THÈSE PRÉSENTÉE
POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE SCIENCES ET ENVIRONNEMENT
SPÉCIALITÉ ECOLOGIE FONCTIONNELLE, EVOLUTIVE ET DES COMMUNAUTES

Par Marylise MARCHAND

BARRIERES ECOLOGIQUES A LA DISSEMINATION DES AGENTS DE L'ENCRE DANS LES CHÂTAIGNERAIES FRANCAISES

Sous la direction de : Cécile ROBIN

Soutenue le 31/03/2022

Membres du jury :

M. MARCAIS, Benoit	Directeur de recherche, INRAE NANCY	Président du jury
M. SACHE, Ivan	Professeur, AgroParisTech	Rapporteur
M. RICHARD, Franck	Professeur, Université de Montpellier	Rapporteur
Mme DELMAS, Chloé	Chargée de recherche, INRAE BORDEAUX	Examinatrice
Mme FOULONGNE-Oriol, Marie	Chargée de recherche, INRAE BORDEAUX	Examinatrice
Mme BUDDE, Katharina	Research associate, Universität Göttingen	Examinatrice
Mme ROBIN, Cécile	Directrice de recherche, INRAE BORDEAUX	Directrice de thèse

Titre: Barrières écologiques à la dissémination des agents causals de l'encre dans les châtaigneraies françaises

Résumé:

Les maladies émergentes des plantes constituent des menaces pour les services écosystémiques et à la santé publique dans le monde entier. Depuis quelques années, le châtaignier fait l'objet d'une recrudescence des signalements de dégâts d'origine pathologique, et en particulier de la maladie de l'encre. Cette maladie est causée par *Phytophthora cinnamomi* et/ou *P. x cambivora,* deux agents pathogènes exotiques qui ont été introduits en Europe au cours du XIX^{ème} siècle. L'objectif de ce travail était d'une part de préciser l'aire de distribution de l'encre du châtaignier en France, et de confirmer le statut d'espèce invasive naturalisée de *P. cinnamomi*. D'autre part, le second objectif était d'identifier les barrières écologiques qui pourraient limiter sa dissémination, des barrières liées aux hôtes, au microbiote du sol et/ou à l'environnement abiotique. Pour répondre à ces problématiques, la mise au point de méthodes de détection et de quantification de *P. cinnamomi* dans le sol (droplet digital PCR ciblée, adaptation d'un pipeline de métabarcoding) a été nécessaire. L'étude d'un dispositif de suivi du dépérissement du châtaignier en France, implémenté par le Département de la Santé des Forêts, a permis de confirmer que *P. cinnamomi* était largement répandu en France. Grâce à un dispositif expérimental dans une forêt de châtaigniers en mélange avec d'autres essences forestières, et où l'encre a été diagnostiquée, nous avons caractérisé les communautés fongiques du sol et quantifié l'inoculum de *P. cinnamomi* dans le sol. Ces communautés se sont avérées modifiées en présence de *P. cinnamomi*. De plus, nous avons mis en évidence que la quantité de *P. cinnamomi* était plus faible dans les parcelles mixtes que pures, suggérant l'existence d'un effet de dilution liée à la diversité des hôtes. La compétence des hôtes, *i.e.* capacité à transmettre l'inoculum, est un trait de l'hôte rarement évalué en phytopathologie, mais qui résulte parfois en des effets de dilution. Dans un premier temps, nous avons cherché à élaborer un cadre conceptuel pour l'étude de ce trait au travers d'une revue bibliographique. Dans un second temps, nous avons obtenu les premières évaluations de compétence pour *P. cinnamomi* de différentes essences forestières, au travers d'un dispositif expérimental en conditions contrôlées. Le chêne sessile (*Quercus petraea*) et le chêne pédonculé (*Q. robur*) se sont avérés très peu compétents pour *P. cinnamomi*. Ces résultats pourraient avoir d'importantes implications pour la gestion des écosystèmes de châtaigniers et de chênes menacés par la maladie de l'encre.

Mots clés : Maladie émergente, *Phytophthora cinnamomi*, compétence

Title: Ecological barrier to the dissemination of the causal agents of the ink disease in French chestnut groves

Abstract:

Emerging plant diseases pose threats to ecosystem services and public health worldwide. In recent years, chestnut has been subject to an upsurge in reports of pathogen damage, particularly ink disease. This disease is caused by *Phytophthora cinnamomi* and/or *P. x cambivora*, two exotic pathogens that were introduced in Europe during the 19th century. The objective of this work was on the one hand to specify the distribution area of chestnut ink disease in France, and to confirm the status of naturalized invasive species for *P. cinnamomi*. On the other hand, the second objective was to identify the ecological barriers that could limit its dissemination, barriers related to the hosts, the soil microbiota and/or the abiotic environment. To address these issues, the development of methods for the detection and the quantification of *P. cinnamomi* in the soil (targeted droplet digital PCR, adaptation of a metabarcoding pipeline) was necessary. The investigation of a monitoring network of chestnut decline in France, implemented by the Department of Forest Health, confirmed that *P. cinnamomi* was widespread in France. Using an experimental set-up in a chestnut forest mixed with other forest species, and where the ink disease was diagnosed, we characterized the soil fungal communities and quantified the soil inoculum of *P. cinnamomi*. These communities were found to be modified in the presence of *P. cinnamomi*. In addition, we found that the abundance of *P. cinnamomi* was lower in mixed than in pure plots, suggesting the existence of a dilution effect related to host diversity. Host competence, *i.e.* the ability to transmit inoculum, is a host trait that is rarely assessed in plant pathology, but which sometimes results in dilution effects. First, we sought to develop a conceptual framework for the study of this trait through a literature review. Secondly, we obtained the first evaluations of the competence for *P. cinnamomi* of different forest species, through an experiment under controlled conditions. The sessile oak (*Quercus petraea*) and the pedunculate oak (*Q. robur*) were found to have very low competence for *P. cinnamomi*. These results could have important implications for the management of chestnut and oak ecosystems threatened by the ink disease.

Keywords : Emerging disease, *Phytophthora cinnamomi*, host competence

Unité de recherche

INRA – Institut National de la Recherche Agronomique

UMR 1202- Biodiversité, Gènes et Ecosystèmes

Site de Recherches Forêt Bois de Pierroton

69, route d'Arcachon

33612 CESTAS Cedex- France

REMERCIEMENTS

La plus juste des analogies que j'ai pu entendre sur ce que c'était de faire une thèse est celle de la montagne russe, où les hauts sont très hauts et les bas peuvent être très bas. Si on ajoute à ça une petite pandémie, l'analogie tient toujours, mais l'ampleur de ces extrêmes est exacerbée. Je souhaiterais donc remercier toutes les personnes qui ont contribué, de près ou de loin, à cette thèse aussi bien scientifiquement mais surtout par leur soutien moral.

Tout d'abord, je voudrais remercier ma directrice de thèse, Cécile Robin. Merci pour toutes ces discussions et la possibilité que tu m'as donné de m'approprier ce projet. Merci pour le soutien, la confiance que tu m'as témoignée lors de cette thèse, pour les relectures interminables et ces moments passés à réfléchir ensemble.

Merci à l'équipe Gemfor pour les discussions scientifiques et pas scientifiques : Laure Villate, Gilles, Olivier, Agathe, Lisa, Marie-Laure, Benoit… Un merci tout particulier à Xavier, pour ton expérience, que tu as su me partager. Merci d'avoir été là pour ces sorties terrains, qu'il pleuve, qu'il vente, qu'il neige. Et surtout, surtout, de m'avoir fait découvrir l'anchot (et les Pyrénées). Merci à Julie pour ces discussions qui m'ont honnêtement remontées le moral. A Arthur et Jean-Paul, pour les pauses clopes les plus stimulantes que j'ai pu avoir. A Laure Villate, pour ton soutien alcoolisé.

