

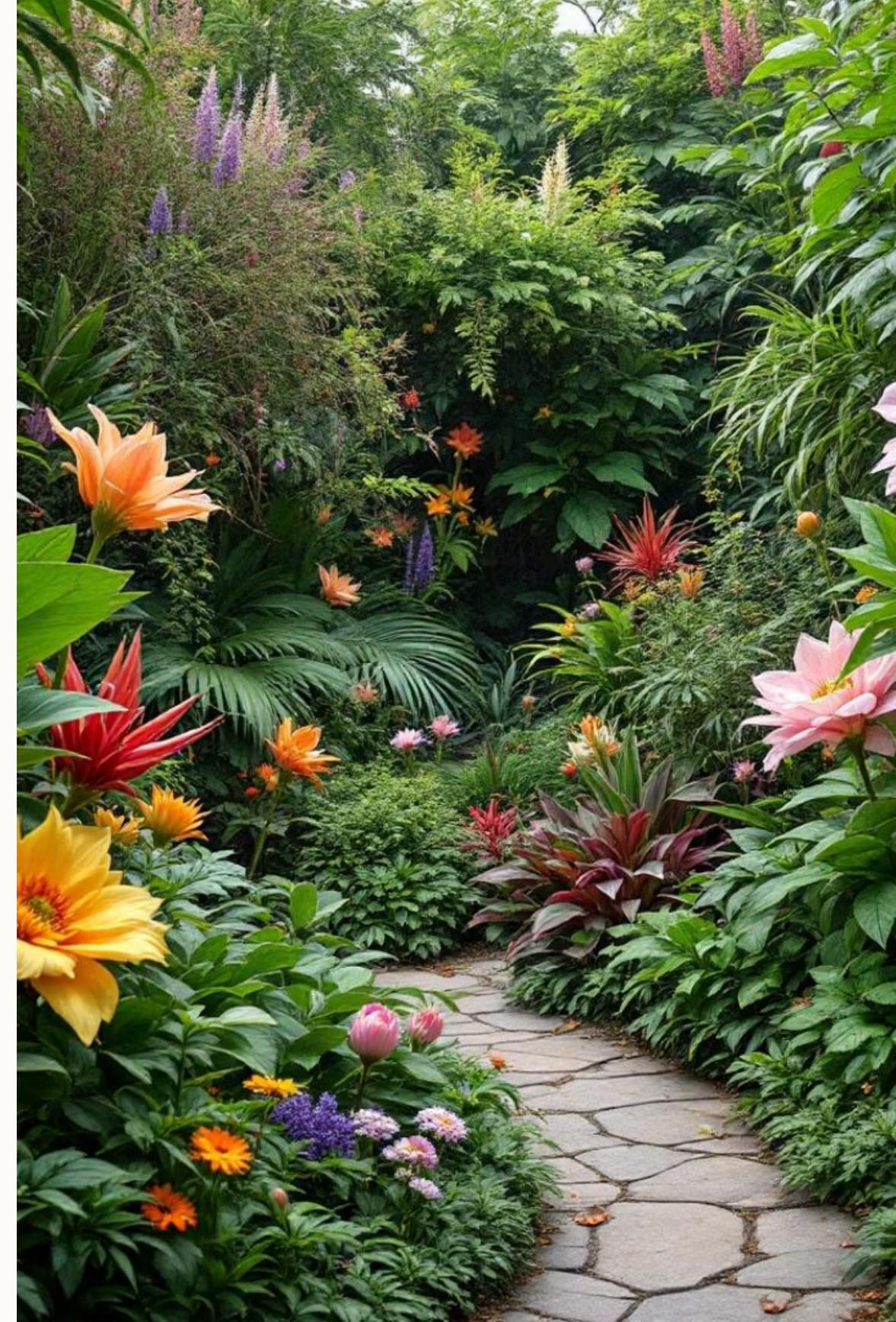
The Global Network of Sentinel Arboreta: Advantages and Limits

A worldwide network of sentinel arboreta for the early detection of plant pests and diseases. This scientific resource is fundamental for global plant biosecurity.

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Plant Biosecurity: Foundation of Conservation

International Cooperation

Essential for preventing the spread of pests and diseases that threaten plant health and biodiversity.

Collection Management

Biosecurity is fundamental for managing living collections in botanical gardens.

Preservation of Diversity

Robust biosecurity policies contribute to global plant conservation efforts.



Sentinel Arboreta: Guardians of Plant Health

Botanical Gardens

Collections of diverse plant species from multiple families and regions.

- Emphasise taxonomic diversity
- Educational and conservation focus
- Display ornamental and rare specimens
- Often include glasshouses for exotic species

Arboreta

Specialised collections focused primarily on woody plants and trees.

- Concentrated on tree species
- Research-oriented arrangements
- Often larger spatial layouts
- Strategic groupings for biosecurity monitoring

Sentinel Function

Arboreta serve as early warning systems in the plant biosecurity network.

- Monitor for emerging threats
- Document host-pest interactions
- Generate baseline ecological data
- Support international surveillance networks



Sentinel Arboreta: Guardians of Plant Health

Diverse Collections

Arboreta and botanical gardens house native and exotic species, creating ideal conditions for observing interactions between plants and potentially harmful organisms.

Critical Function

Their role extends beyond conservation and education; they function as critical nodes in biosecurity networks, facilitating early detection of invasive species.

1. Utility of the Global Sentinel Arboreta Network



Early Detection

Identification of threats before they become epidemics



Preventive Protection

Anticipation of potential threats



International Cooperation

Global scientific collaboration networks



1.1. Early Detection of Pests and Pathogens

Early Identification

Networks allow for the detection of pests, pathogenic fungi, bacteria, viruses and insect vectors at very early stages.

Strategic Plantings

By planting host species outside their natural range and observing them regularly, potential threats are anticipated.

Prevention of Epidemics

This action reduces control costs and prevents significant economic and ecological losses.



1.2. Monitoring of Strategic Species

Sentinel networks prioritize monitoring ecologically and economically significant tree species including European beech, chestnut, and oak varieties to track potential threats and protect forest ecosystems.



Fagus sylvatica

The European beech is a species of high ecological value monitored in sentinel networks.



Castanea sativa

The European chestnut has great economic and cultural importance.



Quercus spp.

Oak species are fundamental in many forest ecosystems.

1.3 Generation of Baseline Data on Interactions



1.4 Supporting Early Warning Systems

Sentinel arboreta feed critical data to international plant health organizations that monitor, alert, and coordinate responses to emerging threats.



EPPO

European and Mediterranean Plant Protection Organisation creates alert lists and recommendations for emerging harmful organisms.



IPPC

International Plant Protection Convention promotes the official exchange of phytosanitary information between countries.



EFSA

European Food Safety Authority uses field data to model risks to crops and biodiversity.



Applications of information from the Sentinel



Rapid Alerts

Issuing warnings about new organisms in regions where they were not previously present



Hotspot Identification

Locating high-risk areas where surveillance should be intensified

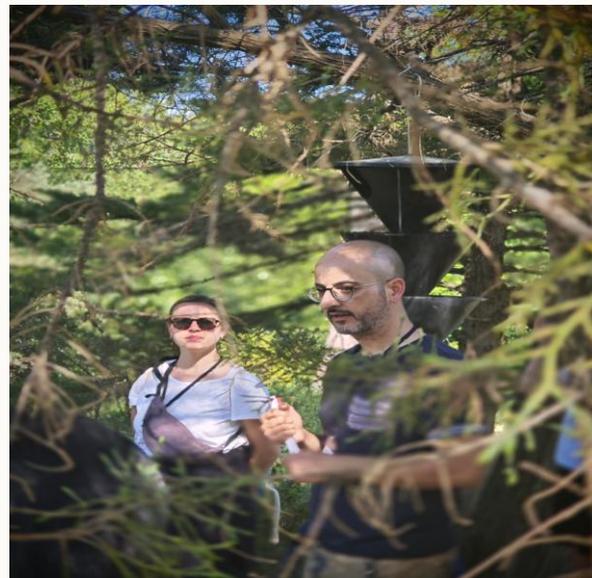


Priority Actions

Establishment of quarantine, containment or eradication measures based on evidence

1.5 Platform for Scientific Research

Sentinel plantations offer ideal semi-structured conditions for scientific studies in plant epidemiology, microbial ecology, vector distribution and integrated management.



2. Scientific and Applied Uses



2.1 Plant Health Risk Assessment



Risk Lists

Establishment of potentially dangerous plant-pathogen combinations for disease-free zones.



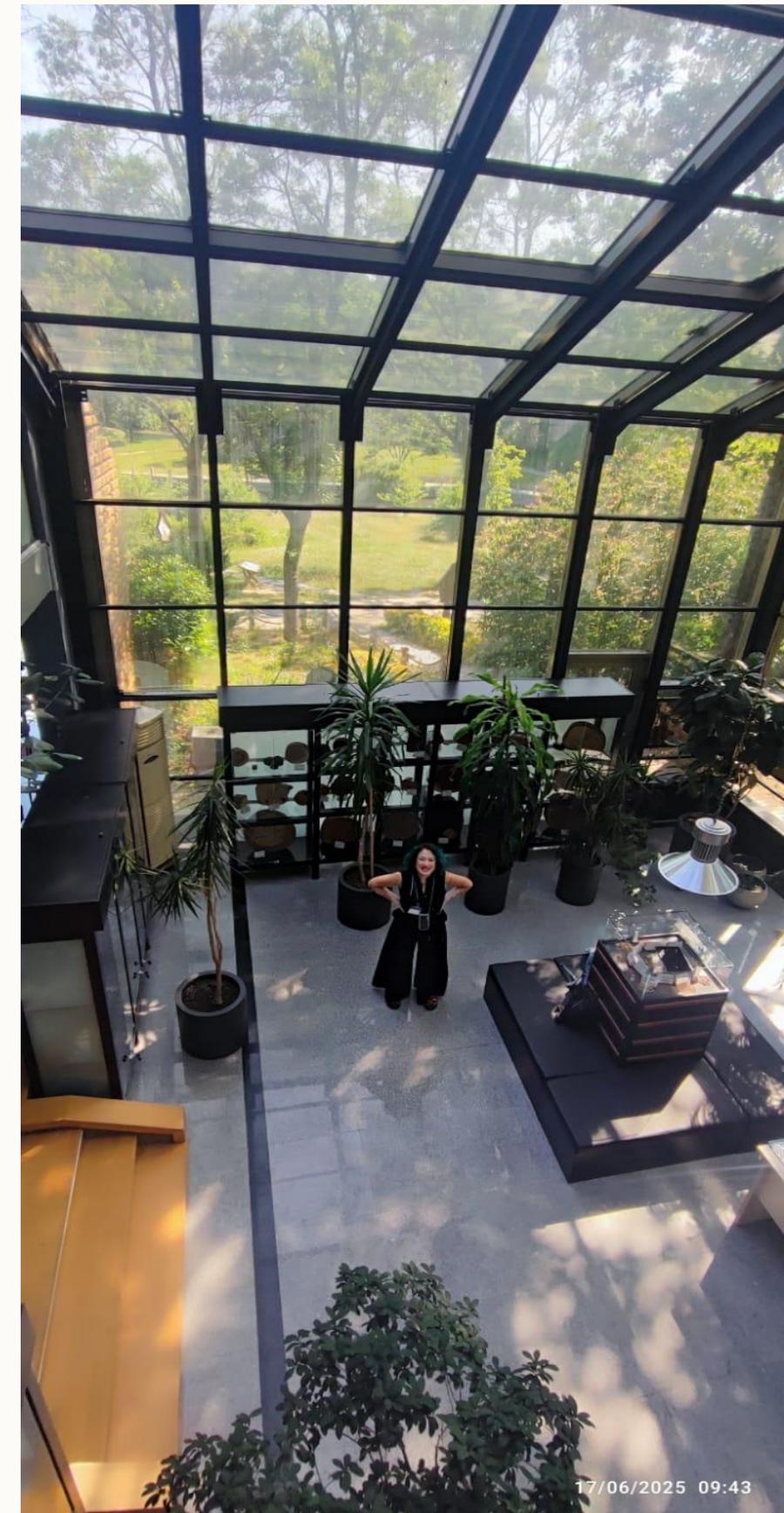
Empirical Evidence

Direct observation of spontaneous infections providing data on compatibility between organisms.



Prevention

Early identification of threats to implement preventive measures.



2.2 Validation of Predictive Models



Field Data Collection

Real observations from sentinel arboreta provide crucial validation points for theoretical models



Predictive Modeling

Theoretical models estimate distribution and ecological suitability of pests and pathogens



Model Refinement

Comparing predictions with field data improves the reliability of risk maps



Future Scenarios

Validated models enable more accurate forecasting of emerging threats under changing conditions

Theoretical models of distribution and climatic or ecological suitability are validated using real data obtained in the field, improving the reliability of risk maps and future scenarios.

2.3 Susceptibility Tests

Species Comparison

Evaluation of different tree species against specific pathogens to identify levels of natural resistance.

- Response to pathogenic fungi
- Tolerance to insects
- Resistance to bacteria

Management Factors

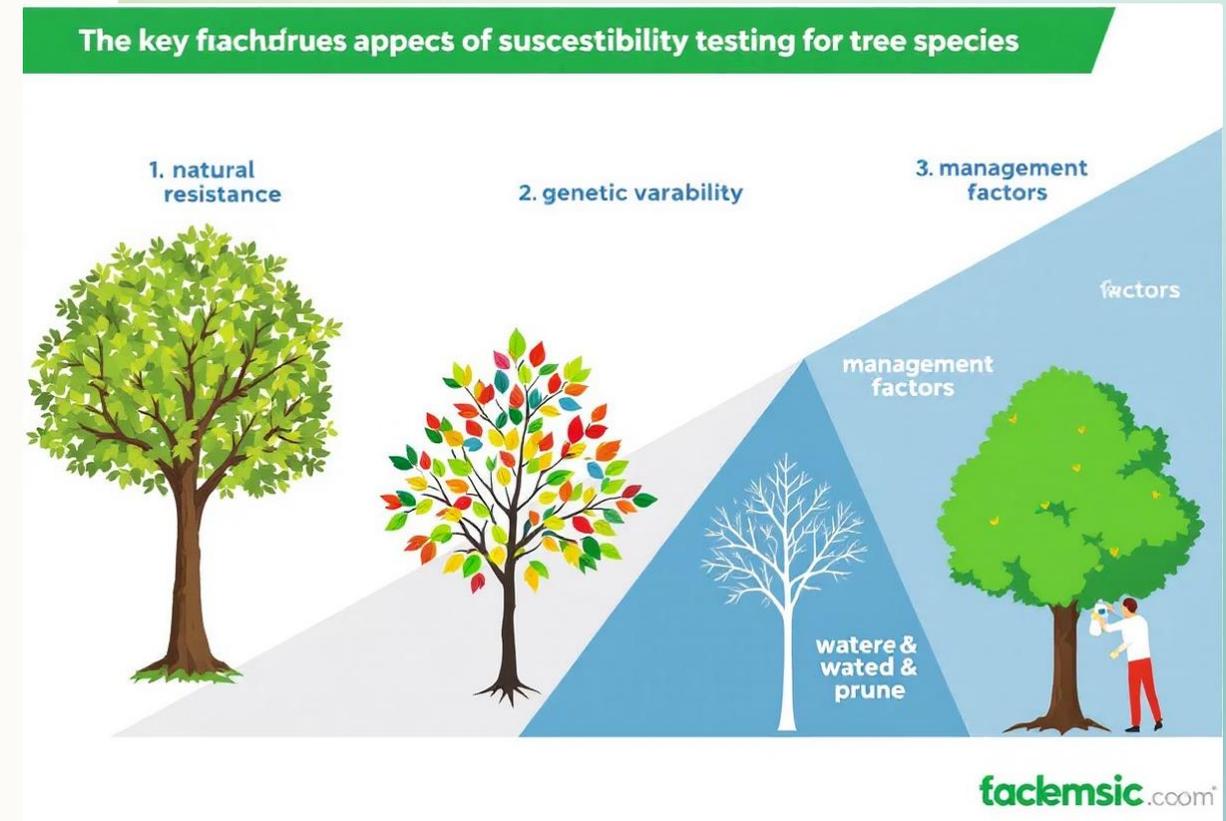
Evaluation of cultural practices and treatments that can influence susceptibility.

- Water regime
- Fertilisation
- Preventive pruning

Genetic Variability

Analysis of different genetic origins to identify populations with greater natural resistance.

- Resistant ecotypes
- Geographical variation
- Adaptive potential



Sentinel networks provide critical data that informs and strengthens regulatory frameworks for plant biosecurity across international borders.

