The invasion framework helps the management of the spread of alien pathogens:

The case of canker stain disease of plane trees.

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***Ceratocystis platani* is an ascomycete causing a lethal disease affecting plane trees called "*canker stain disease*" (CSD).**

The pathogen was introduced into Europe from North America during WWII through wooden packaging of supply material of the Allied army.

CSD is currently reported in Italy, France, Switzerland, Greece, Albania and Turkey

Disease cycle

EFSA, 2021
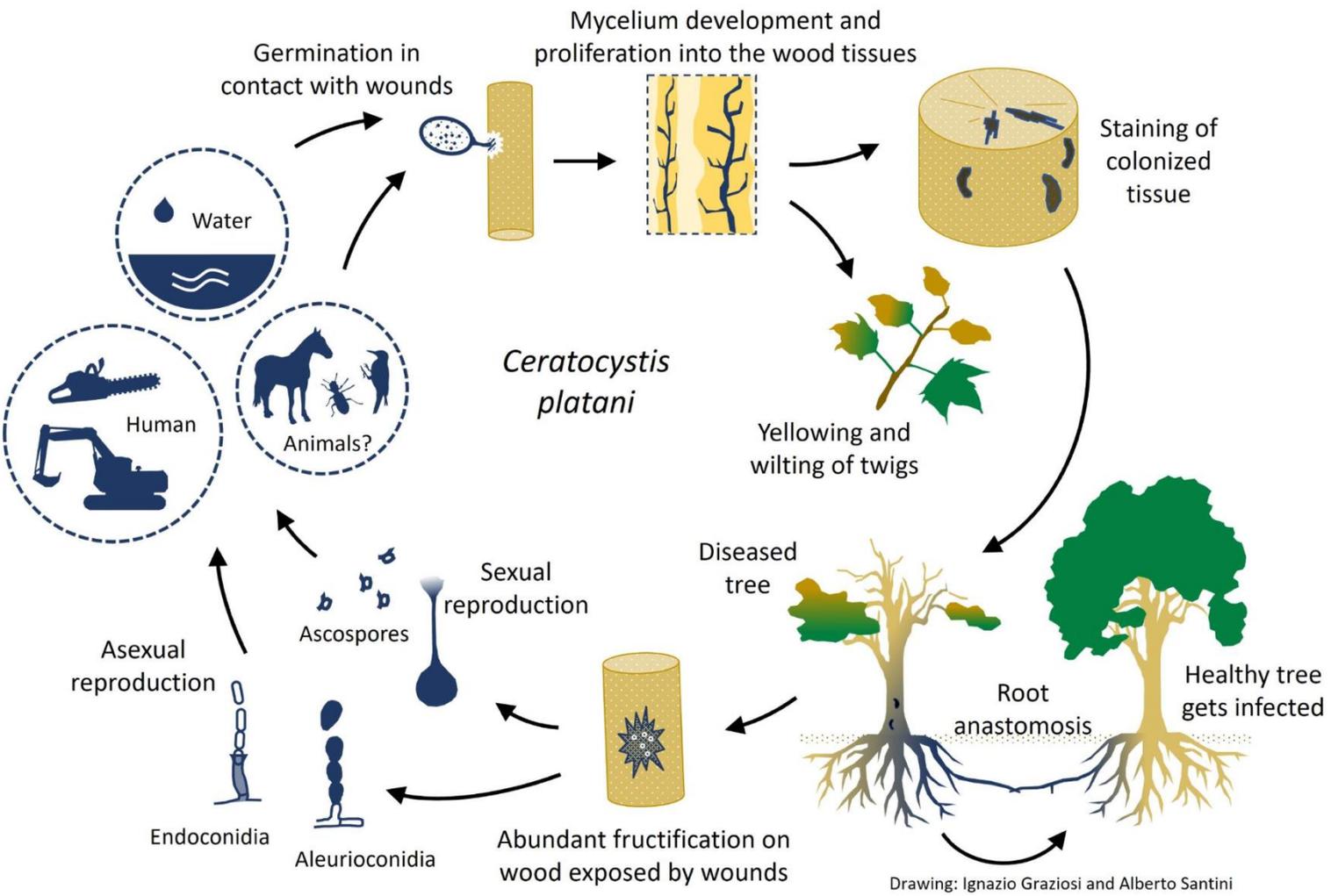
C. platani is a wound pathogen and the disease is spread mainly by human activities as pruning, terracing and road digging. CSD can spread naturally by animals and running water as well as by root anastomosis.

The pathogen is **extremely infectious** and although several eradication attempts have been implemented in all the affected countries, none have been successful.
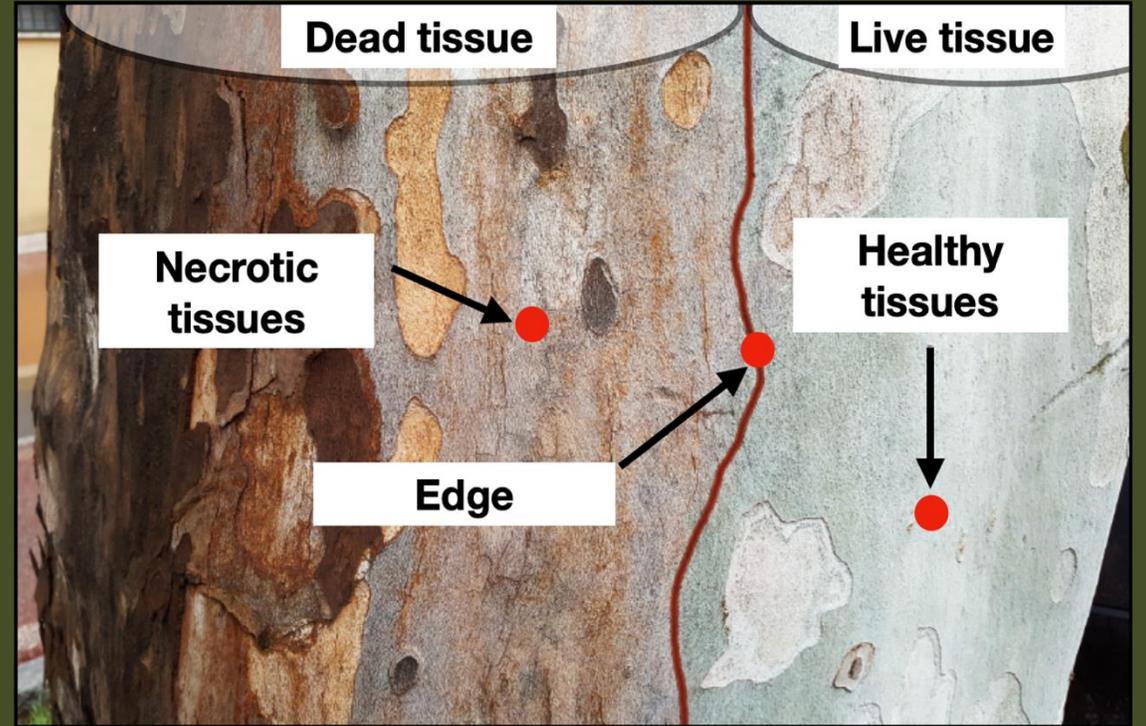
Symptomatic