Merci à mes collègues de bureau, présents et anciens. Le turn-over a été important au cours de ces 3 années (et demi) de thèse, mais j'ai à chaque fois rencontré des gens aussi particuliers, qu'intéressants que drôles. Merci à Tatiana en particulier, pour avoir fait ton stage avec moi, de t'être montré curieuse et d'avoir été aussi dégourdie que tu l'as été tout au long de ce stage.

Un grand merci à mes amis et ma famille d'avoir été là pour me remonter le moral aux moments où ça allait moyen-moyen. A mes potes de collège et à ceux de prépa pour ce qu'on garde d'intact malgré tout ce temps. A Thomas, mon ancien coloc, sans qui le confinement aurait eu un goût beaucoup plus triste. A Gibsette, Sez, Nora, Vic, Titi, pour le flex incroyable que vous apportez dans ma vie. A Marie, merci pour ton soutien indéfectible, tes relectures, pour tout.

Merci au DSF et à la région Nouvelle Aquitaine pour le financement de cette thèse.
Je terminerai sur une citation qui, je trouve, résume plutôt bien la thèse en général :

« Petit à petit, le chameau rentre dans la casserole »

Table des matières

INTRODUCTION

1. La maladie de l'encre du châtaignier

1.1. La santé des forêts

La structure et la composition des forêts sont en perpétuelle évolution puisqu'elles sont fréquemment exposées à des perturbations naturelles comme les feux, les tempêtes, les sécheresses, les épidémies (Jactel *et al.*, 2017). De nouvelles perturbations, médiées par l'homme, altèrent les forêts trop rapidement pour que les processus d'adaptation puissent suivre leur rythme (Figure 1). Typiquement, la plus grande partie des épidémies est causée par des espèces exotiques invasives puisqu'en forêts saines, les pestes et pathogènes sont une part intégrante de l'écosystème (Dajoz, 2000).

Définir la santé d'une forêt, comme en médecine humaine, est difficile à appréhender. En foresterie, il est commun de mesurer la condition d'une forêt à l'échelle d'un peuplement par sa productivité. Cependant, ce proxy néglige des attributs importants d'une forêt comme l'assemblage des espèces, la structure de la végétation, la biomasse, les cycles de nutriments. Il existe des mesures de la santé des forêts qui vont de strictement utilitaires à des définitions plus écologiques (voir Trumbore *et al.*, 2015). La mortalité des arbres demeure un bon indicateur de la santé des écosystèmes forestiers. En effet, une forêt en bonne santé va enregistrer ordinairement un niveau « basal » de mortalité d'arbres. Son augmentation peut traduire un excès de stress subit par la forêt. L'un des paramètres clés d'une forêt saine est sa capacité à se remettre d'une perturbation. Les forêts sont de plus en plus soumises à des stress (climatiques, biotiques ou stochastiques) qui les font basculer vers un état non-sain (Figure 1).

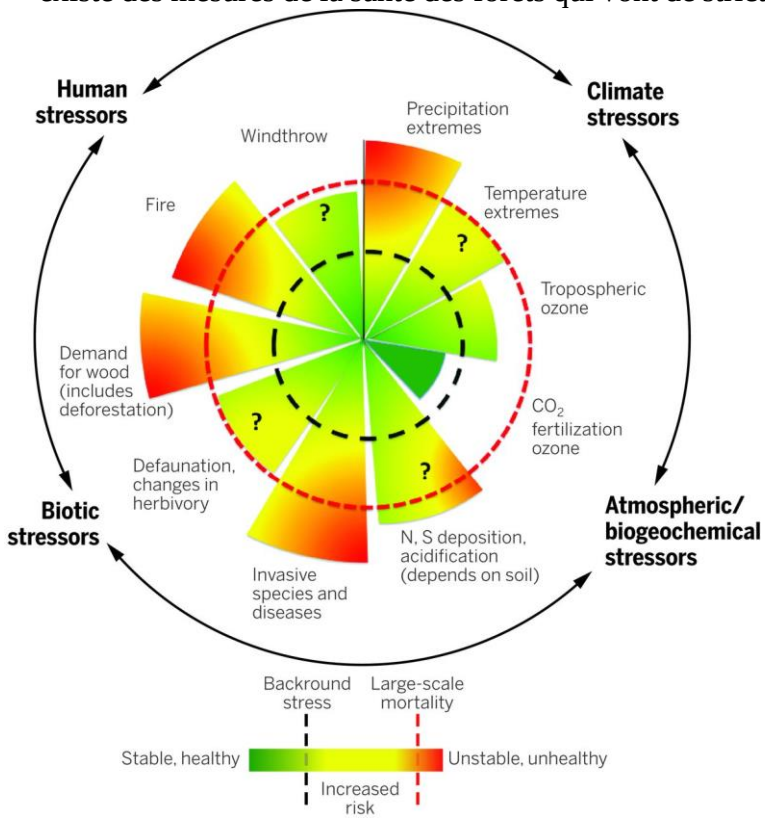


Figure 1: Exemple de différents stress et perturbations qui affectent les forêts et leurs possibles évolutions (Source : Trumbore *et al.*, 2015)

En France, les dernières années se sont révélées plutôt défavorables pour la forêt. En effet, le taux d'arbres morts sur pied a connu une augmentation (IGN, 2021). Plus particulièrement, le châtaignier est l'une des essences pour laquelle la situation s'est le plus dégradée (Figure 2).

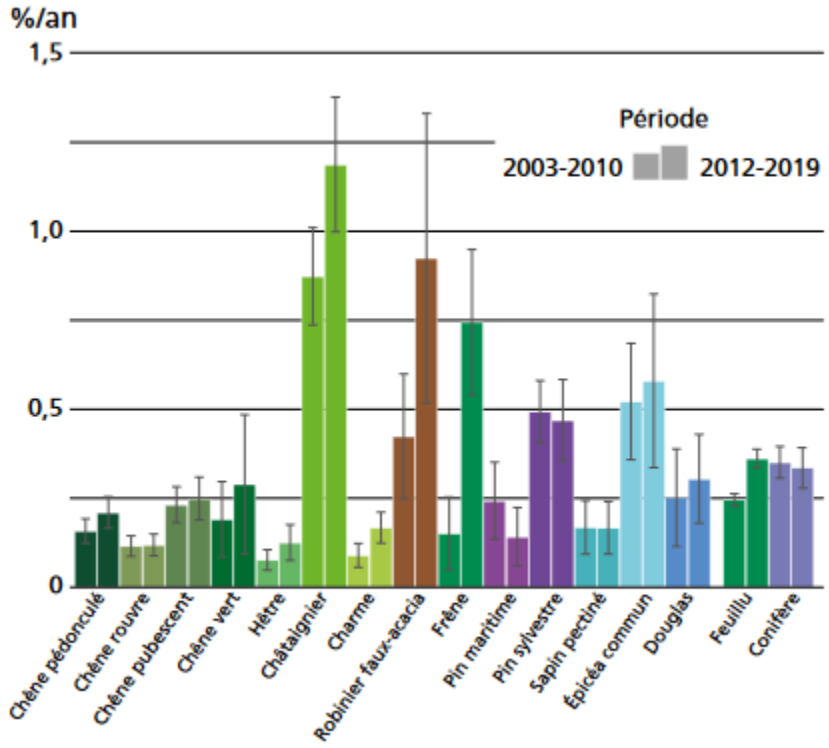


Figure 2: Evolution du taux annuel moyen d'arbres morts de moins de 5 ans selon l'essence (Source : IGN Inventaire Forestier N°47, Novembre 2021).

