathogens on plants and humans: solutions in (from) the antifungal pipeline? *Infection and Drug Resistance* **12**: 3727–3737.

Alvarez-Loayza P, White JF Jr, Torres MS, Balslev H, Kristiansen T, Svenning JC, Gil N. 2011. Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. *PLoS ONE* **6**: e16386.

Antoniou A, Abergel M, Akrivou A, Beale T, Day R, Fielder H, Larenaudie M, MacLeod A, Rosace MC, Sarakatsani E *et al.* 2024. Horizon scanning: tools to identify emerging threats to plant health in a changing world. *EPPO Bulletin* **54**: 73–88.

Aram K, Rizzo DM. 2018. Distinct trophic specializations affect how *Phytophthora ramorum* and Clade 6 *Phytophthora* spp. colonize and persist on *Umbellularia californica* leaves in streams. *Phytopathology* **108**: 858–869.

Baggio JS, Marin MV, Peres NA. 2021. *Phytophthora* crown rot of Florida strawberry: inoculum sources and thermotherapy of transplants for disease management. *Plant Disease* **105**: 3496–3502.

Barrès B, Halkett F, Dutech C, Andrieux A, Pinon J, Frey P. 2008. Genetic structure of the poplar rust fungus *Melampsora larici-populina*: evidence for isolation by distance in Europe and recent founder effects overseas. *Infection, Genetics and Evolution* **8**: 577–587.

Batista E, Lopes A, Alves A. 2021. What do we know about Botryosphaeriaceae? An overview of a worldwide cured dataset. *Forests* **12**: 313.

Batzer JC, Mueller DS. 2020. Soybean fungal endophytes *Alternaria* and *Diaporthe* spp. are differentially impacted by fungicide application. *Plant Disease* **104**: 52–59.

Beaulieu J, Del Castillo Munera J, Balci Y. 2021. Detection of oomycide-insensitive *Phytophthora* isolates in Maryland ornamental nurseries and Mid-Atlantic landscapes provide data for reconsidering management strategies. *Plant Health Progress* **22**: 281–286.

Bell KL, Campos M, Hoffmann BD, Encinas-Viso F, Hunter G, Webber BL. 2024. Environmental DNA methods for biosecurity and invasion biology in terrestrial ecosystems: progress, pitfalls, and prospects. *Science of the Total Environment* **926**: 171810.

Brasier CM. 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* **57**: 792–808.

Brilli F, Luchi N, Michelozzi M, Calamai L, Cencetti G, Pecori F, Nigrone E, Santini A. 2020. Volatile organic compounds (VOC) as biomarkers for detection of *Ceratocystis platani*. *Forest Pathology* **50**: e12618.

Broders K, Boraks A, Barbison L, Brown J, Boland GJ. 2015. Recent insights into the pandemic disease butternut canker caused by the invasive pathogen *Ophiognomonia clavigignenti-juglandacearum*. *Forest Pathology* **45**: 1–8.

Carroll G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* **69**: 2–9.

Carroll GC, Carroll FE. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. *Canadian Journal of Botany* **56**: 3034–3043.

CFIA. 2015. *D-08-04: Plant protection import requirements for plants and plant parts for planting.* [WWW document] URL https://inspection.canada.

ca/en/plant-health/invasive-species/directives/imports/08-04/ [accessed 1 April 2025].

Chang L, Li YL, Gao ZW, Bonello PE, Cleary M, Munck IA, Santini A, Sun H. 2024. First report of *Epicoccum latusicollum* causing leaf spot disease on red maple (*Acer rubrum* L.) in China: insights from a sentinel planting garden. *Crop Protection* 175: 106439.

Chen X, Dou M, Li Y, Su J, Zhao A, Huang X. 2024. Elucidating the endophytic bacterial and fungal community composition and diversity in the tree fern *Alsophila spinulosa* through meta-amplicon sequencing. *Frontiers in Microbiology* 15: 1445315.

