



Sentinel plantations, study cases and methodology

Carmen Morales Rodríguez
cmorales@unitus.it

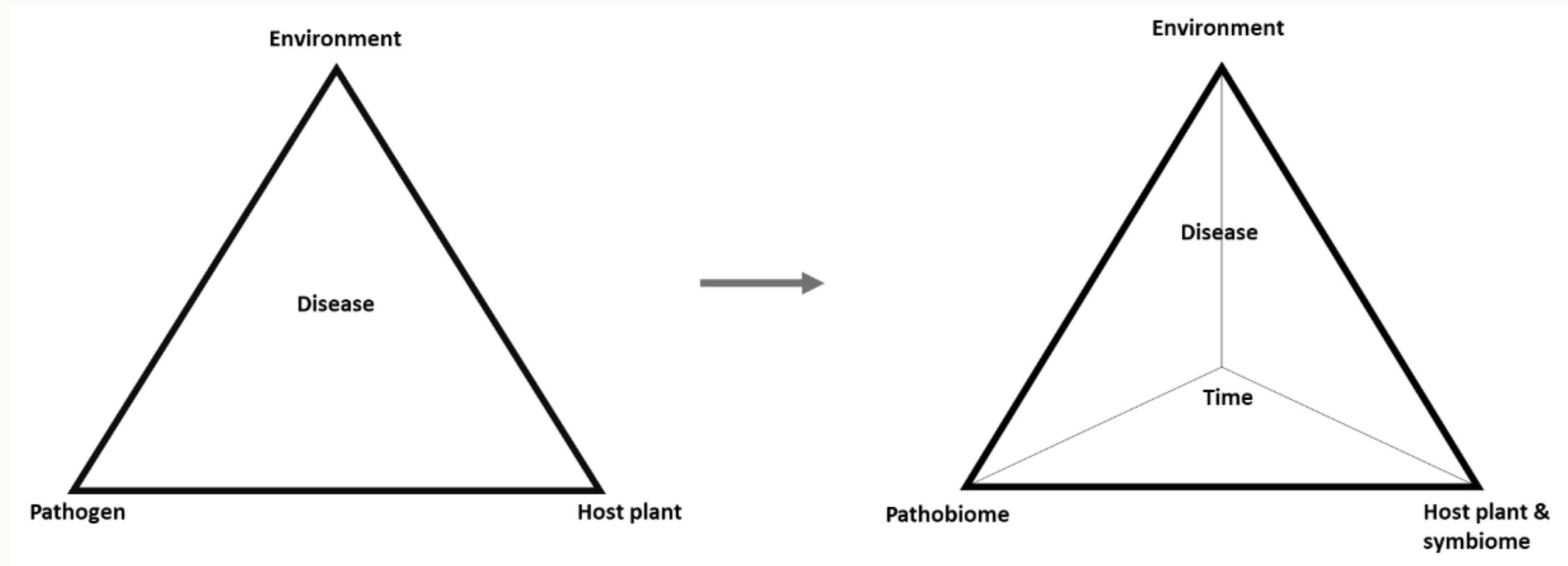


UNIVERSITÀ
DEGLI STUDI DELLA
TUSCIA



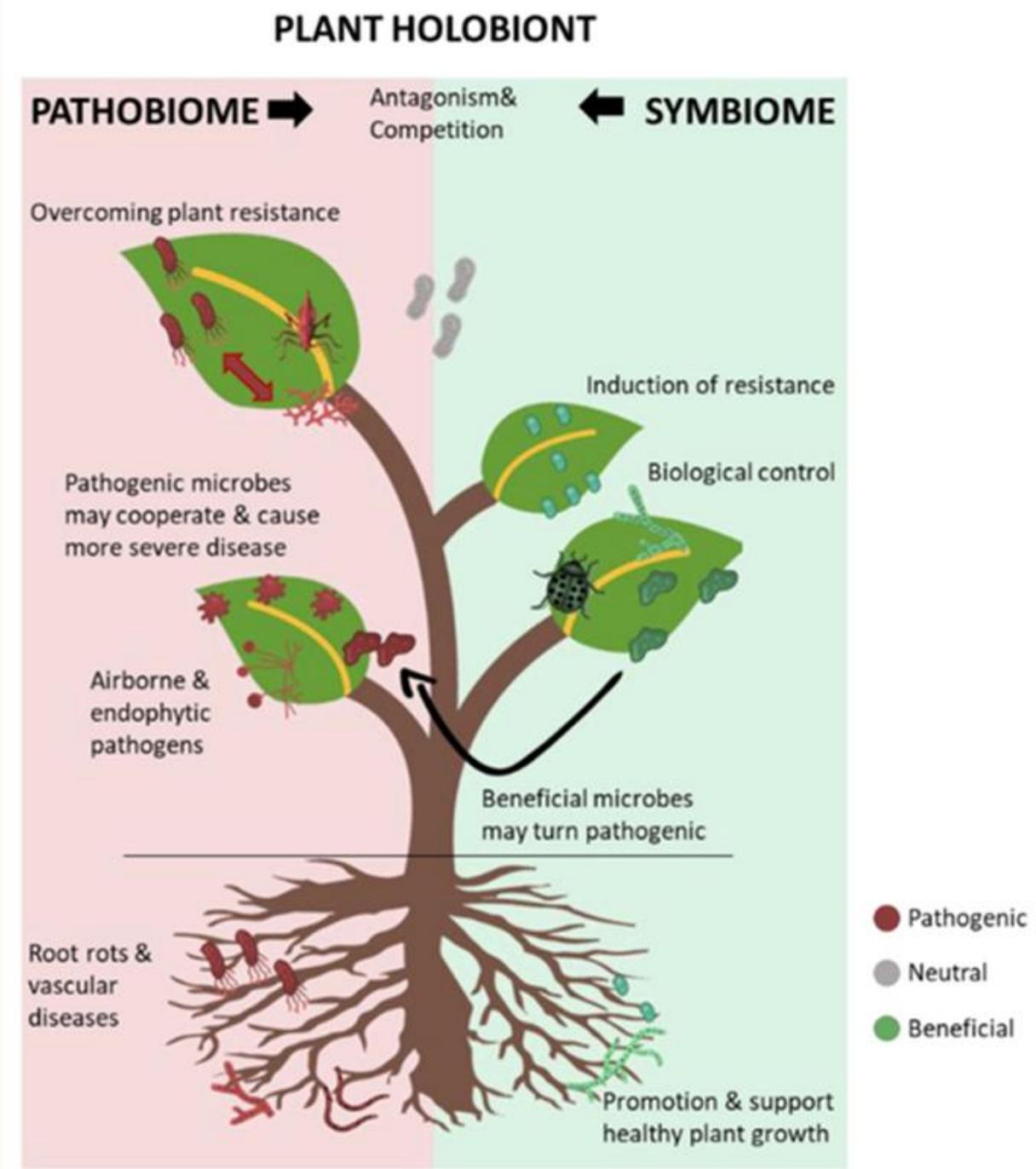
The Disease Triangle in Phytopathology

Modern phytopathology recognizes that plant disease emerges from complex interactions between multiple factors. The traditional model has evolved to include the holobiont concept.



The plant **symbiome** is an emerging concept that describes the set of symbiotic interactions between a plant and its associated microbial community, including bacteria, fungi, actinobacteria, viruses and other microorganisms that live:

on surfaces (rhizosphere, phyllosphere),
inside tissues (endophytes),
in symbiotic roots (e.g. mycorrhizae, rhizobia).



Coevolution in Host-Pathogen Relationships

Plants and pathogens engage in evolutionary arms races spanning millions of years. Native ecosystems develop balanced relationships through this coevolutionary process.

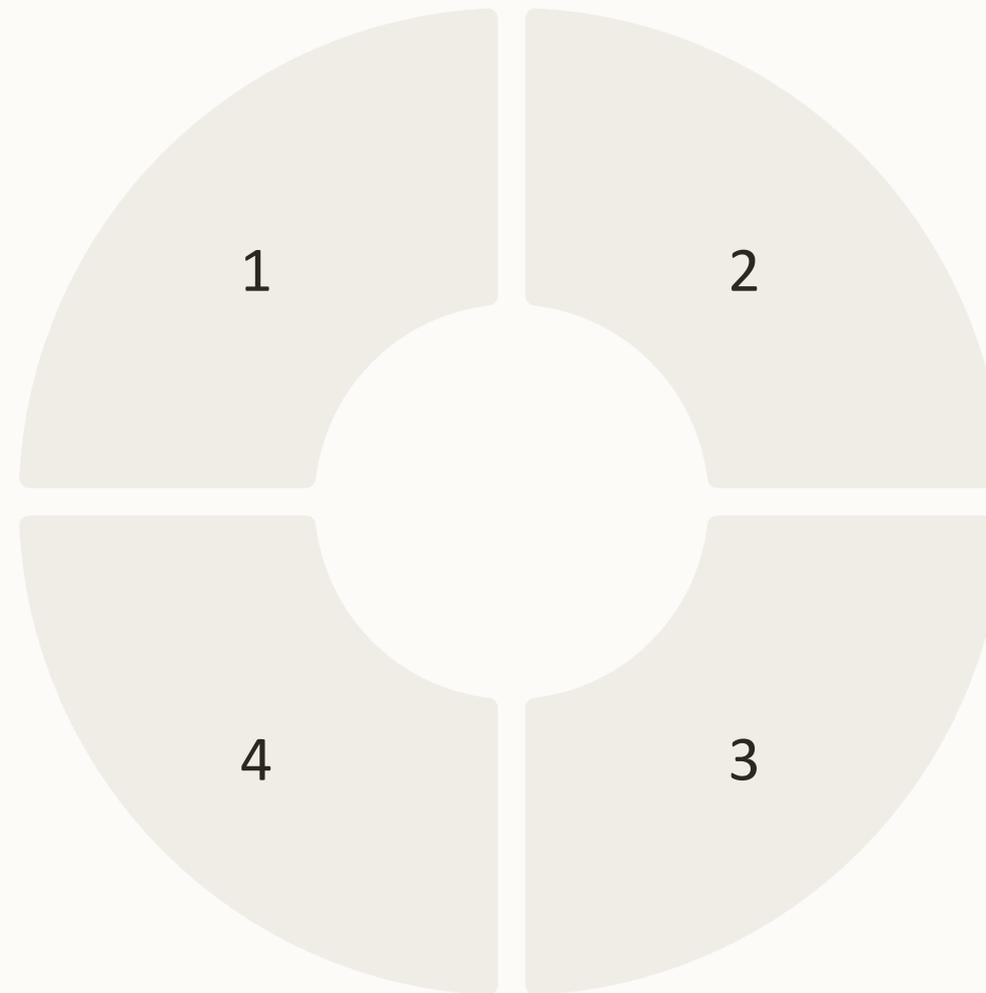
Invasive pathogens disrupt this balance, facing hosts with no evolutionary history of resistance.

Host Defense Evolution

Plants develop biochemical and physical barriers against recurring pathogen attacks.

Geographic Isolation

Separate evolutionary paths create vulnerability when species from different regions meet.



Pathogen Adaptation

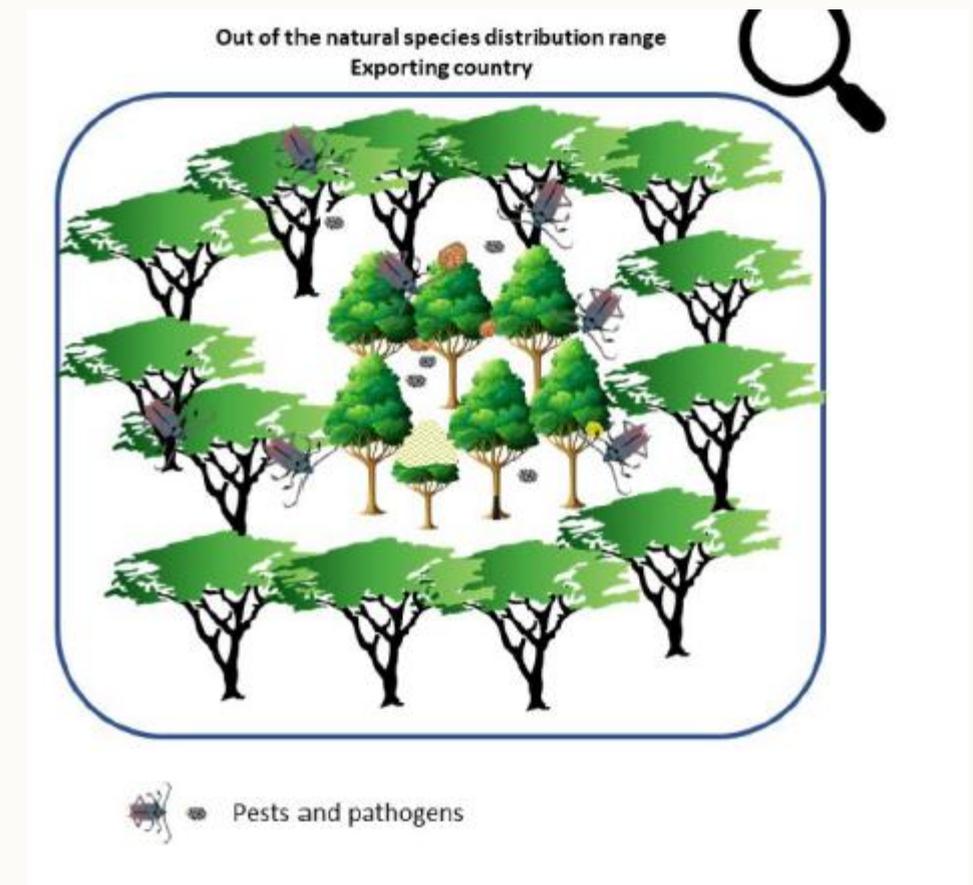
Pathogens evolve mechanisms to overcome host defenses and maintain virulence.