Dead (soon)

Healthy

Replaced with another species

Dead tissue

Live tissue

Necrotic tissues

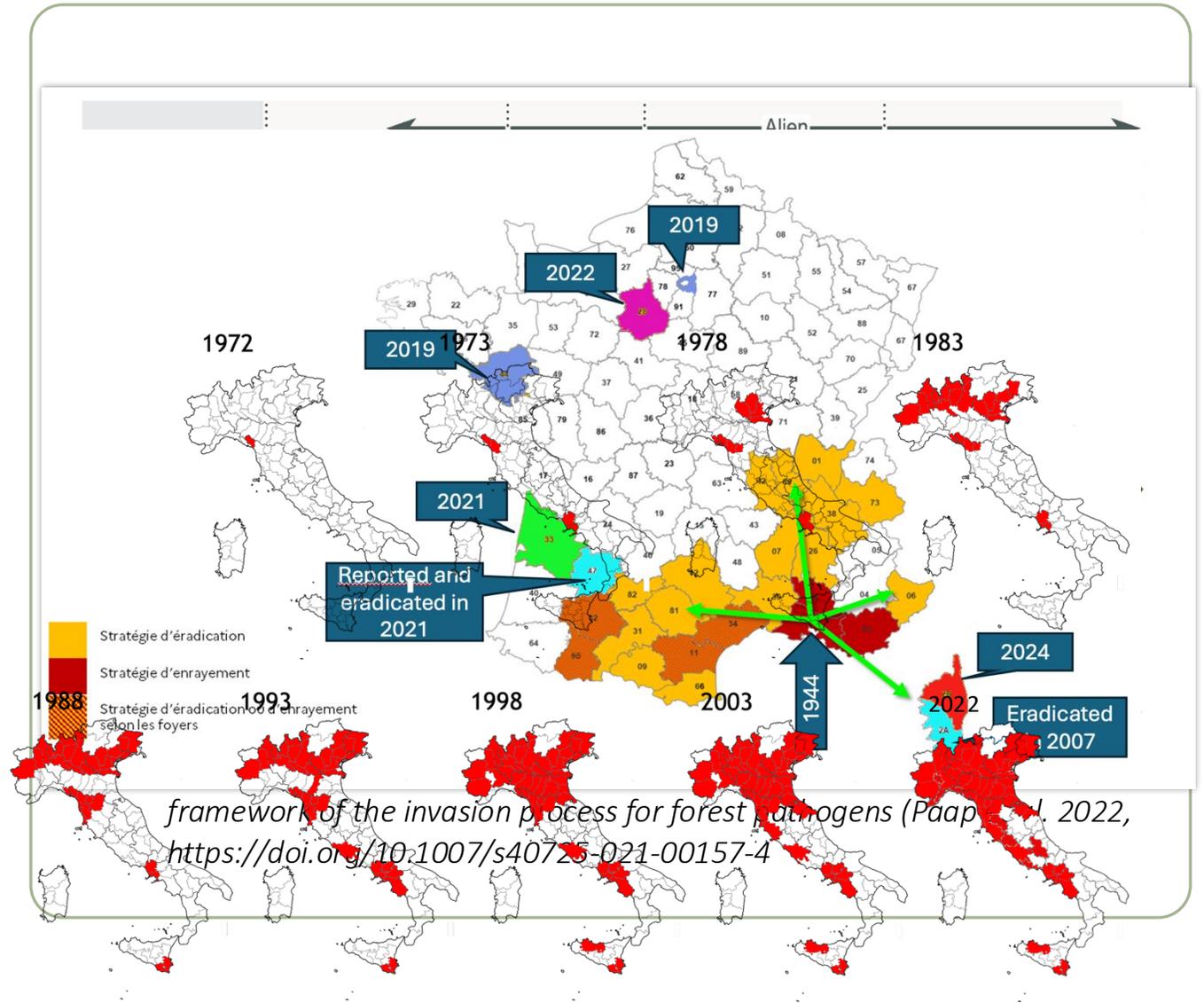Healthy tissues

Edge

Spread in Europe

The invasion process of IFPs consists of four main stages: the arrival of individuals (propagules) in a new territory, sometimes followed by a phase of population establishment, then its development into an epidemic phase, and finally its geographical expansion.
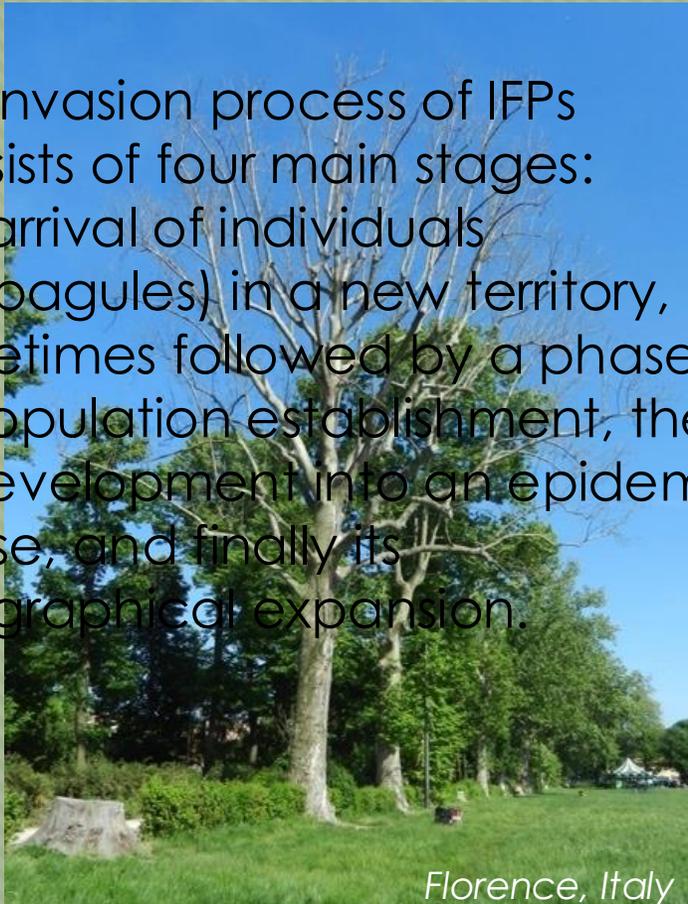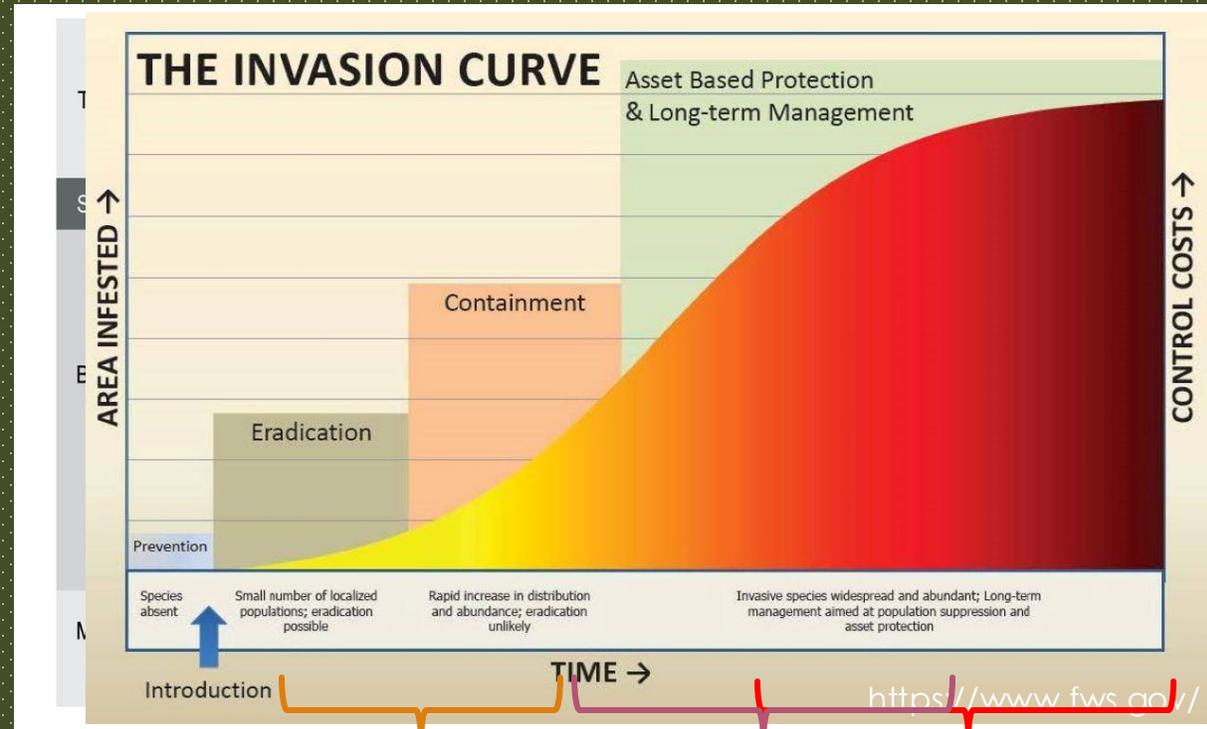
Florence, Italy

The principle of preventing the risks associated with these invasive species is to hinder the transition from one phase to the next as quickly and effectively as possible.

framework of the invasion process for forest pathogens (Paap et al. 2022, https://doi.org/10.1007/s40725-021-00157-4

To avoid the further spread of the disease within an area or the introduction in a new country, there is only one means: Prevention through early detection.

