Consiglio Nazionale delle Ricerche
Istituto per la Protezione Sostenibile delle Piante

New molecular tools for early detection of forest pests and pathogens

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EPPO Conference on Diagnostics of Plant Pests
Recent developments and future trends
2026-04-22/24, Vienna (AT)

The combination of forest pathogens, international trade and climate change poses a significant threat to the health of forests worldwide

Increased trade (P4P) makes it easier for invasive species to be introduced, while climate change makes trees more vulnerable and creates conditions that encourage the development of new pests.

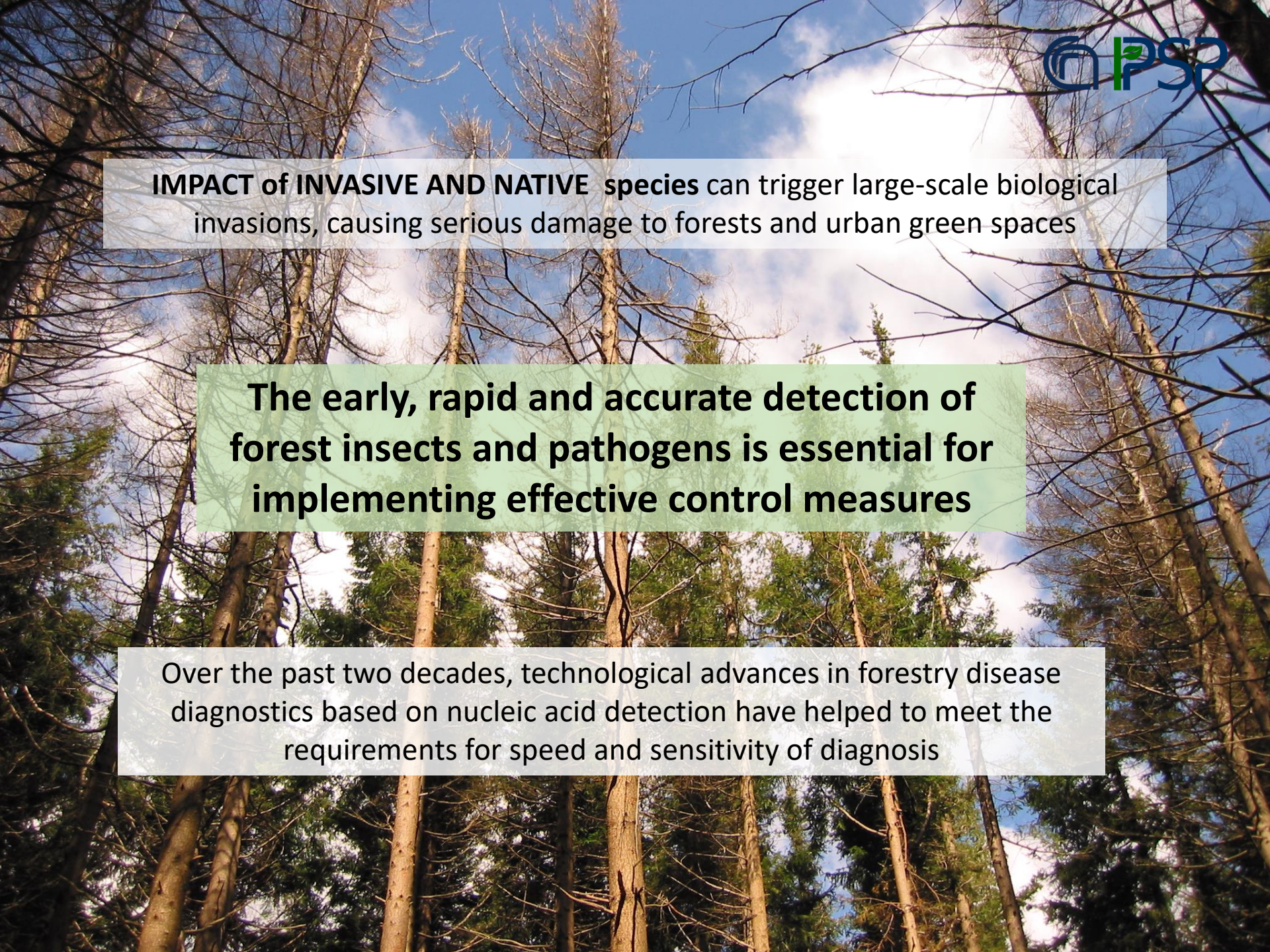
Northward spread
Rising temperatures enable harmful insects and fungi to colonise regions that were previously too cold for them

Increased virulence
Mild autumns and winters extend the reproductive season of pathogens, accelerating their spread

GLOBAL CHANGE

More vulnerable plants
Water and heat stress weaken plants, making them more susceptible to disease

New introductions
Non-native thermophilic species establish themselves more easily when they come into contact with new host



IMPACT of INVASIVE AND NATIVE species can trigger large-scale biological invasions, causing serious damage to forests and urban green spaces

The early, rapid and accurate detection of forest insects and pathogens is essential for implementing effective control measures

Over the past two decades, technological advances in forestry disease diagnostics based on nucleic acid detection have helped to meet the requirements for speed and sensitivity of diagnosis

PCR has proven to be a powerful tool for quantitative analysis of nucleic acids

Detection of a specific microorganism in a mixture of DNA by using reduced quantities of starting plant material