Chiranjeevi S, Saadati M, Deng ZK, Koushik J, Jubery TZ, Mueller D, O'Neal ME, Merchant N, Singh A, Singh AK *et al.* 2024. InsectNet: real-time identification of insects using an end-to-end machine learning pipeline. *PNAS Nexus* 4: pgae575.

Denman S, Kirk SA, Moralejo E, Webber JF. 2009. *Phytophthora ramorum* and *Phytophthora kernoviae* on naturally infected asymptomatic foliage. *EPPO Bulletin* 39: 105–111.

Dunstan WA, Howard K, Grigg A, Shaw C, Burgess TI, Hardy GE. 2020. Towards eradication of *Phytophthora cinnamomi* using a fallow approach in a Mediterranean climate. *Forests* 11: 1101.

EFSA Panel on Plant Health (PLH). 2014. Scientific opinion on the pest categorisation of *Cryphonectria parasitica* (Murrill) Barr. *EFSA Journal* 12: 3859.

Enderle R, Stenlid J, Vasaitis R. 2019. An overview of ash (*Fraxinus* spp.) and the ash dieback disease in Europe. *CABI Reviews* 2019: 1–12.

Eschen R, Britton K, Brockerhoff E, Burgess T, Dalley V, Epanchin-Niell RS, Gupta K, Hardy G, Huang Y, Kenis M *et al.* 2015. International variation in phytosanitary legislation and regulations governing importation of plants for planting. *Environmental Science & Policy* 51: 228–237.

Eschen R, O'Hanlon R, Santini A, Vannini A, Roques A, Kirichenko N, Kenis M. 2019. Safeguarding global plant health: the rise of sentinels. *Journal of Pest Science* 92: 29–36.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186–194.

Franić I, Cleary M, Aday Kaya AG, Bragança H, Brodal G, Cech TL, Chandelier A, Doğmuş-Lehtijärvi T, Eschen R, Lehtijärvi A *et al.* 2024. The biosecurity risks of international forest tree seed movements. *Current Forestry Reports* 10: 89–102.

Gilbert GS, Briggs HM, Magarey R. 2015. The impact of plant enemies shows a phylogenetic signal. *PLoS ONE* 10: e0123758.

Gilbert GS, Webb CO. 2007. Phylogenetic signal in plant pathogen–host range. *Proceedings of the National Academy of Sciences, USA* 104: 4979–4983.

Gladieux P, Feurtey A, Hood ME, Snirc A, Clavel J, Dutech C, Roy M, Giraud T. 2015. The population biology of fungal invasions. *Molecular Ecology* 24: 1969–1986.

Graham HR, Hayward E, Ehlting J, de la Bastide PY, Avis TJ, Feau N, Hamelin R, Bérubé J, McMullin DR, Tanney JB. 2025. *In vitro* suppression of the Swiss needle cast pathogen *Nothophaeocryptopus gaeumannii* by metabolite extracts from endophytes of Douglas-fir. *PhytoFrontiers*. doi: 10.1094/PHYTOFR-07-24-0085-R.

Granados GM, McTaggart AR, Rodas CA, Roux J, Wingfield MJ. 2020. Species of Cryphonectriaceae occupy an endophytic niche in the Melastomataceae and are putative latent pathogens of *Eucalyptus*. *European Journal of Plant Pathology* 156: 273–283.

Hardham AR, Blackman LM. 2018. *Phytophthora cinnamomi*. *Molecular Plant Pathology* 19: 260–285.

Hernandez-Escribano L, Iturritxa E, Elvira-Recuenco M, Berbegal M, Campos JA, Renobales G, García I, Raposo R. 2018. Herbaceous plants in the understory of a pitch canker-affected *Pinus radiata* plantation are endophytically infected with *Fusarium circinatum*. *Fungal Ecology* 32: 65–71.

Herpin-Saunier NY, Sambaraju KR, Yin X, Feau N, Zeglen S, Ritokova G, Omdal D, Côté C, Hamelin RC. 2022. Genetic lineage distribution modeling to predict epidemics of a conifer disease. *Frontiers in Forests and Global Change* 4: 756678.