1.2. Le châtaignier, une espèce ligneuse particulièrement vulnérable

Le châtaignier, *Castanea sativa* (*Castanea*, ancien nom du chêne; -*sativa*, cultivé), appartient à la famille des *Fagacées* (Rameau *et al.*, 2008). Le centre d'origine du châtaignier est supposé être situé dans la région transcaucasienne. Les résultats d'études de génétique de populations suggèrent que plusieurs zones en Europe (région caucasienne mais aussi en Espagne, Italie) ont servi de refuge pendant le dernier maximum glaciaire (Krebs *et al.*, 2004; 2019; Mattioni *et al.*, 2013). *C. sativa* serait naturalisé depuis le néolithique après son introduction par la main de l'homme (Figure 3). Des traces fossiles de tissus datant de 8.5 millions d'années ont été identifiées en France (Ardèche) (Breisch, 1999). Cependant la date et les mécanismes associés à la domestication et la migration de *C. sativa* vers l'Europe occidentale sont toujours débattus.

Figure 3: Carte de la distribution de *Castanea sativa* en Europe. En vert: aire probable d'origine, en jaune : Population introduite et naturalisée depuis le Néolithique (Source : Caudullo *et al.*, 2017).

Le châtaignier a largement été planté sur le territoire français où il semble s'être acclimaté avec une aise relative (Bourgeois, 2004, Figure 4). Le châtaignier est une essence plastique mais pas frugale qui peut se satisfaire de pH comprit entre 4.5 et 6.5 (Bourgeois, 2004). C'est une espèce calcifuge (Bourgeois, 2004), c'est-à-dire qu'elle montre des signes de détresse dans des sols avec du calcaire labile dit "actif" (à un taux de >5%) (Breisch, 1999). C'est la raison pour laquelle on ne le retrouve que rarement sur sols calcaires mais de façon plus privilégiée sur sols siliceux (roches magmatiques) (Bourgeois, 2004). C'est une essence sensible au manque (réserve utile minimum de 120 mm) ou à l'excès d'eau, aussi on le retrouve sur des sols plutôt secs, relativement aérés mais profonds (Bourgeois, 2004). Cette espèce se développe dans des régions avec des températures annuelles moyennes entre

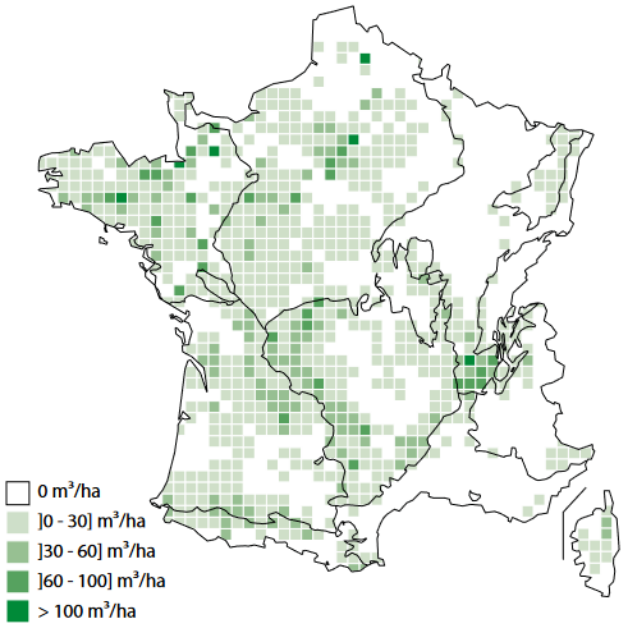


Figure 4: Volume moyen à l'hectare du châtaignier en France (Source : IGN 2019).

8°C et 15 °C et des précipitations entre 600-700 mm/an à 1500-1600 mm /an (Freitas *et al.*, 2021).

Cette essence multiservice a fortement imprégné les coutumes et la vie du terroir. En effet, il était à la fois le pain du pauvre, qui sauva de la disette de nombreux paysans, et le faux marron glacé qui réchauffe les veillées de Noël. Au Moyen-âge, son bois ainsi que sa reprise rapide après coupe et sa facilité d'emploi étaient des qualités appréciées dans la fabrication des échalas. Au XX^{ème} siècle, la culture du châtaignier a décliné du fait de l'exode rural, de la maladie et de sa haute teneur en tannin qui a justifié son exploitation en masse principalement dans la région lyonnaise (Bourdu, 1996). Les châtaigniers qui subsistent sont exploités pour leurs fruits, la châtaigne, dont la production est assurée par deux types de vergers, les vergers traditionnels qui sont majoritaires dans le Sud-Est de la France et les vergers plus récents composés d'hybrides interspécifiques (*C. sativa x C. crenata*) (Bourgeois, 2004), et pour leurs bois. En raison de sa durabilité et de sa haute teneur en tannin, le bois châtaignier est particulièrement adapté pour un usage en extérieur (Rameau *et al.*, 2008). En forêt française, il est essentiellement présent à l'état de taillis denses et ne forme que rarement de belles futaies (Bourdu, 1996). Les grumes de gros diamètre sont exploitées en produits construction (charpente, bardage ...), et ameublement (plots, plateaux ...). Les billons de diamètre moyens souvent issus de taillis sont valorisés dans les lames de parquet, terrasse. Les fleurs de châtaigniers sont particulièrement appréciées par les apiculteurs. C'est une essence dont les différents usages ne tarissent pas. Ce manuscrit se concentre sur les châtaigniers en situation forestière ainsi, le terme « châtaignier » fait uniquement référence à l'espèce *C. sativa*.

En absence de gestion forestière, les peuplements de châtaigniers ont tendance à se faire coloniser par d'autres espèces et à évoluer vers des forêts mixtes (Conedera *et al.*, 2016). Bien qu'étant implantée sur le territoire, c'est une essence particulièrement vulnérable aux bioagresseurs. En effet, il est l'hôte principal du chancre du châtaignier, *Cryphonectria parasitica*, qui fut introduit en France en 1950 et qui a été responsable de l'extinction de la quasi-totalité des châtaigniers américains (Anagnostakis, 2001; Crandall *et al.*, 1945). Il est également l'hôte d'un insecte galligène, le cynips, *Dryocosmus kuriphilus* (Fernandez-Conradi *et al.*, 2018), qui fit son apparition en France en 2007. Bien que des moyens de lutte biologique efficaces, comme le virus CHV1 (*Cryphonectria* HypoVirus 1) pour le chancre et l'insecte antagoniste *Torymus sinensis* pour le cynips, ait été mis en place pour contrôler ces deux

parasites, il n'en demeure pas moins que le châtaignier ait subi et subit encore un lourd bilan. En effet, il est un hôte sensible de la maladie de l'encre.

1.3. La maladie de l'encre : une maladie, deux agents causals

La maladie de l'encre du châtaignier résulte principalement de l'infection par deux espèces de *Phytophthora* : *Phytophthora cinnamomi* et/ou *P. cambivora* (Vettraino *et al.*, 2005) mais ce ne sont pas les seules espèces associées à la maladie. En Europe, *P. plurivora, P. syringae, P. cactorum, P. citricola, P. cryptogae* and *P. heveae* ont été isolées à partir de sol sous des châtaigniers symptomatiques (Vettraino *et al.*, 2005; Akili Şimşek *et al.*, 2019; Sharpe 2017). Etant les plus virulentes et abondantes, seulement *P. cinnamomi* et *P. x cambivora* seront considérées par la suite (Akilli Şimşek *et al.* 2019; Sharpe 2017). Ces espèces de *Phytophthora* appartiennent aux Oomycètes (règne Straménopiles), à deux subclades phylogénétiques distincts des *Phytophthora* (subclades 7c et 7a, respectivement, Figure 5).