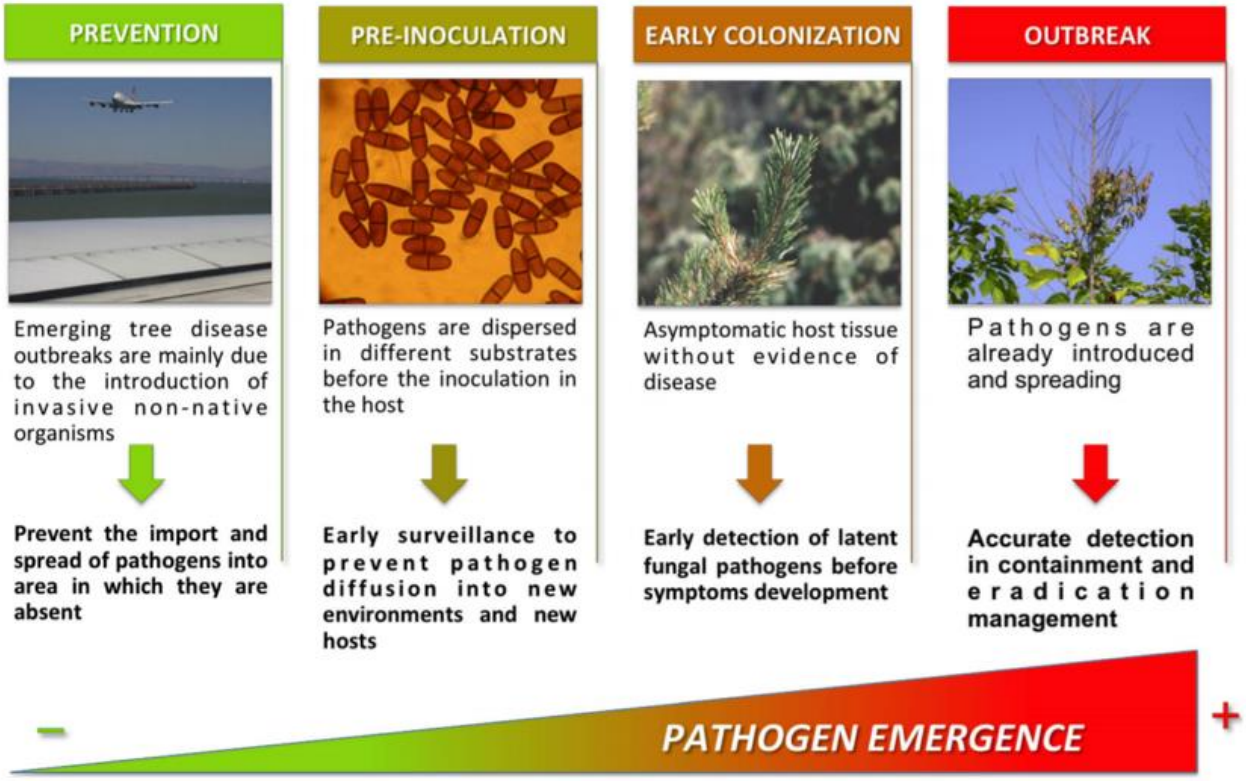


Fig. 3 Application of molecular techniques for plant pathogen detection in different steps of the invasion process

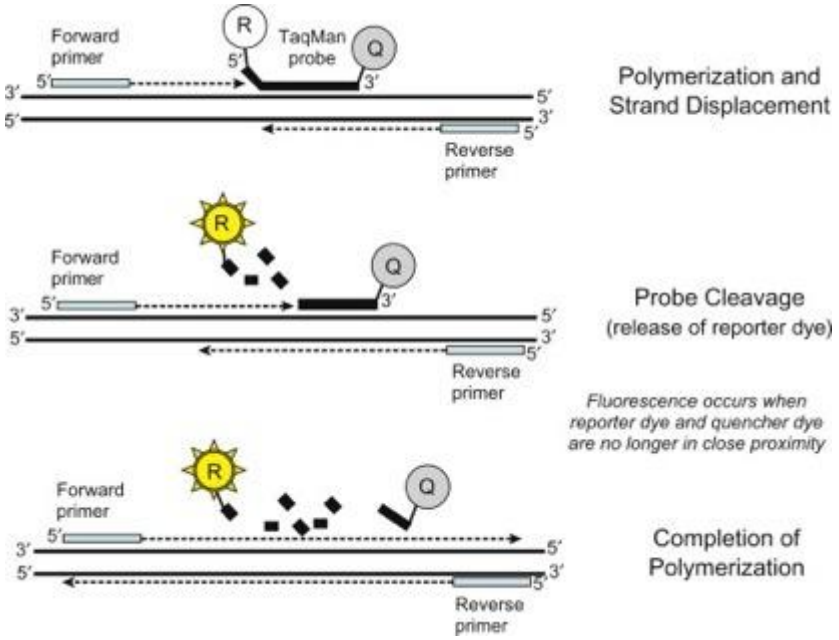
Springer

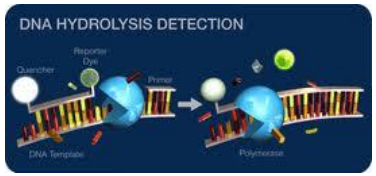
(Luchi et al., 2020)

Main characteristics of PCR approaches:

SENSITIVITY | **RAPIDITY** | **SPECIFICITY** | **REPRODUCIBILITY**

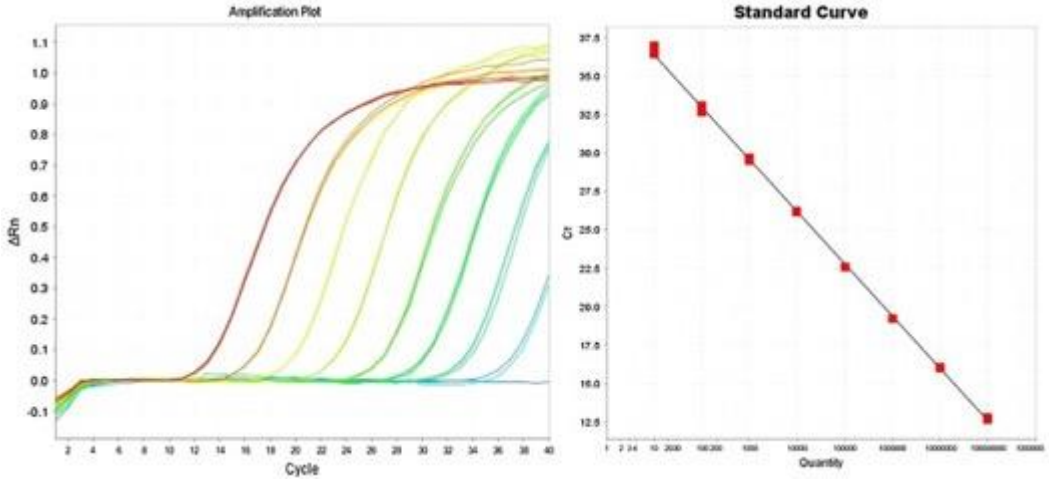
Real-time quantitative PCR





During the amplification reaction, the Taq-polymerase enzyme degrades the probe producing a **fluorescent signal that is directly proportional to the amount of pathogen DNA detected**

By means of the **standard curve**, the DNA of the target pathogen can be quantified for each sample



Ceratocystis platani airborne inoculum detection

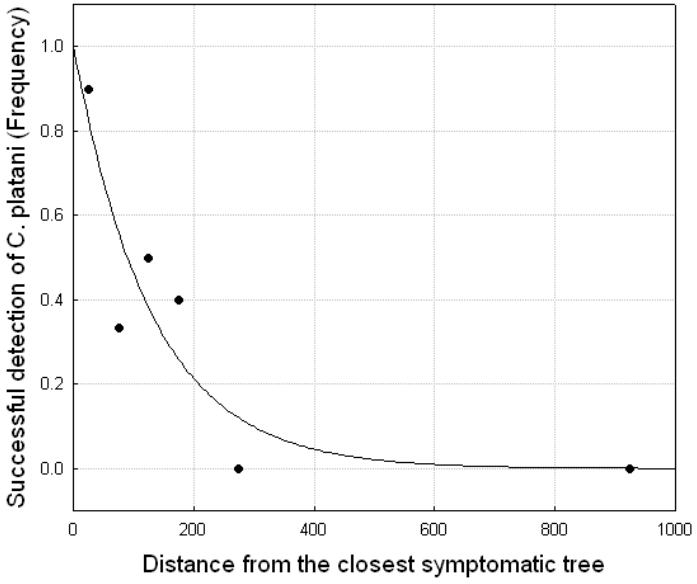
Canker stain disease

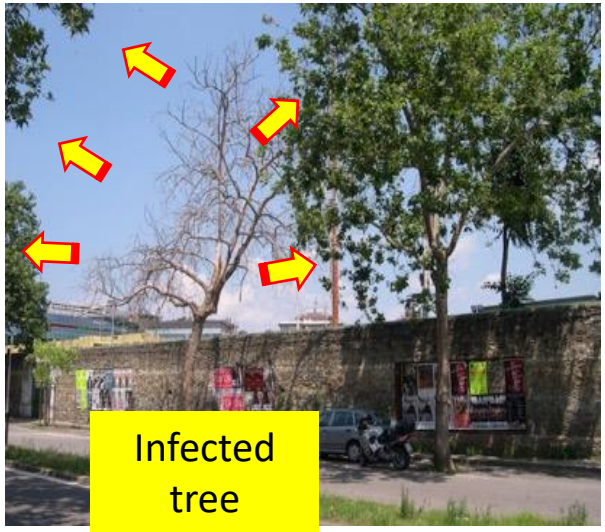
Platanus x acerifolia

Airborne inoculum traps

DNA extraction + Real-time PCR
(Detection of *Ceratocystis platani*)

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

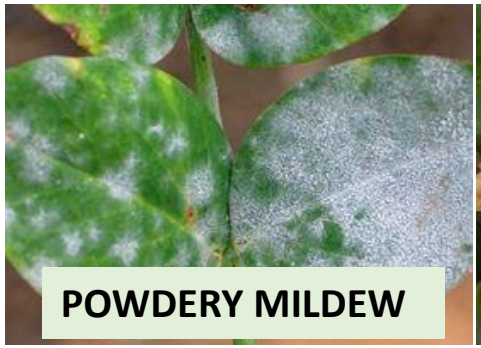




Luchi N., Ghelardini L., Belbahri L., Quartier M., Santini A. (2013). **Rapid detection of *Ceratocystis platani* inoculum by quantitative real-time PCR**. Appl. Env. Microb. 79 (17): 5394-5404.

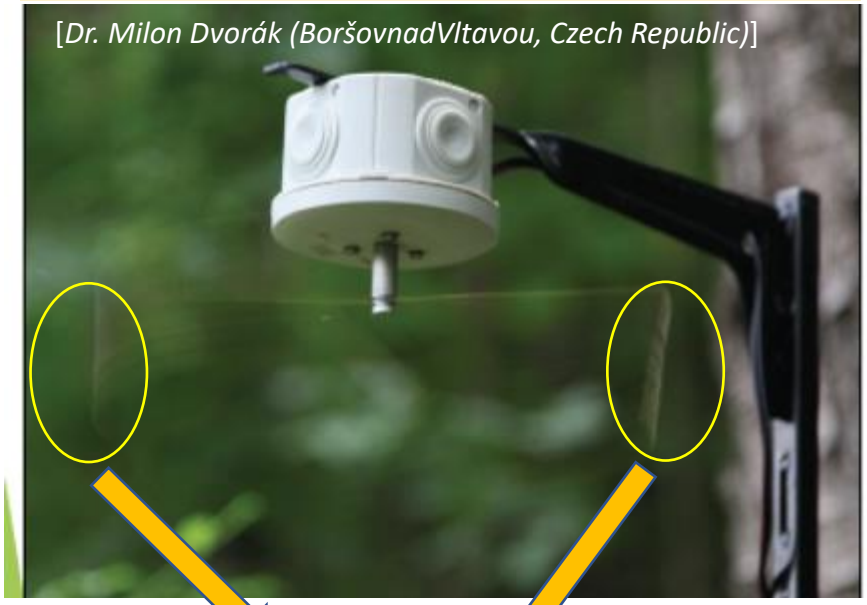
Airborne inoculum detection in nursery

In recent years, most of the diseases that are causing infections of the aerial portions of nursery plants can be traced to two major groups of pathogens