2.4 Support for Regulatory Decisions

100+

Monitored Species

Diversity of plants under surveillance in global sentinel networks

50+

Participating Countries

International collaboration in plant health surveillance

200+

Detected Pathogens

Organisms identified through early warning systems



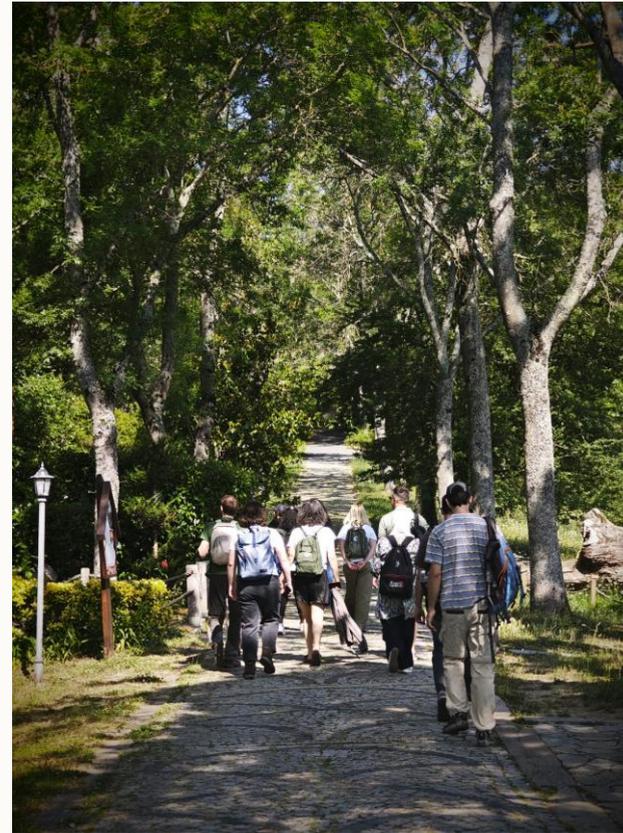
2.5 Training, Awareness and Citizen Science

Sentinel arboreta networks serve as valuable educational platforms that bridge the gap between scientific research and practical applications in plant biosecurity.



Academic Training

These environments provide hands-on experience with real-world plant health challenges.



Citizen Science

Volunteers participate in basic observations through structured methodologies...



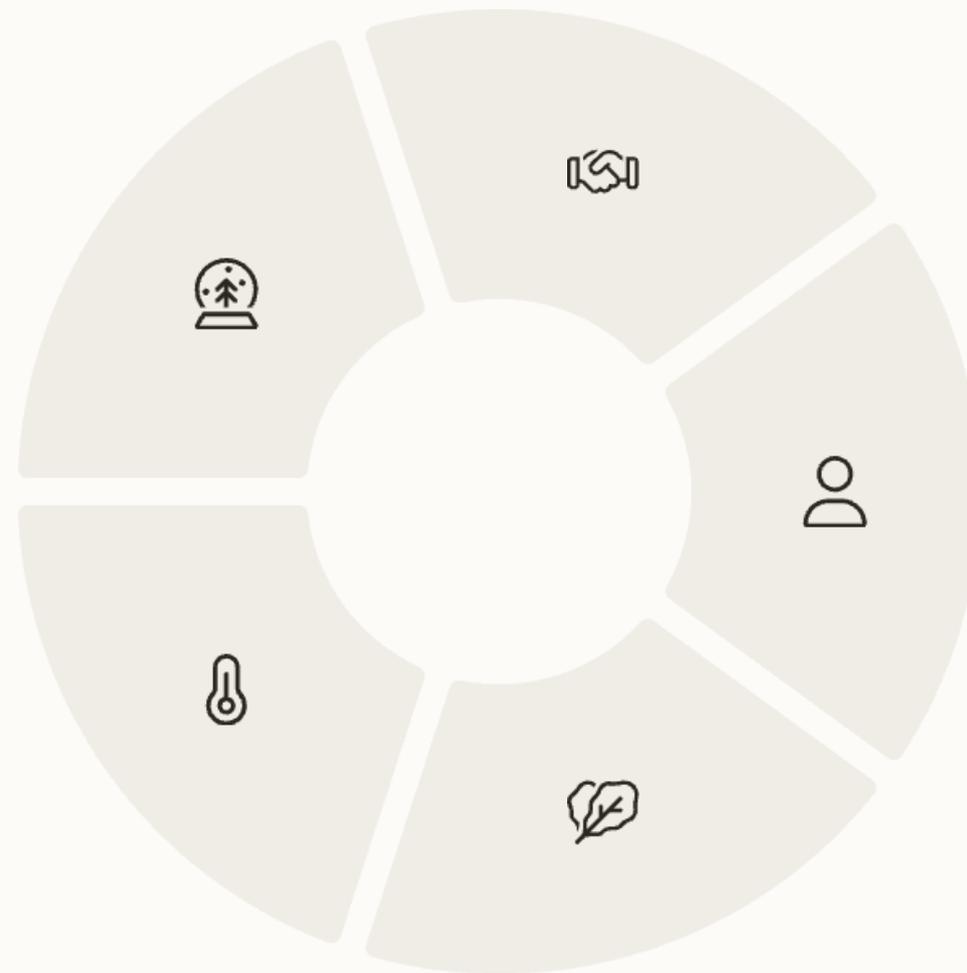
Technical Training

Public administration staff receive practical training on plant health surveillance.

3. Opportunities of the Global Network

Global Cooperation
International scientific collaboration

Climate Change
Observatory of climate effects



Science-Policy

Link between research and regulation

Citizen Participation

Network expansion through volunteers

One Health Approach

Comprehensive ecosystem health

3.1 Internationalisation and Global Cooperation



Protocol Harmonisation

Development of standardised methodologies to ensure data comparability between countries.



Multilateral Projects

Scientific collaboration between institutions from different continents to address common threats.



Comparative Observation

Analysis of the behaviour of the same species in different climatic regions of the world.



3.2 Science-Policy Interaction

Evidence for Decision-makers

Generation of robust scientific data on emerging threats to inform policy-makers.

Investment Justification

Provision of evidence-based arguments to allocate resources for phytosanitary surveillance and control.

Research-Regulation Bridge

Establishment of direct links between applied research and regulatory development.

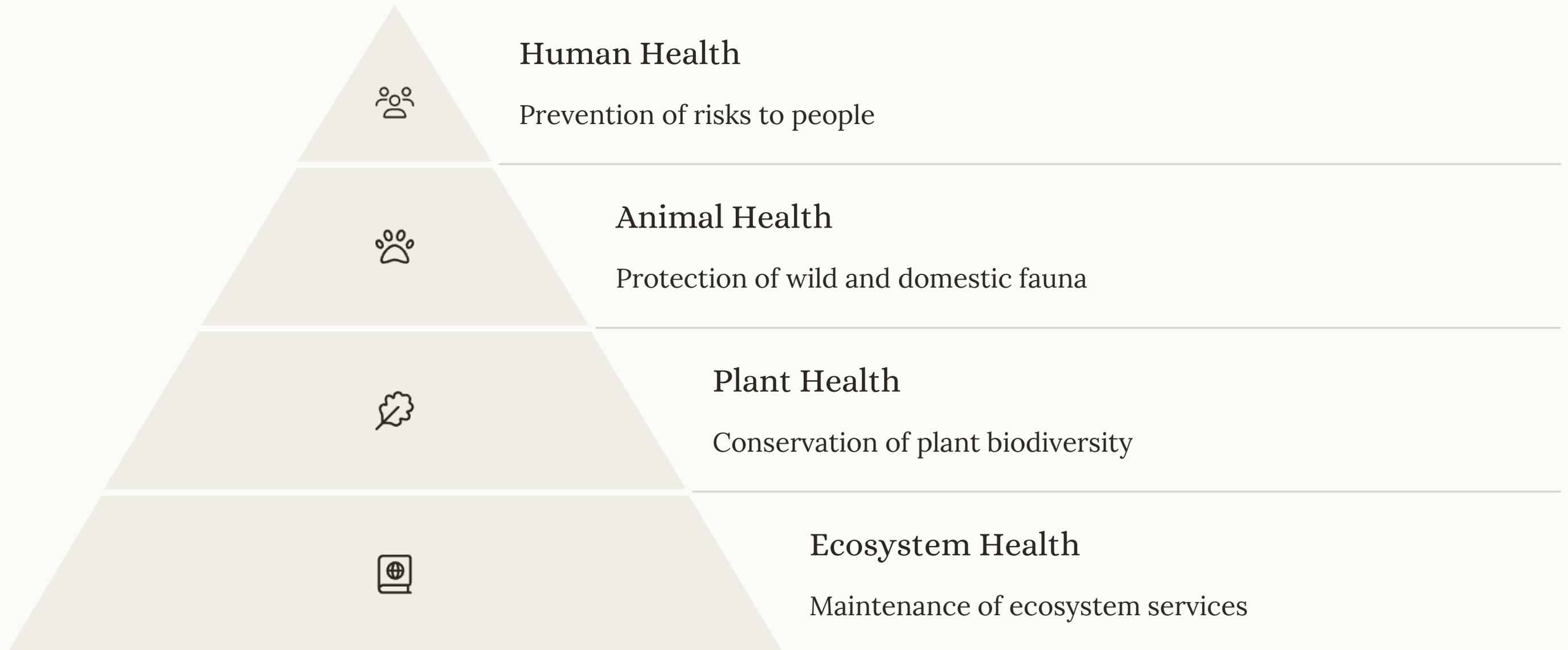


3.3 Citizen Science in Sentinel Networks

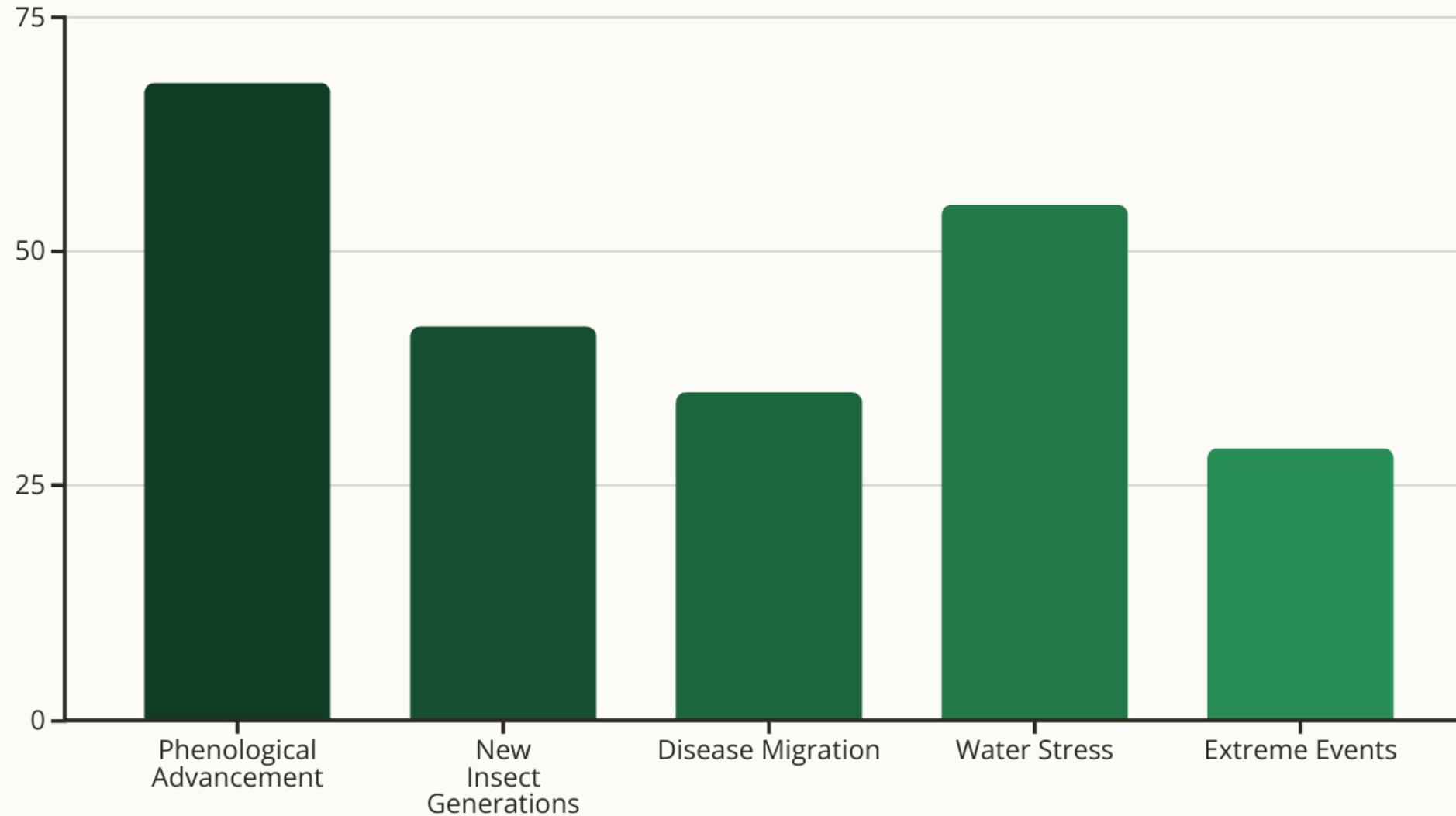


Citizen participation expands the spatial coverage of networks through trained observers, strengthening the culture of prevention and community surveillance.

3.4 One Health Approach in Sentinel Networks



3.5 Climate Change Observatory



The sentinel networks document phenomena related to climate change, such as phenological advancement, new generations of vector insects and the migration of diseases to previously unaffected areas.



4. Limitations and Risks



Limited
Representativeness



Continuous
Investment



Time Factor



Dependency on
Experts

4.1 Limited Ecological Representativeness

Artificial Environments

Botanical gardens and urban plots do not fully replicate the conditions of natural ecosystems.

- Modified soils
- Urban microclimate
- Artificial planting density



Implications for Interpretation

Observations must be contextualised considering these ecological differences.

- Cautious extrapolation
- Field validation
- Complementary models



4.2 Continuous Investment Required

Sentinel networks require substantial and sustained financial commitment to function effectively. The costs include:

- **Long-term Monitoring**
Multiple years of consistent observation are necessary to establish reliable data patterns and identify emerging threats
- **Financial Resources**
Significant annual budget allocation for facilities, equipment, and operational expenses
- **Human Resources**
Dedicated team of specialized personnel with expertise in plant pathology, entomology, and botanical science

Investment requirements vary significantly by network size, geographic scope, and research intensity.



4.3 Slow Response: The Time Factor



Pathogen Introduction

Initial arrival of the organism to the sentinel plantation, frequently asymptomatic.



Latency Period

Asymptomatic phase where the pathogen is present but not visible, can last years.



Favourable Conditions

Environmental changes that trigger symptom expression (temperature, humidity).



Symptom Manifestation

Visible appearance of damage that allows detection and diagnosis.



4.4 Dependency on Specialised Experts



Entomologists

Specialists in insect taxonomy, fundamental for the precise identification of vectors and pests.



Mycologists

Experts in pathogenic fungi, necessary for the diagnosis of fungal diseases.



Plant Pathologists

Scientists specialised in plant diseases, essential for comprehensive diagnosis.

4.5 Risk of Involuntary Vector

Potential risks associated with unwanted organisms.

1

Accidental Introduction

Without strict protocols, sentinel plantations could introduce unwanted organisms.

2

Establishment

Gardens could become pathogen reservoirs if not managed properly.

3

Dispersal

There is a risk they may act as escape points into surrounding natural ecosystems.



Real Network Examples



Various sentinel networks have been established worldwide, demonstrating the practical application of these concepts in international plant health surveillance.

International Sentinel Plant Network (ISPN)



Global Network

Coordinated by BGCI (Botanic Gardens Conservation International) since 2013.



Early Warning

System to detect emerging pests and pathogens before they become established.



Botanical Gardens

Utilises the existing infrastructure of arboreta and botanical collections worldwide.

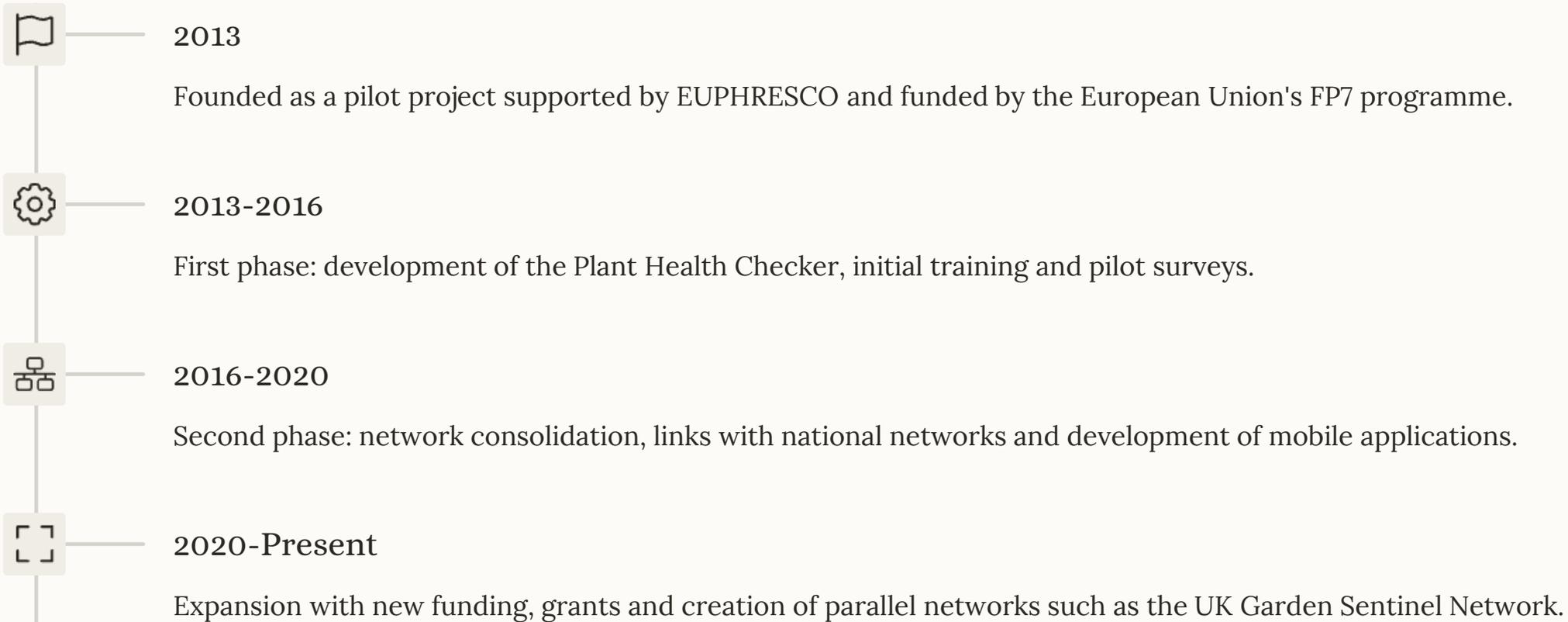
<https://www.bgci.org/>





International Plant Sentinel Network

Origin and Evolution of the IPSN Network





Key Activities and Resources of IPSN

Plant Health Checker

Standardised form for systematic visual monitoring that categorises symptoms as green/yellow/red, facilitating early detection.