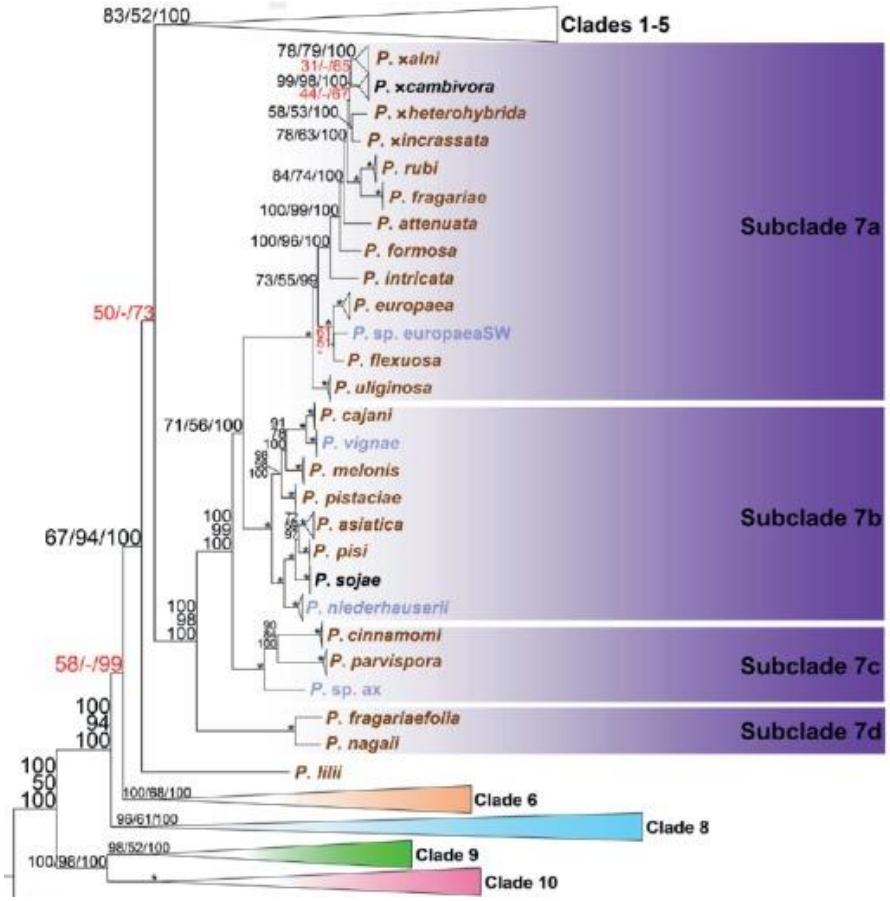


Figure 5: Structure du clade 7 des *Phytophthora* auquel appartiennent *P. cinnamomi* et *P. x cambivora* basé sur sept marqueur génétique nucléaire (Source : Yang et *al.*, 2017)

Les Oomycètes sont un taxon phylogénétique distinct de celui des « vrais » champignons mais proche. Ils présentent certaines similarités physiologiques et biochimiques avec les

champignons, *i.e.* hyphe filamenteux, reproduction par spores, mode de nutrition (Judelson et Blanco, 2005), qui ne seraient que le fruit d'une convergence d'évolution. Ils se distinguent dans la composition de leur paroi, le fonctionnement de leurs voies métaboliques (Erwin et Riberio, 1996) et la production de zoospores asexuée motiles possédant des flagelles (Figure 6B) responsables de leur mouvement qui est le caractère le plus distinctif de ces organismes (Beakes *et al.*, 2012; Cahill *et al.*, 1996).

P. cinnamomi et *P. x cambivora* sont des espèces telluriques qui infectent le système racinaire. *P. cinnamomi* infecte essentiellement les racines principales et fines, mais il est également capable d'infecter les tiges ligneuses par des blessures ou des ruptures naturelles dans le périderme (O'Gara *et al.*, 2015). Quand les conditions sont favorables à leur développement, ces pathogènes entrent en phase de sporulation asexuée (Figure 6A), par la libération de zoospores (Figure 6B) par les sporanges (Figure 6C). Ces zoospores, qui naviguent de façon autonome dans les microporosités du sol, sont attirées par chimiotactismes vers leurs hôtes, *i.e.* les exsudats racinaires. Le contact avec les racines de l'hôte conduit au détachement du flagelle, puis à la fixation des spores sur la surface, *i.e.* enkystement. Les zoospores germent ensuite et pénètrent les tissus de l'hôte grâce à la production d'enzymes qui dégradent la paroi des cellules (Hardham, 2005). En 2-3 jours chez les hôtes susceptibles, comme l'est le châtaignier, des sporanges secondaires se forment à la surface des racines. Ceci peut se répéter plusieurs fois de façon rapide (on parle d'infections multicycliques) amplifiant rapidement le potentiel d'inoculum dans la zone infectée (Hardham, 2005). Les *Phytophthora* sont capables de survivre sur le long-terme dans le sol et tissus infectés grâce à des structures de dormances (Sendall et Drenth, 2001) : les chlamydospores (Figure 6D) et dans une moindre mesure, les oospores (Figure 6E) (Judelson et Blanco, 2005).