Both of these two classes are characterized by organisms that are biotrophic: **they need live hosts to feed themselves**.
This makes their in-vitro cultivation and diagnosis difficult.

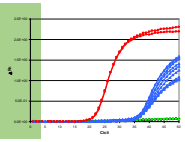
Rotating Arm Spore Traps ROTTRAP 120 (RAST 120)



DNA extraction

Real-time PCR (duplex)

Pathogen DNA quantification



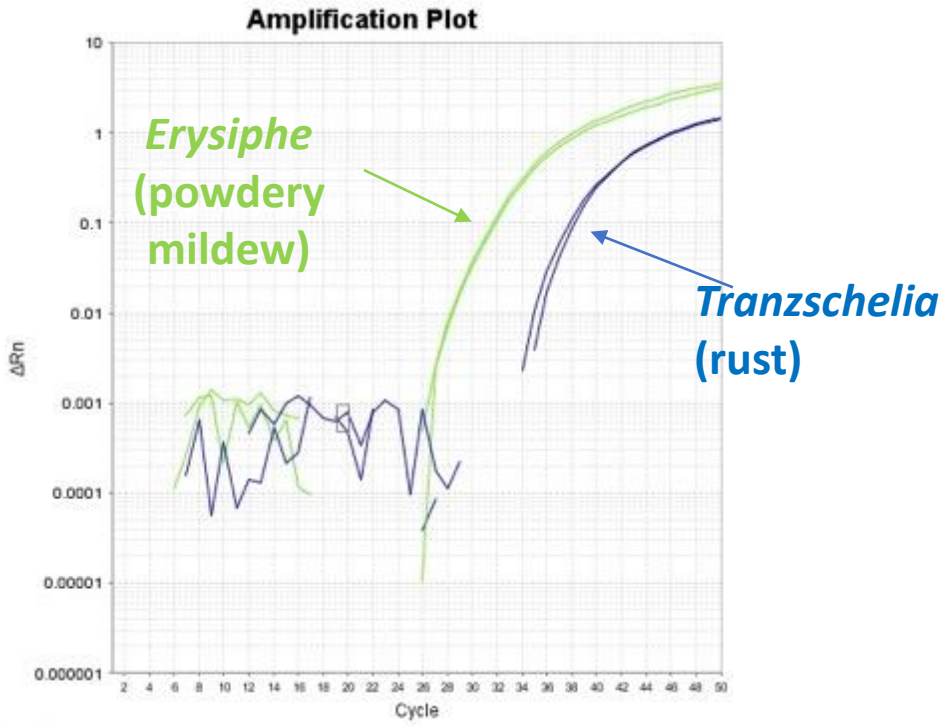
Simultaneous detection of rust and powdery mildew by duplex qpcr

The simultaneous presence of rusts and powdery mildew, in the same reaction volume, was detected

DNA powdery mildew

+

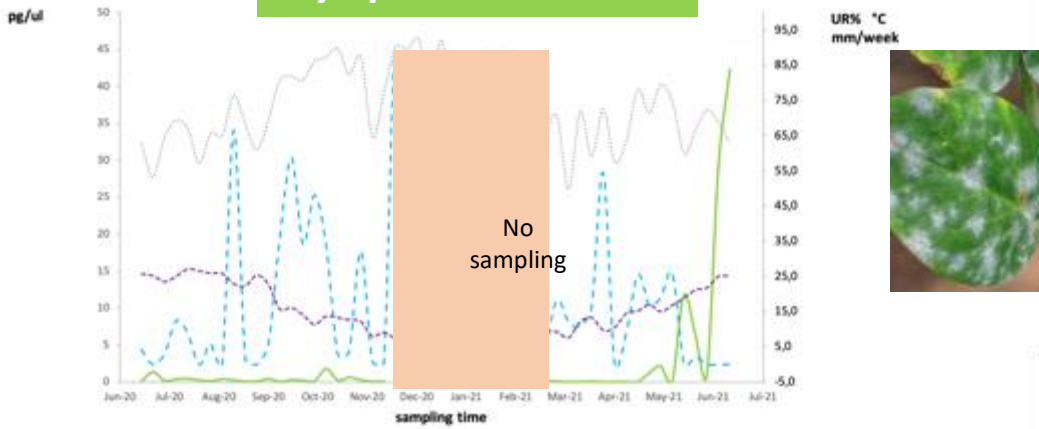
DNA rust



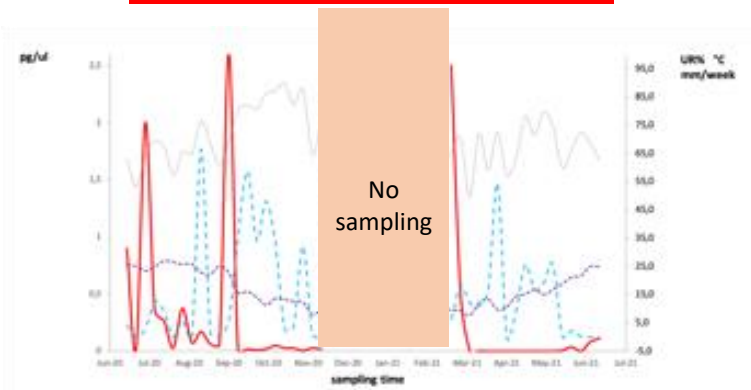
N. LUCHI, E. NIGRONE, F. PECORI, D.MIGLIORINI, G. INCROCCI, A.SANTINI (supported by AUTOFITOVIV project: «Buone pratiche per l'autocontrollo e la gestione fitosanitaria sostenibile nel vivaismo ornamentale» - REGIONE TOSCANA)