Hietala AM, Agan A, Nagy NE, Børja I, Timmermann V, Drenkhan R, Solheim H. 2022. The native *Hymenoscyphus albidus* and the invasive *Hymenoscyphus fraxineus* are similar in their necrotrophic growth phase in ash leaves. *Frontiers in Microbiology* 13: 892051.

Hill L, Jones G, Atkinson N, Hector A, Hemery G, Brown N. 2019. The £15 billion cost of ash dieback in Britain. *Current Biology* 29: R315–R316.

Hill R, Llewellyn T, Downes E, Oddy J, MacIntosh C, Kallow S, Panis B, Dickie JB, Gaya E. 2021. Seed banks as incidental fungi banks: fungal endophyte diversity in stored seeds of banana wild relatives. *Frontiers in Microbiology* 12: 643731.

Inoue T, Okane I, Ishiga Y, Degawa Y, Hosoya T, Yamaoka Y. 2019. The life cycle of *Hymenoscyphus fraxineus* on Manchurian ash, *Fraxinus mandshurica*, in Japan. *Mycoscience* 60: 89–94.

IPPC Secretariat. 2020. *ISPM 36. Integrated measures for plants for planting.* Rome: FAO on behalf of the Secretariat of the International Plant Protection Convention.

IPPC Secretariat. 2024. *Glossary of phytosanitary terms.* International Standard for Phytosanitary Measures No. 5. Rome, Italy: FAO on behalf of the Secretariat of the International Plant Protection Convention.

Jung T, Orlikowski L, Henricot B, Abad-Campos P, Aday AG, Aguín Casal O, Bakonyi J, Cacciola SO, Cech T, Chavarriaga D *et al.* 2016. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *Forest Pathology* 46: 134–163.

Kemler M, Garnas J, Wingfield MJ, Gryzenhout M, Pillay KA, Slippers B. 2013. Ion Torrent PGM as tool for fungal community analysis: a case study of endophytes in *Eucalyptus grandis* reveals high taxonomic diversity. *PLoS ONE* 8: e81718.

Ko KK, Chng KR, Nagarajan N. 2022. Metagenomics-enabled microbial surveillance. *Nature Microbiology* 7: 486–496.

Koukol O, Kolařík M, Kolářová Z, Baldrian P. 2012. Diversity of foliar endophytes in wind-fallen *Picea abies* trees. *Fungal Diversity* 54: 69–77.

Kranjec Orlović J, Drvodelić D, Vukelić M, Rukavina M, Diminić D, Oršanić M. 2021. Impact of thermotherapy and short-term storage on *Quercus robur* L. acorn mycobiota and germination. *Forests* 12: 528.

Kuris AM, Lafferty KD, Sokolow SH. 2014. Sapronosis: a distinctive type of infectious agent. *Trends in Parasitology* 30: 386–393.

Laubray S, Buée M, Marçais B. 2024. *Hymenoscyphus fraxineus* persistence in the ash litter. *Plant Pathology* 73: 1937–1946.

Leal I, Feau N, Uzunovic A, Foord B, Hamelin RC. 2024. A molecular method to assess viability of *Phytophthora* in infected wood following phytosanitary heat treatment. *PhytoFrontiers* 4: 690–699.

Liebhold AM, Brockerhoff EG, Garrett LJ, Parke JL, Britton KO. 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and the Environment* 10: 135–143.

Lin T, Tang J, Li S, Li S, Han S, Liu Y, Yang C, Chen G, Chen L, Zhu T. 2023. Drought stress-mediated differences in phyllosphere microbiome and associated pathogen resistance between male and female poplars. *The Plant Journal* 115: 1100–1113.

Linderman RG, Davis EA. 2008. Evaluation of chemical agents for the control of *Phytophthora ramorum* and other species of *Phytophthora* on nursery crops. *Plant Health Progress* 9: 26.