Ecological Balance

Native ecosystems reach relative equilibrium where disease remains manageable.

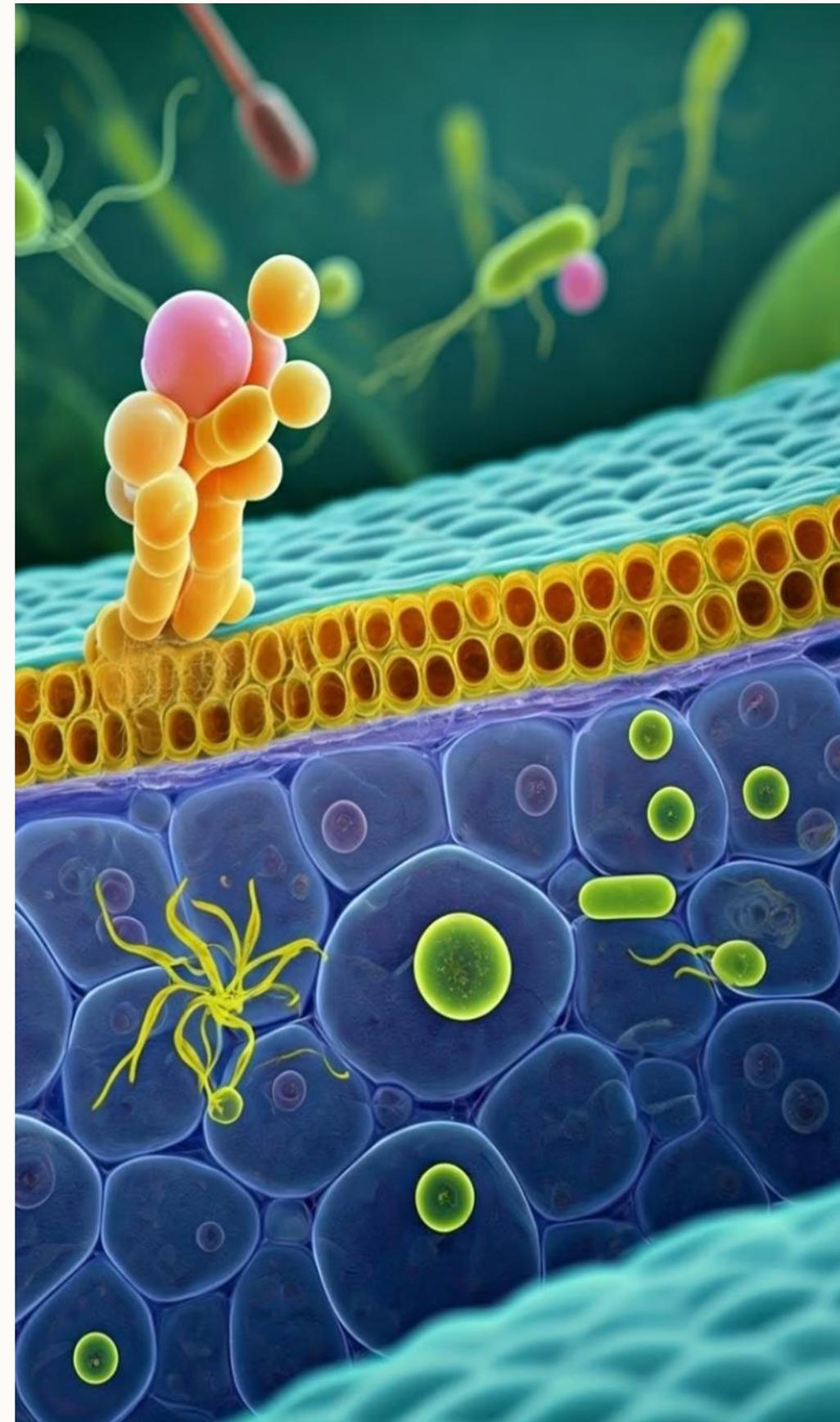
Sentinel plantation

- In sentinel plantations, non-native plants are grown in a country out of their natural distribution range (e.g. native European trees planted in China) and monitored for potentially damaging agents which may provide useful data for PRA.
- If novel pest/pathogen-host plant combinations occur, the plants are likely to develop **symptoms due to a lack of coevolution with the native organism** (Parker and Gilbert 2004; Vettraino et al. 2015).
- The assessment of **symptoms and signs**, along with sampling of symptomatic tissues, and the isolation of potential pest/pathogen organisms, should be prioritized.
- Therefore, methods and protocols used in sentinel plantations should aim to characterize damage morphotypes, followed by **isolation** or collection and **species level identification** of the causal agent(s) (Roques et al. 2017).



Sentinel plantation

- It is necessary to carry out HTS analysis of a representative sample of the propagation material (e.g. seeds) intended to be used before export to the country where the sentinel planting will be located.
- Knowledge of the plant's endophytic community in its native range can give a baseline for interpretation of, for example, fungi contributing to disease.
- In sentinel plantation trials in China, absence of controls in the propagation material did not allow confirmation of the Asiatic origin of detected OTUs (Vettraino et al. 2015).



SENTINEL PLANTATION

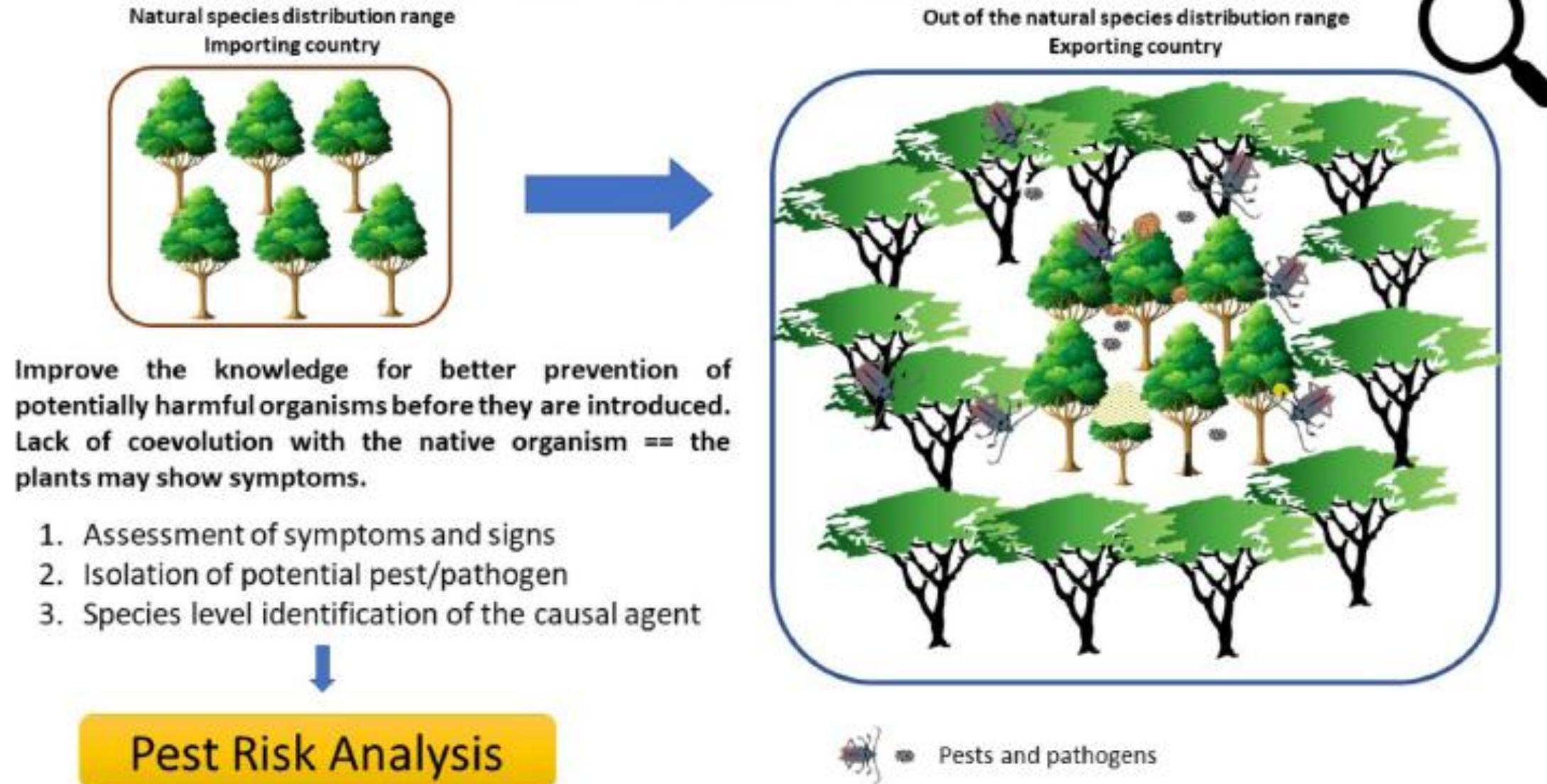
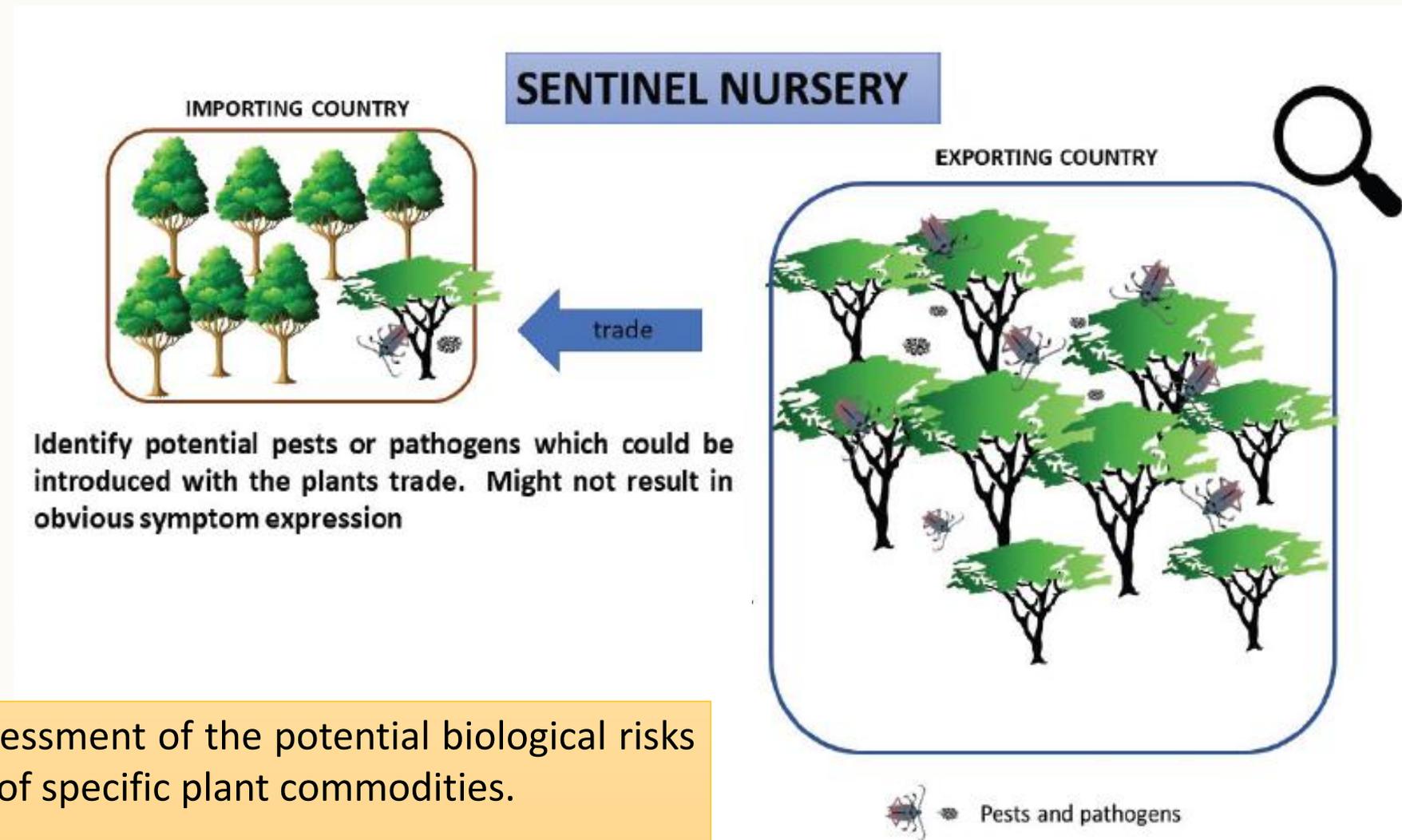


Figure 1. Schematic representation of the sentinel plantation concept. Tree species native to the importing country are planted in the exporting country. Being exposed to the resident pest and pathogens, they should develop visible symptoms.