THE INVASION CURVE

Asset Based Protection & Long-term Management

Containment

Eradication

Prevention

AREA INFESTED →

CONTROL COSTS →

Species absent

Small number of localized populations; eradication possible

Rapid increase in distribution and abundance; eradication unlikely

Invasive species widespread and abundant; Long-term management aimed at population suppression and asset protection

Introduction

TIME →

https://www.fws.gov//

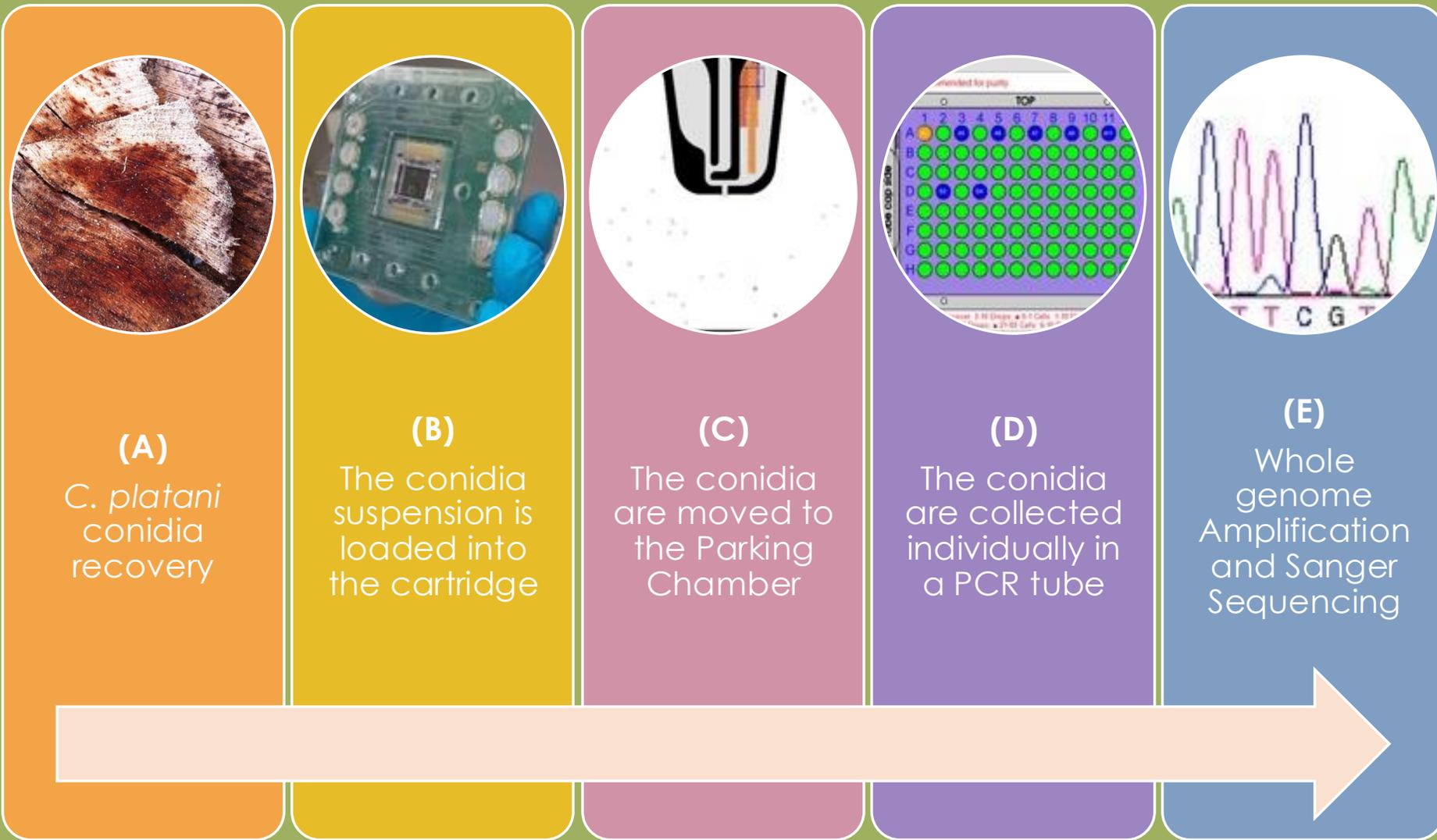| Prevention and Survellance	Eradication	Containment and mitigation
• **Single cell** enabling genome- level analysis of individual propagules • **VOCs** fingerprint characterising C. platani • **Real-time PCR** assays based on ceratoplatanin and ITS genes • **LAMP** assay for in-field detection	• **LAMP** assay for in-field detection • **Real-time PCR** assays based on ceratoplatanin and ITS genes • **Airborne inoculum traps**	• **Aerial remote sensing** of tree damage **at regional scale**

(A) *C. platani* conidia recovery

(B) The conidia suspension is loaded into the cartridge

(C) The conidia are moved to the Parking Chamber

(D) The conidia are collected individually in a PCR tube

(E) Whole genome Amplification and Sanger Sequencing

Schematic representation of the entire workflow for the molecular analysis of a single conidium. The conidial suspension is loaded into the cartridge, and then within the DEPArray single cell- sorting instrument, individual conidia are selected and collected, and then used for subsequent DNA isolation and sequencing analysis (Luchi et al. 2025 https://doi.org/10.1111/ppa.70040).

Microbial Volatile Compounds (MVOCc)

➤ To measure the blend of volatile organic compounds (VOCs) emitted during different phases of disease cycles in target host/pathogen binomial (i.e. *Ceratocystis platani* - *Platanus* spp.).

➤ To make use of VOC 'fingerprints' emitted from target host/pathogen binomial to create libraries and to implement methods for fast and non-invasive routine detection.
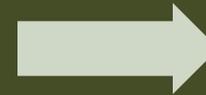

HEADSPACE

MEDIUM & MYCELIUM

Growth
Petri dishes with PDA (1.5 %) at 20 °

Transfer in vials (5ml) with PDA at 20°

Plane trees inoculated to collect VOC released in vivo from the bark

VOC 'headspace' analysis
* Solid Phase Micro Extraction (SPME)
* Thermo-Desorption - Gas Chromatograph -
Mass Spectrometer (TD-GC-MS)

Microbial Volatile Compounds (MVOCc)