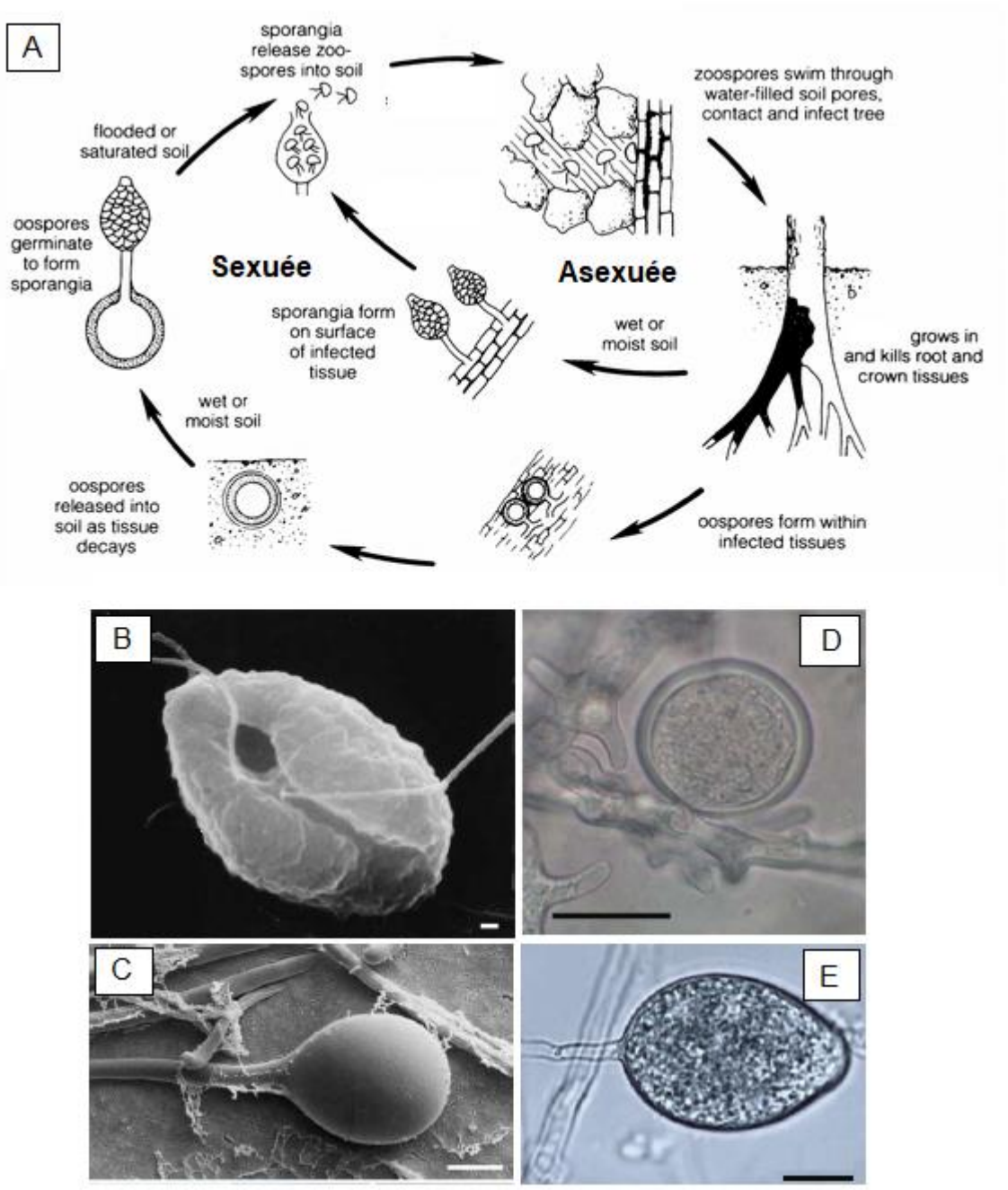


Figure 6: **A**: Cycle de vie des *Phytophthora* (Source : Wilcox, 1992), **B**: Zoospore de *P. cinnamomi* avec les deux flagelles apparents, **C**: Sporange de *P. cinnamomi* se développant sur un apex hyphal, , **D**: chlamydospore de *P. cinnamomi*, **E** : Oospore de *P. cinnamomi*
(Les images B et C issues de microscopie électronique à balayage, source : Hardham, 2005 et les images D et E sont issues de microscopie optique, sources : McCarren et *al.*, 2005; Frisullo *et al.*, 2018)

1.4. Une étiologie plutôt complexe

Les plantes ont la capacité intrinsèque à détecter et répondre à une infection et sont, par conséquent, capables de lutter contre la majorité des pathogènes potentiels (Lapin et Van den Ackerveken, 2013). Cependant, la coévolution entre hôte et agent pathogène a pour conséquence une adaptation perpétuelle des mécanismes de défense du côté de la plante et infectieux du côté du pathogène. Dans certains cas, le système de réponse de la plante se retrouve inefficace face à des pathogènes qui ont évolué de telle sorte qu'ils puissent contourner cette immunité. Les plantes doivent se défendre contre un large panel de champignons et oomycètes pathogènes qui ont mis en place différentes stratégies pour acquérir leurs ressources en énergie et subvenir à leur besoin en nutriments organiques. Il y a des organismes pathogènes qui dégradent et détruisent les cellules hôtes pour se nourrir des tissus morts (on parle de nécrotrophes). Certains ponctionnent les nutriments sans tuer les cellules (biotrophes) et d'autres, comme les *Phytophthora*, commencent leur cycle en infectant les tissus vivants et poursuivent leur développement sur les tissus morts (hémibiotrophes). Ainsi, les plantes doivent posséder et répondre de façon appropriée à chacune de ces différentes stratégies.

Suite aux infections racinaires, le mycélium de *P. cinnamomi* ou *P. cambivora* envahit le cortex, croît rapidement dans le péricycle, détruit le phloème et dans une moindre mesure le xylème (Oßwald *et al.*, 2014). L'apport nutritif et hydrique jusqu'au sommet de l'arbre est alors diminué ce qui se traduit par une diminution de la surface foliaire, la microphyllie, puis le flétrissement des feuilles. Enfin, l'arbre alloue son énergie à un nombre plus limité de feuilles, ce qui se traduit par une défoliation progressive. Finalement, l'arbre ne peut plus fournir d'énergie aux feuilles et la couronne est complètement défoliée, ce qui entraîne la mort de l'individu. Cette dégradation progressive de l'état de l'arbre est qualifiée de « dépérissement ».

C'est ici que réside le premier problème de diagnostic de la maladie. Un arbre soumis à un stress abiotique, par exemple un stress hydrique, présentera un faciès similaire à celui d'un arbre infecté par les agents de l'encre (Maurel *et al.*, 2001) à l'exception du fait que le processus est beaucoup plus rapide dans le cas des arbres atteints de la maladie de l'encre. La réponse physiologique d'un arbre en asphyxie racinaire est par ailleurs similaire à celle induite par un stress hydrique (Bradford et Hsiao, 1982). De plus, en France, le châtaignier se trouve souvent dans des sites qui ne lui sont pas favorables, tels que des sols calcaires, peu profonds ou hydromorphes (Bourgeois, 2004), ce qui peut entraîner un dépérissement suite à des stress

abiotiques et à la présence de bioagresseurs secondaires. Au sens strict, le dépérissement est une situation pathologique multifactorielle complexe qui implique un large spectre de facteurs de stress abiotiques et biotiques (Manion, 1991) et qui se traduit par une dégradation progressive de l'état de santé de l'arbre. La difficulté du diagnostic du dépérissement réside dans le fait que si l'impact d'un facteur de stress individuel peut être évalué, il est très difficile d'extrapoler l'impact interactif et potentiellement additif de multiples facteurs de stress, qui peuvent se produire séquentiellement ou simultanément (Niinemets, 2010). Ainsi, il est probable que de nombreux événements de dépérissement du châtaignier ont été attribués à des stress abiotiques lorsque *P. cinnamomi* et/ou *P. x cambivora* seraient impliqués. Contrairement à la spirale de mort inéluctable de Manion (1991), Whyte *et al.* (2016) décrivent le déclin par le biais d'un modèle de rétablissement du déclin de l'arbre (Tree Decline Recovery Seesaw) qui prend en compte la possibilité de récupération (Figure 7). La santé de l'arbre peut fluctuer en fonction des différents stress qu'il rencontre, en tenant compte de sa résilience et de sa capacité d'acclimatation aux stress précédents (Whyte *et al.*, 2016).