Sentinel nursery

In a sentinel nursery, native plants are grown in their natural distribution range to identify potential pests or pathogens which could be spread with the international trade of these plants. In this case, the results obtained will be helpful in **CRA** (Kenis et al. 2018).

- **Commodity Risk Analysis (CRA)** is a structured assessment of the potential biological risks associated with the international or regional trade of specific plant commodities.
- In the context of invasive alien plant species, CRA focuses on evaluating the likelihood that a traded plant or plant product may act as a pathway for the unintentional introduction and spread of invasive plants or their propagules (e.g., seeds, fragments).



Sentinel nursery

Assuming that **host-parasite co-evolution** of native species might **not result in obvious symptom** expression, a host shift to a taxonomically similar plant species in the final location of the plant may give rise to novel host-parasite interactions.

- Therefore, diagnostic methods that can detect **endophytic or latent pathogens** must be employed in addition to standardized diagnostics for symptomatic tissue.
- Thus, sampling must be oriented to both symptomatic and nonsymptomatic material.
- In this system, the use of HTS is useful for screening of the microbial communities even in the absence of symptoms.
- One possible way to filter large datasets arising from HTS is to group the OTUs/AVs according to their functional guild, focusing the sampling and identification on what are grouped as pathogens or opportunistic pathogens.

Sentinel nursery

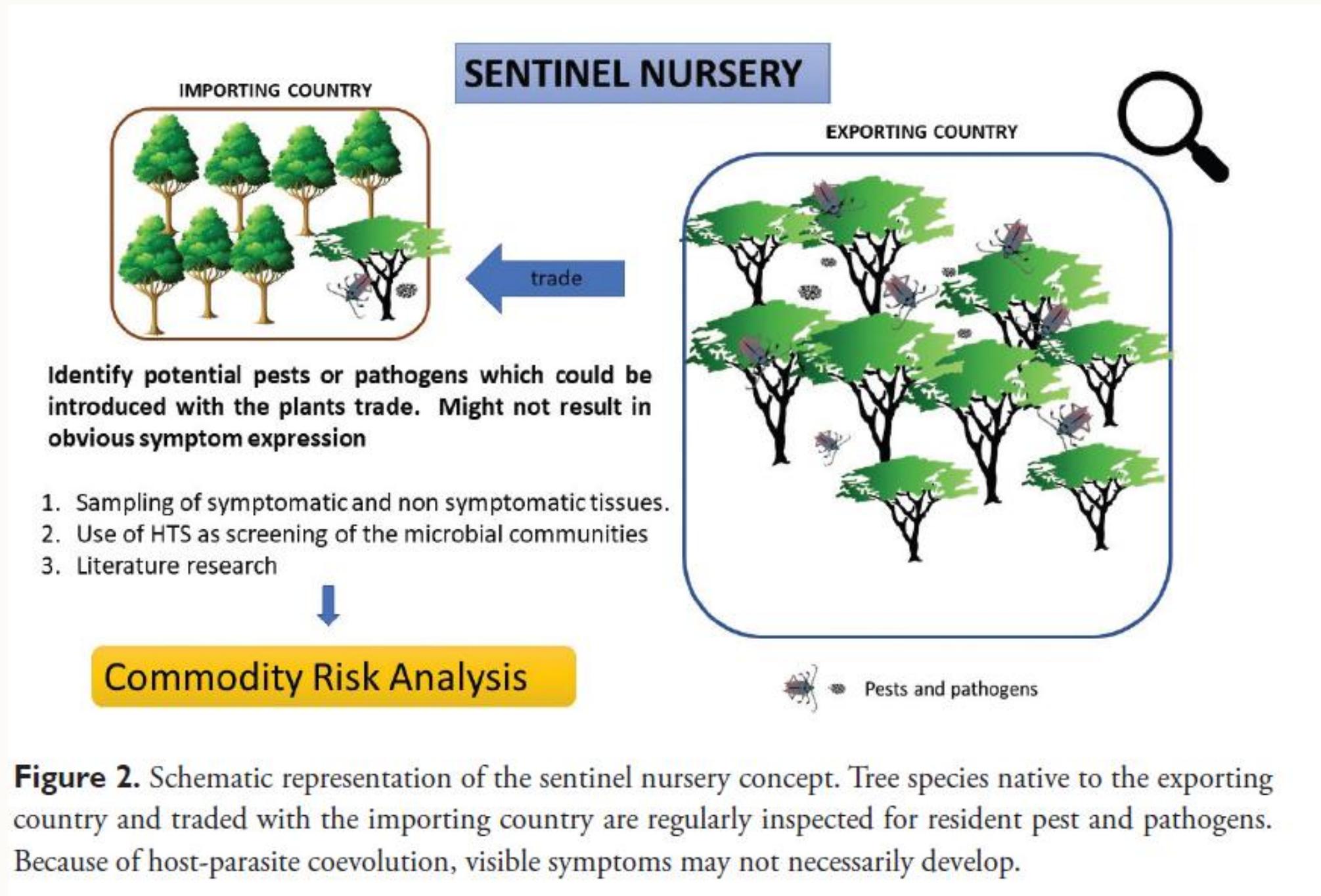


Figure 2. Schematic representation of the sentinel nursery concept. Tree species native to the exporting country and traded with the importing country are regularly inspected for resident pest and pathogens. Because of host-parasite coevolution, visible symptoms may not necessarily develop.

Sentinel Plantations vs Sentinel Nurseries

Sentinel Plantations

Established collections of mature trees in their destination environment.

- Long-term monitoring capabilities
- Natural exposure to local pathogens
- Represents established trade pathways

Sentinel Nurseries

Young trees from export regions grown in controlled settings.

- Early detection of potential threats
- Higher diversity of species possible
- More controlled observation environment

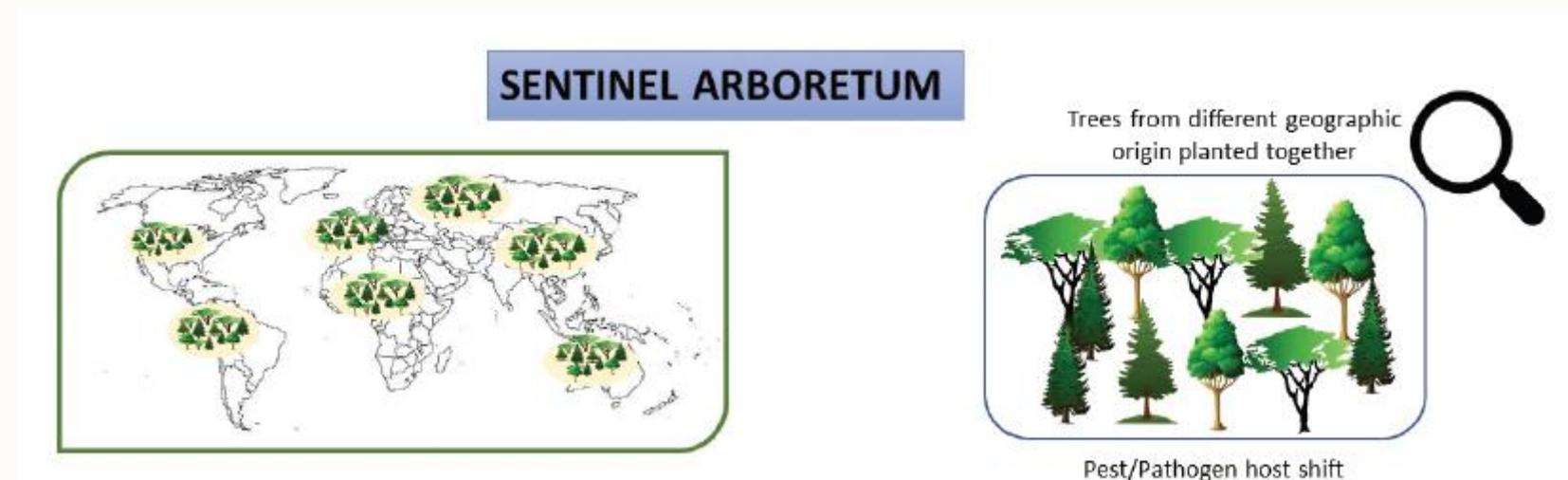
Complementary Approaches

Both systems provide crucial but different biosecurity insights.

- Different life stages monitored
- Varying pathogen susceptibility profiles
- Together form comprehensive detection network

Sentinel arboretum

- A sentinel arboretum comprises a broad range of **both native and non-native tree species** from diverse regions around the world, which can allow testing of various ecological hypotheses on biological invasions, as possible host-shifts, one of the main barriers to establishment of alien plant pests and pathogens, can be examined.
- Non-native species are exposed to inoculum of native, potentially pathogenic organisms harboured by native trees species growing in the same or nearby environment. An expanded assumption here is that all native and non-native tree species planted in the same area are cross-exposed to inoculum harboured by each of the tree species in a latent native-to-native interaction.



Sentinel arboretum

- Protocols used in sentinel arboreta should aim to characterize damage morphotypes, followed by isolation or collection, and species level identification of the organisms causing these symptoms.
- The non-native trees might harbour endophytic microflora since the time of their introduction into arboreta as propagation material (e.g. seeds, seedlings, cuttings).
- HTS can be useful in detecting non-symptomatic native host endophytic species or latent infections, contributing to characterization of the donor host microbiome and to the description of a novel host-shift event.
- Differing from sentinel plantations, sentinel arboreta may also allow surveys of the recruitment of insects by mature trees, and especially of particular groups, such as xylophagous pests (Roques et al. 2015).

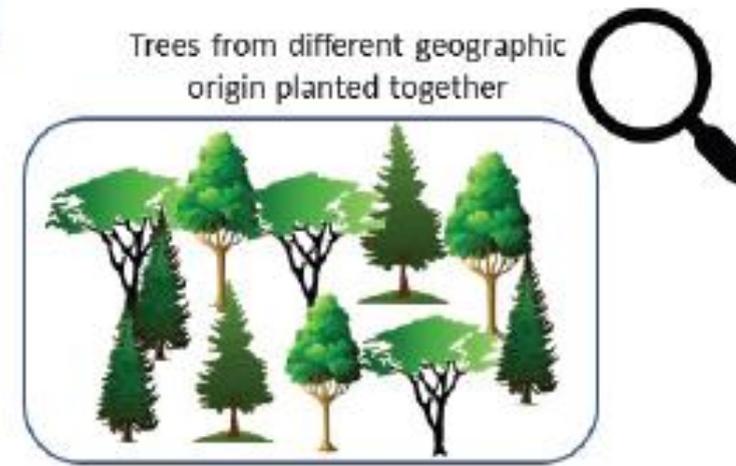


SENTINEL ARBORETUM



Opportunity to monitor host shift events and to evaluate the behaviour of a tree species vs alien organisms.

1. Assessment of symptoms and signs
2. Isolation of potential pest/pathogen
3. Species level identification of the causal agent
4. Sampling of symptomatic and non symptomatic tissues
5. Use of HTS as screening of the microbial communities
6. Literature research



Pest/Pathogen host shift

Assessment of novel
host-pathogen interactions before
introduction in the host native area

**Pest Risk Analysis
Commodity Risk Analysis**

Figure 3. Schematic representation of the sentinel arboretum (botanical garden) concept. The exotic and native tree species cultivated in the same area/environment are cross-exposed to inoculum harbored by each of the species. The identification of causal agents of different symptomatologies provides a list of new pests or pathogens potentially harmful to those plants in their native environments.



The Sentinel Concept

Sentinel systems act as living laboratories for monitoring potential threats before they spread.





The Challenge of Detection

Co-Evolution

Pathogens often don't produce striking symptoms on their native hosts due to co-evolution.

Endophytic Microbes

Woody plants harbor hundreds of endophytic microorganisms that don't cause symptoms.

Host Jumps

Pathogens can jump to new host species with which they haven't co-evolved, causing severe disease.



Sentinel Plantings: Sampling Methods for Early Warning

Sentinel plantings serve as early warning systems against alien tree pests. The COST Action FP1401 Global Warning network provides field guides for identifying damage on woody plants.