Weekly sampling
(from July 2020 to June 2021)

Erysiphe detection



Tranzschelia detection



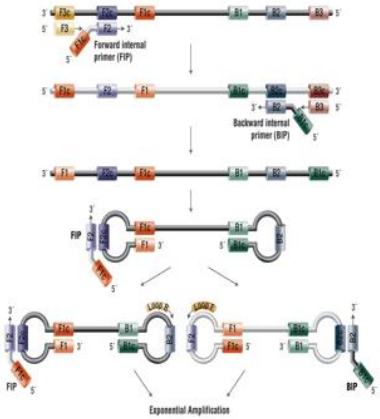
Mean Temperature (°C)

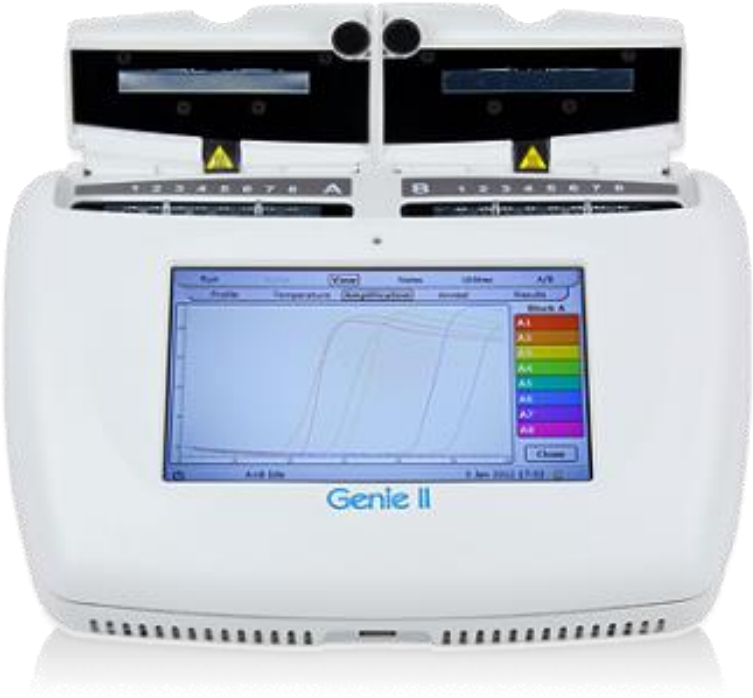
Relative Humidity (UR%)

Weekly Rainfall (mm/week)

Loop-mediated isothermal amplification (LAMP)

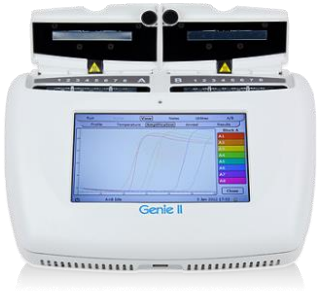
is a gene amplification method which amplifies DNA with high specificity and efficiency under isothermal conditions





- ✓ LAMP method is based on the use of a set of four to six specially designed primers spanning six to eight distinct sequences on the target DNA.
- ✓ The amplification is performed by DNA polymerase, which has strand-displacing activity.
- ✓ The whole reaction occurs in a single tube and is divided into two steps: non-cyclic and cyclic.

Several LAMP assays have been developed for **human and animal diseases** and **food safety control…BUT ALSO FOR PLANT DISEASES**



Aglietti et al. AMB Expr (2019) 9:50
https://doi.org/10.1186/s13568-019-0774-9

AMB Express

ORIGINAL ARTICLE Open Access

Real-time loop-mediated isothermal amplification: an early-warning tool for quarantine plant pathogen detection

Chiara Aglietti[1,2], Nicola Luchi[1*], Alessia Lucia Pepori[1], Paola Bartolini[1], Francesco Pecori[1], Aida Raio[1], Paolo Capretti[2] and Alberto Santini[1]

Phytophthora ramorum
Xylella fastidiosa
Ceratocystis platani
Fusarium circinatum
Gnomoniopsis smithogilvyi
Diplodia sapinea
Agrilus planipennis
A. anxius

Reports

BioTechniques

Real-time loop-mediated isothermal amplification assay for rapid detection of *Fusarium circinatum*

Dagmar Stehlíková[1,2], Nicola Luchi[*,2], Chiara Aglietti[2,3], Alessia Lucia Pepori[2], Julio Javier Diez[4] & Alberto Santini[2]

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AMB Express

ORIGINAL ARTICLE Open Access

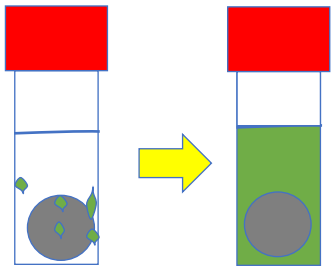
Rapid diagnostics for *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae*) in chestnut nuts: new challenges by using LAMP and real-time PCR methods