Lovell-Read FA, Parnell S, Cunniffe NJ, Thompson RN. 2023. Using 'sentinel' plants to improve early detection of invasive plant pathogens. *PLoS Computational Biology* 19: e1010884.

Luchi N, Ioos R, Santini A. 2020. Fast and reliable molecular methods to detect fungal pathogens in woody plants. *Applied Microbiology and Biotechnology* 104: 2453–2468.

Lynch SC, Eskalen A, Gilbert GS. 2021. Host evolutionary relationships explain tree mortality caused by a generalist pest–pathogen complex. *Evolutionary Applications* 14: 1083–1094.

Mahnert A, Ortega RA, Berg C, Grube M, Berg G. 2018. Leaves of indoor ornamentals are biodiversity and functional hotspots for fungi. *Frontiers in Microbiology* 9: 2343.

Marsberg A, Kemler M, Jami F, Nagel JH, Postma-Smidt A, Naidoo S, Wingfield MJ, Crous PW, Spatafora JW, Hesse CN *et al.* 2017. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Molecular Plant Pathology* 18: 477–488.

Mausse-Sitoe SN, Rodas CA, Wingfield MJ, Chen S, Roux J. 2016. Endophytic Cryphonectriaceae on native Myrtales: possible origin of *Chrysoporthe* canker on plantation-grown *Eucalyptus*. *Fungal Biology* 120: 827–835.

McTaggart AR, van der Nest MA, Steenkamp ET, Roux J, Slippers B, Shuey LS, Wingfield MJ, Drenth A. 2016. Fungal genomics challenges the dogma of name-based biosecurity. *PLoS Pathogens* **12**: e1005475.

Mehl J, Wingfield MJ, Roux J, Slippers B. 2017. Invasive everywhere? Phylogeographic analysis of the globally distributed tree pathogen *Lasiodiplodia theobromae*. *Forests* **8**: 145.

Migliorini D, Ghelardini L, Tondini E, Luchi N, Santini A. 2015. The potential of symptomless potted plants for carrying invasive soilborne plant pathogens. *Diversity and Distributions* **21**: 1218–1229.

Mishcherikova V, Lynikienė J, Marčiulynas A, Gedminas A, Prylutskyi O, Marčiulynienė D, Menkis A. 2023. Biogeography of fungal communities associated with *Pinus sylvestris* L. and *Picea abies* (L.) H. Karst. along the latitudinal gradient in Europe. *Journal of Fungi* **9**: 829.

Mohandoss J, Suryanarayanan T. 2009. Effect of fungicide treatment on foliar fungal endophyte diversity in mango. *Sydowia* **61**: 11–24.

Müller MM, Hamberg L. 2021. Eradication of endophytes from needles, their inoculation with *Lophodermium piceae* and persistence of this fungus in needles of Norway spruce. *Mycological Progress* **20**: 981–992.

Nelson A, Vandegrift R, Carroll GC, Roy BA. 2020. Double lives: transfer of fungal endophytes from leaves to woody substrates. *PeerJ* **8**: e9341.

Newcombe G, Harding A, Ridout M, Busby PE. 2018. A hypothetical bottleneck in the plant microbiome. *Frontiers in Microbiology* **9**: 1645.

Nicoletti R, Beccaro GL, Sekara A, Cirillo C, Di Vaio C. 2021. Endophytic fungi and ecological fitness of chestnuts. *Plants* **10**: 542.

Niskanen T, Lücking R, Dahlberg A, Gaya E, Suz LM, Mikryukov V, Liimatainen K, Druzhinina I, Westrip JR, Mueller GM *et al.* 2023. Pushing the frontiers of biodiversity research: unveiling the global diversity, distribution, and conservation of fungi. *Annual Review of Environment and Resources* **48**: 149–176.