Field Guide for the Identification of Damage on Woody Sentinel Plants

Edited by Alain Roques, Michelle Cleary,
Iryna Matsiakh and René Eschen



Field Guide for the Identification of Damage on Woody Sentinel Plants

Edited by

Alain Roques

Institut National de la Recherche Agronomique,
Unité de Zoologie Forestière, Orleans, France

Michelle Cleary

Swedish University of Agricultural Sciences, Alnarp, Sweden

Iryna Matsiakh

Ukrainian National Forestry University, Lviv, Ukraine

René Eschen

CABI, Delémont, Switzerland





General Sampling Considerations

Experimental Design

How sentinel plantings are organized, including replication of tree species.

Sampling Design

How, when and what should be sampled for efficient and reliable detection.

Diagnostic Challenges

Similar-looking symptoms might have different causes, requiring careful procedures.



Key Sampling Principles

1 Whole Plant Examination

Check the entire plant for different damage morphotypes. Take samples from representative symptomatic organs.

2 Photographic Documentation

Take high-resolution photos of the whole plant, damaged organs, and visible agents before collecting samples.



Key Sampling Principles

3 Avoid Cross-contamination

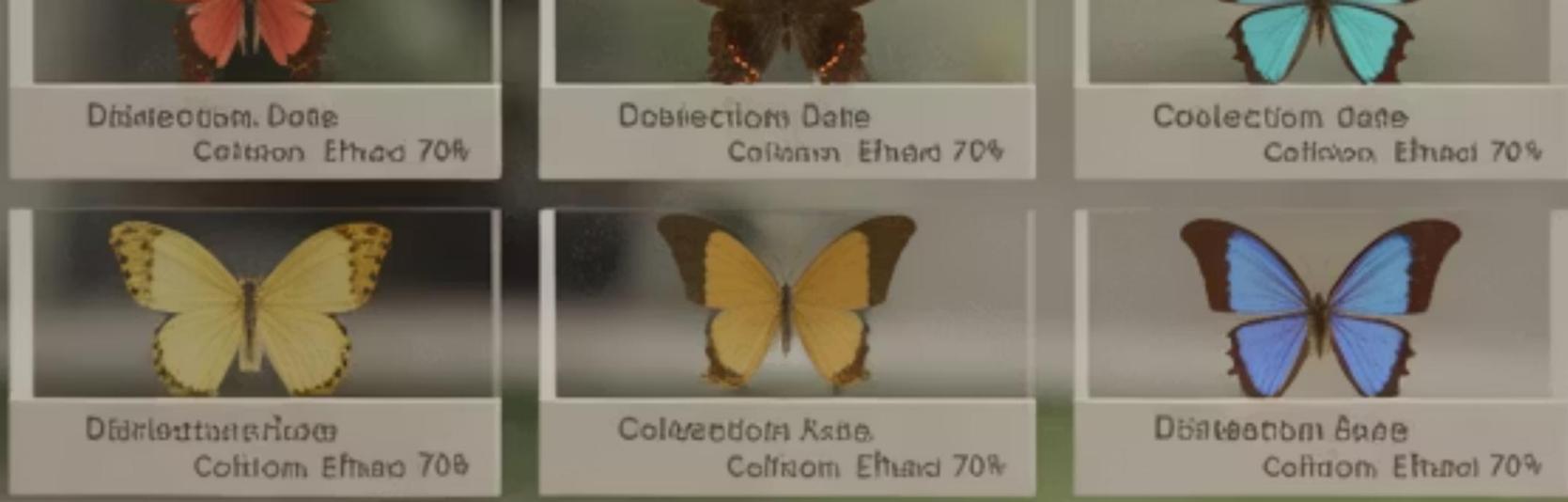
Ensure sampling instruments are clean, especially when sampling for pathogens.

4 Timing

Conduct at least three samplings per year (spring, summer and fall).

5 Healthy Tissue

Sample apparently healthy tissue to establish baselines and detect latent infections.



Final Sampling Considerations

Proper Labelling

Essential for scientific value. Include:

- Locality and GPS coordinates
- Host plant information
- Collection date
- Collector name
- Unique identifying number

Sample Disinfection

Consider whether to disinfect samples before processing.

Superficial contamination may represent additional pathways for alien microorganisms.



Study cases

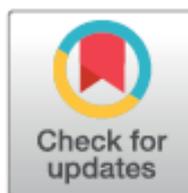
RESEARCH ARTICLE

The sentinel tree nursery as an early warning system for pathway risk assessment: Fungal pathogens associated with Chinese woody plants commonly shipped to Europe

Anna Maria Vettrai¹, Hong-Mei Li², Rene Eschen³, Carmen Morales-Rodriguez¹, Andrea Vannini¹*

1 DIBAF-University of Tuscia, Viterbo, Italy, **2** CABI, Chinese Academy of Agricultural Sciences, Beijing, China, **3** CABI, Delémont, Switzerland

* vannini@unitus.it



 OPEN ACCESS

Citation: Vettrai AM, Li H-M, Eschen R, Morales-Rodriguez C, Vannini A (2017) The sentinel tree nursery as an early warning system for pathway risk assessment: Fungal pathogens associated with Chinese woody plants commonly shipped to

Abstract

Introduction of and invasion by alien plant pathogens represents the main cause of emerging infectious diseases affecting domesticated and wild plant species worldwide. The trade in living plants is the most common pathway of introduction. Many of the alien tree pathogens recently introduced into Europe were not previously included on any quarantine lists. To help determine the potential risk of pest introduction through trading of ornamental plants, a sentinel nursery was established in Beijing, China in 2008. The sentinel nursery

Beijing Sentinel Nursery Study



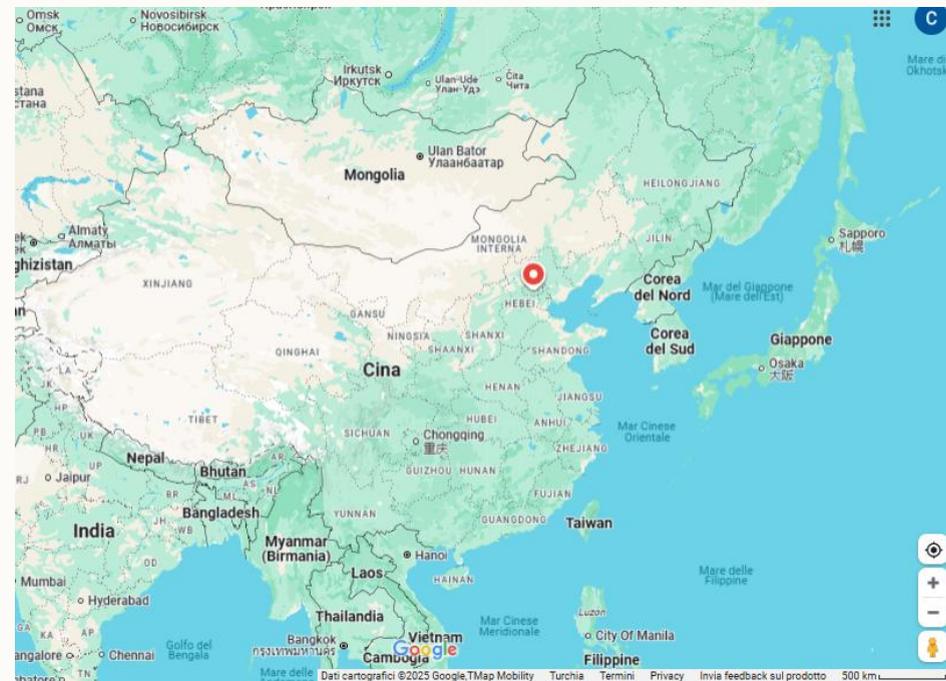
Four Species

Ilex cornuta,
Buxus microphylla,
Fraxinus chinensis, and
Zelkova schneideriana



Regular Inspection

Plants monitored for symptoms and sampled for pathogen analysis



Established 2012

Located in Shunyi district,
Beijing (40°14'27"N 116°49'47"E)

- The nursery was established in April 2012 with plants bought in the same exporting nursery.
- Since their purchase, plants were not protected against potential pests.
- Five blocks of 20 plants were planted for each species, i.e. 100 plants per species.
- No plant was taller than 80 cm, to correspond to classical exported plants.
- The distance between plants was 50 cm and between blocks 1.5 to 2 m.
- The nursery was regularly inspected in 2012 and 2013 for presence of symptoms and signs of diseases.





Research Methodology



Sample Collection

Symptomatic and healthy tissues collected from all four species



Fungal Isolation

Cultures grown from symptomatic tissues on potato dextrose agar



DNA Extraction

Genomic DNA extracted from fungal colonies and healthy plant tissues



Analysis

Molecular identification through sequencing and phylogenetic analysis. 454 Life Sciences GS-FLX System (Macrogen)

Symptoms Observed in Sentinel Nursery

Buxus microphylla

Necrotic spots on leaves



Fraxinus chinensis

Canker



*Zelkova
schneideriana*

bark necrosis

Ilexcornuta var. *fortunei*
leaf necrosis

- Six fungal species were isolated from symptomatic tissues.
- *Alternaria alternata* was found on all hosts, while other species showed more specific host associations.