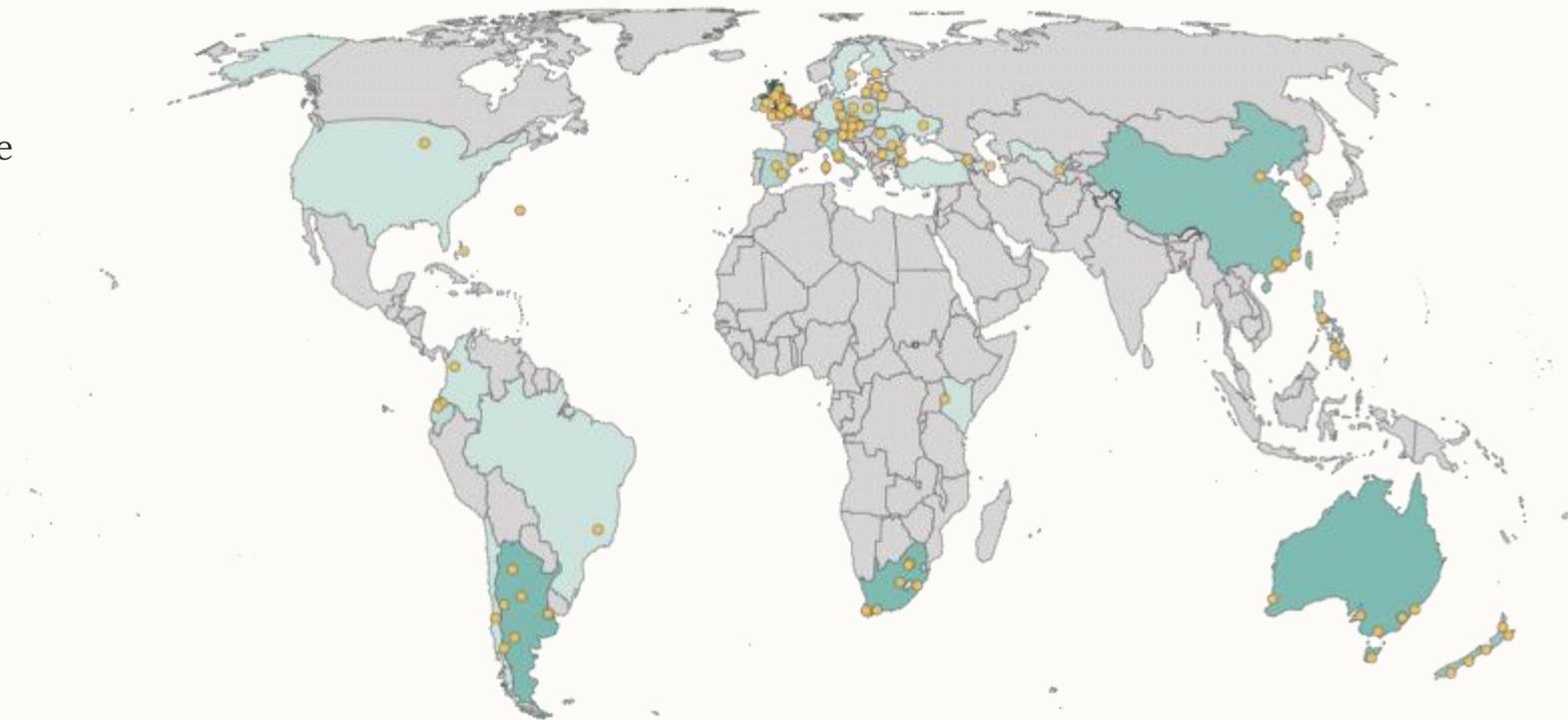
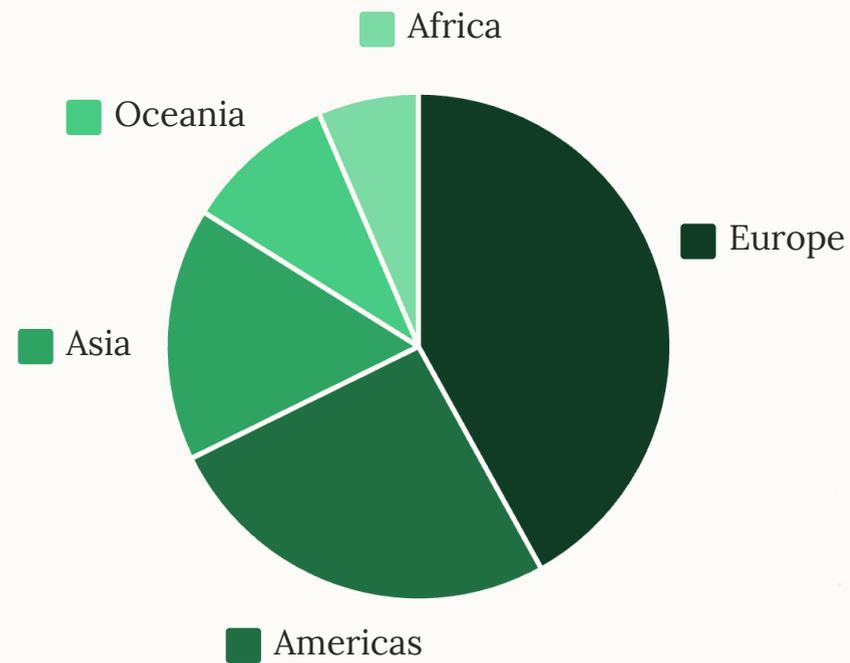
Sampling Protocols

Specific guidelines for pests such as Emerald Ash Borer, poplar blight and *Xylella fastidiosa*, among other priority pathogens.

Visual Resources

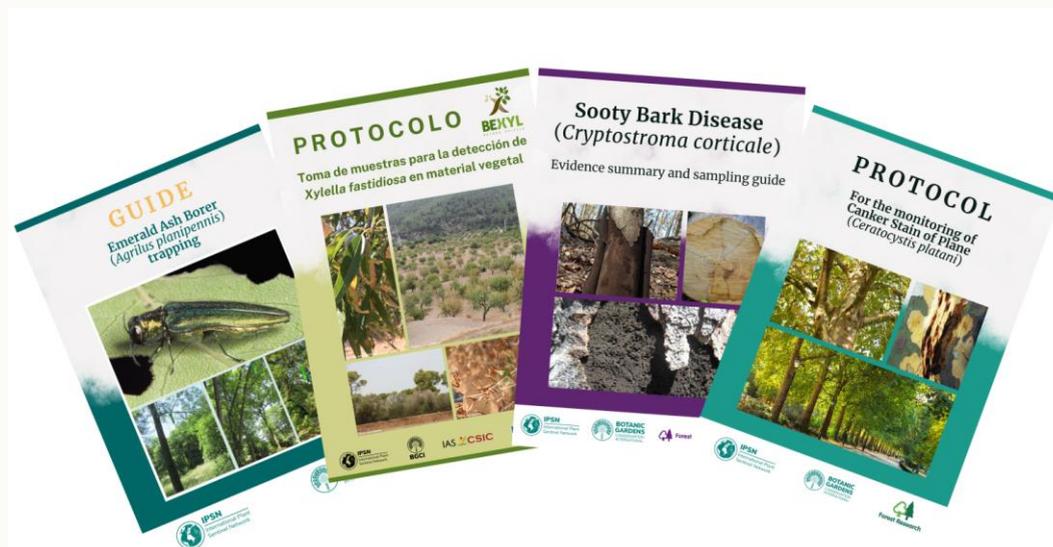
Explanatory videos, diagnostic posters, technical sheets and biosecurity manuals available in multiple languages.

Global Reach and Institutional Links



The IPSN network integrates more than 150 institutions across five continents, collaborating with National Plant Protection Organisations (NPPOs), research institutes and international organisations such as EPPO and IPPC. With **109 Botanic Gardens and Arboreta**, we now count with members across **39 countries** from around the globe.

- IPSN member gardens will monitor the non-native species, the ‘sentinels’, in their collection for damage by pests and diseases and report back to the plant’s country of origin.
- The IPSN has already produced a standard form for botanic gardens and arboreta (the IPSN Plant Health Checker) to record general health problems observed in sentinel (exotic) plants.
- General guidance is also provided on how to observe plant pests and diseases, and on how to prepare samples for diagnostic purposes.
- Posters on emerging pest and disease threats to trees in the UK have been prepared for oak (*Agrilus auroguttatus*, *Ceratocystis fagacearum*, *Enaphalodes rufulus*), ash (*Agrilus planipennis*, *Hymenoscyphus fraxineus*, *Xylosandrus germanus*), and pine (*Thaumetopoea pityocampa*, *Dothistroma septosporum*, *Monochamus galloprovincialis*).



<https://www.bgci.org/resources/bgci-tools-and-resources/ipsn-plant-health-checker/>

The PHC consists of a user-friendly 2-step form that allows individuals, whether botanic garden staff, horticultural students, or enthusiasts, to comprehensively document plant health observations.

STEP1:

This initial step compiles essential general data about the survey, plant details, and environmental conditions using a simple traffic light system (red-amber-green) to indicate the health status of the specimen. No specialized knowledge is required for this step, making it accessible to all users.

STEP2:

For more detailed observations, Step 2 guides users through a thorough survey of the specimen, focusing on specific symptoms and signs of pest or disease presence in different parts of the plant. This step is recommended for individuals with some knowledge of plant pests and diseases or appropriately trained plant health officers.





Plant Health Checker - Step 1

Name of Botanic Garden / Arboretum:			
Country:			
Address:			
Name of IPSN contact:			
Survey details			
Survey carried out by:			
Date of survey:			
Best description of season:			
Main reason for surveying this particular individual:			
Plant details			
Species (Cultivar):			
Accession number:			
GPS			
Country/region species is native to:			
Age/amount of time plant has been present in gardens:			
General Comments:			
General description (please tick)			
Generally healthy	<input checked="" type="checkbox"/>	Some damage	<input checked="" type="checkbox"/>
Dying	<input checked="" type="checkbox"/>	Dead	<input checked="" type="checkbox"/>
Any recent changes in health or overall look:			

General description of environment

Any management issues (e.g. irrigation, soil pH, sun bleaching) or any recent use of pesticides/ fungicides/ herbicides:

Description of environment (focusing on recent changes and individuals in close proximity):

For each section of the plant give it a rating dependent on how healthy it appears:

Red (R) = In very poor health and of imminent concern due to significant damage potentially resulting in death of individual

Orange (O) = Not currently a concern but could develop; should be checked frequently to monitor progress

Green (G) = As would be expected on a 'healthy plant'

Black (X) = Absent/not applicable

Where an **orange or red** rating is given, ensure you give a description of why you've given it this rating in notes.

1.) Crown
 R O G X

2.) Flowers / Fruits (circle)
 R O G X

3.) New growth
 R O G X

4.) Leaves
 R O G X

5.) Trunk & branches
 R O G X

6.) Base and Roots (if exposed)
 R O G X

Notes:

What do you think is wrong with this plant? <i>(give an indication of how sure you are of this diagnosis)</i>		Reference/file name of any photographs taken:	
1.) Is a re-survey required?	2.) If yes, in what timeframe <i>(include a suggested date)</i>	3.) Should this be escalated to an appropriate staff member to carry out STEP 2 ?	4.) Name of person escalated to <i>(if applicable)</i> :
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	5.) Date:



Accession number: _____

Survey completed by: _____ Date: _____

Plant Health Checker – Step 2

Please read: This section should be completed if escalation is specified by STEP 1. It should be carried out by an appropriately trained staff member who has the relevant knowledge concerning the plant's history and/or pest and pathogen identification skills.

Tick all signs/symptoms that are at abnormal levels or are unexpected for the individual, and are thus cause for concern (e.g. are out of the ordinary/new to the plant). Give a description and an indication of severity/abundance in the **notes**, plus note anything else of importance or interest.

1. Crown			
Thin /sparse	<input checked="" type="checkbox"/>	Notes:	
Yellow leaves	<input checked="" type="checkbox"/>		
Dead wood	<input checked="" type="checkbox"/>		
2. Blossom/Flowers			
Dead	<input checked="" type="checkbox"/>	Notes:	
Malformed	<input checked="" type="checkbox"/>		
Swollen	<input checked="" type="checkbox"/>		
3. New Growth (Shoots and Buds)			
Dead	<input checked="" type="checkbox"/>	Dieback	<input checked="" type="checkbox"/>
Wilted	<input checked="" type="checkbox"/>	Malformed	<input checked="" type="checkbox"/>
Notes:			
4. Leaves			
Dead	<input checked="" type="checkbox"/>	Malformed	<input checked="" type="checkbox"/>
Smaller than expected (stunted)	<input checked="" type="checkbox"/>	Mosaics / mottled / variation in colour	<input checked="" type="checkbox"/>
Sticky	<input checked="" type="checkbox"/>	Galls	<input checked="" type="checkbox"/>
Rust	<input checked="" type="checkbox"/>	Mildew	<input checked="" type="checkbox"/>

4. Leaves continued (leaf spots)			
Single	<input checked="" type="checkbox"/>	Numerous	<input checked="" type="checkbox"/>
Present only at the edge	<input checked="" type="checkbox"/>	All over leaf	<input checked="" type="checkbox"/>
Only on old growth	<input checked="" type="checkbox"/>	Only on new growth	<input checked="" type="checkbox"/>
Yellowing (chlorotic leaves)	<input checked="" type="checkbox"/>	Brown/blackening (necrotic leaves)	<input checked="" type="checkbox"/>
Notes:			
5. Trunk & Branches			
Canker or lesion	<input checked="" type="checkbox"/>	Approx. number	
Dry	<input checked="" type="checkbox"/>	Gummy/sticky	<input checked="" type="checkbox"/>
Approx. height of canker from ground (m)			
Galls	<input checked="" type="checkbox"/>	Approx. size (m)	
Trunk bleeding ('weeping patches')			<input checked="" type="checkbox"/>
Approx. height of bleed from ground (m)			
Approx. number of bleeds over trunk			
Vertical bleeds (in a line up the trunk)	<input checked="" type="checkbox"/>	Horizontal bleeds (around the trunk)	<input checked="" type="checkbox"/>
Loose Bark / bark flaking / comes off easily			<input checked="" type="checkbox"/>
Notes:			
6. Base and Roots (if exposed)			
Bootlaces/black strands (1-2mm wide)			<input checked="" type="checkbox"/>
Fungal mycelium/white strands			<input checked="" type="checkbox"/>
Mushrooms/toadstools on plant			<input checked="" type="checkbox"/>
Damage by mammals	<input checked="" type="checkbox"/>	Notes:	
Decay / Rotting		<input checked="" type="checkbox"/>	
Wet	<input checked="" type="checkbox"/>	Dry	<input checked="" type="checkbox"/>

7. General pest damage		Location (e.g. leaf)	
Insect galleries under loose bark	<input checked="" type="checkbox"/>		
Insect eggs	<input checked="" type="checkbox"/>		
Chewing damage	<input checked="" type="checkbox"/>		
Insect webbing	<input checked="" type="checkbox"/>		
Insect mines	<input checked="" type="checkbox"/>		
Frass	<input checked="" type="checkbox"/>		
Bore holes (circle below)			
<5mm	5-10mm	>15mm	<input checked="" type="checkbox"/>
Notes:			
8. Pest sightings		Location (e.g. leaf)	Photo (file name)
<i>(give an indication of how sure you are of this identification)</i>			
	<input checked="" type="checkbox"/>		
9. General Observations and Additional Notes			
Reference/file name of any photographs taken:			