Noseworthy MK, Allen EA, Dale AL, Leal I, John EP, Souque TJ, Tanney JB, Uzunovic A. 2024. Evidence to support phytosanitary policies – the minimum effective heat treatment parameters for pathogens associated with forest products. *Frontiers in Forests and Global Change* **7**: 1380040.

Ormsby MD. 2022. Elucidating the efficacy of phytosanitary measures for invasive alien species moving in wood packaging material. *Journal of Plant Diseases and Protection* **129**: 339–348.

Paini DR, Bianchi FJJA, Northfield TD, De Barro PJ. 2011. Predicting invasive fungal pathogens using invasive pest assemblages: testing model predictions in a virtual world. *PLoS ONE* **6**: e25695.

Philibert A, Desprez-Loustau ML, Fabre B, Frey P, Halkett F, Husson C, Lung-Escarmant B, Marçais B, Robin C, Vacher C *et al.* 2011. Predicting invasion success of forest pathogenic fungi from species traits. *Journal of Applied Ecology* **48**: 1381–1390.

Pisetta M, Albertin I, Petriccione M, Scortichini M. 2016. Effects of hot water treatment to control *Xanthomonas arboricola* pv. *corylina* on hazelnut (*Corylus avellana* L.) propagative material. *Scientia Horticulturae* **211**: 187–193.

Puertolas A, Bonants PJ, Boa E, Woodward S. 2021. Application of real-time PCR for the detection and quantification of oomycetes in ornamental nursery stock. *Journal of Fungi* **7**: 87.

Quiring D, Flaherty L, Adams G, McCartney A, Miller JD, Edwards S. 2019. An endophytic fungus interacts with crown level and larval density to reduce the survival of eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), on white spruce (*Picea glauca*). *Canadian Journal of Forest Research* **49**: 221–227.

Ramsfield TD, Ball RD, Gardner JF, Dick MA. 2010. Temperature and time combinations required to cause mortality of a range of fungi colonizing wood. *Canadian Journal of Plant Pathology* **32**: 368–375.

Richardson SN, Nsiama TK, Walker AK, McMullin DR, Miller JD. 2015. Antimicrobial dihydrobenzofurans and xanthenes from a foliar endophyte of *Pinus strobus*. *Phytochemistry* **117**: 436–443.

Rooney-Latham S, Blomquist CL, Kosta KL, Gou YY, Woods PW. 2019. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. *Plant Disease* **103**: 448–455.

Santini A, Ghelardini L, De Pace C, Desprez-Loustau ML, Capretti P, Chandelier A, Cech T, Chira D, Diamandis S, Gaitniekis T *et al.* 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. *New Phytologist* **197**: 238–250.

Schulz B, Boyle C. 2006. What are endophytes? In: Schulz B, Boyle C, Sieber T, eds. *Microbial root endophytes*. Berlin, Germany: Springer, 1–13.

Seebens H, Essl F, Hulme PE, van Kleunen M. 2022. Development of pathways of global plant invasions in space and time. In: Pyšek P, Pergl J, Essl F, eds. *Global plant invasions*. Cham, Switzerland: Springer, 53–69.

Serrano Y, Iturritxa E, Elvira-Recuenco M, Raposo R. 2017. Survival of *Fusarium circinatum* in soil and *Pinus radiata* needle and branch segments. *Plant Pathology* **66**: 934–940.

Sherwood P, Nordström I, Woodward S, Bohman B, Cleary M. 2024. Detecting pathogenic *Phytophthora* species using volatile organic compounds. *Molecules* **29**: 1749.

Siripatrawan U, Makino Y. 2024. Hyperspectral imaging coupled with machine learning for classification of anthracnose infection on mango fruit. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **309**: 123825.

Slippers B, Wingfield MJ. 2007. Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90–106.

Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ. 2011. Climate change and forest diseases. *Plant Pathology* **60**: 133–149.

Swett CL, Gordon TR. 2012. First report of grass species (Poaceae) as naturally occurring hosts of the pine pathogen *Gibberella circinata*. *Plant Disease* **96**: 908.