C. platani growth *in vitro* has a strong **'fruity aroma'** (i.e. 'banana') and emitts a blend of VOC similar to that of the phylogenetically closed *C. populicola*
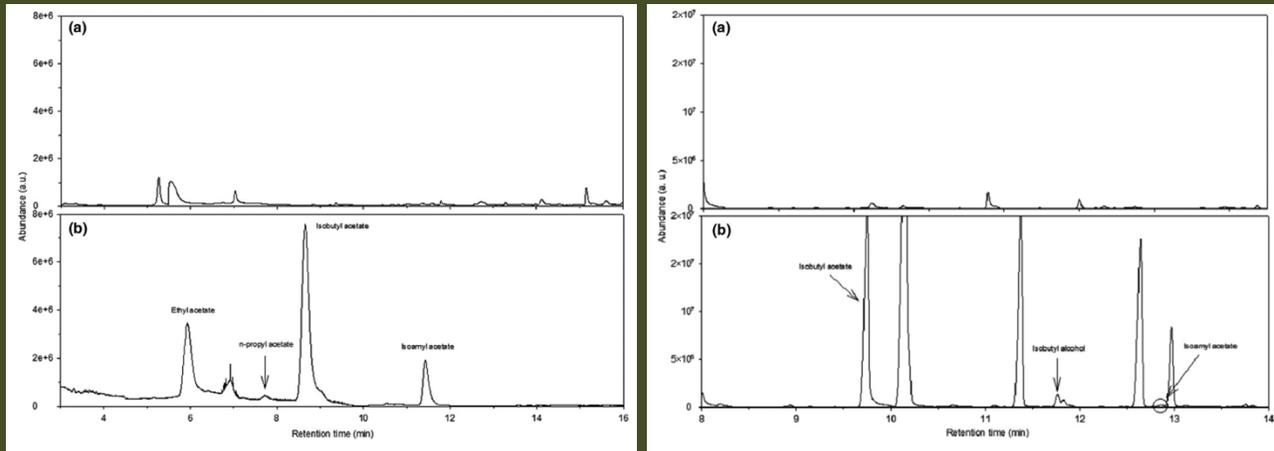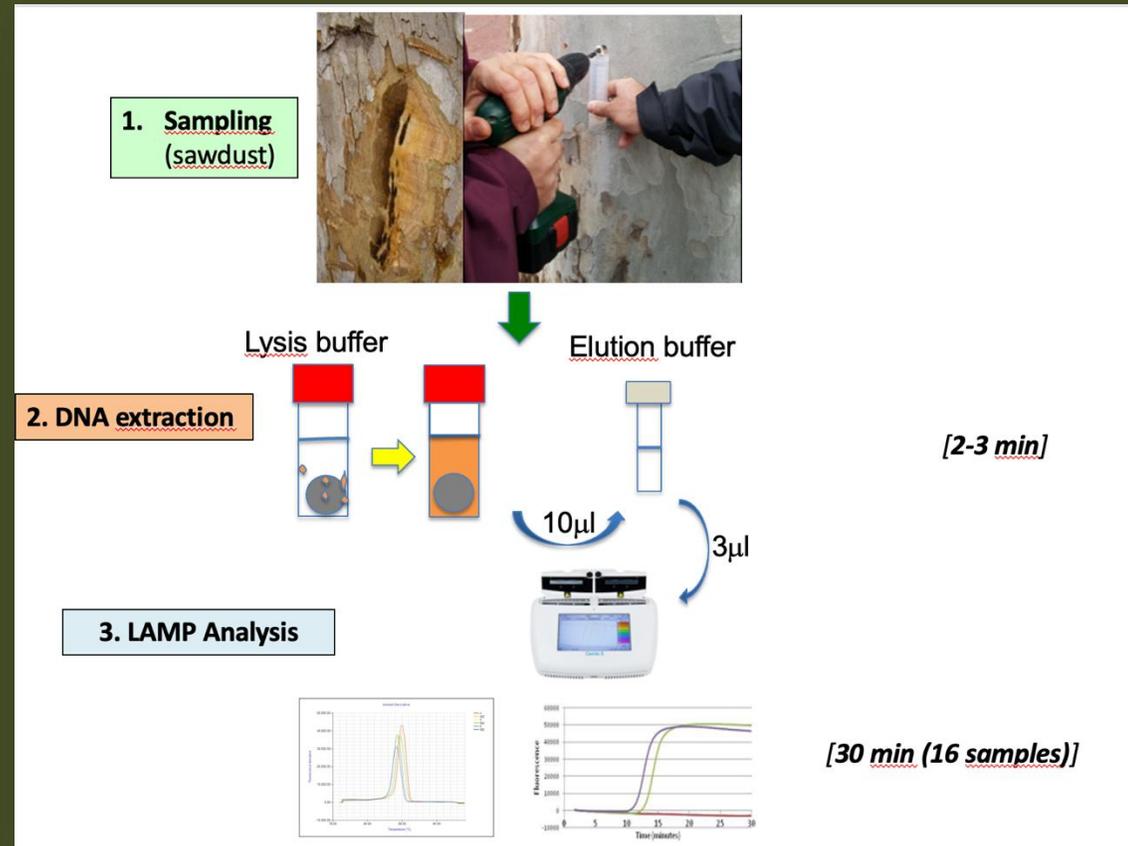

VOC fingerprint specific to *C. platani* has been identified and can be detected just 15 d.a.i. and it could be implemented for fast and non-invasive routine detection.

Brilli et al., 2020 https://doi.org/10.1111/ppa.70040

	ethyl acetate (%)	n-propyl acetate (%)	isobutyl acetate (%)	isobutyl alcohol (%)	isoamyl acetate (%)	isoamyl alcohol (%)
Fusarium circinatum	---	---	---	---	---	---
* Ceratocystis populicola	51.1 ± 10.0	2.2 ± 0.8	42.1 ± 7.7	---	14.5 ± 3.1	---
Geosmithia langdonii	---	---	---	58.0 ± 12.2	---	42.0 ± 12.2
** Ceratocystis platani	9.4 ± 2.4	2.2 ± 2.2	38.4 ± 0.6	---	45.0 ± 1.6	5.0 ± 0.8
Diplodia sapinea	---	---	---	---	---	100.0 ± 0.0
Chalara fraxinea	---	---	---	---	---	---
Ophiostoma novo-ulmi	---	---	---	35.9 ± 29.3	---	76.1 ± 23.9
Heterobasidion annosum	---	---	---	---	---	---
Seiridium cardinale	---	---	---	---	---	---