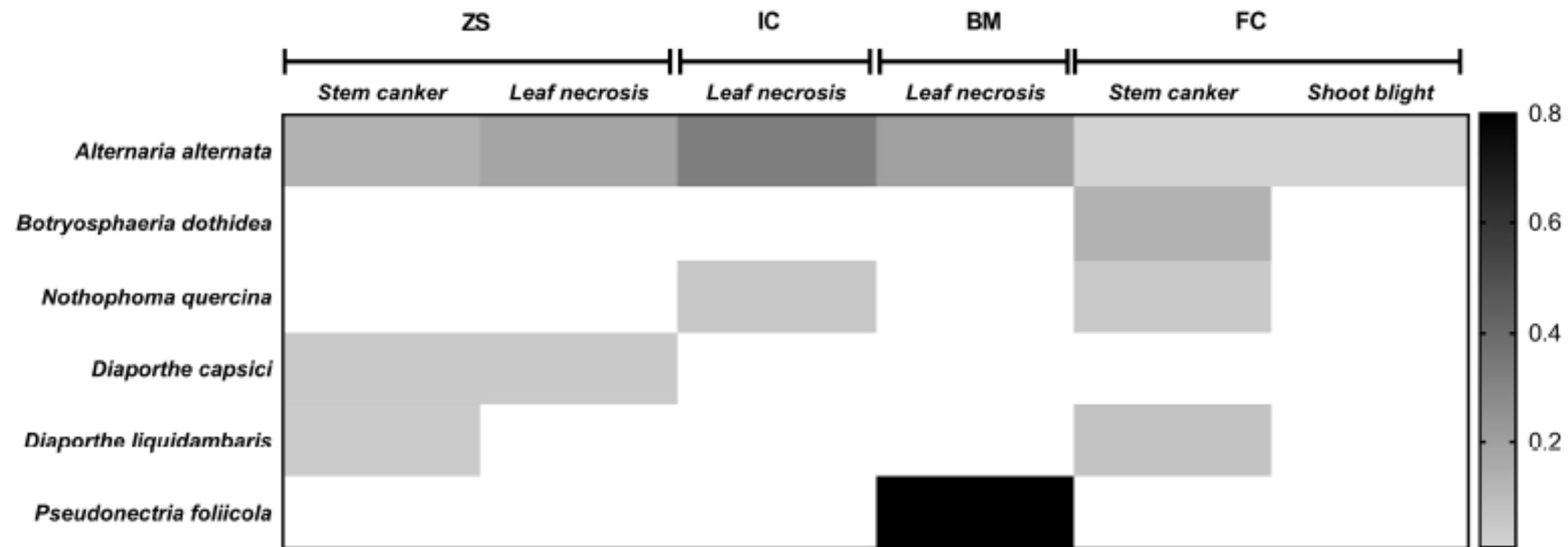
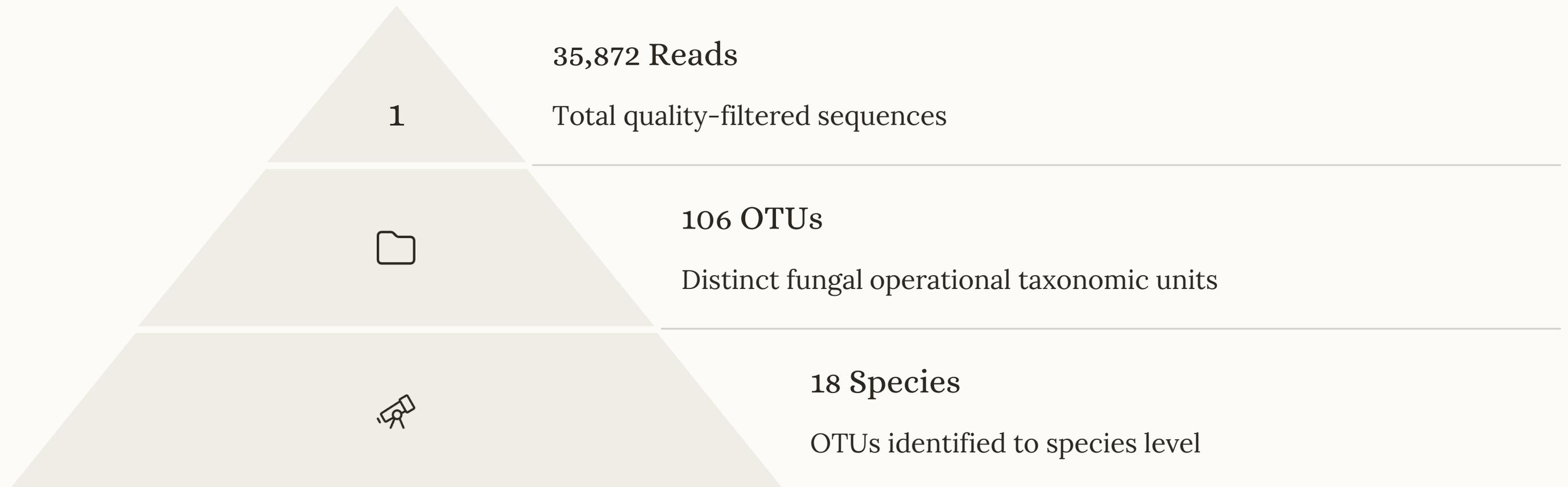
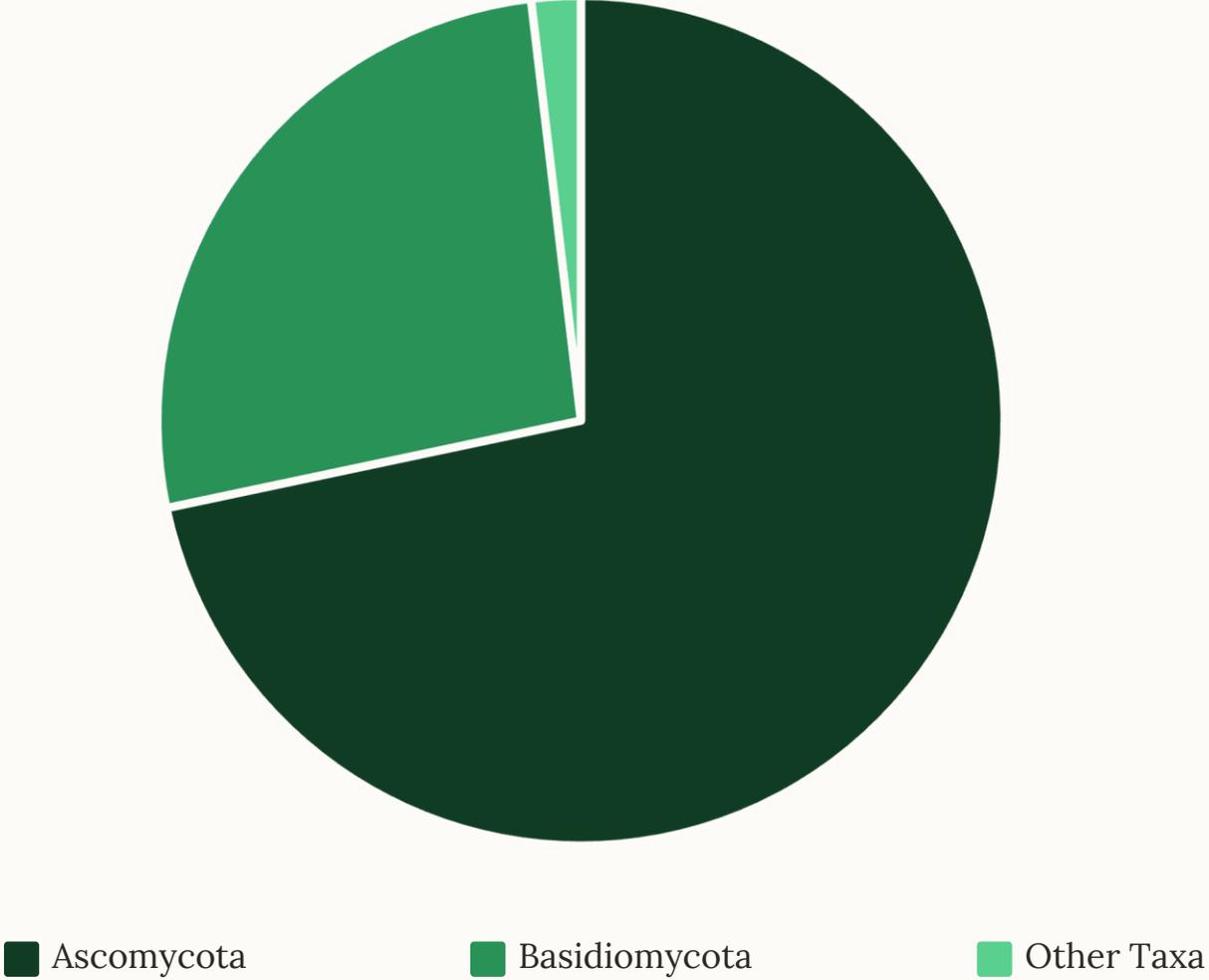


Fig 2. Heatmap of the abundance of fungal species identified by culturing analysis from symptomatic host tissues. Darker colour indicate a higher abundance of the isolates. *Zelkova schneideriana* (ZS); *Ilex cornuta* var. *fortunei* (IC); *Buxus microphylla* (BM); *Fraxinus chinensis* (FC).

NGS Results Overview



Taxonomic Distribution of Fungal OTUs



The majority of fungal OTUs belonged to Ascomycota (76 OTUs, 33,184 reads), followed by Basidiomycota (28 OTUs, 2,676 reads). Other taxa represented less than 0.03% of sequences.



OTUs by Host Plant

25

*Buxus
microphylla*

Moderate fungal
diversity

48

Ilex cornuta
Second highest
diversity

56

*Fraxinus
chinensis*

Highest diversity of
fungal OTUs

24

*Zelkova
schneideriana*

Lowest fungal
diversity

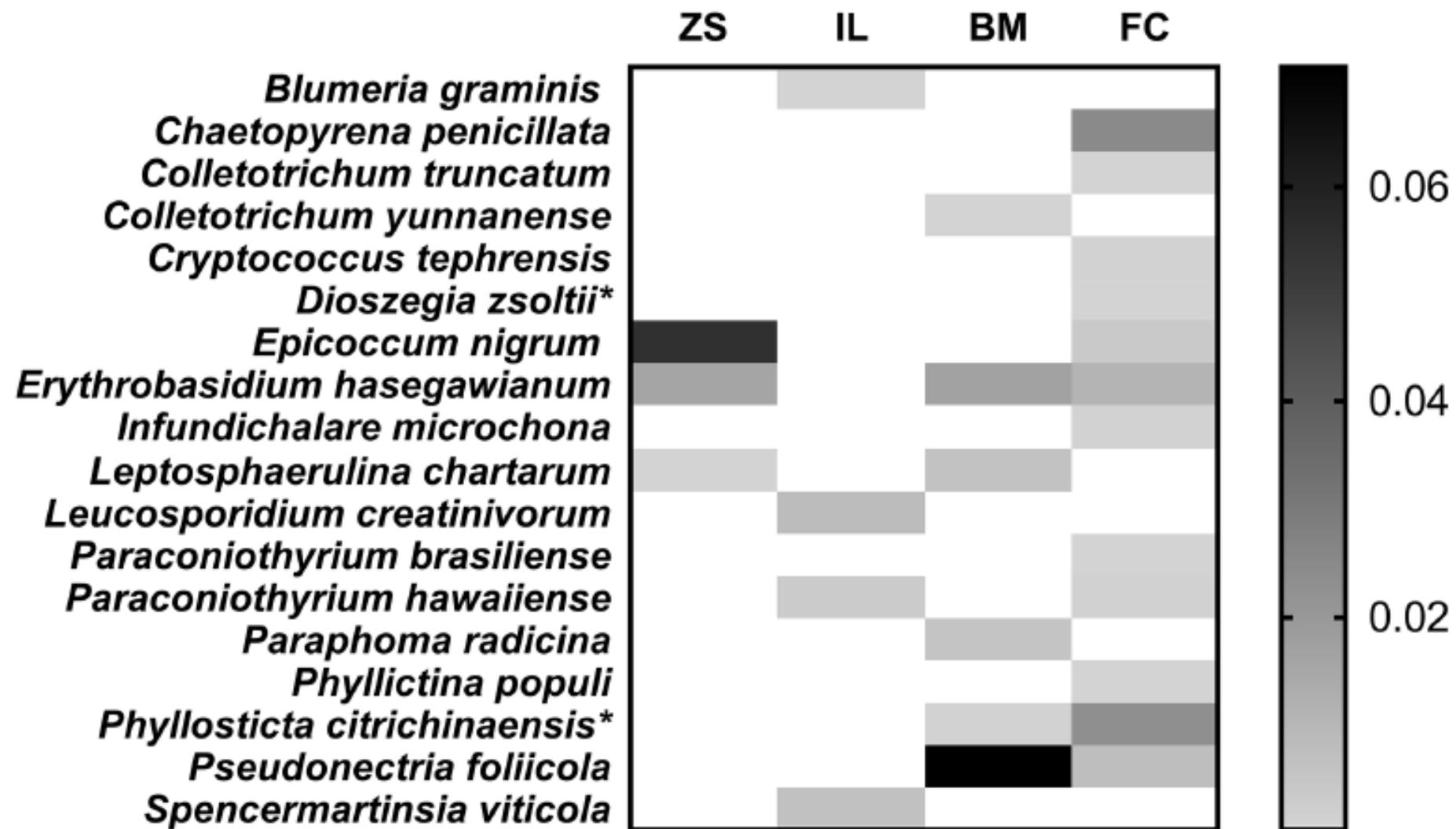
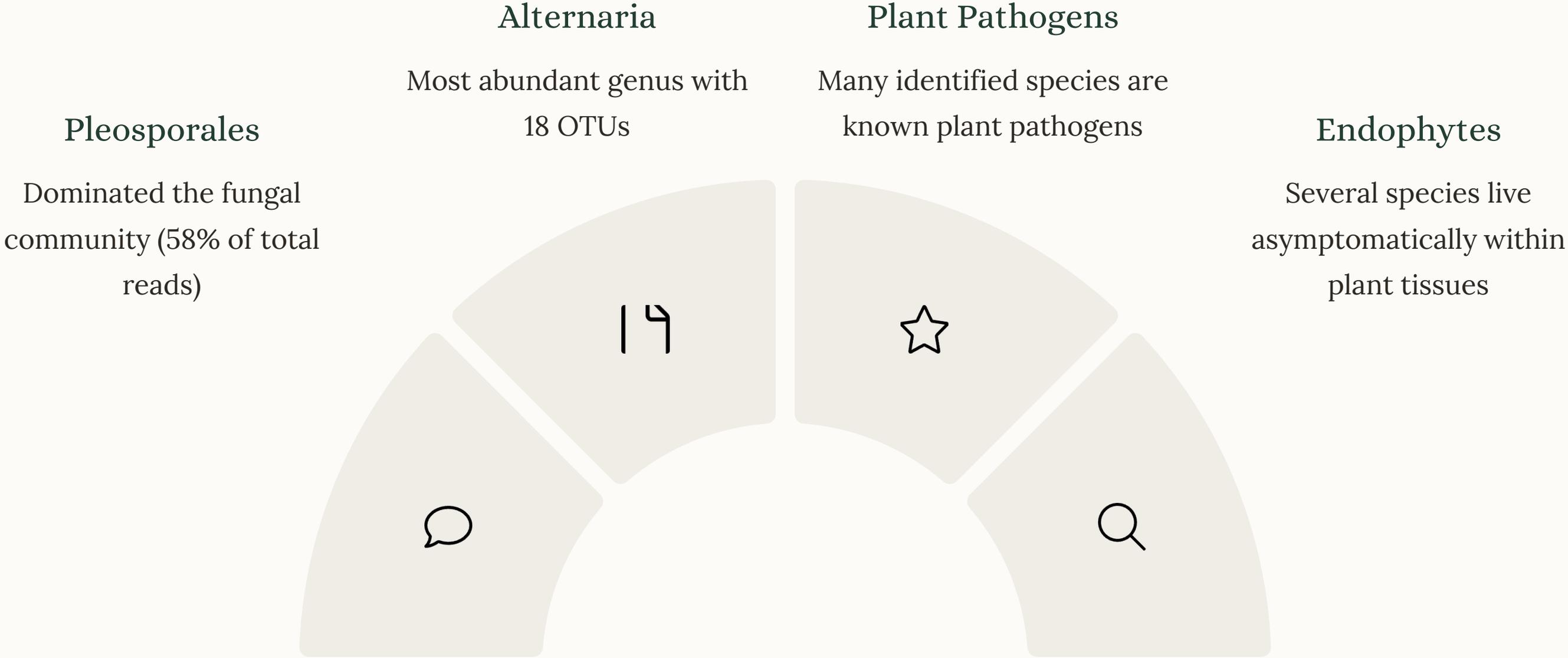


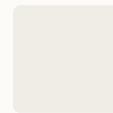
Fig 5. Heatmap of the abundance of the fungal taxa identified as species by NGS analysis from sentinel nursery plants. Higher intensities of the colour reveal higher abundances of the isolates. *Zelkova schneideriana* (ZS); *Ilex cornuta* var. *fortunei* (IC); *Buxus microphylla* (BM); *Fraxinus chinensis* (FC). * Ambiguous species identification, possible new species.