Swett CL, Kirkpatrick SC, Gordon TR. 2016. Evidence for a hemibiotrophic association of the pitch canker pathogen *Fusarium circinatum* with *Pinus radiata*. *Plant Disease* **100**: 79–84.

Swiecki TJ, Bernhardt EA. 2022. *Can thermotherapy be used to eliminate Phytophthora from infected root systems of container plants?* Final report for task 3 of: An experimental management project to protect against the sudden oak death and other plant pathogens in SFPUC watersheds. Forest service agreement no. 18-JV-11272139-029 Mod. 1. Prepared for USDA Forest Service, Pacific Southwest Research Station, Berkeley, CA, 42.

Tanney JB, Feau N, Shamoun SF, Kope HH, Dicaire A, Drugmand B, Walker J, Burlakoti P, Joshi V. 2024. *Cryptostroma corticale* (Ellis & Everh.) PH Greg. & S. Waller causing sooty bark disease in British Columbia, Canada. *Canadian Journal of Plant Pathology* **46**: 596–610.

Thomas DC, Vandegrift R, Ludden A, Carroll GC, Roy BA. 2016. Spatial ecology of the fungal genus *Xylaria* in a tropical cloud forest. *Biotropica* **48**: 381–393.

Toda M, Beer KD, Kuivila KM, Chiller TM, Jackson BR. 2021. Trends in agricultural triazole fungicide use in the United States, 1992–2016 and possible implications for antifungal-resistant fungi in human disease. *Environmental Health Perspectives* **129**: 055001.

Turbelin AJ, Malamud BD, Francis RA. 2017. Mapping the global state of invasive alien species: patterns of invasion and policy responses. *Global Ecology and Biogeography* **26**: 78–92.

USDA-APHIS. 2024. *Not authorized pending pest risk analysis (NAPPRA)*. [WWW document] URL https://www.aphis.usda.gov/plant-imports/nappra/ [accessed 1 April 2025].

Vaz ABM, Fonseca PLC, Silva FF, Quintanilha-Peixoto G, Sampedro I, Siles JA, Carmo A, Kato RB, Azevedo V, Badotti F *et al.* 2020. Foliar mycoendophytome of an endemic plant of the Mediterranean biome (*Myrtus communis*) reveals the dominance of basidiomycete woody saprotrophs. *PeerJ* **8**: e10487.

Wang MR, Cui ZH, Li JW, Hao XY, Zhao L, Wang QC. 2018. *In vitro* thermotherapy-based methods for plant virus eradication. *Plant Methods* **14**: 1–18.

Wang QT, Guo MJ, Lv T, Zhou H, Wang S, Wang SJ, Lin YR, Gronefeld S, Kirschner R, Piepenbring M *et al.* 2023. Phylogeny and taxonomy of *Rhytisma*-like species worldwide. *Fungal Diversity* **120**: 77–119.

Wilson D. 1995. Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos* **73**: 274–276.

Win PM, Matsumura E, Fukuda K. 2021. Effects of pesticides on the diversity of endophytic fungi in tea plants. *Microbial Ecology* **82**: 62–72.

Wingfield MJ, Brockerhoff EG, Wingfield BD, Slippers B. 2015. Planted forest health: the need for a global strategy. *Science* **349**: 832–836.

Wingfield MJ, Slippers B, Barnes I, Duong TA, Wingfield BD. 2025. The pine pathogen *Diplodia sapinea*: expanding frontiers. *Current Forestry Reports* **11**: 1–14.

Würth DG, Dahl MB, Trouillier M, Wilmking M, Unterseher M, Scholler M, Sørensen S, Mortensen M, Schnittler M. 2019. The needle mycobiome of *Picea glauca*–a dynamic system reflecting surrounding environment and tree phenological traits. *Fungal Ecology* **41**: 177–186.

Supporting Information

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

Table S1 Examples of invasive pathogens with latent/endophytic life histories that were introduced outside of their native range via P4P pathway, with records of first detections.