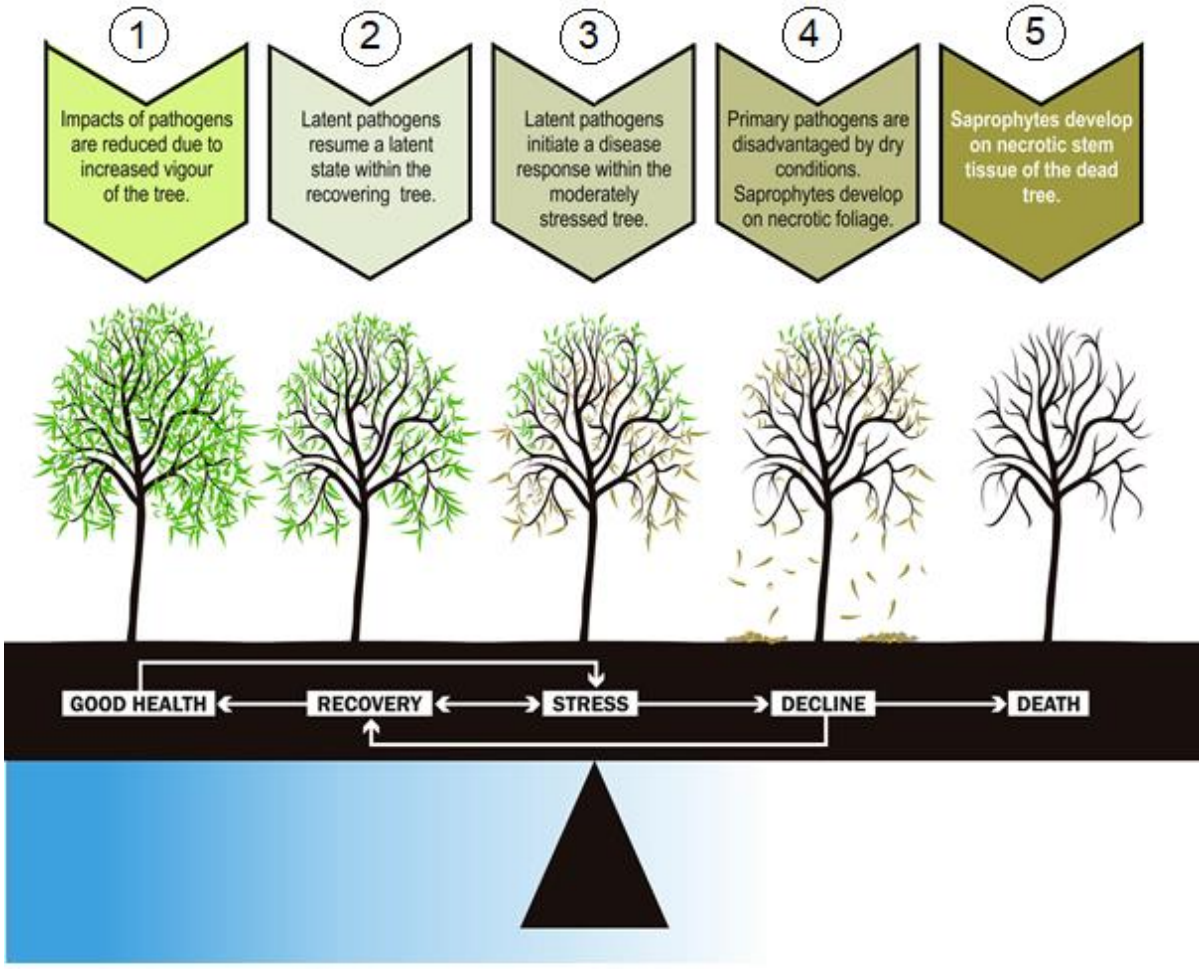


Figure 7: Modèle de rétablissement du déclin de l'arbre (adapté de Whyte *et al.*, 2016)

Les agents de l'encre sont considérés comme étant des agents primaires d'infection qui vont provoquer le passage du châtaignier de la phase saine 1 aux étapes suivantes. Dans un premier temps, l'inoculum au pied de l'arbre augmentera de façon proportionnelle à l'état de dégradation de l'arbre. Cependant, un stress abiotique de type sécheresse peut faire basculer l'arbre infecté et stressé (3) à l'étape suivante (4). En effet, les épisodes de sécheresses peuvent diminuer la capacité des arbres à résister aux agresseurs. De plus, un arbre dont le système racinaire est en partie détruit suite aux infections est plus sensible à un stress hydrique (Maurel *et al.,* 2001). La bascule vers l'étape 5 dépend de la répétition des stress abiotiques qui peut résulter en la mort de l'arbre. Par ailleurs, comme un stress hydrique a aussi un effet négatif sur le développement de *P. cinnamomi* dans les tissus végétaux et dans le sol, un retour à un niveau de précipitations favorables à la croissance de l'arbre hôte peut permettre un rétablissement, en l'absence d'inoculum viable dans le sol. Si à une période de sécheresse succèdent de fortes précipitations, l'excès d'eau étant défavorable au châtaignier mais favorable aux *Phytophthora,* l'arbre hôte ne se rétablira pas et son état de dépérissement s'accentuera encore. Ce schéma

illustre pourquoi la présence de *P. cinnamomi* (et *P. x cambivora*) pourrait ne pas être directement proportionnelle à l'intensité du dépérissement. En effet l'inoculum ne sera pas forcément à son maximum aux étapes où l'arbre est le plus défolié.

Figure 8: Symptômes liées à l'infection par *P. cinnamomi* (et *P. x cambivora*)
(Source : INRAE et DSF)

La croissance de *P. cinnamomi* (ou *P. x cambivora*) dans les tissus de l'hôte peut se traduire par des nécroses corticales (Figure 8), qui sont le prolongement de nécroses racinaires. Cependant, elles ne s'observent pas systématiquement car l'arbre (sensible) meurt des dommages racinaires avant que les lésions ne puissent atteindre le collet (Maurel *et al.*, 2001). Ces nécroses corticales du collet sont parfois accompagnées d'écoulements noirâtres, similaires à de l'encre, dont la maladie tient son nom. Lorsque les semis issus de régénération naturelle sont présents dans la parcelle, il est facile d'observer des nécroses sur les plants qui sont en train de flétrir (Figure 8). Etant une réaction de défense, le symptôme « d'encre » n'est pas spécifique à *Phytophthora* et peut être imputé à d'autres bioagresseurs. De plus, sur châtaignier, ce type de symptôme est loin d'être systématique. En revanche, du fait de la dissémination des *Phytophthora* dans le sol, la distribution de la maladie est agrégée en « foyers » (Figure 8). Une telle dynamique de dépérissement permet de suggérer la présence de ces agents pathogènes et

leur implication dans les symptômes de dépérissements observés, même si *Armillaria mellea* est reconnu pour provoquer des symptômes similaires.

1.5. Des pathogènes difficiles à identifier et à quantifier

Comme il vient d'être mentionné précédemment, le diagnostic de la maladie est difficile à mettre en place. A cela s'ajoute les difficultés liées à la détection et à la quantification des agents pathogènes dans le sol.

Des tests *in situ* permettent rapidement (5 minutes) de confirmer la présence du pathogène et de réduire le nombre d'échantillons envoyé au laboratoire. Ces tests de dépistage, le kit ELISA (Enzyme-Linked ImmunoSorbant Assay, Agdia, Elkhart, IN), le dispstick ou des dispositifs de flux latéraux (LFD, Pocket Diagnostic, Central Science Laboratory, York, UK), sont basés sur une affinité anticorps-antigène (O'Brien *et al.*, 2009). Un problème inhérent à cette approche est qu'hormis pour le dipstick, difficile à obtenir en Europe, le test n'est pas spécifique à une espèce de *Phytophthora.* En effet, il y a un risque de faux positif à autre genre d'oomycète, les *Pythium* (Hardham, 2005) et le test ne détecte pas toujours la présence du pathogène même lorsqu'il est présent (O'Brien *et al.*, 2009). Tomlinson *et al.* (2010) propose une identification efficace de *Phytophthora* en un temps très réduit à partir des composés de ces tests d'immunodétection. Un problème supplémentaire provient du fait qu'il nécessite de prélever des tissus nécrosés pour exécuter ces tests : soit de racines (difficiles à prélever sauf sur semis), soit de tissus corticaux du tronc (rare dans le cas des châtaigniers). Les méthodes classiques de détection de *Phytophthora* comprennent l'isolement du pathogène de tissus et de sols infectés par piégeage biologique (Erwin et Ribeiro, 1996). L'isolement de *Phytophthora* à partir de tissus nécrosés ou non symptomatiques nécessite la mise en milieu sélectif afin d'empêcher la croissance d'autres micro-organismes antagonistes (Sendall et Drenth, 2001). Cette méthode nécessite des observations minutieuses en laboratoire et permet la détection les *Phytophthora* vivants uniquement: le mycélium doit être actif dans les tissus pour pouvoir se développer sur le milieu de culture. Or, il est reconnu que *P. cinnamomi et P. x cambivora* ont une durée de vie limitée dans les tissus corticaux de châtaignier. Il est donc communément admis qu'un échec de détection par ces méthodes ne signifie pas une absence du pathogène (Cooke *et al.*, 2007). En cas d'échec, il est possible d'isoler *P. cinnamomi et P. x cambivora* dans le sol récolté au pied des arbres malades. La technique la plus usuelle demeure alors le piégeage biologique qui, d'une façon simple et peu onéreuse, utilise la formation et la migration des zoospores à partir

de l'inoculum présent dans le sol dans une solution aqueuse du sol vers des tissus sains, les « pièges », qui sont soit des disques foliaires soit des fruits de plantes sensibles (*Quercus sp.*, *Castanea sp*.) desquels les espèces de *Phytophthora* sont isolées (Figure 9A).