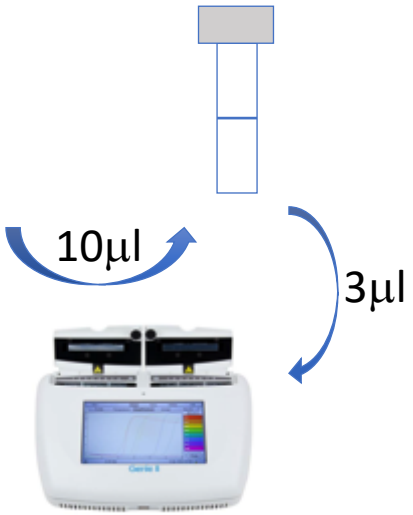
Anna Maria Vettraino[1†], Nicola Luchi[2*†], Domenico Rizzo[3], Alessia Lucia Pepori[2], Francesco Pecori[2] and Alberto Santini[2]

Ceratocystis platani detection (LAMP portable assay)

1. Sampling

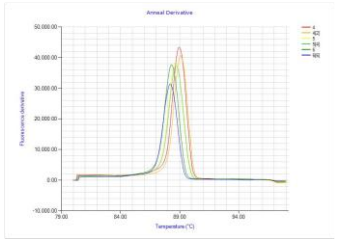
2. DNA extraction

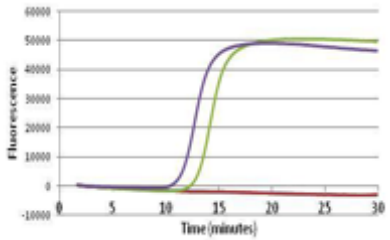




[2-3 min]

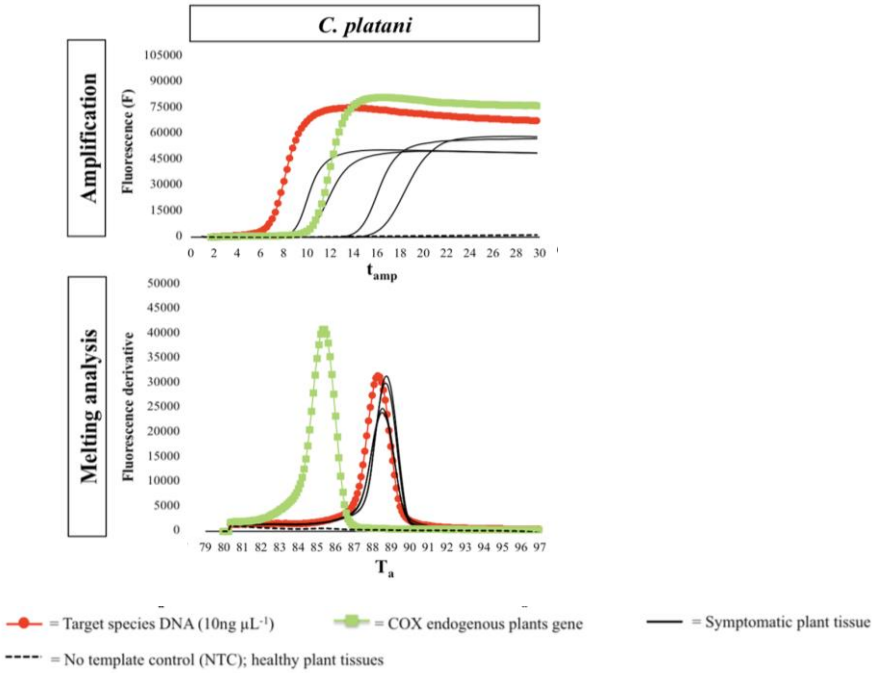
3. LAMP Analysis





[30 min (16 samples)]

LAMP Portable detection method



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ORIGINAL ARTICLE Open Access

Real-time loop-mediated isothermal amplification: an early-warning tool for quarantine plant pathogen detection

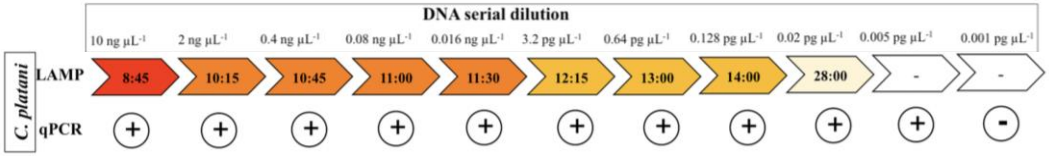
Chiara Aglietti[1,2], Nicola Luchi[1*], Alessia Lucia Pepori[1], Paola Bartolini[1], Francesco Pecori[1], Aida Raio[1], Paolo Capretti[2] and Alberto Santini[1]

C. platani LAMP detection

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

Limit of detection (LOD)

- The limit of detection (LOD) of LAMP assays was very low (0.02 pg/µL)



Detection of latent pathogens

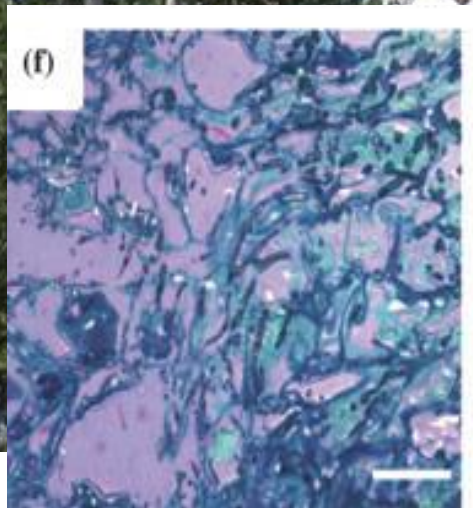
Diplodia sapinea is a fungal pathogen that causes shootblight, crown wilt and canker diseases

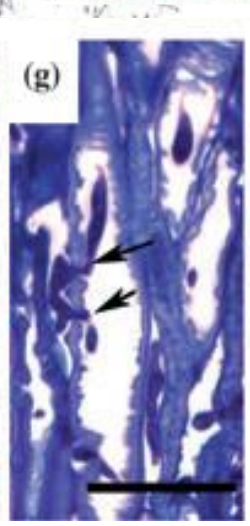
D. sapinea is most widespread in temperate regions, where warm and humid conditions, such as those associated with heavy rainfall during the summer, promote cone colonization and pycnidia production.