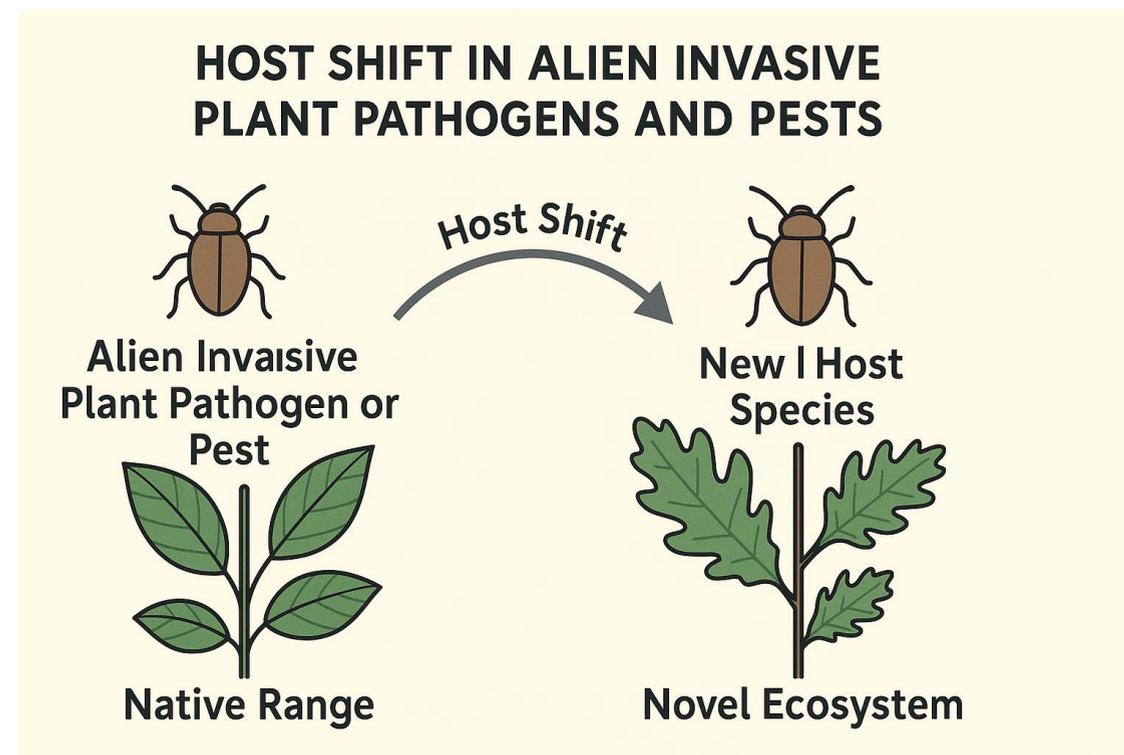
What do you think is wrong with this plant? <i>(give an indication of how sure you are of this diagnosis)</i>		1.) Is a re-survey required? <input checked="" type="checkbox"/>		2.) If yes, in what timeframe <i>(include a suggested date)</i>	
3.) Should this be reported to the local diagnostic laboratory - a physical sample may be required <i>(this is only if symptoms are severe or if a pest of concern)</i>		3.) Date reported:		4.) Should this be escalated to local National Plant Protection Organisation (NPPO)? <i>(as advised by local diagnostic laboratory)</i>	
<input checked="" type="checkbox"/>				<input checked="" type="checkbox"/>	
				5.) Date reported:	

Revealing Novel Interactions Between Oak and *Tubakia* Species

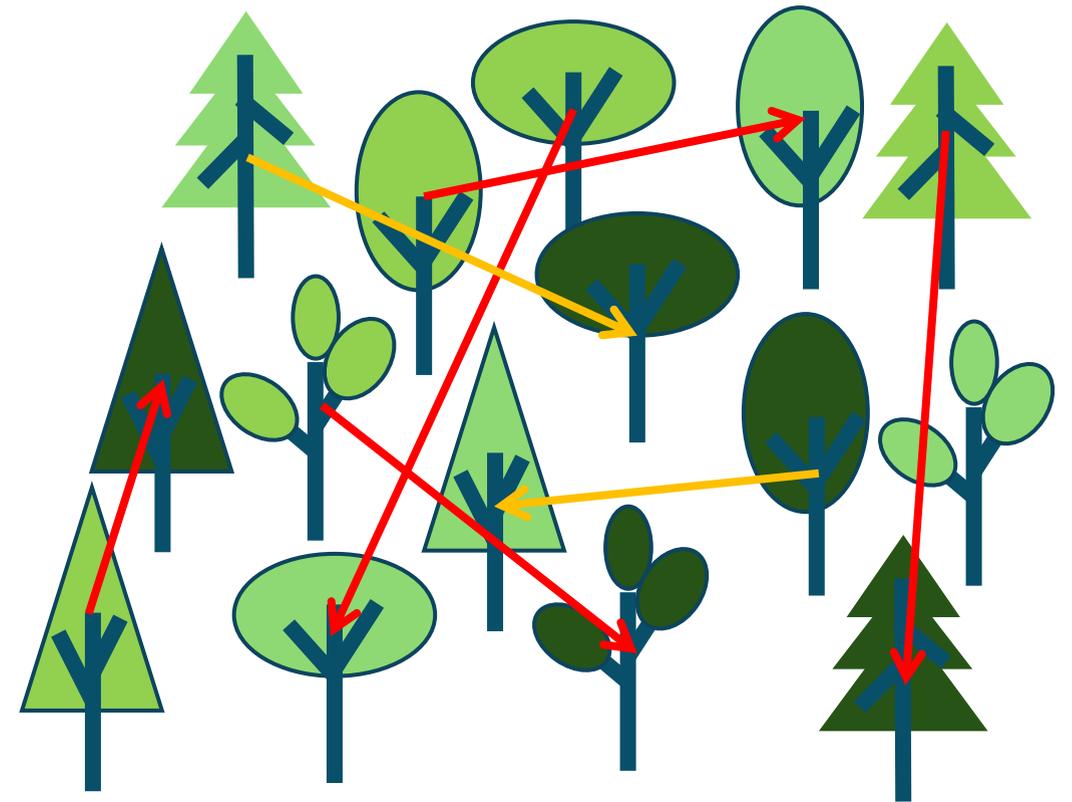


Botanical gardens/arboreta, as collection of woody species from different continents, represent one existing opportunity to monitor host-shift events and to evaluate the behaviour of a tree species vs alien organisms.

“**Host shift events**” in the context of alien invasive plant pathogens and pests refer to situations where a pathogen or pest jumps from its original host species to a new, often unrelated host, frequently in a novel ecosystem (e.g., outside its native range). This phenomenon can have severe ecological and economic consequences, particularly when it involves introduced (alien) organisms that find susceptible hosts in new regions.



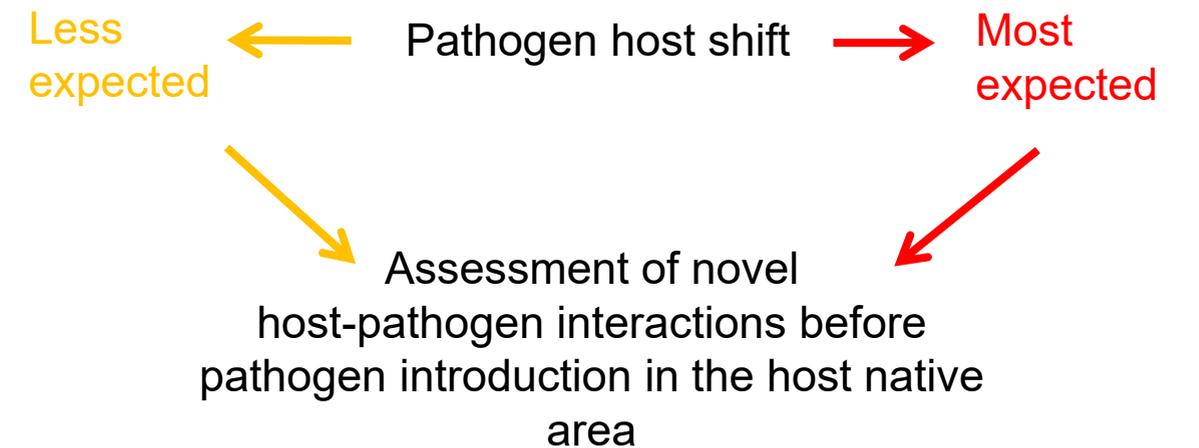
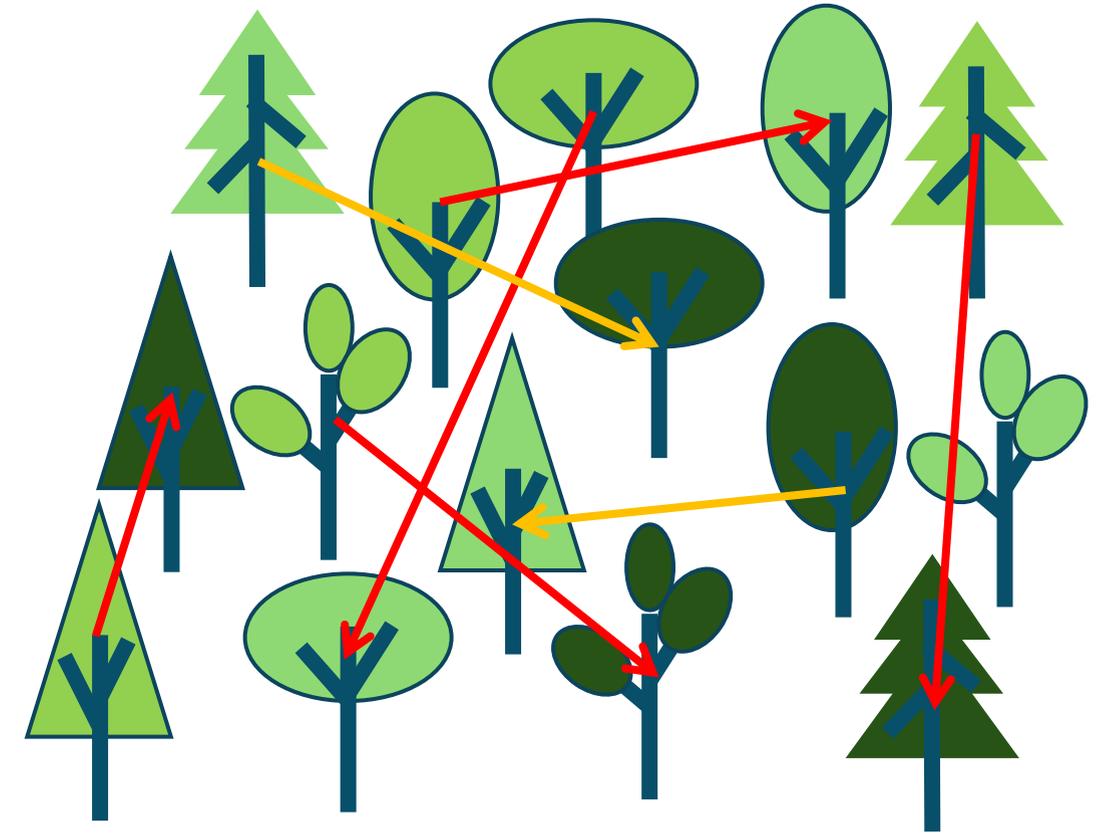
The assumption is that cultivated exotic trees are exposed to inoculum of native potentially pathogenic organisms harboured by native species in the same environment or from neighbour environments



Less expected ← Pathogen host shift → Most expected

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

A further expanded assumption is that all the exotic and native tree species cultivated in the same area/environment are cross-exposed to inoculum harboured by each of the species in a latent native-to-native interaction.





The Atatürk Arboretum

- These assumptions have been tested in one of the largest and biodiverse arboretum established in Istanbul with a collection of hundreds of different tree species from different continents, each of which characterized for the geographic origin of the propagation material.
- The tree collection in the arboretum is organized according to large taxonomic groups. As a consequence related species from different continents grow together in the same plot in strict contact, favouring interactions and inter-changes



Revealing novel interactions between oak and *Tubakia* species: evidence of the efficacy of the sentinel arboreta strategy

Carmen Morales-Rodríguez · Giorgia Bastianelli · MariaPia Aleandri ·
H. Tuğba Doğmuş-Lehtijärvi · Funda Oskay · Andrea Vannini 

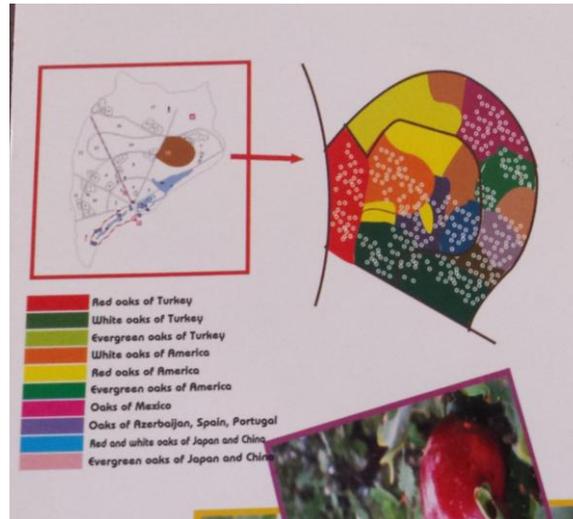
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Abstract In the present study, the sentinel arboreta strategy was applied, and its efficacy was evaluated at the Atatürk Arboretum (Istanbul, Turkey), having as a

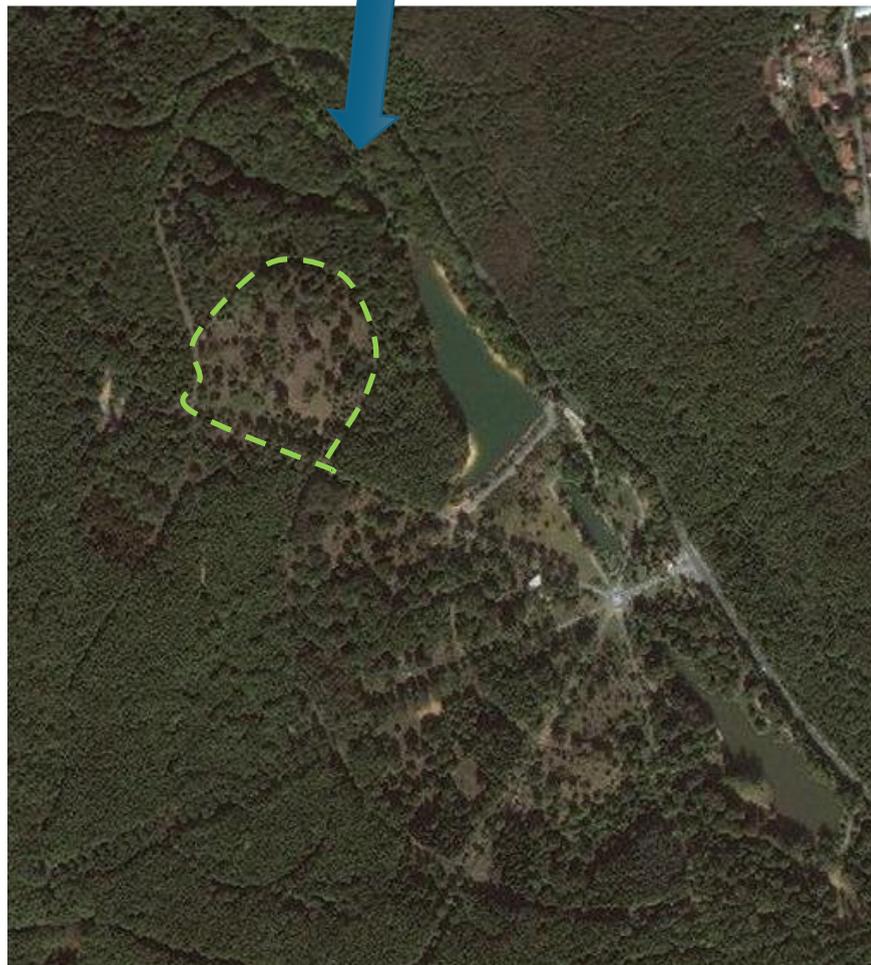
possible movement across geographic areas of these species and on the risk posed in case of introduction in the distribution range of susceptible host species. As a