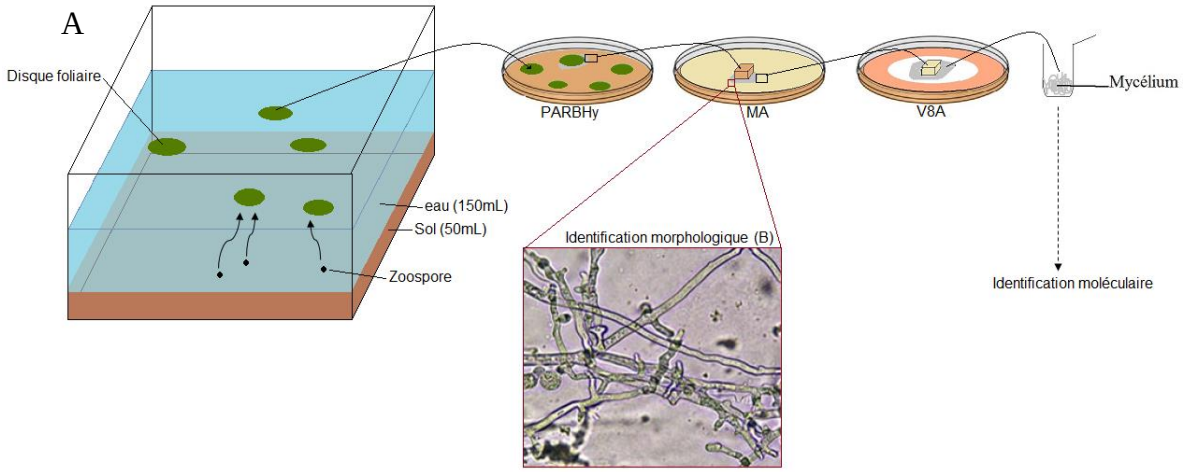


Figure 9: **A** : Procédure d'isolement biologique à partir du sol (PARHBy : Milieu sélectif, MA : Milieu de culture malt agar, V8A : Milieu de culture composé de jus de 8 légumes), **B** : Zoom sur la caractéristique forme en corail du mycélium de *P. cinnamomi* (Source : Akili *et al.*, 2012).

Cependant, la formation de zoospores dans le milieu (favorisée par la présence d'eau) n'est pas toujours suffisante pour un résultat positif (O'Brien *et al.*, 2009). Après l'obtention de cultures pures de *Phytophthora spp*., l'espèce est identifiée soit par ses caractéristiques morphologiques (Figure 9B, exemple pour *P. cinnamomi*), soit par séquençage. Dans le premier cas, l'identification morphologique requiert une expertise importante et la variation des caractères morphologiques des isolats d'une même espèce sont autant de freins au succès de cette méthode (Hardham, 2005). Dans le second cas, un ou plusieurs gènes sont amplifiés (en utilisant des amorces spécifiques des *Phytophthora spp.*) par la technique dite de Polymerase Chain Reaction (PCR). Les séquences obtenues sont comparées à celles présentes dans les banques de données et permettent d'identifier l'isolat étudié. Pour rappel, l'amplification PCR est une réaction qui permet d'obtenir de l'ADN en quantité suffisante pour analyse, en amplifiant de l'ADN à partir d'un court fragment de nucléotides spécifique à l'ensemble du groupe d'espèces ciblées (ici *Phytophthora spp.)* appelé amorce. La plupart des protocoles et kits commerciaux utilisés pour l'extraction d'ADN du sol ou de tissus sont calibrés pour des quantités très faibles de substrats (0.2g). Le sol constitue un excellent réservoir d'inoculum à *Phytophthora* (Elliot *et al.*, 2015) grâce à leur structure de dormances (Oßwald *et al.*, 2014). Cependant, le sol étant une matrice particulièrement hétérogène dans la distribution des micro-organismes (Baveye *et al.*, 2016), le fait d'analyser un échantillon de faible taille amenuise les probabilités d'obtenir

de l'ADN de *Phytophthora* telluriques. Langrell *et al.* (2011) ont proposé de ce fait un protocole d'extraction à partir de 10g de sol. Cependant, le traitement d'une telle charge n'est pas possible dans tous les laboratoires qui doivent être équipés (rotor de centrifugeuse pour tubes adapté, broyeuse gros calibre …). De plus, de nombreux contaminants présents dans le sol ou dans les différents tampons sont co-purifiés dans le processus d'extraction ADN, ceux-ci peuvent compromettre l'efficacité de la PCR, rendant nécessaire la purification des produits post-extraction.

Tous ces problèmes qui sont un frein à la détection des agents pathogènes dans le sol le sont également pour leur quantification. A l'heure actuelle, il existe des méthodes de quantification sur tissus (Engelbrecht *et al.*, 2013; Eshragi *et al.*, 2011). Cependant, malgré quelques tentatives utilisant un système de piégeage semi-quantitatif (Eden *et al.*, 2000) ou de PCR en temps-réel directement sur les pièges (Sena *et al.*, 2018), la quantification des pathogènes dans le sol est encore loin d'être aboutie.

Au vu de ce qui vient d'être présenté, le besoin pour une méthode de détection et de quantification des agents pathogènes dans le sol est nécessaire. Le premier chapitre de cette thèse est dédié à la mise au point d'une telle méthode.

RESUME

- L'infection du châtaignier par les agents de l'encre provoque un état de dépérissement et des symptômes de nécroses corticales suintantes (liées à des écoulements d'encre)

- Le diagnostic terrain de la maladie est difficile à réaliser au vu du seul symptôme de dépérissement

- La détection et la quantification des pathogènes dans le sol nécessitent d'être améliorées

2. Le processus des invasions biologiques en forêts

Au cours des derniers siècles, une redistribution sans précédent des espèces terrestres s'est opérée (Seebens *et al.*, 2017) et ce, par l'intermédiaire de l'homme. En effet, les activités humaines entraînent involontairement la migration d'espèces hors de leur aire biogéographique par le biais des échanges mondiaux (Sikes *et al.*, 2018), de la fragmentation des habitats et de la modification de l'usage des terres (Burdon et Thrall, 2008), des voyages humains et des changements climatiques (Seebens *et al.*, 2017). Le taux d'accumulation d'espèces exotiques dans de nouveaux environnements ne montre aucun signe de saturation et ce, malgré la mise en place de lois de régulation de la biosécurité (Seebens *et al.*, 2017). L'introduction des espèces exotiques est un phénomène largement reconnu pour ses effets néfastes sur les écosystèmes (McGeoch *et al.*, 2010). Le processus d'invasion des espèces dites « alien » est à la fois un composant et un moteur des changements globaux (Dickie *et al.*, 2017). En 2011, Blackburn et ses collaborateurs ont élaboré un modèle simple qui décrit le processus invasif en différentes étapes successives (Figure 10).