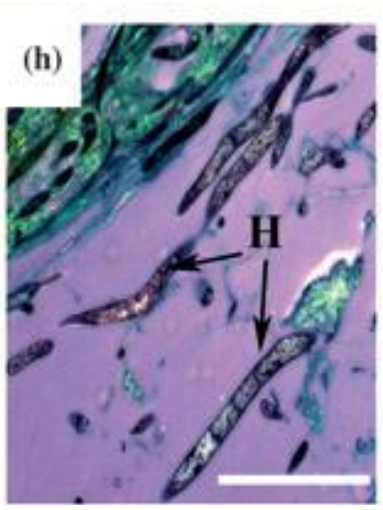
The fungus spreads to healthy plants primarily by wind- and insect-dispersed conidia (Munck and Stanosz, 2009; Feci et al. 2003; Luchi et al. 2012).

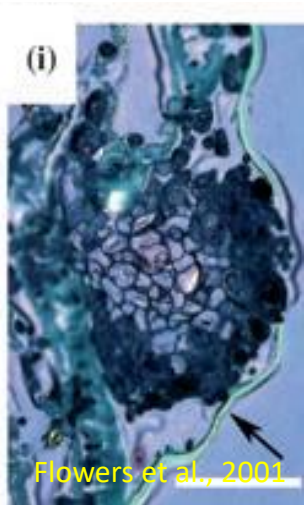
D. sapinea is able to infect host tissue and spend long periods of latency before visible symptoms occur

Flowers et al., 2001







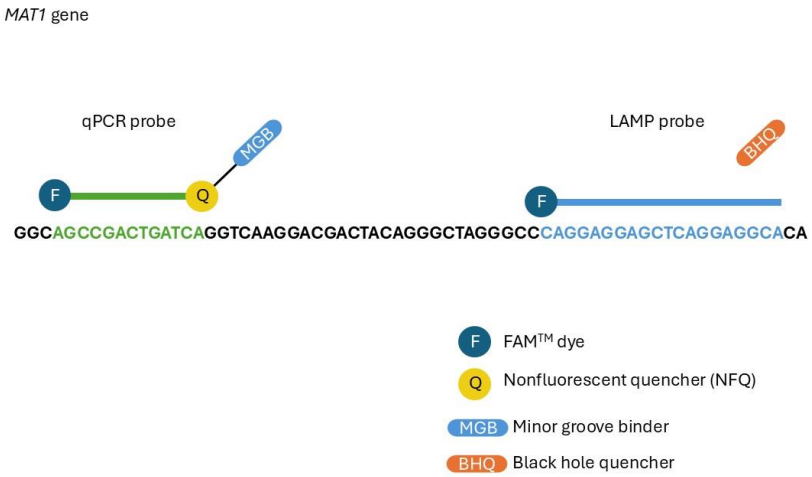


Flowers et al., 2001

Trees might be debilitated by drought or other stress inducing *D. sapinea* to become pathogenic and to produce visible damages

In the last decade, likely as a consequence of climate change, damage caused by ***D. sapinea*** **has increased even in Northern Europe**, especially on *Pinus sylvestris* (Terhonen et al. 2025; Brodde et al. 2023).

We developed two molecular detection methods for the rapid, specific, and sensitive diagnosis of *D. sapinea*: a newly designed qPCR assay with enhanced specificity and a LAMP assay suitable for on-site detection



Using assimilating probe further improves LAMP specificity, enabling even closely related species to be distinguished

The combination of primers and an assimilating probe enabled *D. sapinea* to be distinguished from closely related species such as *D. seriata* and *D. scrobiculata*, thus making the LAMP assay specific to *D. sapinea*

Table 2. LAMP and qPCR primers and probes designed in this study for *D. sapinea*

Molecular assay	Oligonucleotide name	Oligonucleotide type	Sequence[a] (5'-3')	Length (bp)
LAMP	Dsap_MAT1-2-1_F3	F3	GCCTTCATGCTGTACCGC	18
	Dsap_MAT1-2-1_B3	B3	TGATGTCAGACGACTTGCG	19
	Dsap_MAT1-2-1_FIP	FIP	GCCCACATCTGGCCAATGATCTAGGACAACCACGCTACTGT	41
	Dsap_MAT1-2-1_BIP	BIP	AGGTCAAGGACGACTACAGGGCAGCGATACTCCGGGTGAG	39
	Dsap_MAT1-2-1_LF	LF	CCCGGGTTGTTCGCTACA	18
	Dsap_MAT1-2-1_LB_Probe	Fluorescent strand	[*FAM*]ACGCTGAGGACCCGGATGCGAATGCGGATGCGGATGCCGACA GGAGGAGCTCAGGAGGCA	60
	Dsap_BHQ	Quencher strand	TCGGCATCCGCATCCGCATTCGCATCCGGGTCCTCAGCGT[*BHQ*]	40
qPCR	Dsap_qPCR_F	Forward	TCAGATCATTGGCCAGATGTG	21
	Dsap_qPCR_R	Reverse	CCCTAGCCCTGTAGTCGTCCTT	22
	Dsap_qPCR_Probe	TaqMan Probe	[*FAM*]AGCCGAGACTGATCA[*MGB*]	15