LAMP (Loop-mediated isothermal amplification) for early detection of C. *platani*

Loop-mediated isothermal amplification (LAMP) is a gene amplification method which amplifies DNA with high specificity and efficiency under isothermal conditions

1. Sampling (sawdust)

Lysis buffer   Elution buffer

2. DNA extraction   [2-3 min]

10µl   3µl

3. LAMP Analysis

[30 min (16 samples)]

Aglietti et al., 2019
https://doi.org/10.111
1/ppa.70040

LAMP assay was able to amplify DNA from mycelium and infected plant tissue

The limit of detection (LOD) of LAMP assays was very low (0.02 pg μL^{-1})

LAMP is a portable diagnostic tool, requiring minimum equipment and a few, specific scientific skills:
- could be used to check the health status of live plants at the points of entry or in field;
- reducing time of analyses, thus allowing a prompt reaction.

Aglietti et al., 2019

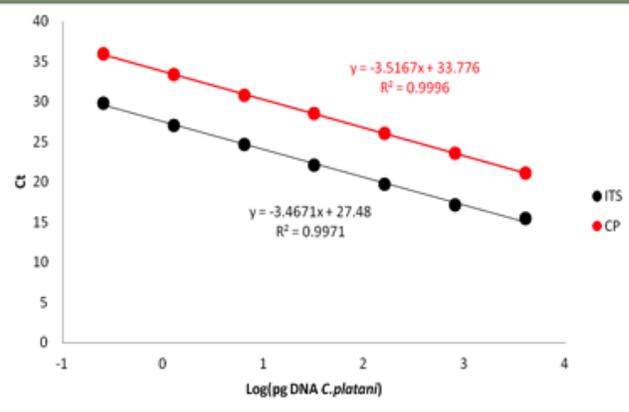|	**DNA serial dilution**										
	10 ng μL^{-1}	2 ng μL^{-1}	0.4 ng μL^{-1}	0.08 ng μL^{-1}	0.016 ng μL^{-1}	3.2 pg μL^{-1}	0.64 pg μL^{-1}	0.128 pg μL^{-1}	0.02 pg μL^{-1}	0.005 pg μL^{-1}	0.001 pg μL^{-1}
C. platani LAMP	8:45	10:15	10:45	11:00	11:30	12:15	13:00	14:00	28:00	-	-
qPCR	+	+	+	+	+	+	+	+	+	+	-

Design of specific Real time qPCR assay (MGB TaqMan Probe)
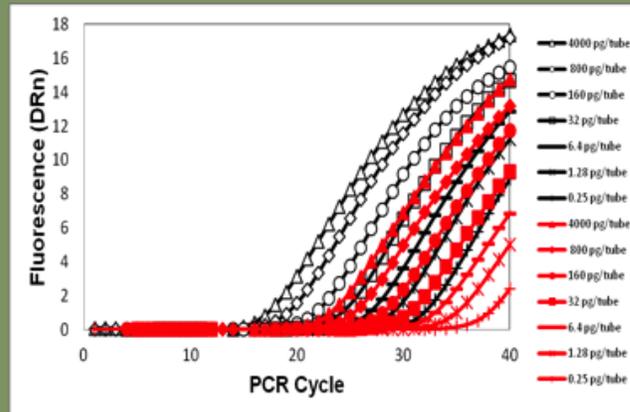
Target genes : Ceratoplatanin (CP) and
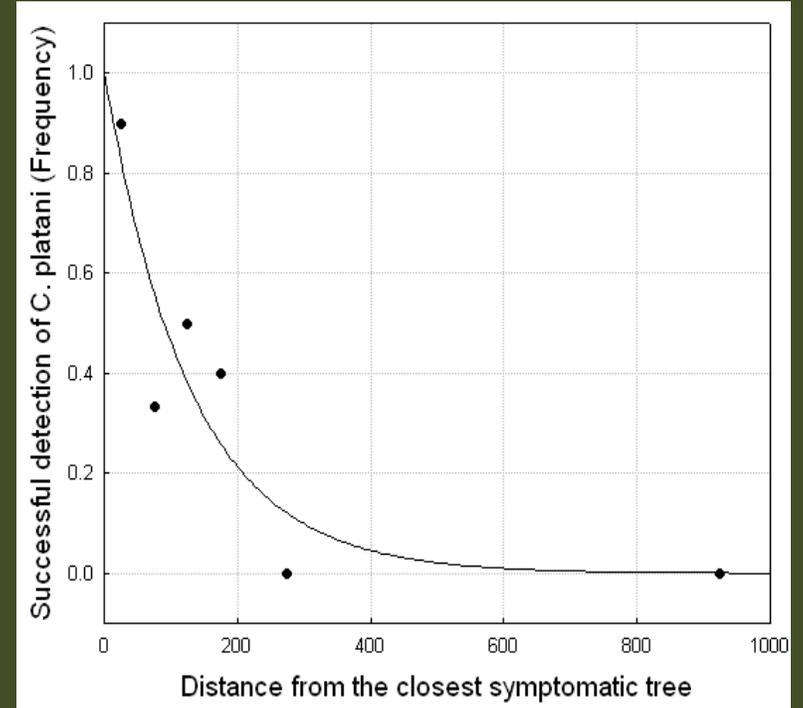Internal Transcribed Spacer (ITS2)

ITS2 vs CP

Standard curves   Amplification plots

LOD= 0.05 pg/l and 2 fg/l DNA for the CP and ITS primer-probe
combinations, respectively

Luchi et al. 2013 https://doi.org/10.1128/AEM.01484-13

# Detection of the inoculum from eDNA samples

The inoculum dispersal during the sanitation cuts was evaluated about 200m from the closest symptomatic tree

The combination of airborne trapping and real-time quantitative PCR assay provides a rapid and sensitive method for the specific detection of a *C. platani* inoculum.