Oak collection

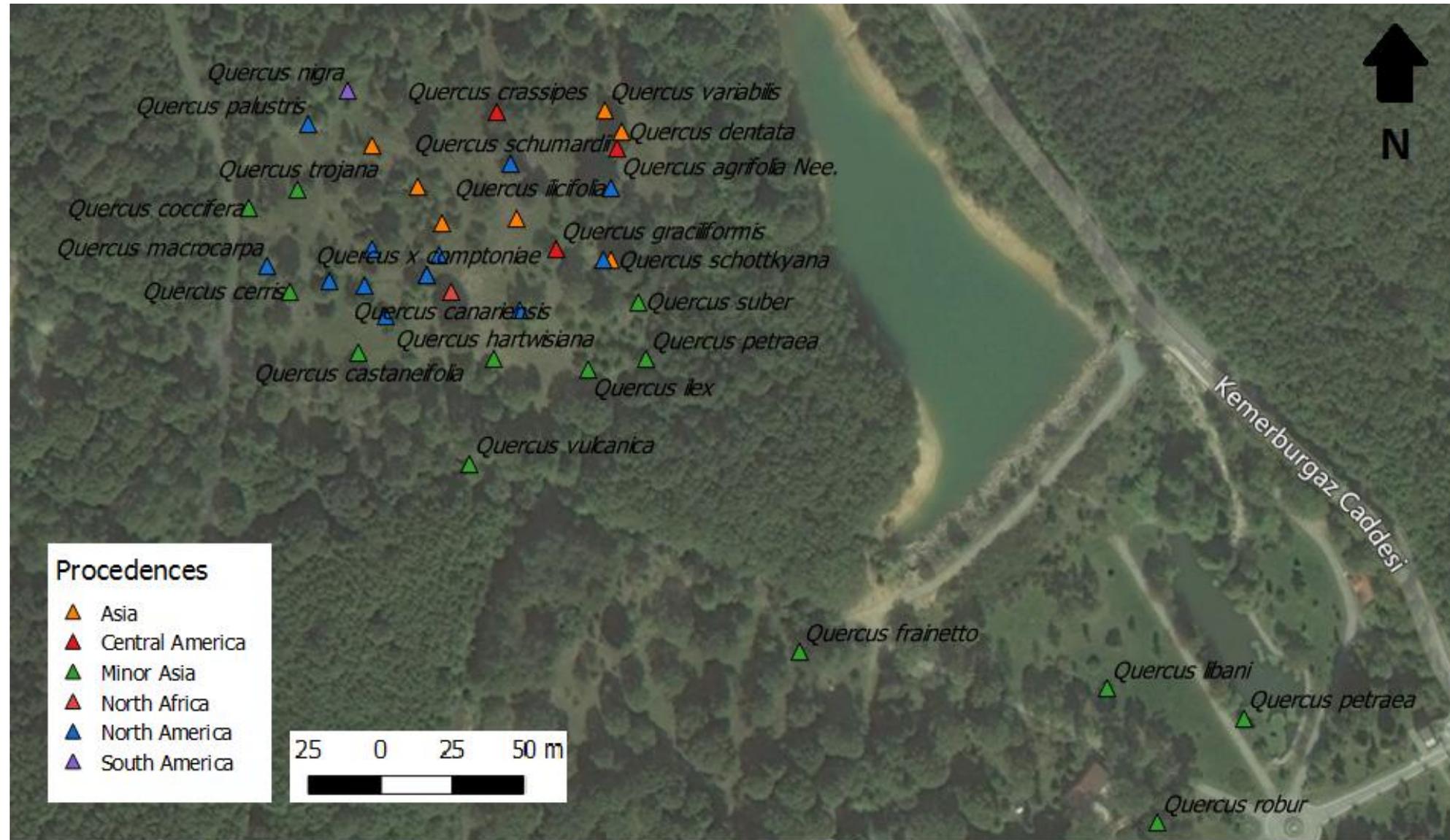


Oak exhibition plot with a size of 2.5 ha at the arboretum



- This oak plantation consists in more than hundred different species started from seedlings grown in the arboretum nursery from about 50 different arboreta and botanical gardens around the world.
- In this space; red, white and evergreen oak of Turkey share the same environment with white, red and evergreen oaks of America, Asia and Europe.

The Atatürk Arboretum



Totally 38 *Quercus* spp. were sampled: 18 from America (North, Central and South), 6 from Asia (China and Japan), 13 from Asia Minor and 1 from North Africa (Canary Islands)

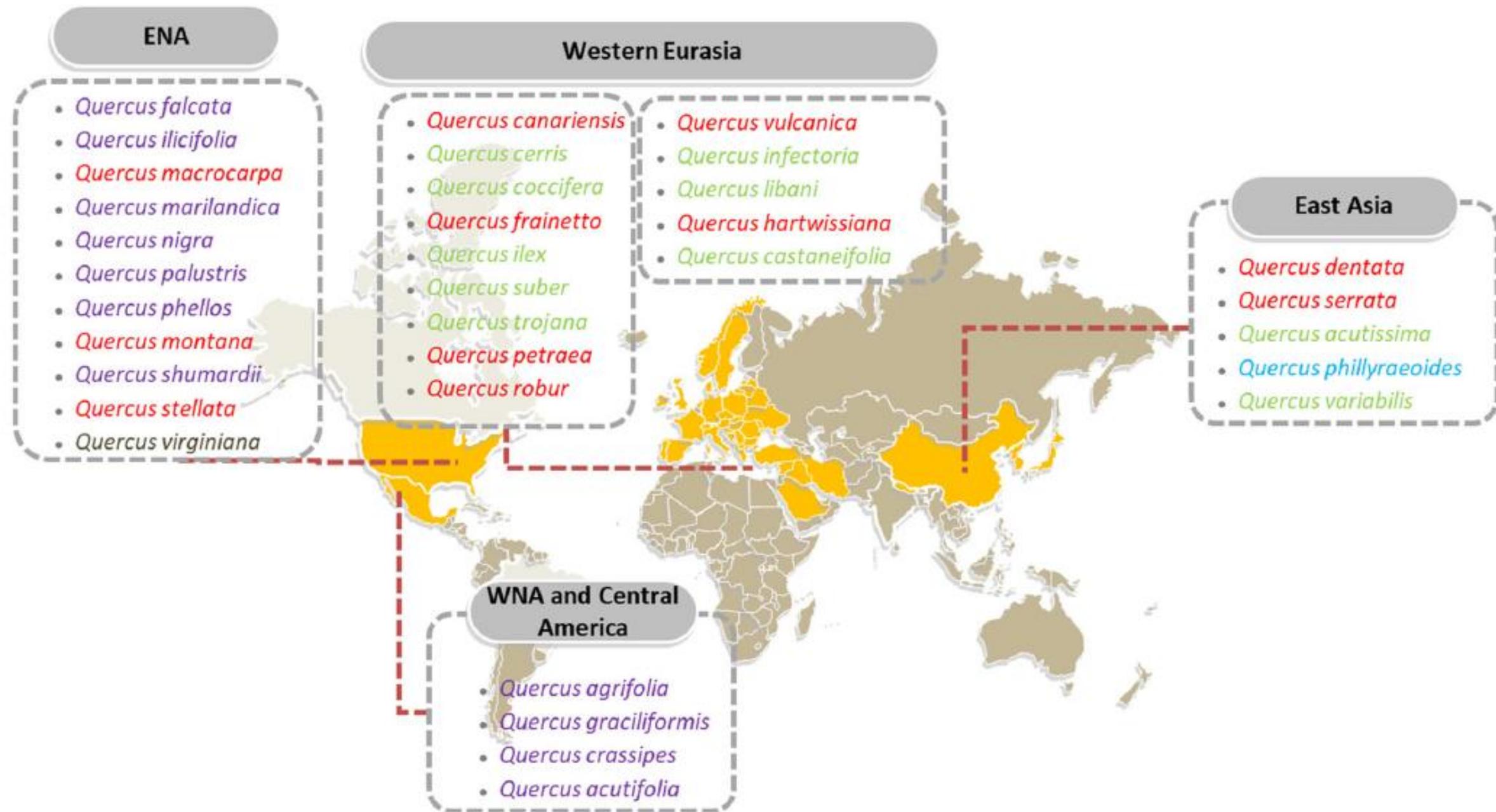
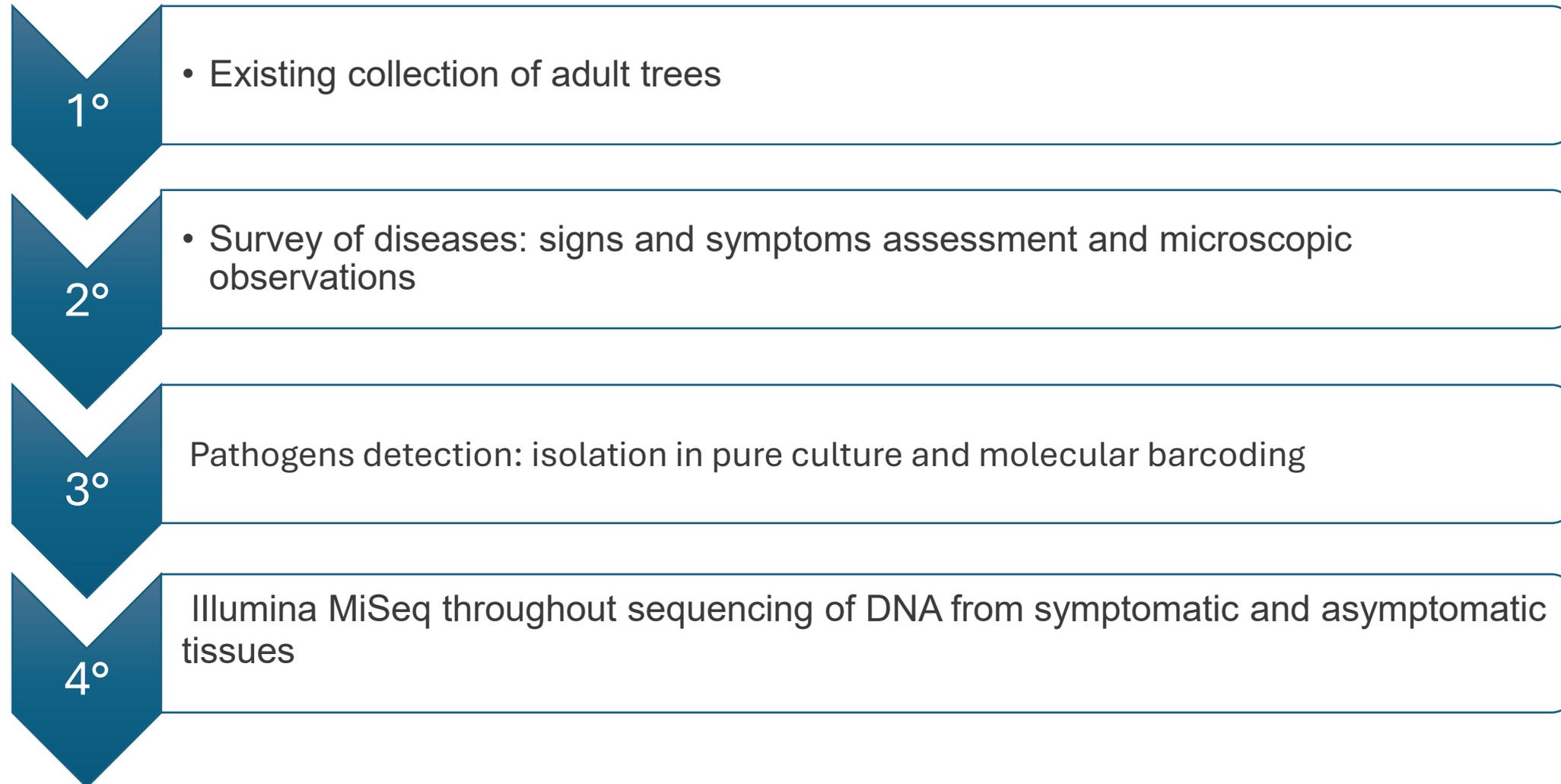


Fig. 2 Geographic distribution of the 34 oak species sampled at the Atatürk Arboretum. Colors refer to the section: Lobatae (purple); Quercus (red); Cerris (green); Ilex (blue), and Virentes (brown)

Sentinel arboreta



Symptoms/signs assessment

- General appearance of the tree:
 - Decline
 - Presence of fruiting body on the base of the trunk
 - Growth anomalies
 - Trunk: presence of cankers
- Assessment and record of foliar, shoot and branch symptoms present on each of the oak tree in the plot.

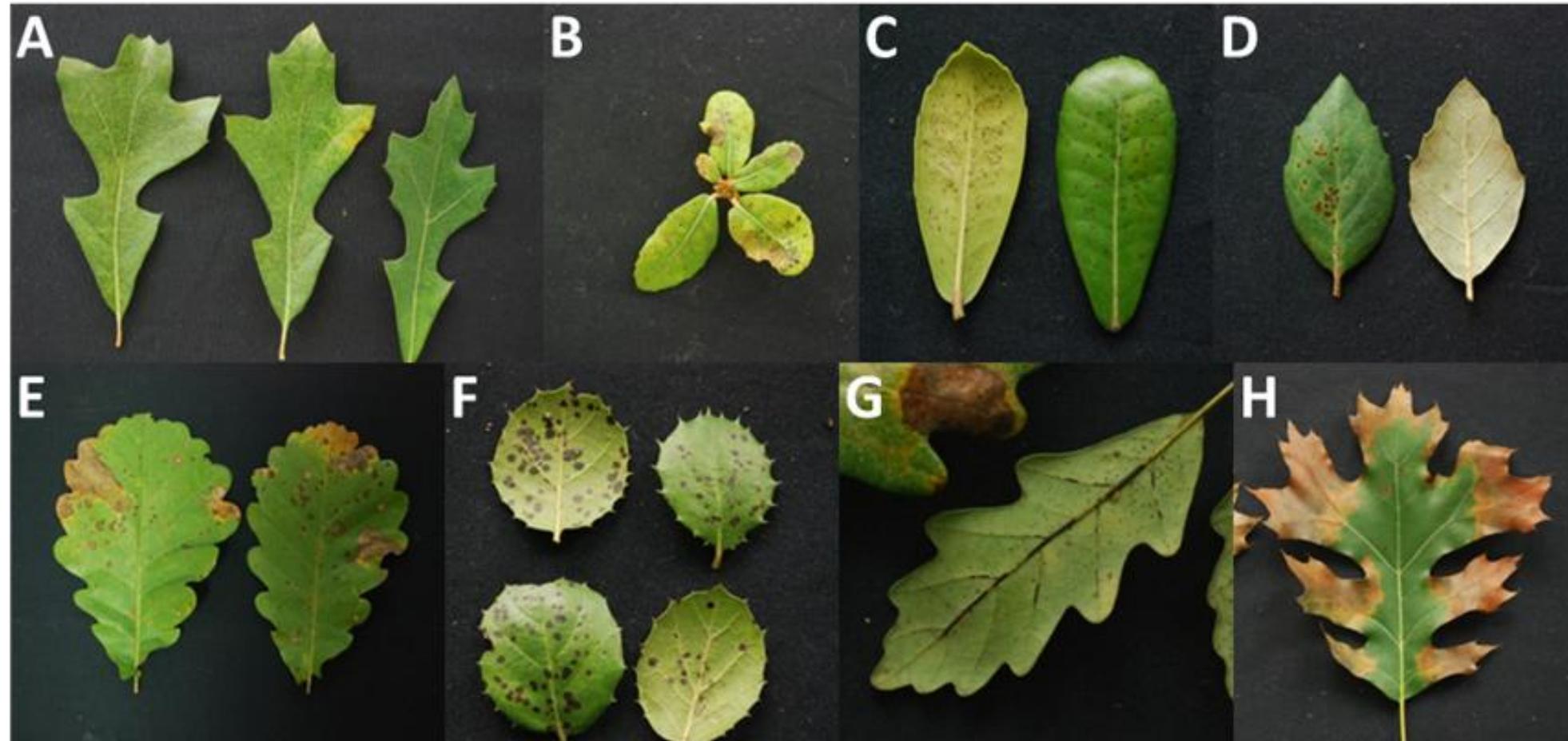


Symptoms/signs assessment

- From each species symptomatic and non-symptomatic leaves and/or shoots and bark were taken from different trees.
- The material was divided in two sub-samples: one for isolation and other for the Illumina MiSeq analysis. The samples were kept at the Faculty of Forestry, Istanbul University.



- The symptoms on leaves and twig/branch for each host species were described and recorded by taking photographs.



A. Discoloration on *Q. graciliformis*. **B.** Deformation on *Q. phillyraeoides*. **C.** Mottling on *Q. phillyraeoides*. **D.** Spots *Q. suber*. **E.** Leaf blotch on *Q. hartwisiana*. **F.** Tar spot on *Q. agrifolia*. **G.** Necrosis on veins of *Q. petraea*. **H.** Leaf Scorch on *Q. schumardii*.

Isolation in pure culture

- Samples from were processed as follow:
 - Small fragments were obtained (1 square cm max) at the interface of healthy and necrotic tissues
 - Fragments were sterilized as follow, 1 min in 75% EtOH; 3 min in 2% sodium hypochloride; 30 s in 75% EtOH; 3 washings in sdH₂O.
 - After sterilization the fragments were cutted in smaller pieces and plated onto PDAsa.
 - Plates were incubated at 24°C



DNA barcoding: Sanger sequencing

Isolates were grown in PDB, the mycelia were harvested, lyophilized and grinded with a ball mills. The genomic DNA was extracted from 100 mg of mycelium using the Nucleospin Plant II kit according to the manufacturer's protocols.

The variable internal transcribed spacer 1 (ITS1) was amplified according to White et al. (1990). The primer pairs for amplifying internal transcribed spacers were ITS1 and ITS4.

The β -tubulin gene was amplified according to O'Donnell et al. (1997). The primer pairs for amplifying β -tubulin gene were T1 and T2.