Note.
[a] FAM = 6-carboxyfluorescein; MGB = Minor groove binder; BHQ = Black Hole Quencher-1; The underlined fragment of the fluorescent strand acts as backward Loop primer

Novel DNA based assays to detect *Diplodia sapinea*, the causal agent of shoot blight on pine trees
F. Pecori, N. Luchi, D. Stehlíková, A.L.Pepori, I. Matsiakh, M. Cleary, A. Santini. *Submitted to Plant Pathology*

Table 3: Results of *Diplodia sapinea* detection on pine samples by using qPCR and LAMP assays designed in this study

Pine sample	Source	N. sample	*D. sapinea* detection (N. positive)		
			Isolation	qPCR	LAMP
Symptomatic shoots	*P. sylvestris* artificial inoculation (Ebbemåla, Sweden)	10	10	10	10
Symptomatic shoots	*P. nigra* plantation (Monte Morello, Italy)	10	10	10	10
Asymptomatic shoots	*P. nigra* plantation (Monte Morello, Italy)	10	3	10	10
Cones (with pycnidia)	*P. nigra* Campus arboreta (Alnarp, Sweden)	5	5	5	5
Cones (with pycnidia)	*P. nigra* public garden (Cortona, Italy)	5	5	5	5
Asymptomatic shoot	*P. nigra* sentinel plantation (Florence, Italy)	10	0	0	0

The limit of detection (LoD) for LAMP and qPCR resulted as 0.64 pg/µL and 0.025 pg/µL, respectively

Novel DNA based assays to detect *Diplodia sapinea*, the causal agent of shoot blight on pine trees
F. Pecori, N. Luchi, D. Stehlíková, A.L.Pepori, I. Matsiakh, M. Cleary, A. Santini. *Submitted to Plant Pathology*

Detection of invasive insects


© Ken Gray Insect Image Collection

Bronze birch borer (*Agrilus anxius*, BBB) poses a serious threat to European birch species if the insect were to be introduced

Received: 18 April 2023 | Revised: 17 November 2023 | Accepted: 29 November 2023
DOI: 10.1002/edn3.503

METHOD

Environmental DNA WILEY

Development of novel LAMP and qPCR assays for rapid and specific identification of Bronze birch borer (*Agrilus anxius*)

Donnie L. Peterson[1] | Francesco Pecori[2] | Nicola Luchi[2] | Duccio Migliorini[2] | Alberto Santini[2] | Kathleen E. Kyle[3] | Claire Rutledge[4] | Aurélien Sallé[5] | Sezer Olivia Kaya[1] | Tod Ramsfield[6] | Michelle Cleary[1]

We developed **novel qPCR and LAMP assays** for BBB and investigated the specificity and sensitivity for their use as early detection tools in European forests

LAMP and qPCR SENSITIVITY

Both assays varied in sensitivity:
- LOD qPCR assay: 20 fg/µL
- LOD LAMP assay: 3.2 pg/µL

The LAMP and qPCR assay was field validated on eDNA samples collected from trees attacked by *A. anxius* in western Canada.

TABLE 5 qPCR and LAMP detection of *Agrilus anxius* from frass samples collected in Canada with variable starting weights (20, 40, and 80 mg) and extract dilutions (100 µL and 200 µL).

	Tested variables of DNA frass extracts					
	20 mg		40 mg		80 mg	
Site	100 µL	200 µL	100 µL	200 µL	100 µL	200 µL
Smoky Lake, AB						
LAMP (t_a)	26:30 (2/2)	33:15 (1/2)	31:00 (2/2)	22:45 (1/2)	n.d. (0/2)	35:00 (1/2)
qPCR (Ct)	30.69 ± 0.19 (3/3)	29.07 ± 0.51 (3/3)	29.67 ± 0.29 (3/3)	29.62 ± 0.26 (3/3)	29.75 ± 0.29 (3/3)	29.56 ± 0.46 (3/3)
Bailey Seed Orchard, BC						
LAMP (t_a)	30:00 (2/2)	36:00 (1/2)	39:15 (2/2)	45:00 (1/2)	42:00 (1/2)	25:30 (2/2)
qPCR (Ct)	33.85 ± 0.74 (3/3)	34.44 ± 0.15 (3/3)	30.13 ± 0.19 (3/3)	29.58 ± 0.14 (3/3)	28.33 ± 0.16 (3/3)	28.15 ± 0.25 (3/3)
Skimikin Seed Orchard, BC						
LAMP (t_a)	24:45 (2/2)	31:45 (2/2)	44:00 (1/2)	32:00 (2/2)	25:15 (2/2)	24:00 (2/2)
qPCR (Ct)	31.24 ± 0.14 (3/3)	31.12 ± 0.05 (3/3)	31.01 ± 0.05 (3/3)	29.64 ± 0.27 (3/3)	30.88 ± 0.30 (3/3)	31.74 ± 0.27 (3/3)

Note: LAMP and qPCR samples were run in duplicate and triplicate, respectively. LAMP: t_a = amplification time; qPCR: Ct mean ± SE; replications shown in brackets.

Frass and larvae resulted in positive detection of *A. anxius* from paper birch and silver birch

Galleries on silver birch filled with BBB frass

Peterson et al. (2023)

Conclusions

qPCR is a highly sensitive, rapid and specific method for the early detection of pests and pathogens. It can detect them even when they are present in small amounts or are asymptomatic, and it can analyse challenging sample, such as insect frass or airborne inoculum

LAMP is a fast and cost-effective alternative to qPCR, operating under isothermal conditions. This makes it suitable for field-based 'point-of-care' diagnosis, with results obtainable in less than 40 minutes

Early diagnosis is part of a decision-making process which in the case of plant health may prevent the spread of invasive species and assist in their eradication


THANK YOU FOR YOUR ATTENTION !