Luchi et al. 2013

Ongoing research
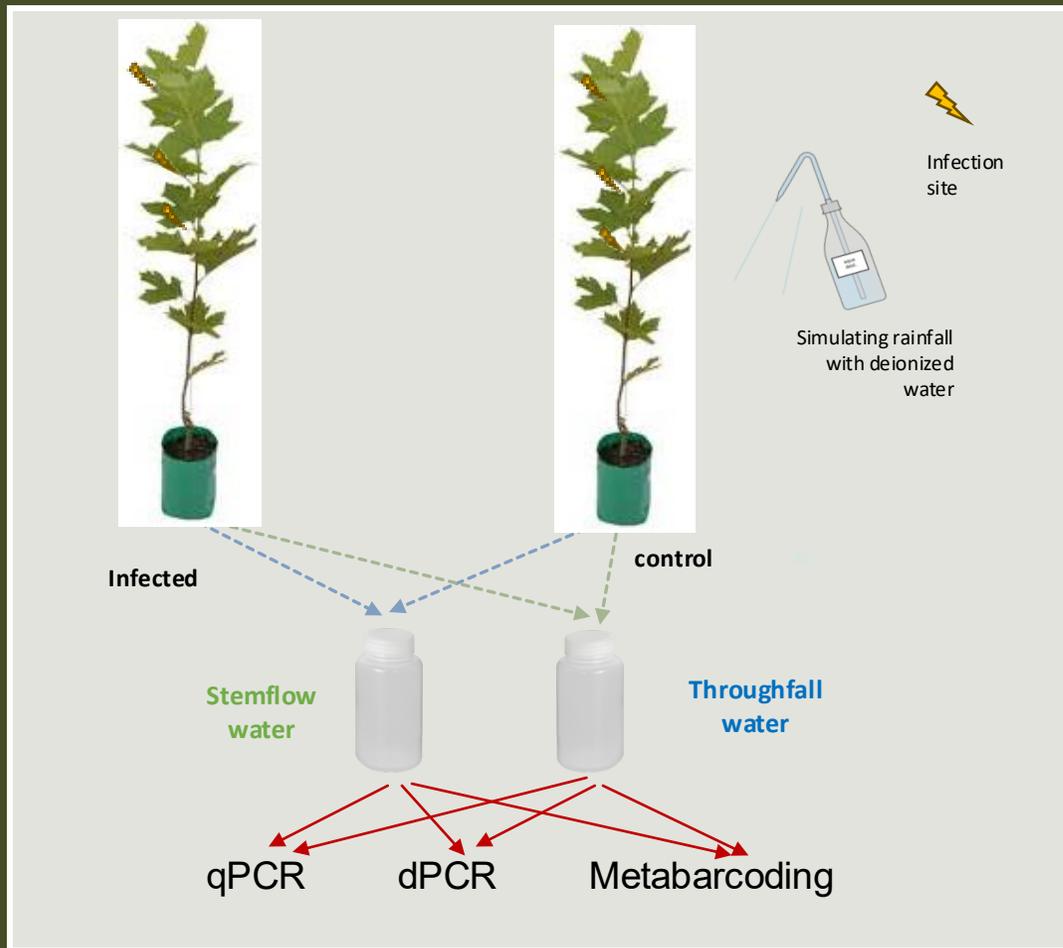
Water transport of the inoculum will be experimentally tested (mod. from Prospero et al., presentation)
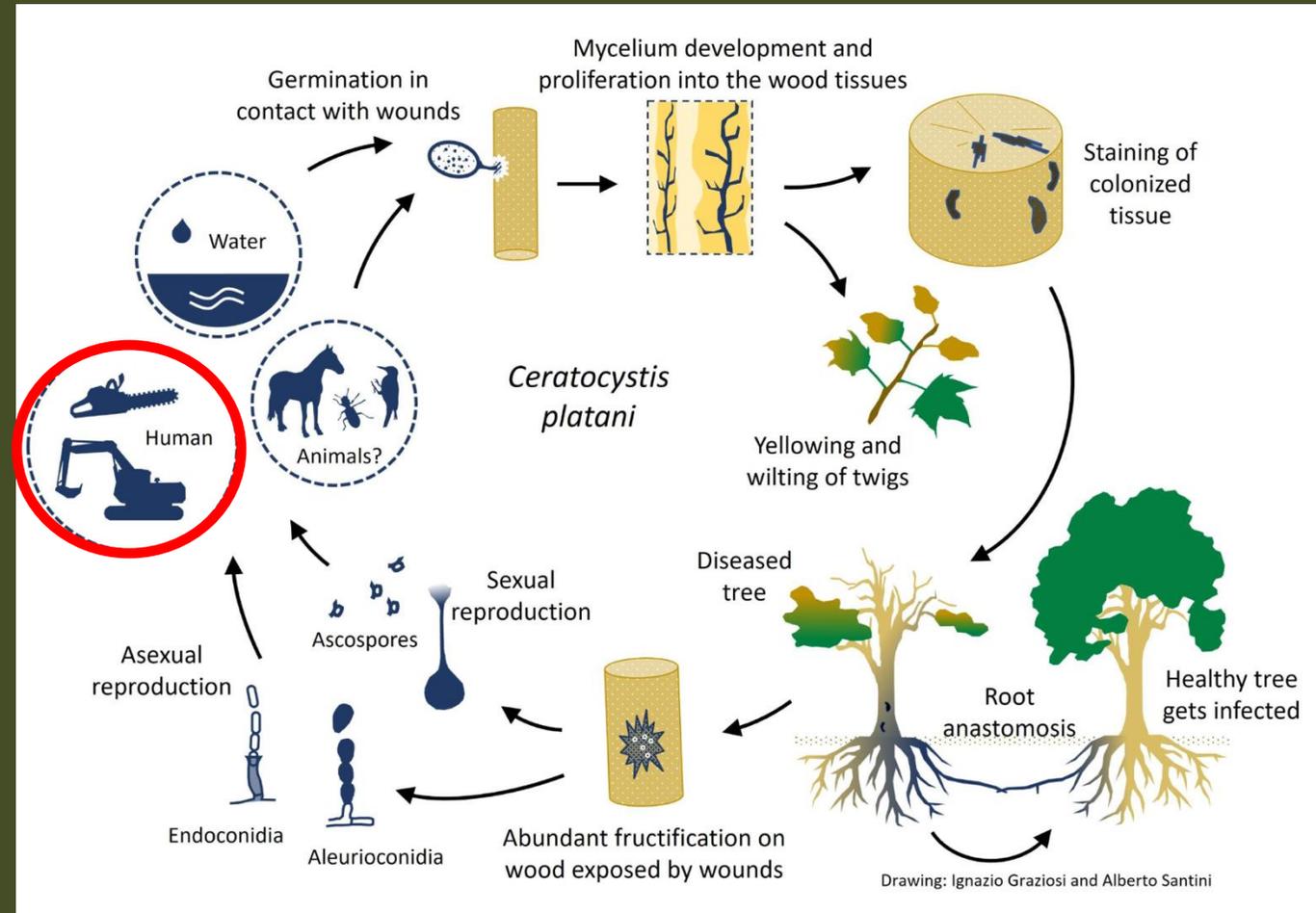
Persistence of inoculum on pruning tools will be evaluated by RNA-based LAMP