Dominant Fungal Groups



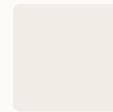


Notable Fungal Species Identified



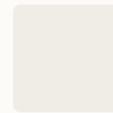
Pseudonectria foliicola

Buxus pathogen found in both isolation and NGS analysis. New to China and not yet recorded in Europe (2013). **Already in Europe**



Nothophoma quercina

Found on *Fraxinus* and *Ilex*. Known to cause cankers and leaf spots on various woody species worldwide.



Diaporthe liquidambaris

Asian endophytic species found on *Zelkova*. Its endophytic behavior could facilitate accidental introduction.

Potential Threats to European Plants



Colletotrichum yunnanense

Asian endophyte of Buxus that could potentially affect European buxus



Phyllosticta citrichinaensis

Citrus pathogen found on non-citrus hosts



Phyllactinia populi

Powdery mildew that could threaten European poplar species

Fraxinus chinensis: A Key Pathway

Highest Diversity

One-third of all fungal OTUs (38) were associated with *Fraxinus chinensis*.

Species Richness

12 of 18 species identified by NGS and 5 of 6 species from culturing were found on this host.

Ash Dieback Connection

Hymenoscyphus fraxineus, which causes ash dieback in Europe, is hemi-biotrophic on *F. chinensis* in Asia.





Challenges in Sentinel Nursery Implementation



Sample Processing

All plant samples should be processed at origin to avoid unintentional introductions.



Multiple Markers

NGS protocols should use multiple genetic markers for accurate species identification.



Susceptibility Testing

Native plants from importing countries should be screened for susceptibility to identified pathogens.



Database Enrichment

Fungal sequence databases need expansion, especially for underrepresented loci.

Benefits of the Sentinel Nursery Approach

Early Detection

Identifies potential pathogens before they enter international trade.

- Reveals asymptomatic infections
- Detects novel host-pathogen interactions
- Provides time for risk assessment

Risk Mitigation

Enables development of targeted prevention strategies.

- Informs quarantine regulations
- Guides inspection protocols
- Supports pest risk analysis



Limitations of the Study

Taxonomic Resolution

rDNA markers cannot always separate closely related species.

Database Gaps

Many fungal species remain undescribed or absent from molecular databases.

Pathogenicity Unknown

Detection doesn't confirm pathogenicity to European native plants.

Limited Host Range

Only four plant species were tested from the many traded internationally.



RESEARCH ARTICLE

Sentinel Trees as a Tool to Forecast Invasions of Alien Plant Pathogens

AnnaMaria Vettrai¹, Alain Roques², Annie Yart², Jian-ting Fan³, Jiang-hua Sun⁴,
Andrea Vannini^{1*}

1 DIBAF, University of Tuscia, Viterbo, Italy, **2** INRA-UR633, Zoologie Forestière, Centre de recherche d'Orléans, Orléans, France, **3** School of Forestry and Bio-technology, Zhejiang A & F University, Lin'an, China, **4** State key laboratory of Integrated Management of pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

* vannini@unitus.it



Abstract

Recent disease outbreaks caused by alien invasive pathogens into European forests posed a serious threat to forest sustainability with relevant environmental and economic effects. Many of the alien tree pathogens recently introduced into Europe were not previously included on any quarantine lists, thus they were not subject to phytosanitary inspections. The identification and description of alien fungi potentially pathogenic to native European flora before their introduction in Europe, is a paramount need in order to limit the risk of invasion and the impact to forest ecosystems. To determine the potential inva-

 OPEN ACCESS

Citation: Vettrai A, Roques A, Yart A, Fan J-t, Sun J-h, Vannini A (2015) Sentinel Trees as a Tool to Forecast Invasions of Alien Plant Pathogens. PLoS

The Sentinel Tree Concept



Plant European Trees in Foreign Locations

European tree species are planted in regions with similar climate conditions but different pathogen communities.



Monitor for Disease Development

Trees are regularly surveyed for symptoms and signs of diseases that develop when exposed to local pathogens.



Identify Potential Invaders

Molecular and morphological techniques identify pathogens that successfully colonize the sentinel trees.



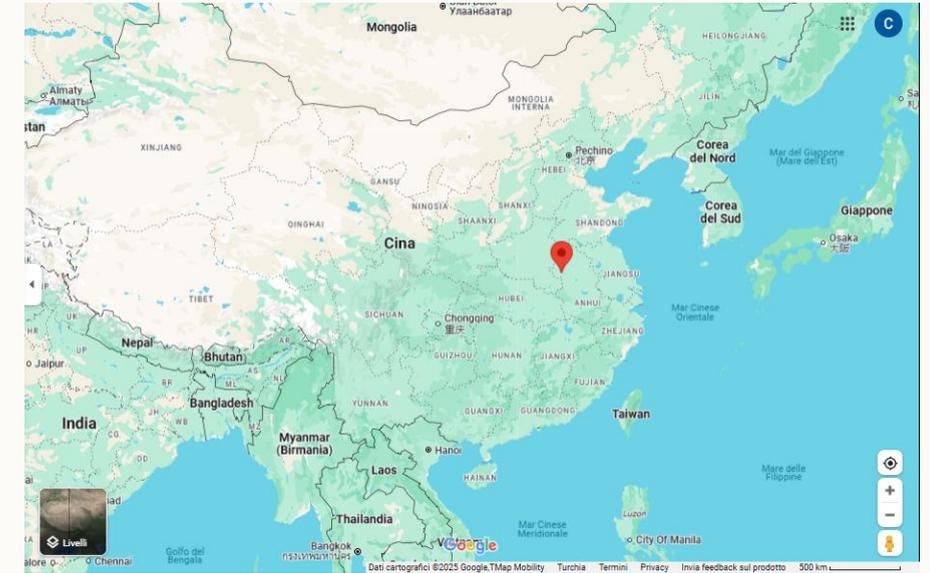
Assess Invasion Risk

Pathogens that colonize sentinel trees represent potential invaders that could threaten European forests if introduced.

This approach improves upon the International Sentinel Plant Network (ISPN) concept by strategically placing trees in ecologically similar environments to their native range, increasing the likelihood of detecting relevant pathogens.

- The sentinel plot was established near Fuyang (30.003333 N; 119.799722 E; 110m elevation), approximately 40 km southwest of Hangzhou in Zhejiang province, southeastern China.

- The site was surrounded by a mixed conifer-broadleaved forest composed primarily of *Pinus massoniana*, *Cunninghamia lanceolata*, various *Fagaceae* species, and *bamboos*. Nearby agricultural land included rice fields.

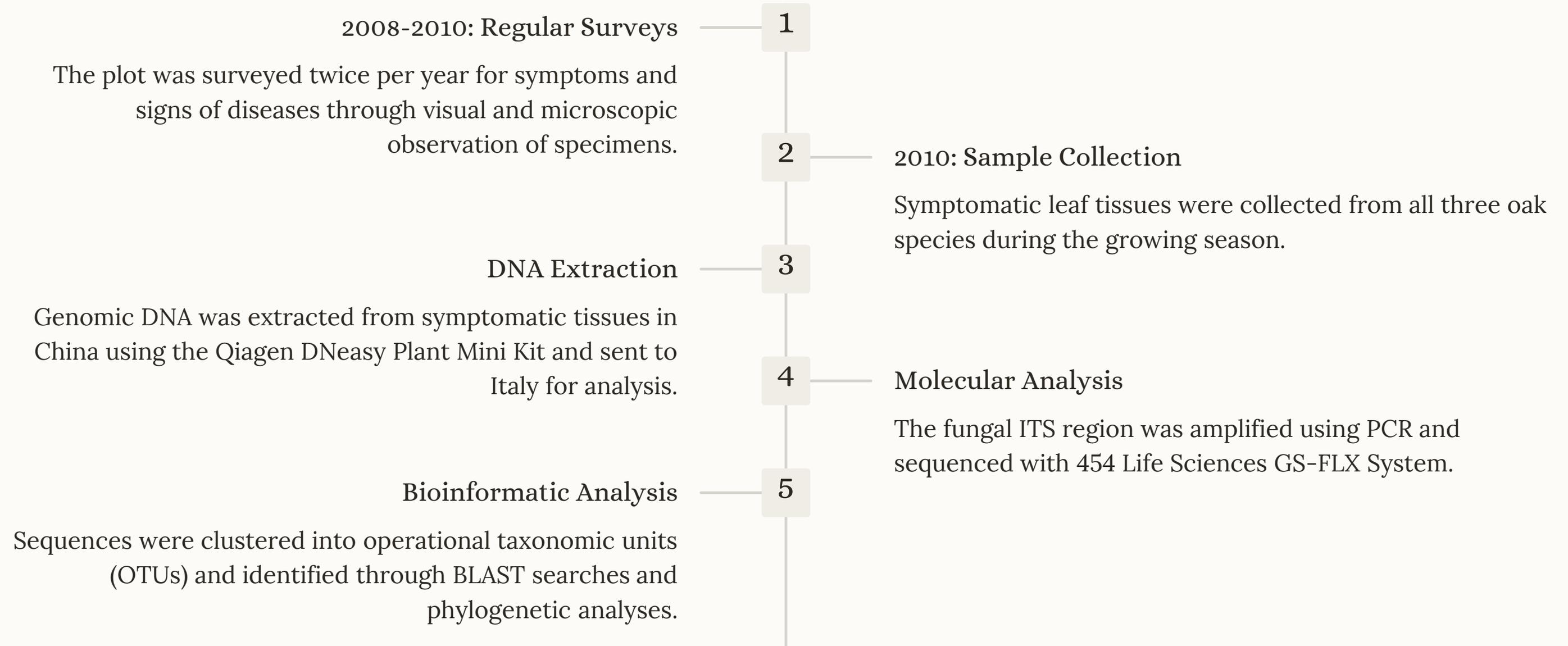


- The study focused on **European oak species planted in China** to detect potential fungal pathogens that could threaten European forests if introduced through global trade pathways.

❖ Three European oak species were selected: *Quercus petraea*, *Q. suber*, and *Q. ilex*. Twenty-five one-year-old bare-rooted seedlings per species were planted in randomized blocks in May 2008.

- Before export to China, all seedlings underwent thorough phytosanitary inspections and treatments. They were kept in quarantine for 3 weeks at the port of entry before planting.

Materials and Methods: Monitoring and Analysis



At the end of the experiment in autumn 2010, all seedlings were removed, including roots, and destroyed by burning to prevent any potential pathogen escape.

Disease Symptoms Observed on Sentinel Oaks

No symptoms or signs were observed on sentinel oaks in 2008-2009 surveys. However, during the 2010 growing season, several disease symptoms appeared:

1 Powdery Mildew

White, talcum powder-like coating on *Q. petraea* leaves, sometimes causing leaf distortion (Fig. 1A).

