TOWARD A NEW SOIL DETECTION METHOD OF THE CAUSAL AGENT OF CHESTNUT INK DISEASE

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BACKGROUND

Phytophthora cinnamomi is the causal agent of the chestnut ink disease and yet in France, reports of the pathogen remain sparse despite heavy suspicions of its ancient presence on the territory [1]. The detectability of microorganisms is a keystone in the study of their invasiness and in the case of *P. cinnamomi*, current detection methods, *e.g.* baiting-isolation, do not finely screen potentially infested soils [2]. DNA-based methods can offer suitable alternatives as they are highly sensitive [3] but one major shortcoming is their range of application, *i.e.* the very small amount of soil used, leading to tenuous probabilities of finding the pathogen in such quantity. Recently, metabarcoding techniques have been developped to study *Phytophthora* diversity in various matrices [4] and their revelance as a screening method for *P. cinnamomi*-infested soil is yet to be deciphered.

The aim of the study is to assess the efficiency of the nested PCR [5] that was shown to be the most sensitive among a pool of several DNA-based methods [3] and the metabarcoding compared to the traditional baiting method.

METHODOLOGY

Study material : 21 environmental soil samples collected under chestnut trees in French forests of two departments (87, 24)

The baiting as a reference

50g of each soil sample was subjected to baiting [6] using chestnut leaf disks incubated for 7 days. The presence of *P. cinnamomi* was evaluated through isolation in selective media of the leaf disks, morphological identification, followed by ITS sequencing.

DNA-based methods

The DNA from 0.2g of these 21 soils was extracted using DNeasy PowerSoil kit (Qiagen) . Water negative controls and replicates were performed

9 soils	6 soils	6 soils
2 replicates	3 replicates	6 replicates

The metabarcoding pipeline

1. **ITS1 amplicon library production**
   Catala et al., 2015
2. **Sequencing** (Illumina MiSeq)
   Reagent kit v2 chemistry, 2x250pb
3. **DADA2 processing**
4. **Taxonomic assignment**
   MEGABLAST against 13 670 ITS1 sequences
5. **Data filtering**
   removal of contaminant sequences and <10 reads

The nested PCR

[5]

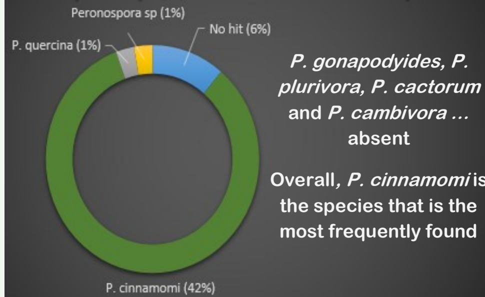
Nested : Second round PCR on the ITS4/ITS5 first round PCR products

Touchdown : Hybrizidation from 71°C to 64°C

Multiplex : Primers specific to *P. cinnamomi* and *P. cambivora*

RESULTS and DISCUSSION

The samples lack microbial diversity



> The metabarcoding is well-known to have biaises [7,8] therefore, those unexpected results could be byproducts of the technique but as no mock community was implemented, there is no way to assess the depth of the PCR biases.
> Another hypothesis is that *P. cinnamomi* invades the whole ecological niche and suppress all other species.

Nonetheless, in our case, the metabarcoding is not useful to study diversity, but it could be useful as a method of detection for *P. cinnamomi*

What method is the most revelant for the detection of *P. cinnamomi ?*

	Number of soils positive for P. cinnamomi			Number of soils negative for P.cinnamomi		
Method	Baiting	Nested PCR	Metabarcoding	Baiting	Nested PCR	Metabarcoding
2 replicates	3	2	5	6	7	4
3 replicates	3	0	1	3	6	5
6 replicates	6	3	4	0	3	2
Total	12	5	10	9	16	11

Baiting > Metabarcoding > Nested PCR

Most efficient

But the efficiency of the methods depend upon climatic conditions as it allows only the detection viable propagules [6].

Most convenient

But it appears rather anticlimatic to use a method that was designed for whole community studies as the most effective tool for the detection of a single pathogen, especially cost-wise.

Less efficient

The protocole was originally designed to be conducted on much larger quantity of soil (10g) than those used by standard DNA extraction kit (0.2-0.5g).

PERSPECTIVES

How about another method ?

AIM

The sensitive and accurate detection of both *P. cambivora* and *P. cinnamomi* in soil samples

TOOL

Droplet digital PCR aiming Ypt1

- ✓ Sensitivity
- ✓ Low sensitivity to inhibitors
- ✓ No replicates

STRATEGY

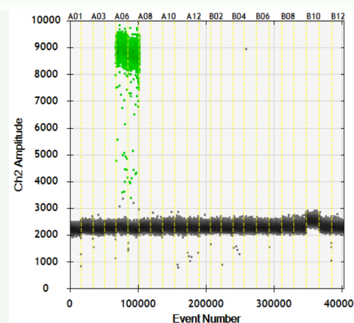
P. cambivora

Transposition from qPCR to ddPCR of primers/probe designed by Liao et al. (2019) [9]

P. cinnamomi

Design of primers and probe (Geneious v9)

PRIMARY RESULTS



Specificity for *P. cambivora* validated on pure mycelium (tested on 22 different species)

Still pending for *P. cinnamomi*

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