Orthomosaics of RPAS images acquisitions, showing different vegetation indexes.

Aerial remote sensing of tree damage at regional scale

Remotely piloted aerial systems (RPAS) will be employed to detect **CSD infections** at canopy level.

Different parameters will be collected and georeferenced, and their correlation with the present conditions found on plants at ground level will be evaluated.

In conclusion…

- **Single-cell technology**
- **VOC fingerprinting**;
- **LAMP assay**;
- **eDNA trapping** and **real-time PCR assay**;
- **aerial remote sensing**
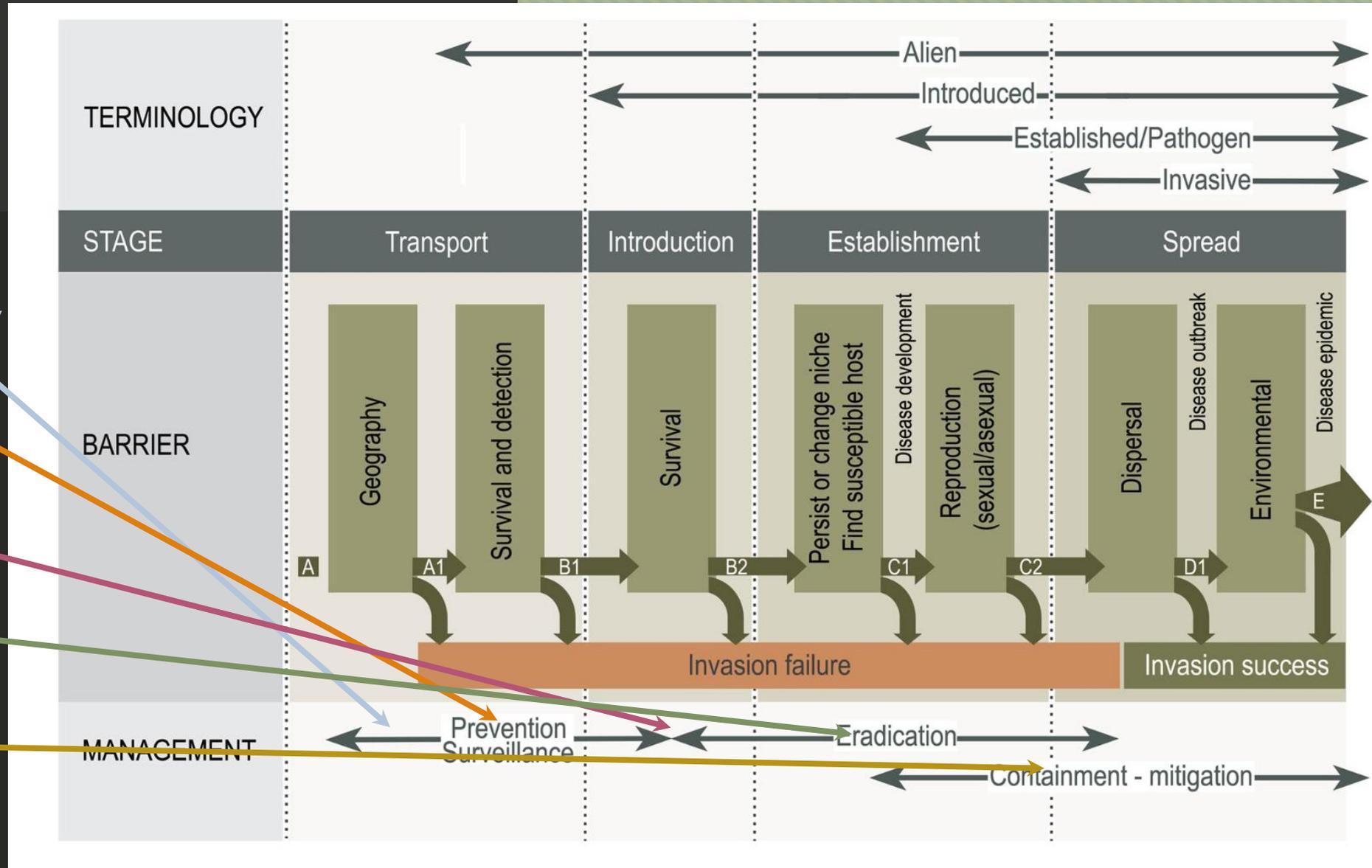
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Rebekka Schloesser
Giovanni Marino

FORSAID

Thank you for your attention!

REGIONE TOSCANA

UB3GUARD

HOMED

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