2 Leaf Spots

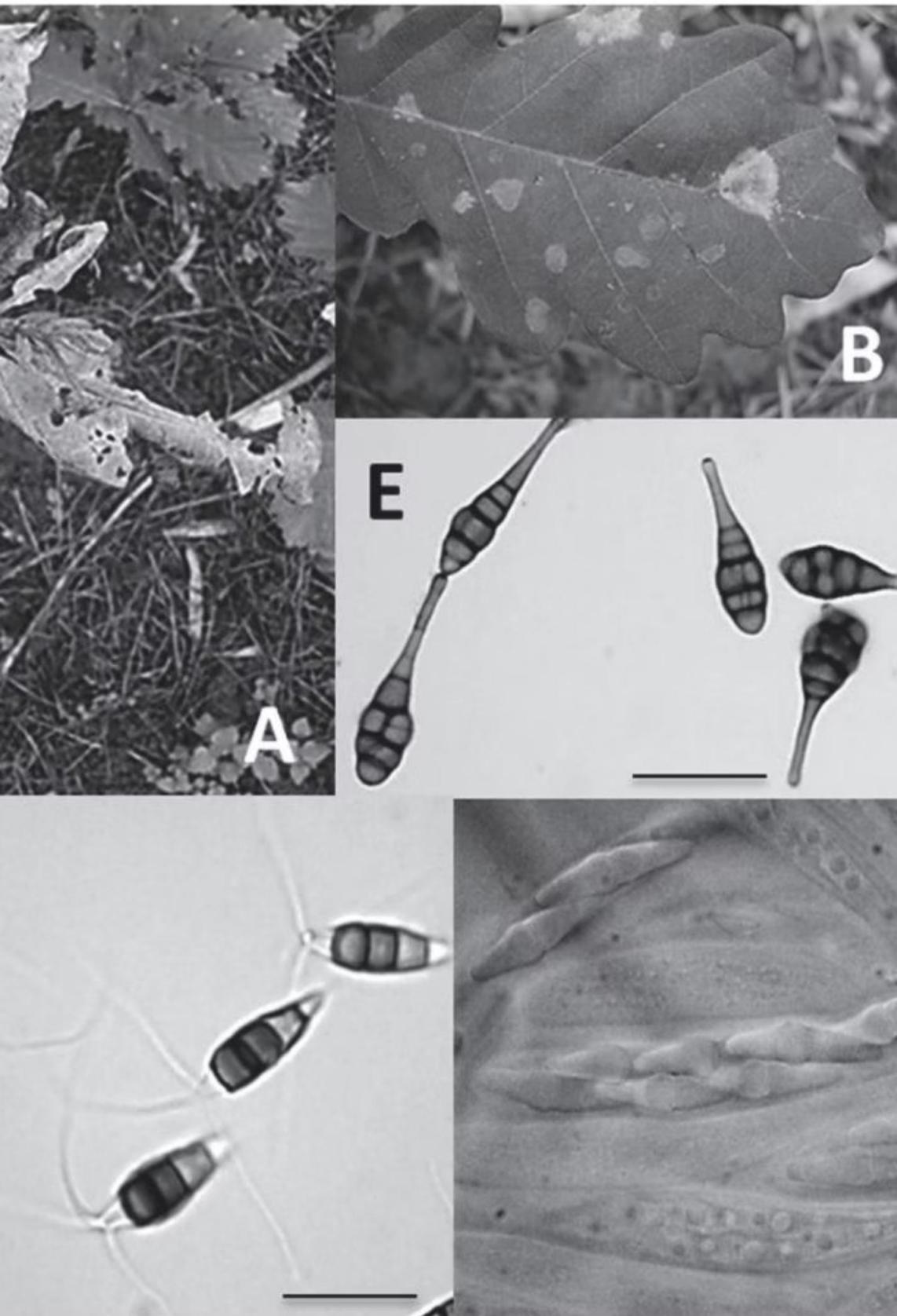
Necrotic spots detected on all three *Quercus* species (Fig. 1B).

3 Branch Cankers

Signs of *Leptosphaeria* cankers on *Q. petraea* branches with perithecia containing bitunicate asci with 3-septate ascospores (Fig. 1C, 1D).

4 Fungal Fruiting Bodies

Alternaria-like conidia observed on all *Quercus* specimens (Fig. 1E) and *Pestalotiopsis*-like conidia found in leaf spots of *Q. petraea* (Fig. 1F).



Molecular Analysis Results: Overview

- After quality filtering and removal of singletons, a total of 14,069 reads were retained for analysis: 746 from *Q. ilex*, 2,959 from *Q. suber*, and 10,364 from *Q. petraea*.
- A total of 106 operational taxonomic units (OTUs) were identified across the three oak species: 30 from *Q. ilex*, 59 from *Q. suber*, and 93 from *Q. petraea*.
- Four OTUs accounted for the largest fraction of reads (59%). OTUs 1-3 were found on all three host species and identified as *Alternaria* sp., unidentified Basidiomycota, and *Epicoccum nigrum*. OTU4 was specific to *Q. petraea* and identified as *Erysiphe quercicola*.

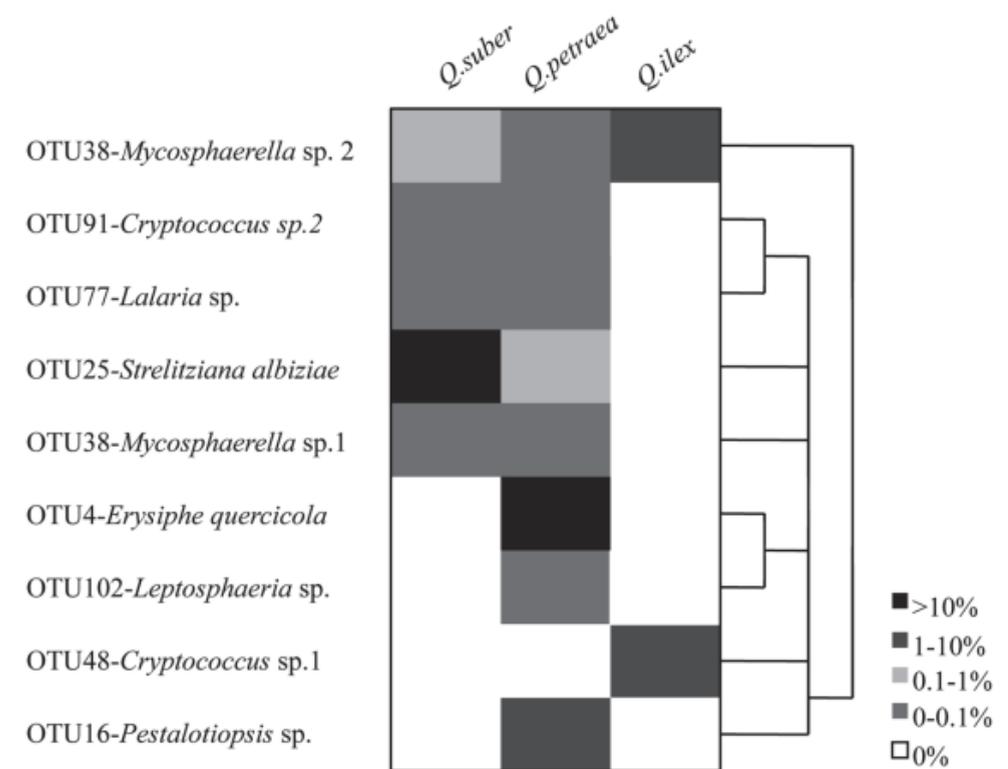


Fig 7. Hierarchically clustered heat map of the taxa distribution with Asian origin. The relationship among samples was determined by Bray-Curtis distance. The heat map plot depicts the relative percentage of each fungal taxa (variables clustering on the vertical-axis) within each tree host (horizontal-axis clustering). The relative abundance for fungal taxa are indicated by color intensity with the legend indicated at the bottom of the figure.

Potentially Invasive Asian Fungi Detected

A total of 9 OTUs were identified as having possible Asian origin, based on clustering in phylogenetic trees with sequences of taxa mostly reported from Asia:



Erysiphe quercicola (OTU4)

Powdery mildew pathogen detected on *Q. petraea*.

Previously described from *Q. phillyraeoides* in Asia and Australia. Recently detected in France on *Q. robur* and *Q. petraea*.



Leptosphaeria sp. (OTU102)

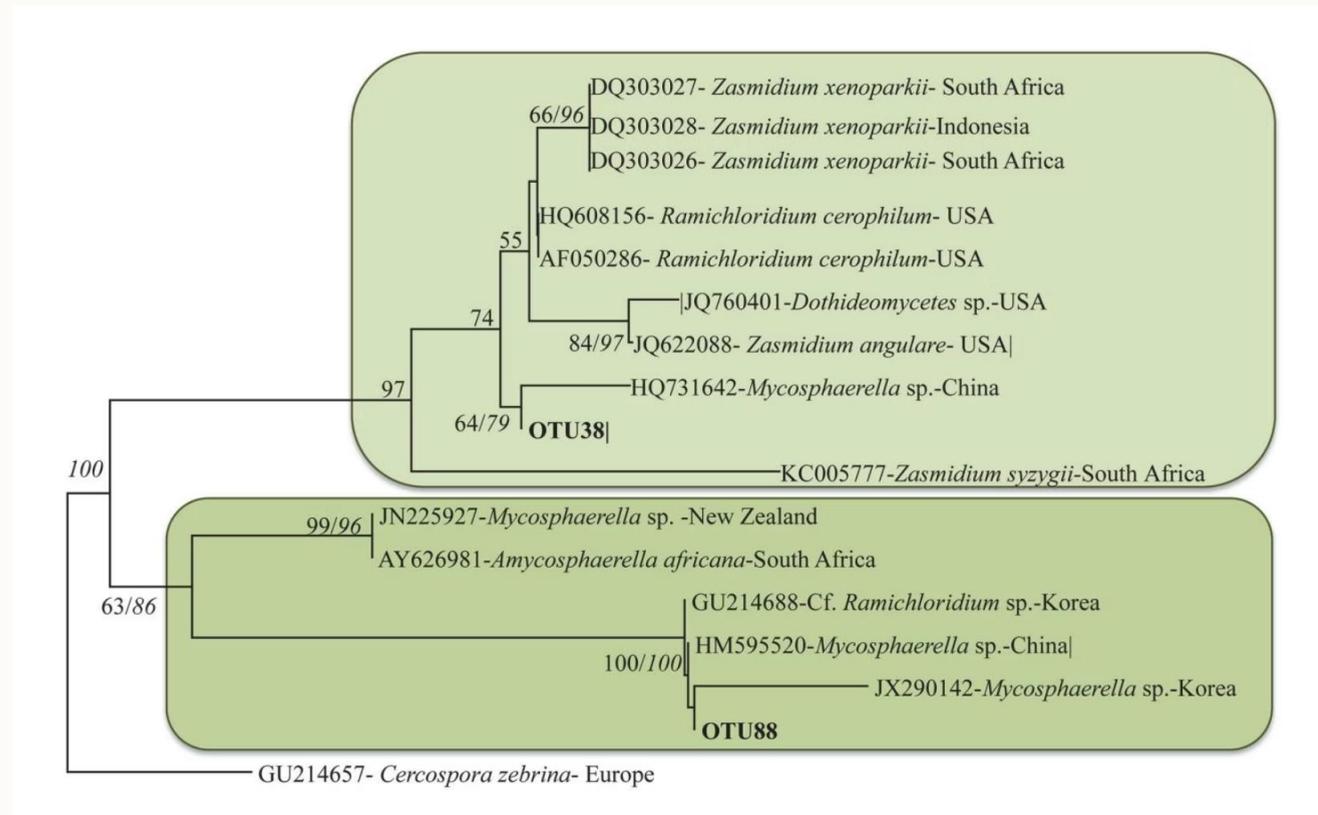
Detected on *Q. petraea* and associated with branch cankers. Phylogenetically related to undescribed *Leptosphaeria* species from China.



Pestalotiopsis sp. (OTU16)

Found on leaf spots of *Q. petraea*. Likely represents a new species based on sequence analysis and morphological observations.

Mycosphaerella Species Complex



Phylogenetic relationship between OTU88, OTU38 and related Mycosphaerella species.

Significant Findings

Two OTUs (38 and 88) were identified as Mycosphaerella species of Asian origin and associated with leaf spots on the sentinel oaks:

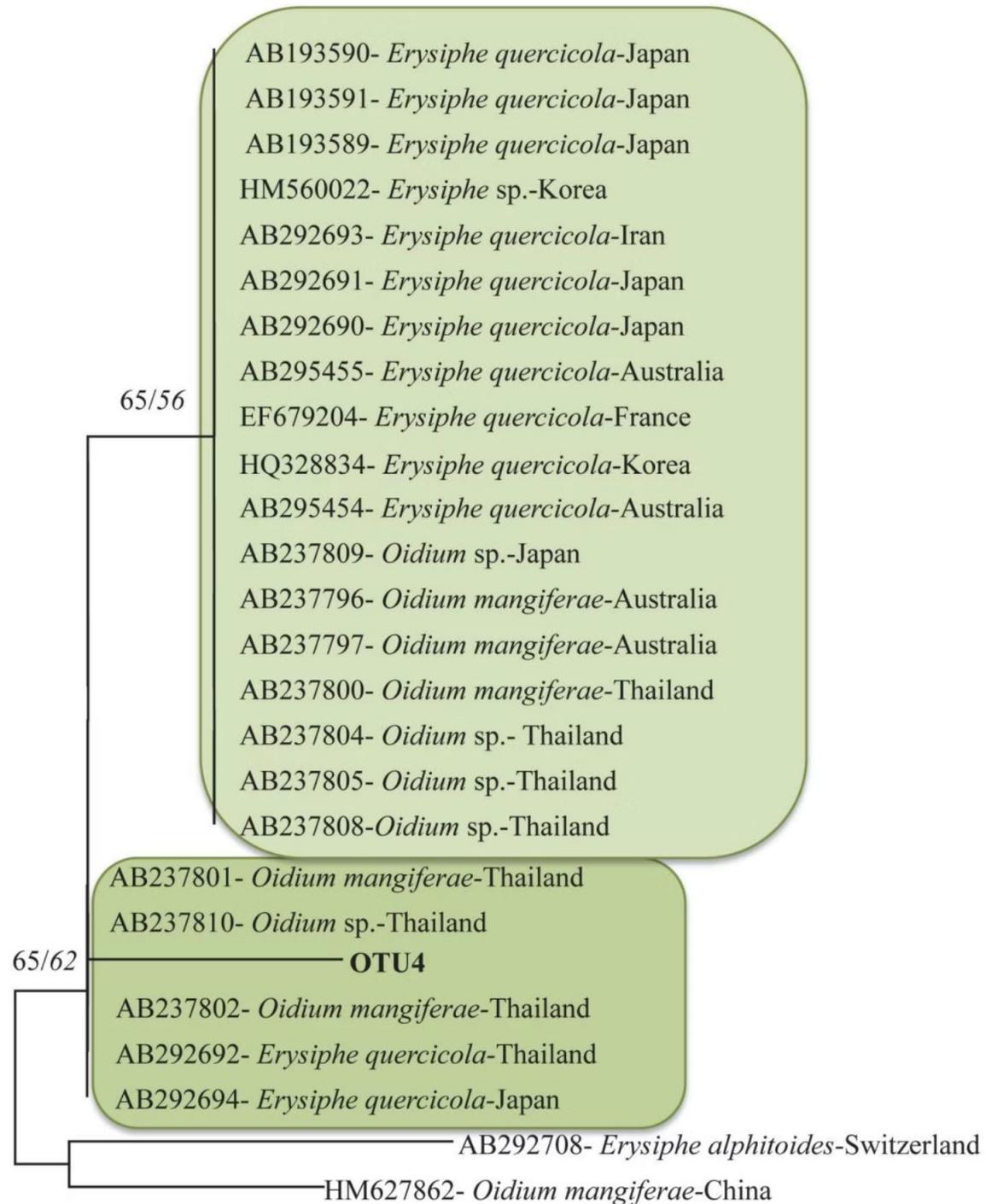
- OTU88 was detected on *Q. petraea* and *Q. suber*, related to *Mycosphaerella* species isolated from *Abies beshanzuensis* in China and *Toona sinensis* in Korea
- OTU38 was detected primarily on *Q. ilex* (3.2% of total reads), identified as *Mycosphaerella* sp. isolated from *Populus* in China
- ***Mycosphaerella* is one of the most common and destructive plant pathogen genera worldwide, with approximately 10,000 species**

Erysiphe quercicola: A Powdery Mildew Pathogen

Significant Findings

OTU4 was identified as *Erysiphe quercicola*, a biotrophic powdery mildew pathogen:

- Detected only on *Q. petraea* (1,214 reads), not on *Q. suber* or *Q. ilex*
- Originally described from *Q. phillyraeoides* in Asia and Australia
- Phylogenetic analysis confirmed the Asian origin of the strain found in the sentinel plantation



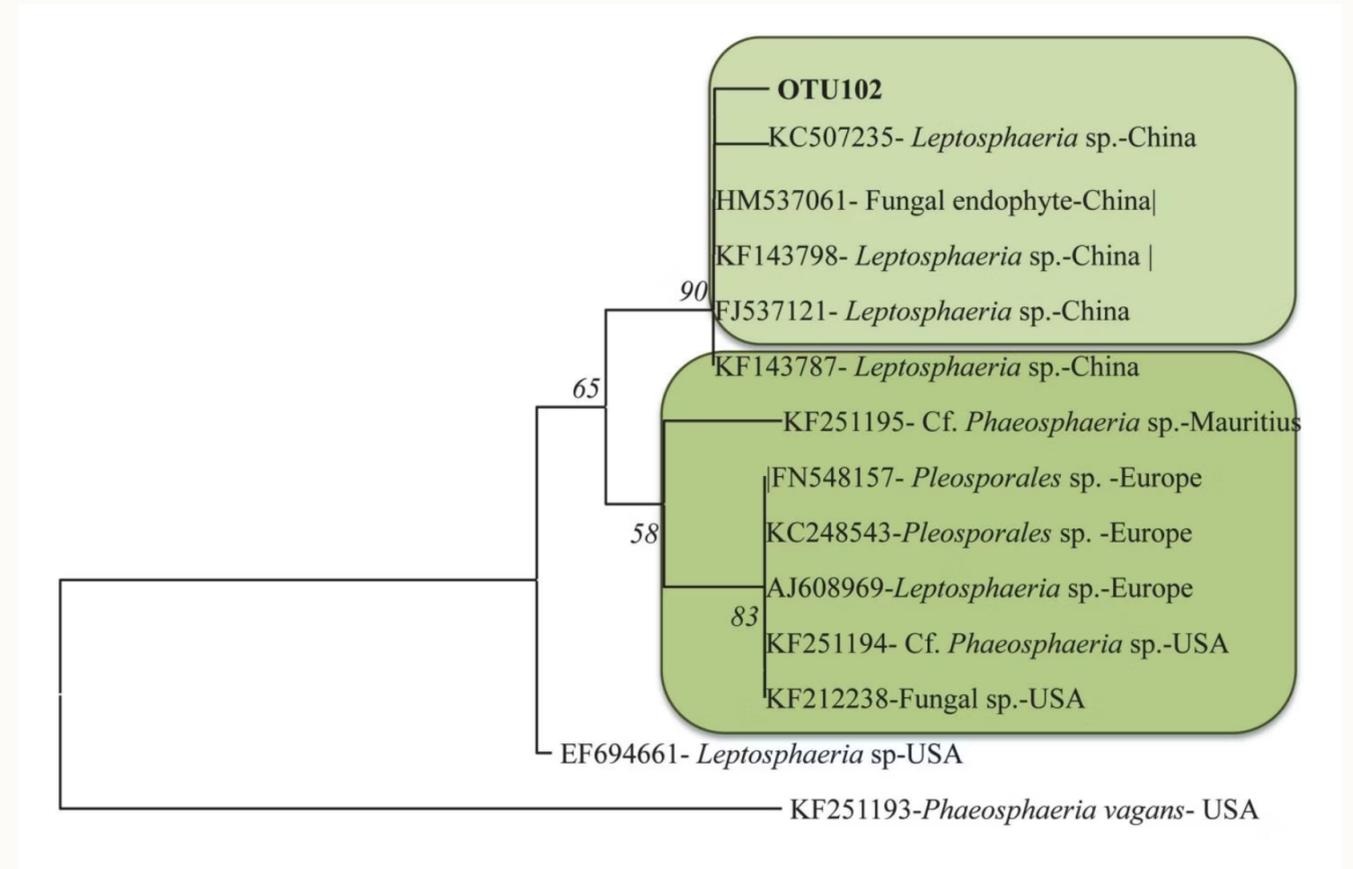
Leptosphaeria Species: Branch Canker Pathogen

Significant Findings

OTU102 was identified as a *Leptosphaeria* species of Asian origin:

- Associated with **branch cankers** on *Q. petraea*
- Perithecia containing bitunicate asci with 3-septate ascospores were observed
- Phylogenetically related to undescribed *Leptosphaeria* species from China
- The genus includes both saprobic and highly pathogenic species

The association of typical symptoms and signs with molecular detection suggests a pathogenic interaction with *Q. petraea*, although further studies are needed to confirm pathogenicity.



Phylogenetic relationship between OTU102 (*Leptosphaeria* sp.) and related species, showing its clustering with Chinese isolates.

Summary of Potential Pathogens

| OTU | Taxa | Molecular Identification | Morphological Identification | Association with Symptoms |
|--------|------------------------------|--------------------------|------------------------------|---------------------------|
| 4 | <i>Erysiphe quercicola</i> | YES | YES | YES |
| 16 | <i>Pestalotiopsis</i> sp. | YES | YES | YES |
| 102 | <i>Leptosphaeria</i> sp. | YES | YES | YES |
| 38, 88 | <i>Mycosphaerella</i> spp. | YES | NO | YES |
| 25 | <i>Strelitziana albisiae</i> | YES | NO | NO |
| 48, 91 | <i>Cryptococcus</i> spp. | YES | NO | NO |
| 77 | <i>Lalaria</i> sp. | YES | NO | NO |

Four out of the nine taxa of Asian origin were associated with symptoms and signs typical of infection on the sentinel trees, suggesting their potential as pathogens of European oak species.

The Case of *Erysiphe quercicola*: Validation of the Sentinel Approach

Detection in Sentinel Plantation

Erysiphe quercicola was detected on *Quercus petraea* in the Fuyang sentinel plantation in 2010, showing its ability to colonize European oak species.



Subsequent Detection in Europe

The pathogen was later detected in France on *Q. robur* and *Q. petraea*, associated with flag-shoot symptoms.



Confirmation of Invasion Risk

This real-world example validates the sentinel tree approach as an effective tool for identifying potential invasive pathogens before they become established in new regions.

Challenges and Bottlenecks

1 Propagation Material Selection

The use of **seedlings imported from Europe** risks introducing European fungi. Using certified seeds would minimize the risk of endophytic infection by European taxa. **Baseline** characterization of the microbial community in propagation material is essential.

3 Sample Processing Limitations

Symptomatic material cannot be shipped abroad and must be processed in specialized **laboratories "in loco."** In this study, biological detection and isolation of candidate pathogenic fungi failed due to collaborative agreement bottlenecks.

2 Proving Pathogenicity

While symptom assessment and molecular detection are necessary, they are not sufficient to prove pathogenicity, especially for taxa that can be both saprophytic and pathogenic. Biological characterization and inoculation trials are required.

4 Regulatory and Logistic Issues

The quality of **reciprocal agreements between collaborating countries**, including shared protocols for plantation establishment and sample processing, is crucial for success.

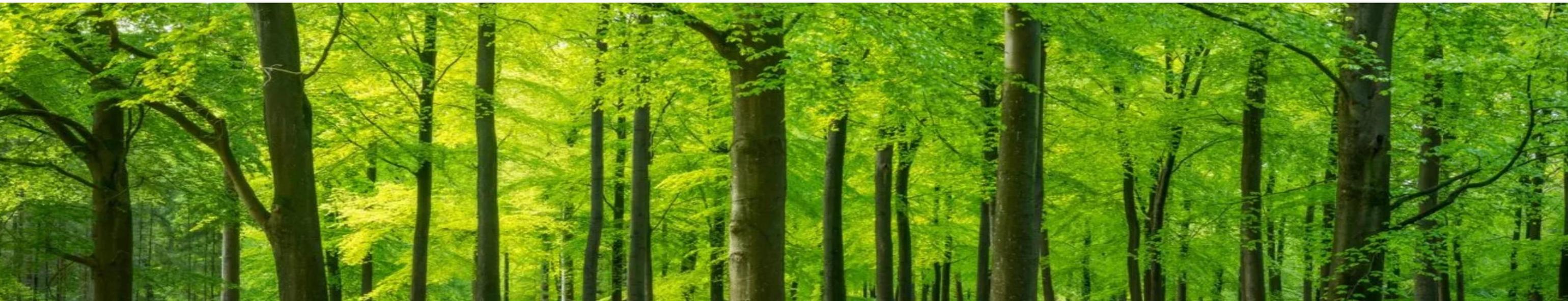
Comparison with Traditional Biosecurity Approaches

Traditional Approaches

- Reactive: Respond to invasions after they occur
- Limited scope: Focus on known pests and pathogens
- Pathway-based: Target known introduction pathways
- Inspection-based: Rely on visual inspection at borders

Sentinel Tree Approach

- Proactive: Identify potential invaders before introduction
- Broader scope: Can detect unknown or emerging pathogens
- Host-based: Focuses on susceptibility of potential hosts
- Science-based: Combines field observations with advanced molecular techniques



Reciprocal Sentinel Plantations

Bidirectional Approach

- The sentinel tree concept can and should be applied reciprocally, with Asian trees planted in Europe to identify European pathogens that might threaten Asian forests.
- This reciprocal approach would provide mutual benefits for biosecurity in both regions and foster international collaboration in forest protection.

Mutual Benefits

Reciprocal sentinel plantations would:

- Strengthen international cooperation in forest health protection
- Provide a more comprehensive understanding of host-pathogen interactions
- Help develop shared protocols and standards for biosecurity
- Create a global early warning system for forest pathogens



Conclusions: The Future of Sentinel Trees in Biosecurity

Sentinel plantations represent a powerful proactive approach to identifying potential invasive pathogens before they arrive in new regions. The Fuyang experiment successfully demonstrated this concept by detecting several Asian fungi capable of colonizing European oak species.

1 Proven Concept

The detection of *Erysiphe quercicola*, which has subsequently invaded European forests, validates the sentinel tree approach as an effective biosecurity tool.

3 Integration Needed

Sentinel plantations should be integrated with other biosecurity tools as part of a comprehensive strategy to protect forests from invasive pathogens.

2 Implementation Challenges

Despite its potential, the approach faces logistic and technical challenges that must be addressed through improved international collaboration and standardized protocols.

4 Global Potential

A global network of reciprocal sentinel plantations could revolutionize our approach to forest biosecurity, shifting from reactive to proactive management of invasive threats.