The sequencing is being performed by GATC Biotech (Germany)

Fungal identification

- 118 isolates

Procedence	Host	Fungal
Minor Asia	<i>Quercus petrea</i>	<i>Pestalotiopsis sp.</i>
		<i>Tubakia dryina</i>
		<i>Ustilaginoidea virens</i>
	<i>Quercus hartwisiana</i>	<i>Preussia intermedia</i>
		<i>Aspergillus sp.</i>
		<i>Nigrospora sp.</i>
	<i>Quercus vulcanica</i>	<i>Diplodia corticola</i>
		<i>Discula quercina</i>
		<i>Tubakia dryina</i>
		<i>Cladosporium cladosporioides</i>
		<i>Cladosporium ramotenellum</i>
	<i>Quercus castaneifolia</i>	<i>Cladsporium sp.</i>
	<i>Quercus castaneifolia</i>	<i>Phyllosticta capitalensis</i>
		<i>Tubakia sp.</i>
		<i>Nigrospora sphaerica</i>
	<i>Quercus infectoria (branch)</i>	<i>Epicoccum nigrum</i>
		<i>Diplodia corticola</i>
	<i>Quercus frainetto</i>	<i>Discula quercina</i>
		<i>Nigrospora sphaerica</i>
		<i>Alternaria sp.</i>
<i>Nigrospora sp.</i>		
		<i>Tubakia sp.</i>

Procedence	Host	Fungal
Minor Asia	<i>Quercus suber</i>	<i>Simplicillium lamellicola</i>
		<i>Nigrospora oryzae</i>
	<i>Quercus coccifera</i>	<i>Tubakia dryina</i>
		<i>Discula quercina</i>
	<i>Quercus trojana</i>	<i>Paraconiothyrium brasiliense</i>
		<i>Tubakia sp.</i>
		<i>Paraconiothyrium brasiliense</i>
		<i>Diaporthe sp.</i>
	<i>Quercus libani</i>	<i>Cladosporium sp.</i>
		<i>Cladosporium sp.</i>
	<i>Quercus robur</i>	<i>Phomopsis sp.</i>
		<i>Nigrospora sphaerica</i>
		<i>Cladosporium sp.</i>
		<i>Tubakia dryina</i>
		<i>Diaporthe sp.</i>
		<i>Diaporthe sp.</i>
	<i>Quercus ilex</i>	<i>Tubakia dryina</i>
		<i>Discula quercina</i>
		<i>Alternaria alternata</i>
		<i>Cosmospora butyri</i>

Fungal identification

Procedence	Host	Fungal
North America	<i>Quercus lyrata</i>	<i>Alternaria alternata</i>
		<i>Diaporthe sp.</i>
		<i>Penicillium tricolor</i>
	<i>Quercus ilicifolia</i>	<i>Tubakia sp.</i>
		<i>Pestalotiopsis sp.</i>
		<i>Discula quercina</i>
		<i>Paraphaeosphaeria sporulosa</i>
		<i>Alternaria alternata</i>
		<i>Phyllosticta capitalensis</i>
		<i>Nigrospora sphaerica</i>
		<i>Cladosporium sp</i>
	<i>Alternaria sp.</i>	
	<i>Quercus stellata</i>	<i>Monochaetia kansensis</i>
		<i>Simplicillium lamellicola</i>
		<i>Monochaetia kansensis</i>
		<i>Cladosporium sp.</i>
		<i>Discula quercina</i>

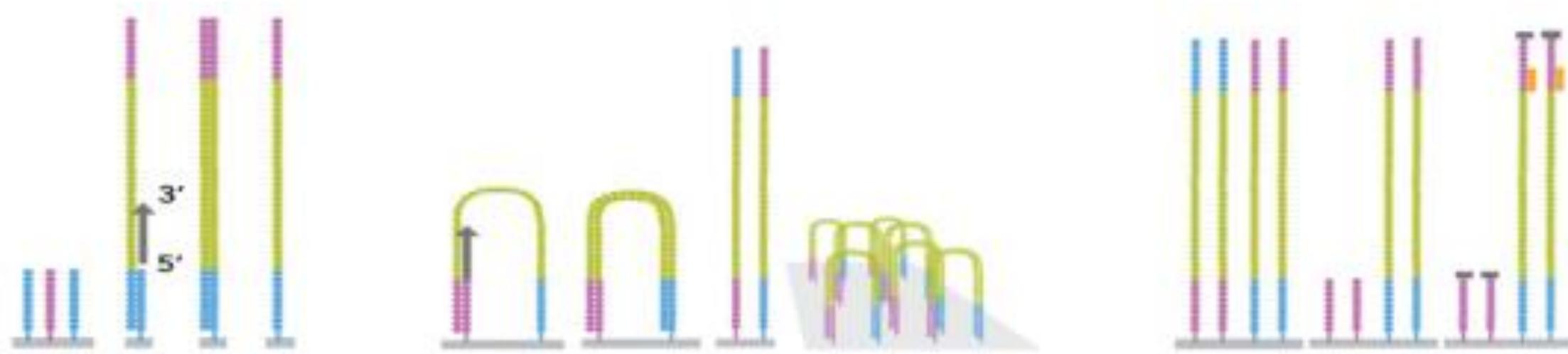
Procedence	Host	Fungal
North America	<i>Quercus schumardii</i>	<i>Phyllosticta capitalensis</i>
	<i>Quercus macrocarpa</i>	<i>Alternaria alternata</i>
		<i>Tubakia sp.</i>
		<i>Alternaria tenuissima</i>
		<i>Epicoccum nigrum</i>
	<i>Quercus marilandica</i>	<i>Tubakia seoraksanensis</i>
		<i>Alternaria alternata</i>
		<i>Nigrospora sphaerica</i>
	<i>Quercus virginiana</i>	<i>Diaporthe foeniculina</i>
		<i>Penicillium tricolor</i>
		<i>Discula quercina</i>
		<i>Alternaria alternata</i>
	<i>Quercus x comptoniae</i>	<i>Alternaria alternata</i>
		<i>Epicoccum nigrum</i>
	<i>Quercus falcata</i>	<i>Alternaria alternata</i>
		<i>Epicoccum nigrum</i>
<i>Quercus palustris</i>	<i>Nigrospora sphaerica</i>	
	<i>Simplicillium lamellicola</i>	
<i>Quercus phellos</i>	<i>Aureobasidium pullulans</i>	

Fungal identification

Procedence	Host	Fungal
Central America	<i>Quercus agrifolia</i>	<i>Simplicillium lamellicola</i>
		<i>Nigrospora sp.</i>
	<i>Quercus crassipes</i>	<i>Phomopsis sp.</i>
		<i>Pestalotiopsis sp.</i>
	<i>Quercus acutifolia</i>	<i>Tubakia sp.</i>
		<i>Tubakia sp.</i>
	<i>Quercus graciliformis</i>	<i>Tubakia dryina</i>
<i>Pestaliopsis sp.</i>		
South America	<i>Quercus nigra</i>	<i>Phyllosticta capitalensis</i>

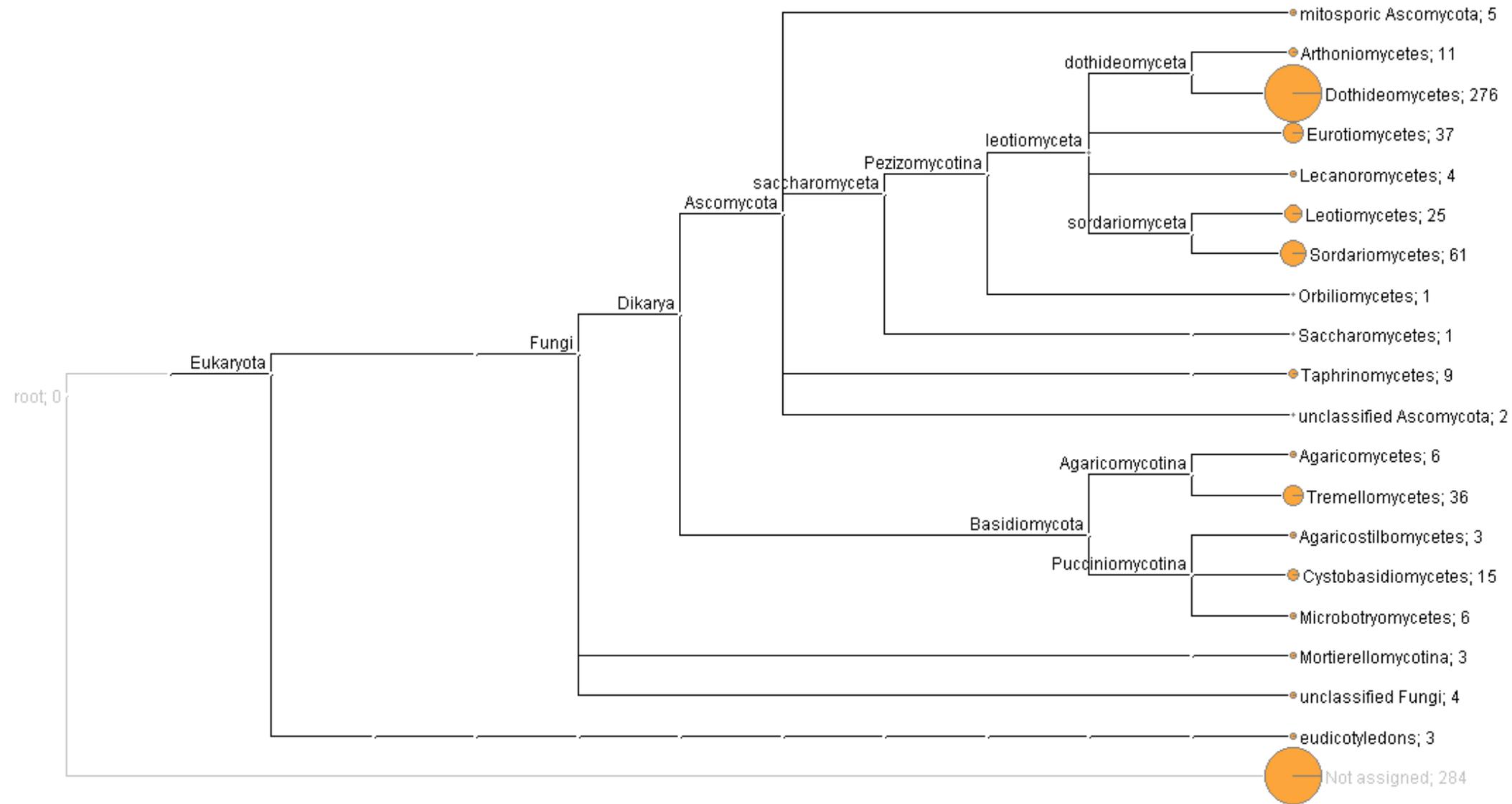
Procedence	Host	Fungal
North Africa	<i>Quercus canariensis</i>	<i>Tubakia sp</i>

Procedence	Host	Fungal
Asia	<i>Quercus dentata</i>	<i>Cladosporium sp.</i>
		<i>Diaporthe eres</i>
		<i>Tubakia sp.</i>
		<i>Tubakia sp.</i>
		<i>Discula quercina</i>
	<i>Quercus glandurifera</i>	<i>Cladosporium sp</i>
		<i>Epicoccum nigrum</i>
		<i>Nigrospora sphaerica</i>
	<i>Quercus variabilis</i>	<i>Cladosporium sp.</i>
	<i>Quercus acutissima</i>	<i>Nigrospora sp.</i>
	<i>Quercus schottkyana</i>	<i>Alternaria sp.</i>
		<i>Alternaria alternata</i>
		<i>Diaporthe rudis</i>
		<i>Pestalotiopsis sp.</i>
		<i>Pleosporales</i>
		<i>Epicoccum nigrum</i>
		<i>Simplicillium lamellicola</i>
	<i>Quercus phillyraeoides</i>	<i>Nigrospora sphaerica</i>
		<i>Biscogniauxia nummularia</i>
		<i>Discula quercina</i>
<i>Pestaliopsis sp.</i>		
<i>Cladosporium sp.</i>		
<i>Penicillium chrysogenum</i>		



Illumina MiSeq DNA mass-sequencing

- Symptomatic tissues were lyophilized and grinded with a ball mills. The DNeasy Plant Mini Kit (Quiagen, Germany) was used for the DNA extraction.
- Amplification of the ITS1 with the primers ITS1F and ITS2 both marked at its ends with a different tag sequence.
- IlluminaMiSeq sequencing was done for the company Eurofins Genomic (Germany)

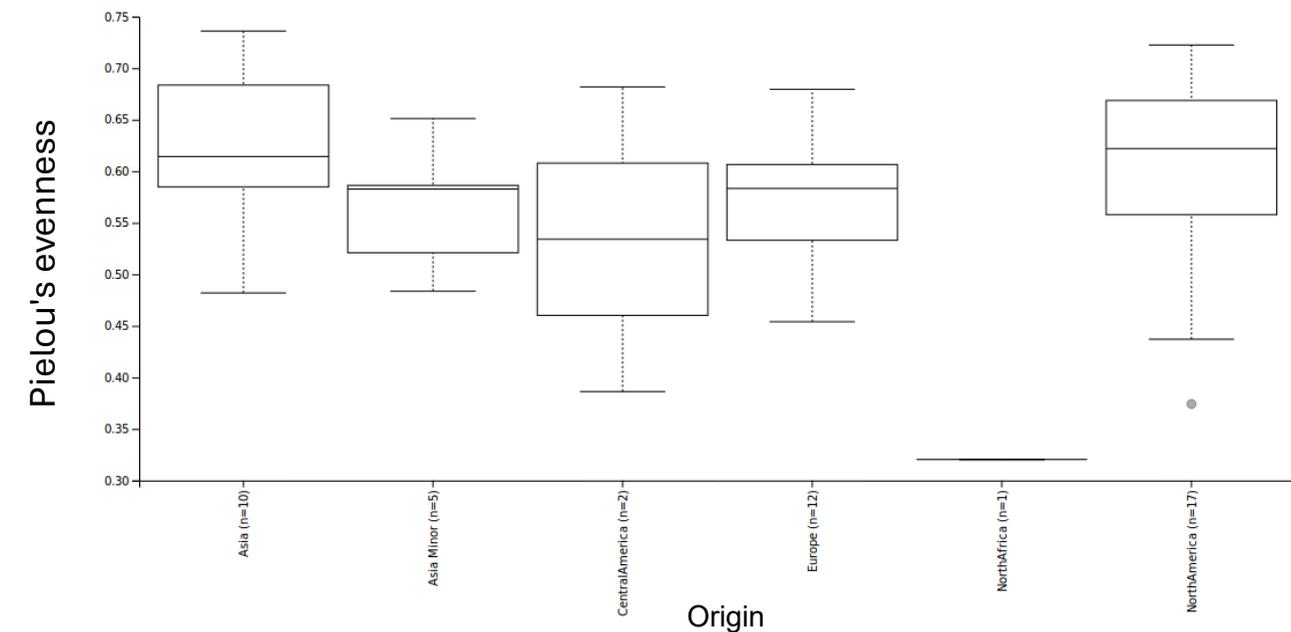


NGS analysis of the fungal community

- More than 3.5 millions of reads passed the trimming and reads preparation
- After the chimera filtering, singletons removed and with a 98% in the clustering process **793 OTUs** were assigned.

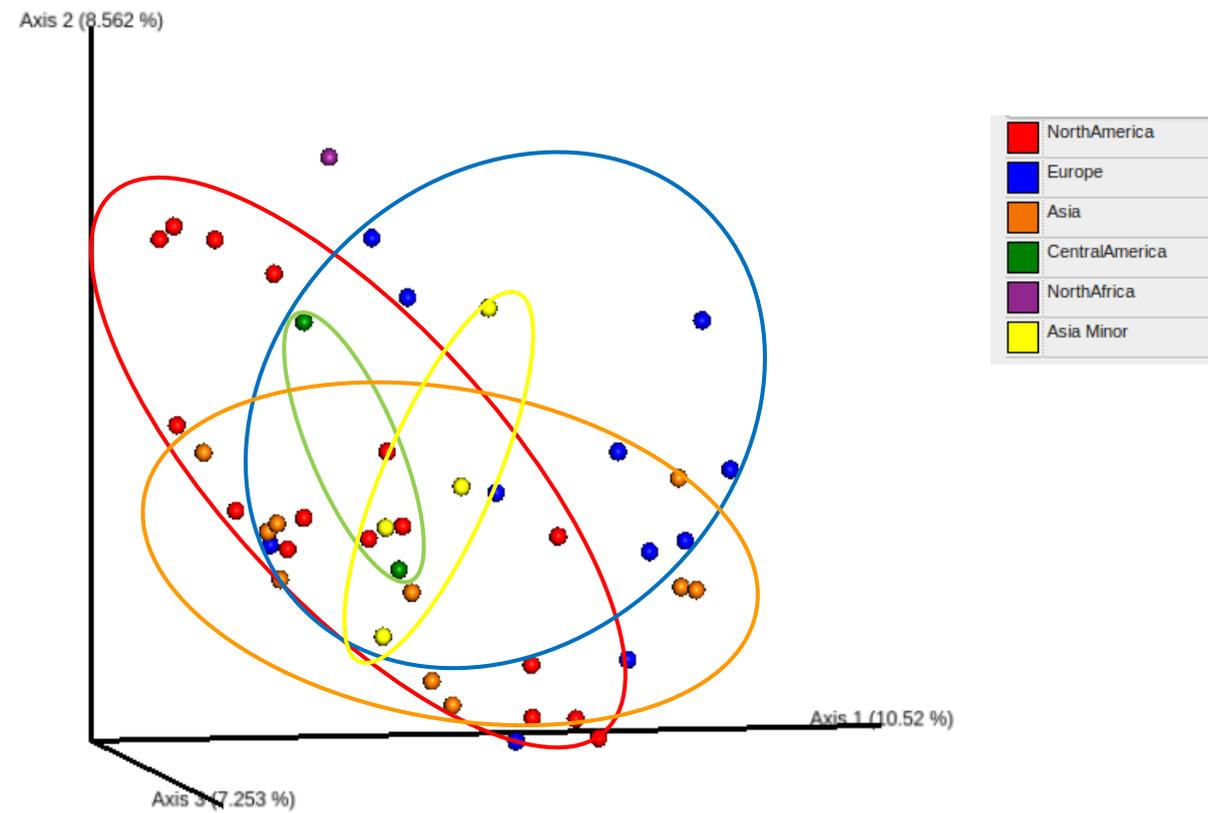
NGS analysis of the fungal community

- **Alpha diversity:** The evenness of the samples were similar and no significant differences ($P < 0.05$) between the continent of origin of the *Quercus* species were found

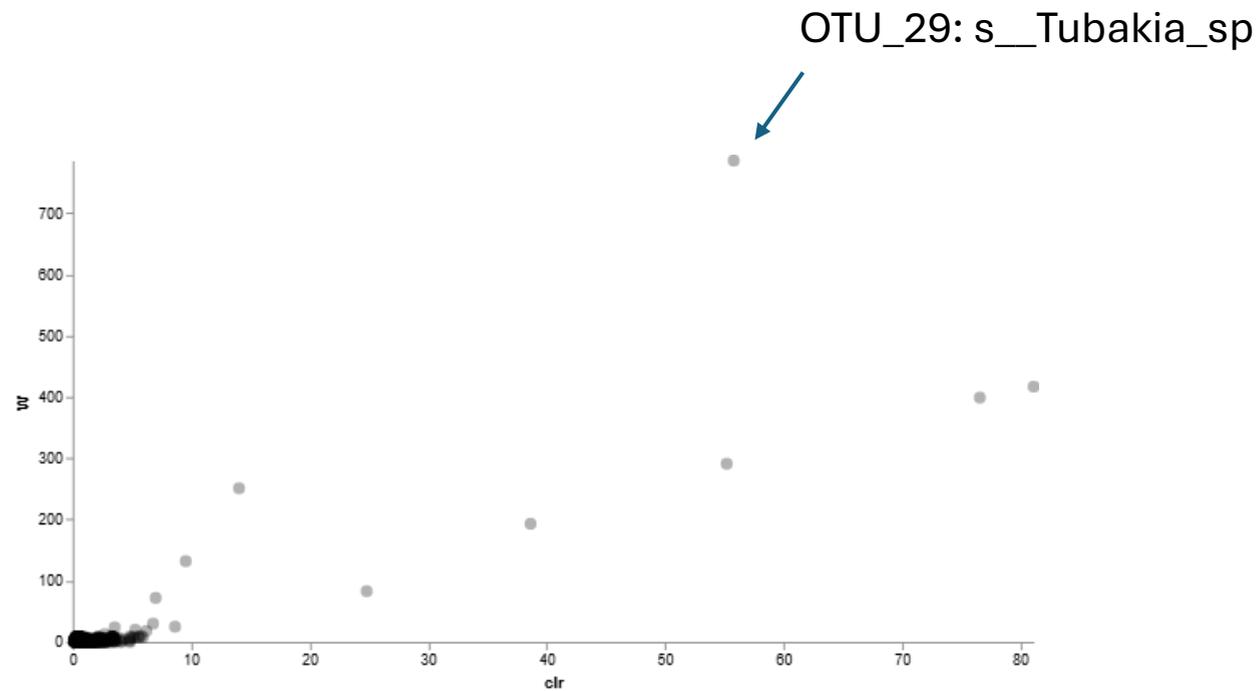


NGS analysis of the fungal community

- Beta diversity: Jaccard distance (a qualitative measure of community dissimilarity)



ANCOM Volcano plot (Analysis of Composition of Microbiomes)



- **X-axis (CLR mean difference or effect size):** represents the centered log-ratio difference in abundance between two groups.
- **Y-axis (W-statistic or detection level):** indicates how many pairwise comparisons were significantly different for each taxon.

- ✓ Each point represents a microbial taxon (OTU).
- ✓ Points that are far from zero on the X-axis and high on the Y-axis are taxa with strong and consistent differential abundance → **these are candidates for biomarkers.**

ANCOM statistical results

	W
OTU_29	786

Practical case: *Tubakia* sp.

- The assumption: the close contact **in the same arboretum of tree species from different areas in the world** could facilitate **host-shift** by potential plant pathogens and expression of novel host-pathogen interactions.



Practical case: *Tubakia* sp.

- ▶ Tubakia leaf spot (formerly called Actinopelte leaf spot), is a common late-season fungal disease of oaks.
- ▶ Symptoms include small to large dark brown or reddish-brown spots or blotches. Spotting that occurs on leaf veins may cause large extended areas of dead leaf tissue along the veins. If trees are heavily infected with Tubakia leaf spot, premature defoliation may occur.



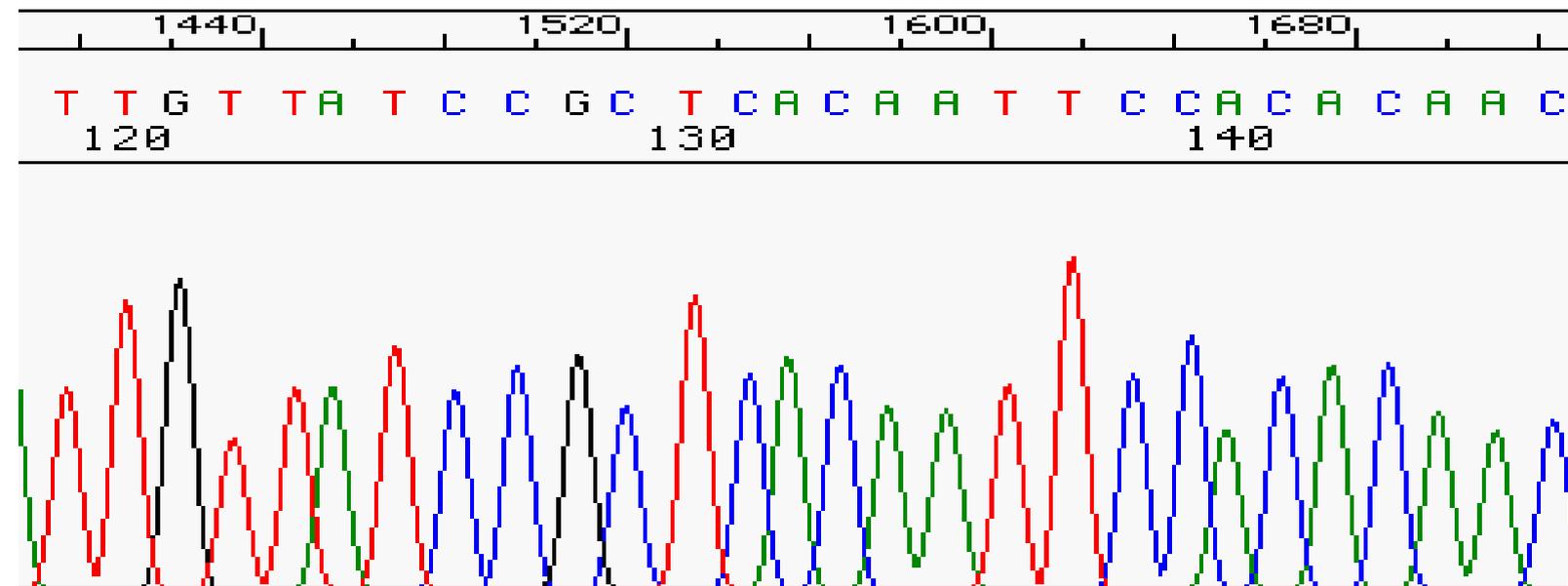
Red oak leaves with spots. *Tubakia dryina*

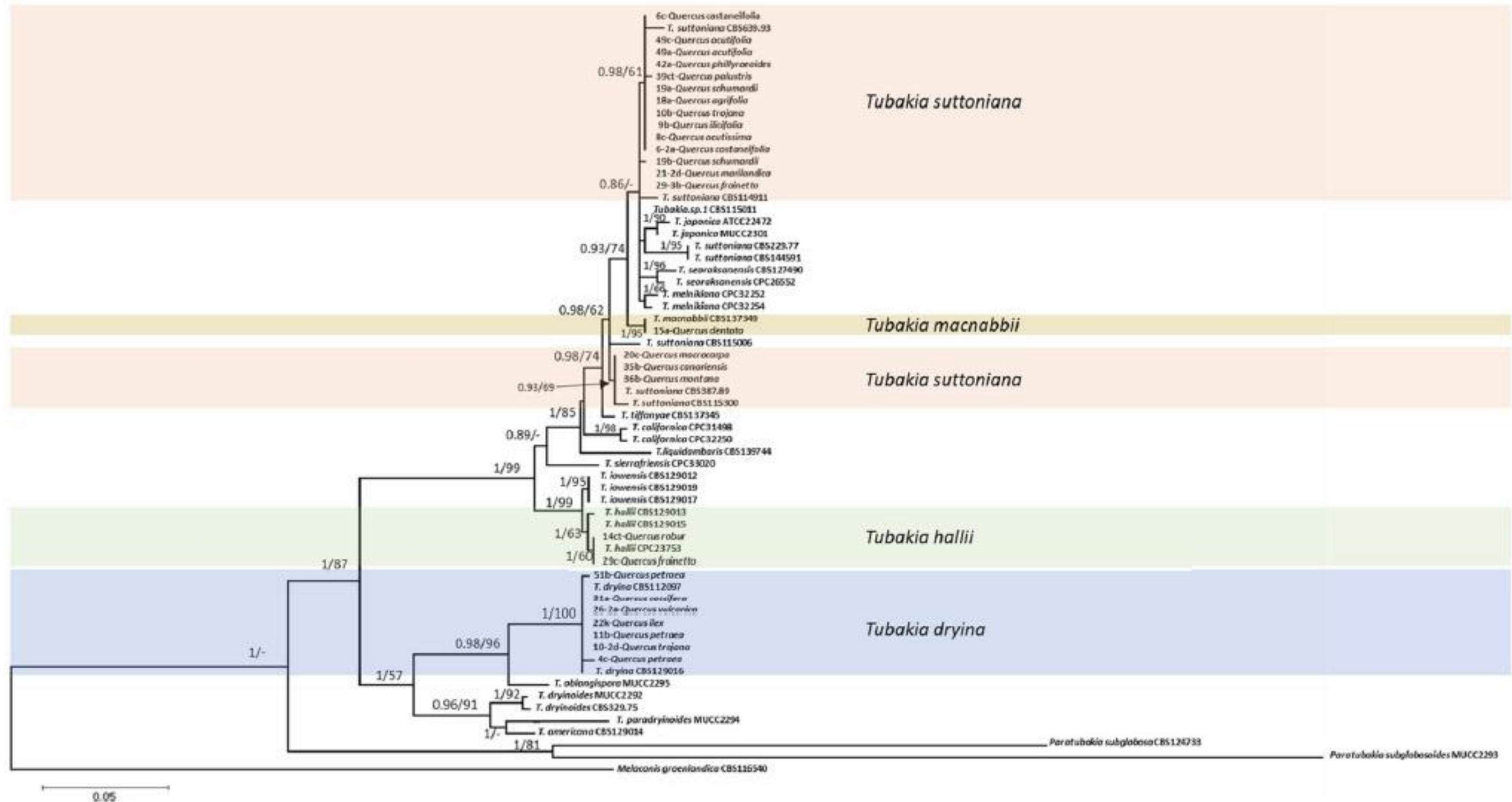


Burn oak blight. *Tubakia iowensis*

Practical case: *Tubakia* sp.

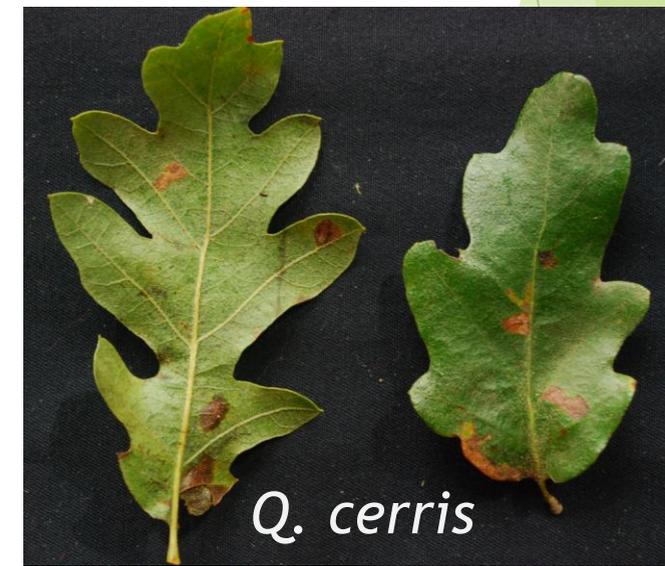
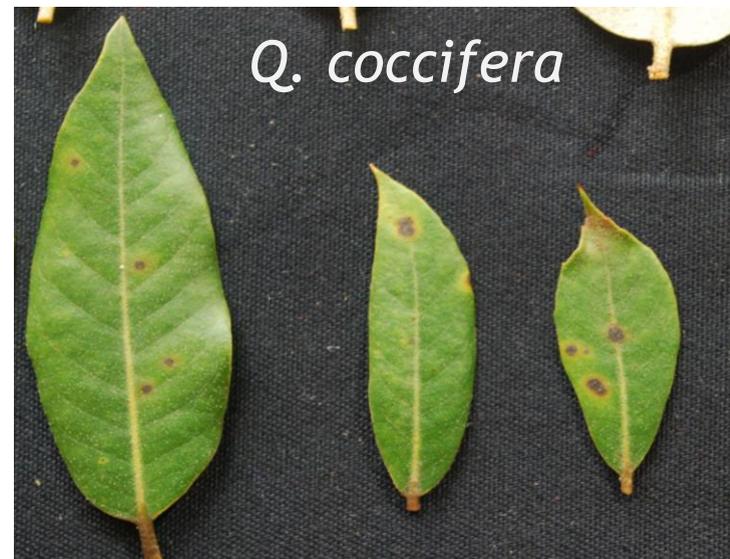
- ▶ *Tubakia* cultures were among the most abundant obtained from isolation activities
- ▶ Sequences were obtained from *Tubakia* pure cultures: ITS, Tef1 and Btub
- ▶ Additional sequences from Genbank were downloaded, jModelTest was used to determine the best nucleotide substitution model and phylogenetic trees were done for the isolates identification.





Tubakia dryina (Sacc.) B. Sutton

- ▶ Only isolated from European species.
- ▶ *Host range and distribution: on Fagus sylvatica, Quercus alba, Q. macrocarpa, Q. robur):*
First records on *Q. ilex*, *Q. coccifera*, *Q. vulcanica* and *Q. trojana*
- ▶ Europe (Germany, Italy, Netherlands, Poland, Romania, Russia, UK), North America (USA), Mexico and New Zealand. ***First record in Turkey***



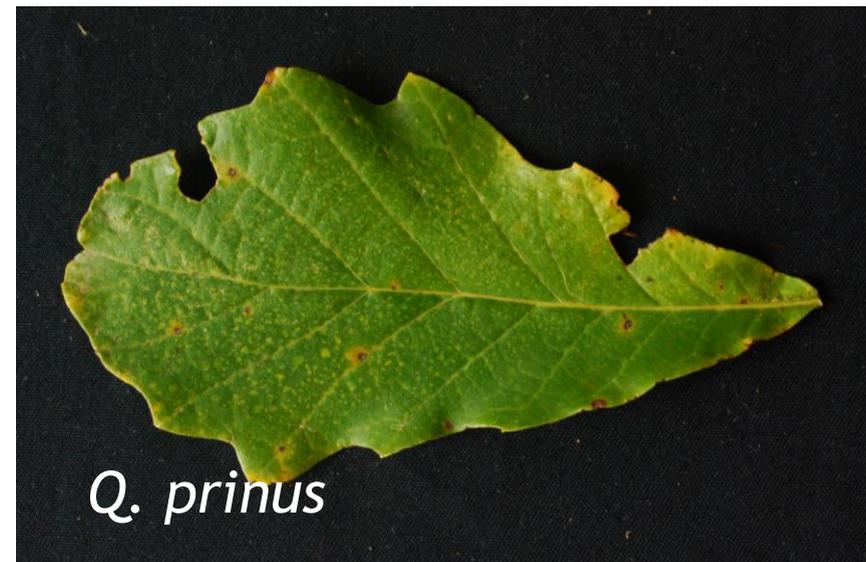
Tubakia macnabbii T.C. Harr.& McNew

- ▶ *Host range:* On *Castanea* sp., *Quercus* (*alba*, *hemisphaerica*, *imbricaria*, *kelloggii*, *laurifolia*, *macrocarpa*, *marilandica*, *muehlenbergii*, *nigra*, *palustris*, *rubra*, *stellata*, *velutina*, *virginiana*). **First record in *Q. dentata* (Asia)**
- ▶ *Distribution:* North America (USA, Arkansas, Florida, Illinois, Iowa, Kansas, Louisiana, Maryland, Minnesota, Missouri, New Hampshire, Ohio, Oklahoma, Wisconsin). **First record in Turkey**



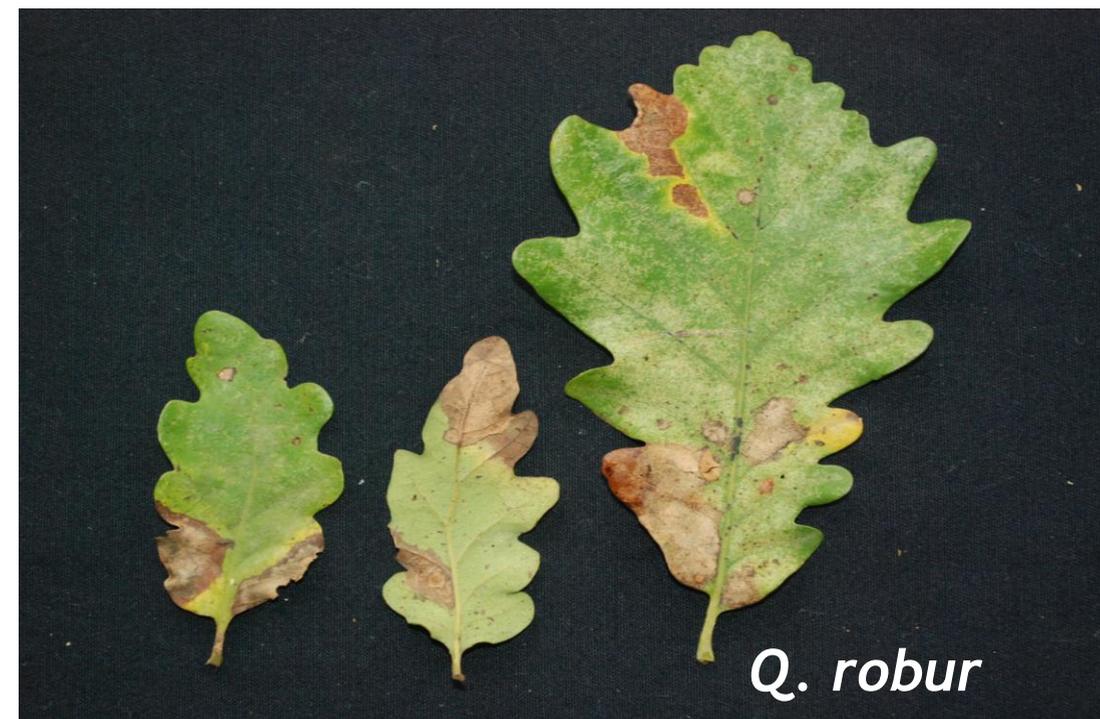
Tubakia suttoniana U.Braun & Crous

- ▶ *Host range* :*Quercus cerris*, *Q. robur* and *Q. rubra*. *First records in Q. macrocarpa and Q. prinus=Q. montana (North America) , Q. canariensis (North Africa), Quercus phillyraeoides (East Asia), Quercus frainetto (Western Eurasia)*
- ▶ *Distribution: Italy, Netherlands and New Zealand. First record in Turkey*



Tubakia hallii T.C. Harr.& McNew

- ▶ Host range and distribution: On *Quercus alba*, *Q. bicolor*, *Q. macrocarpa*, *Q. muehlenbergii*, *Q. stellata*: **First records in *Q. frainetto* and *Q. robur* (Europe and Asia Minor)**
- ▶ Distribution: North America (USA, Arkansas, Iowa, Kansas, Minnesota, Missouri, Wisconsin). **First record in Turkey**



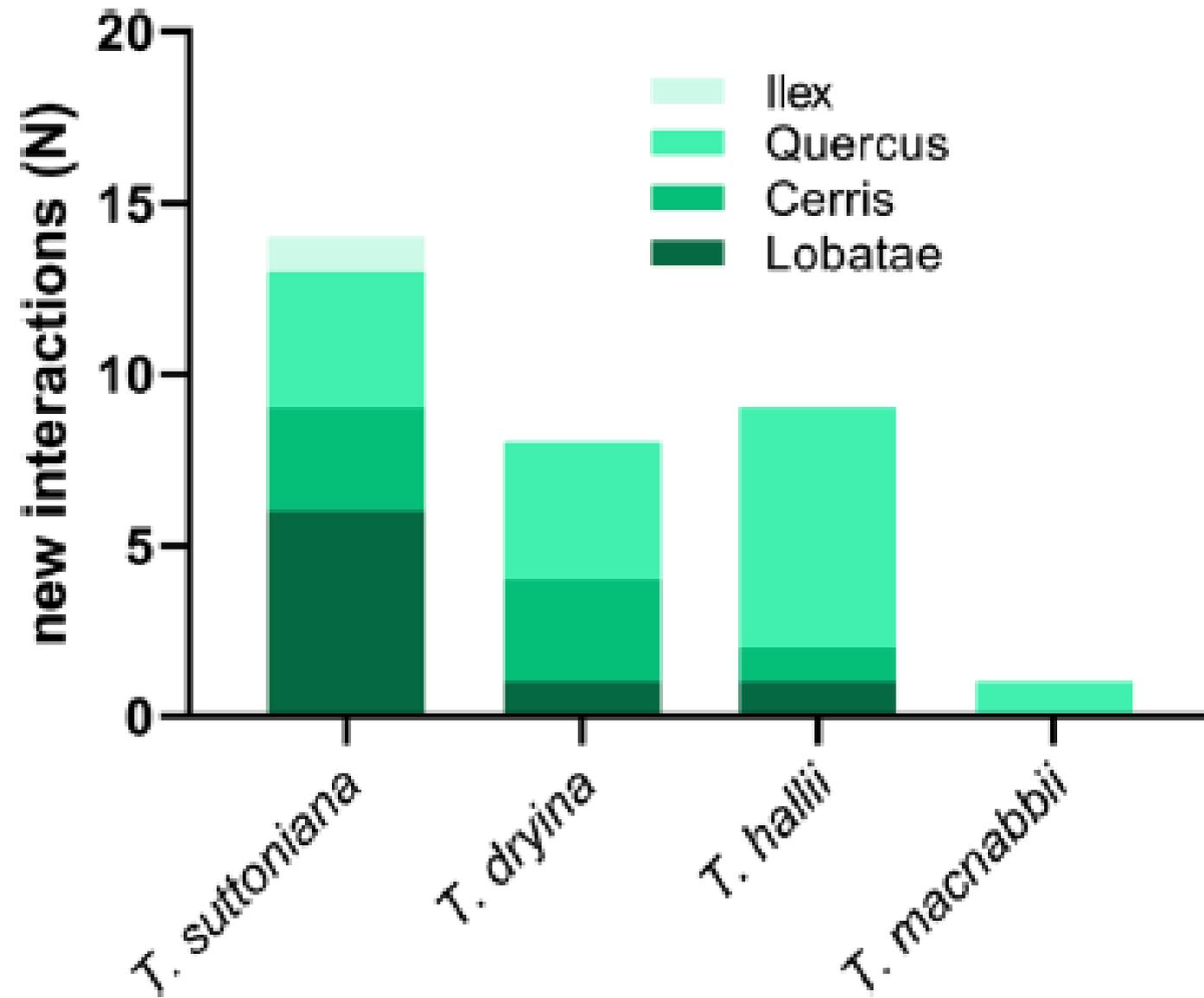


Fig. 7 Number of interactions new to science per *Tubakia* sp. and per *Quercus* section determined with the isolation/detection trials from leaves at the Atatürk Arboretum

Pathogenicity test

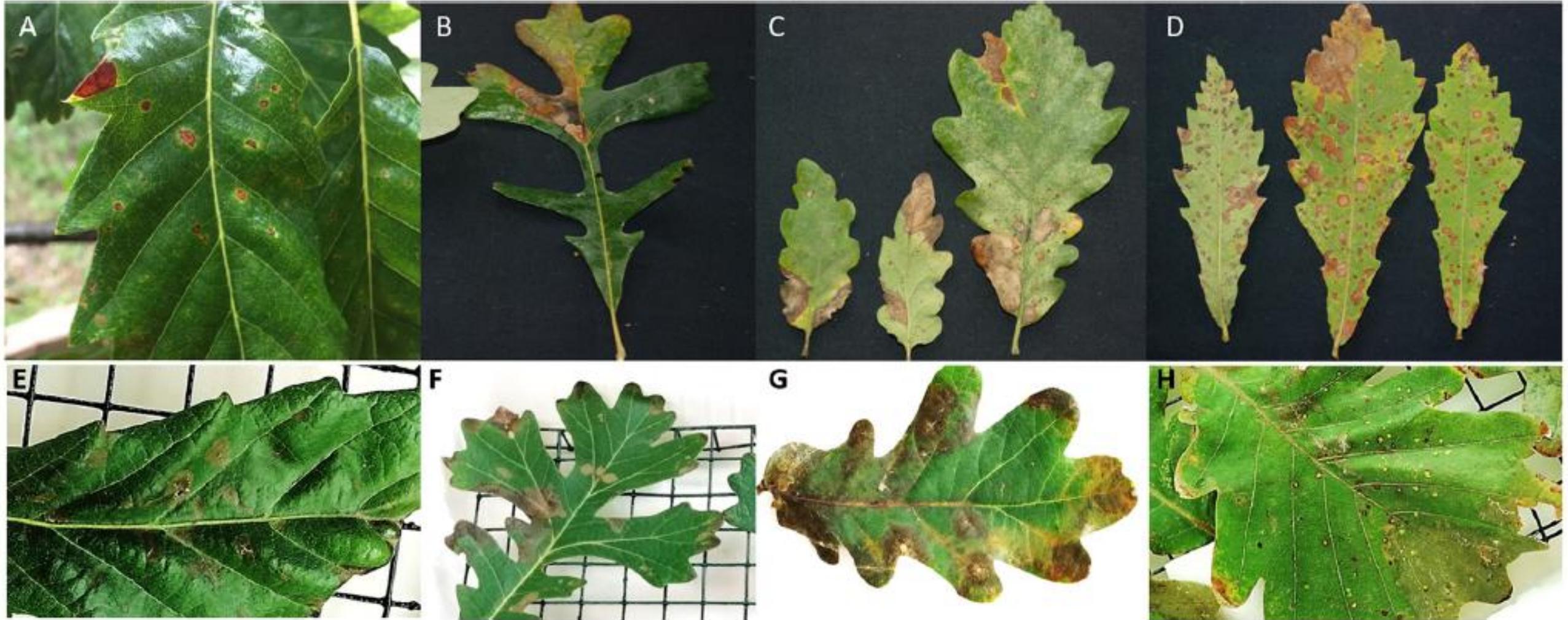


Fig. 3 Examples of leaf symptoms observed at the Atatürk Arboretum (A–D) and as results of artificial inoculation with *Tubakia* spp. (E–H). *Q. castaneifolia* (A); *Q. castaneifolia* x *T.*

suttoniana (E); *Q. macrocarpa* (B); *Q. macrocarpa* x *T. hallii* (F); *Q. robur* (C); *Q. robur* x *T. dryina* (G); *Q. dentata* (D); *Q. dentata* x *T. macnabbii* (H)

Table 2 Results of pathogenicity tests carried out with 14 combinations of 10 *Quercus* spp. and the 4 *Tubakia* spp. All the host species—*Tubakia* spp. combinations tested were

previously detected with the isolation trials from leaves of the trees at the Atatürk Arboretum

<i>Isolate/host</i>	<i>Tubakia</i> spp.	Host	Section	Symptoms	Re-isolation	Pathogenicity new to science
4c/ <i>Q. robur</i>	<i>T. dryina</i>	<i>Q. trojana</i>	Cerris	Yes	Yes	Yes
		<i>Q. robur</i>	Quercus	Yes	Yes	No
14c/ <i>Q. robur</i>	<i>T. halli</i>	<i>Q. robur</i>	<i>Quercus</i>	No	Nd	Nd
		<i>Q. macrocarpa</i>	<i>Quercus</i>	Yes	Yes	No
15a/ <i>Q. dentata</i>	<i>T. macnabbi</i>	<i>Q. dentata</i>	Quercus	Yes	Yes	Yes
		<i>Q. marilandica</i>	Lobatae	Yes	Yes	No
39ct/ <i>Q. palustris</i>	<i>T. suttoniana</i>	<i>Q. acutissima</i>	Cerris	Yes	Yes	Yes
		<i>Q. macrocarpa</i>	Quercus	Yes	Yes	Yes
		<i>Q. montana</i>	Quercus	Yes	Yes	Yes
		<i>Q. robur</i>	Quercus	Yes	Yes	Yes
		<i>Q. palustris</i>	Lobatae	Yes	Yes	Yes
		<i>Q. graciliformis</i>	Lobatae	Yes	No	Nd
		<i>Q. castaneifolia</i>	Cerris	Yes	Yes	Yes
		<i>Q. trojana</i>	Cerris	No	Nd	Nd

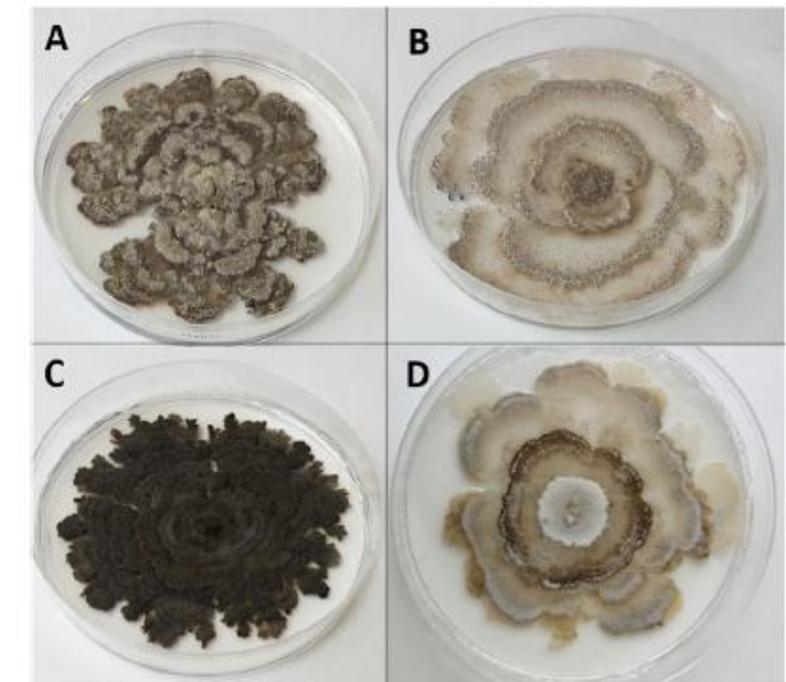


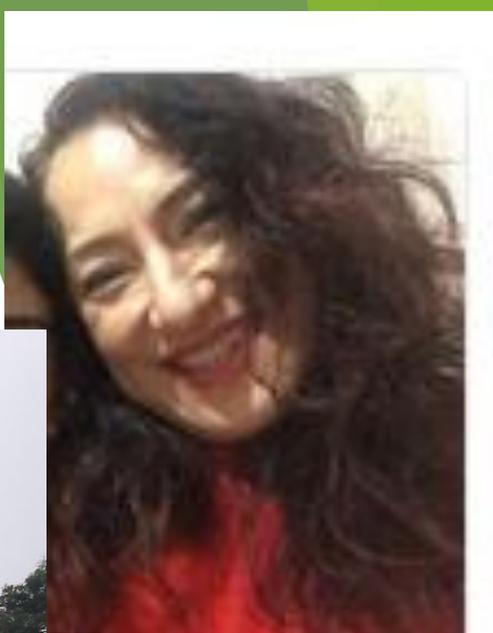
Fig. 5 Colony morphology on PDAs of the four *Tubakia* spp. Isolated from necrotic lesion on oak leaves: *Tubakia suttoniana*, isolate 39ct from *Quercus palustris* (A); *Tubakia hallii*, isolate 14c from *Quercus robur* (B); *Tubakia dryina*, isolate 4c from *Quercus robur* (C); *Tubakia macnabbi*, isolate 15a from *Quercus dentata* (D)

Conclusive remarks

- ▶ Arboreta and botanical gardens have the potentiality to act as sentinel plantations to study and identify hidden pathogens and novel host-pathogen interactions.
- ▶ Specifically native-to-exotic interactions are expected that might reveal risk for new hosts and environments
- ▶ The example of *Tubakia* sp. evidences the potentiality of the strategy
- ▶ In analogy with 'on purpose' established sentinel plantations and nurseries, arboreta and botanical gardens inspections must be considered a preliminary screening to identify pathogens for which a PRA is recommended



Warning
Pest
Disease
Sentinel
Monitor
PRA
Identification
Plant
Tree
Country
Nursery
Organism
Regulate
Database
Cost
Early
System
Import
Trade
Risk
Establish
Community
Global Network
Phytosanitary Seed
Nathal



Cost action FP1401-Global Warning