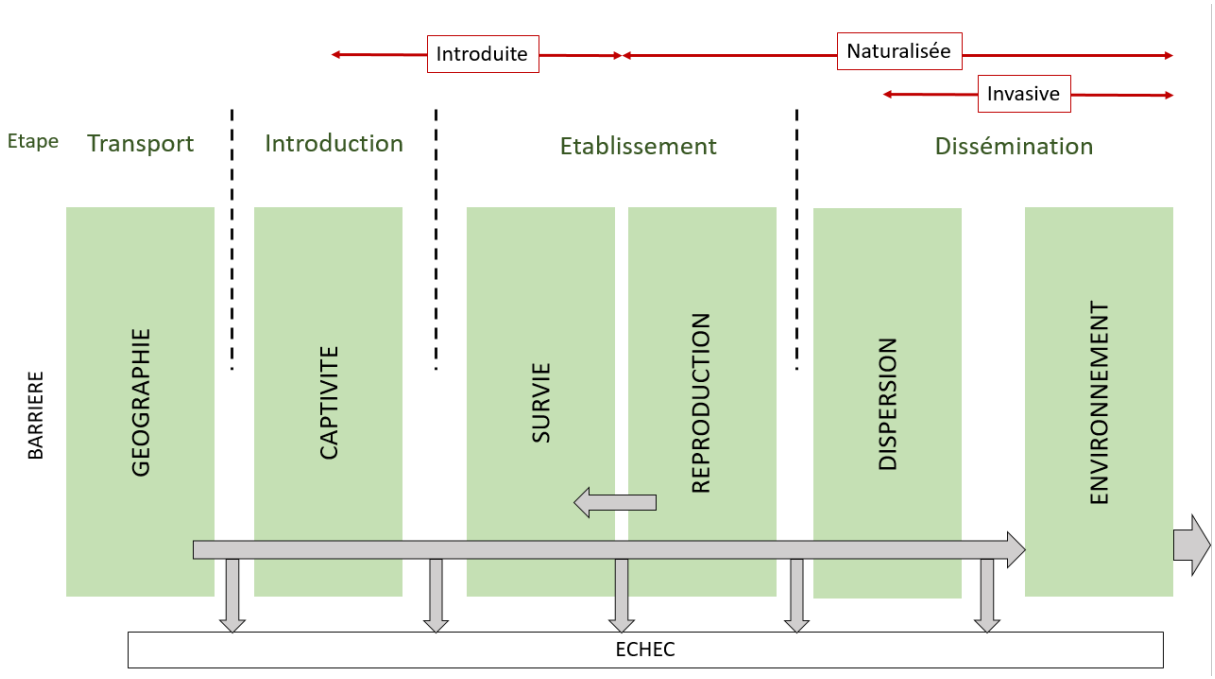


Figure 10: Modèle du processus d'invasion (adapté de Blackburn *et al.*, 2011)

A chaque étape, l'espèce doit surmonter une barrière afin de passer à la phase suivante pour *in fine* devenir invasive dans l'écosystème exogène dans lequel elle a été introduite. L'échec d'invasion peut se produire à n'importe quelle étape du *continuum* d'invasion. Seulement entre 5 et 20% des espèces arriveraient à surmonter les barrières (Williamson et Fitter, 1996). Les populations peuvent être incapables de survivre, de se reproduire, ou de se maintenir à un niveau

de population adéquat les empêchant ainsi de devenir invasives. Dans d'autres cas, les populations peuvent s'établir et ne pas se disséminer (échec d'invasion après naturalisation). Les espèces peuvent stagner à un stade précédent ou bien revenir à un état antérieur menant à une extinction locale ou régionale (Simberloff et Gibbons, 2004). Le processus d'invasion réussi peut être décrit comme l'introduction d'une espèce dans un environnement récipient et qui a atteint le stade de la dissémination (Blackburn *et al.*, 2011) car c'est le stade où les impacts écologiques des organismes exotiques deviennent apparents (Thakur *et al.*, 2019). Il existe plusieurs hypothèses pour expliquer le succès d'invasion d'espèces exotiques. Par exemple, l'hypothèse de ERH[1] (Enemy Release Hypothesis) qui, appliquée aux espèces fongiques ou oomycètes, expliquerait que les espèces pourraient atteindre une plus grande fitness dans leur nouvelle aire d'introduction en raison de l'absence d'ennemis naturels.

Nous allons présenter ici le modèle de Blackburn *et al.* (2011) appliqué à *P. cinnamomi* et P. *x cambivora* lorsque cela est possible.

2.1. Le transport des *Phytophthora,* le franchissement des barrières géographiques

Le franchissement des barrières géographiques nécessite une phase de transport de l'aire d'origine à l'aire d'introduction. Le transport des *Phytophthora* est notamment possible par l'introduction de matériel végétal infecté où les *Phytophthora* pathogènes se trouvent sous forme latente (phase hémibiotrophe) ou dans le sol sous forme de chlamydospores.

Le centre d'origine exact de *P. cinnamomi* est largement débattu puisqu'il situerait possiblement en Nouvelle-Guinée, en Indonésie, à Sumatra, en Malaisie, mais aussi potentiellement en Afrique du Sud (Zentmyer, 1988). Plus récemment, la Papouasie-Nouvelle Guinée a été identifiée comme le centre d'origine de *P. cinnamomi* (Arentz, 2017). L'étude des mating types[2] de *P. cinnamomi* est employée pour retracer leur historique géographique. Au cours des 4 500 dernières années, le mating type A1 de *P. cinnamomi* aurait probablement migré de la Papouasie-Nouvelle-Guinée vers l'Asie à n'importe quel moment lors de voyages commerciaux (Moore, 2003). La présence d'isolats A1 d'une structure similaire à celle d'Australie suggère que l'agent pathogène aurait migré de Taïwan à l'Australie par la suite (Vannini *et al.*, 2014). En revanche, le centre d'origine du mating type A2 demeure obscur. Il

[1] Initialement postulée pour les plantes invasives par Keane et Crawley, 2002.
[2] Voir la partie 2.3. L'établissement des espèces

a été suggéré que ce mating type soit apparu suite à une modification du A1 (Arentz, 2017). Dans tous les cas, le A2 est plus largement répandu que le A1 dans le monde et pourrait avoir été transporté d'Asie vers les Amériques latines (ou autres) via le transport de plantes par les Espagnols ou autres exploreurs au début du XVII^{eme} siècle (Zentmyer, 1988). Quant à *P. x cambivora* aucune étude n'a été menée pour déterminer l'origine de cette espèce, mais son transport, tout comme *P. cinnamomi*, s'est opéré par inadvertance via le transport de terre ou le transport de matériel végétal infecté (McNeill *et al.*, 2011; Meurisse *et al.*, 2019; Jung *et al.*, 2016).

2.2. L'introduction des espèces pathogènes, le franchissement de la barrière de captivité

Pour les microorganismes qui sont involontairement introduits sur des plantes ou des animaux vecteurs, cette phase est liée à celle des vecteurs. Pour des agents phytopathogènes, cette phase se déroulerait lorsque l'agent phytopathogène s'échappe des pépinières (ou/et parcs et jardins), dans lesquelles la plante exotique porteuse du pathogène est cultivée, vers un écosystème naturel. Les pépinières jouent un rôle crucial puisque les plants forestiers sont nécessaires à la régénération artificielle des forêts. En effet, dans certains cas, la plantation est la seule alternative viable pour le renouvellement des forêts lorsque la régénération naturelle est infaisable. L'étape d'introduction est déterminante pour l'installation réussite du pathogène. Une enquête menée à l'échelle de l'Europe a révélé la présence d'espèces de *Phytophthora* dans 91.5% de ces pépinières (Jung *et al.*, 2016) et dans une moindre mesure en France à 31% (Decourcelle et Robin, 2009). Plus inquiétant encore, la présence des pathogènes ne se révèle pas de façon évidente puisque certains hôtes, en fonction de leurs sensibilités, sont asymptomatiques (Crone *et al.*, 2013). De plus, les réglementations en vigueur ne font pas figurer *P. cinnamomi* ou *P. x cambivora* comme organismes de quarantaine ou réglementés, les faisant échapper à tout contrôle officiel (Robin et Husson, 2018). Cependant, l'échappement des *Phytophthora* des pépinières ne garantit pas nécessairement leur établissement dans un environnement naturel. En effet, seules les espèces écologiquement compatibles peuvent s'établir, se reproduire et se disséminer. Une étude menée en Suède a en effet montré que si une large diversité d'espèces de *Phytophthora* se trouvait en pépinières, seulement une partie se retrouvait en forêt, avec une plus large diversité en forêt anthropogénique qu'en forêt naturelle (Figure 11).

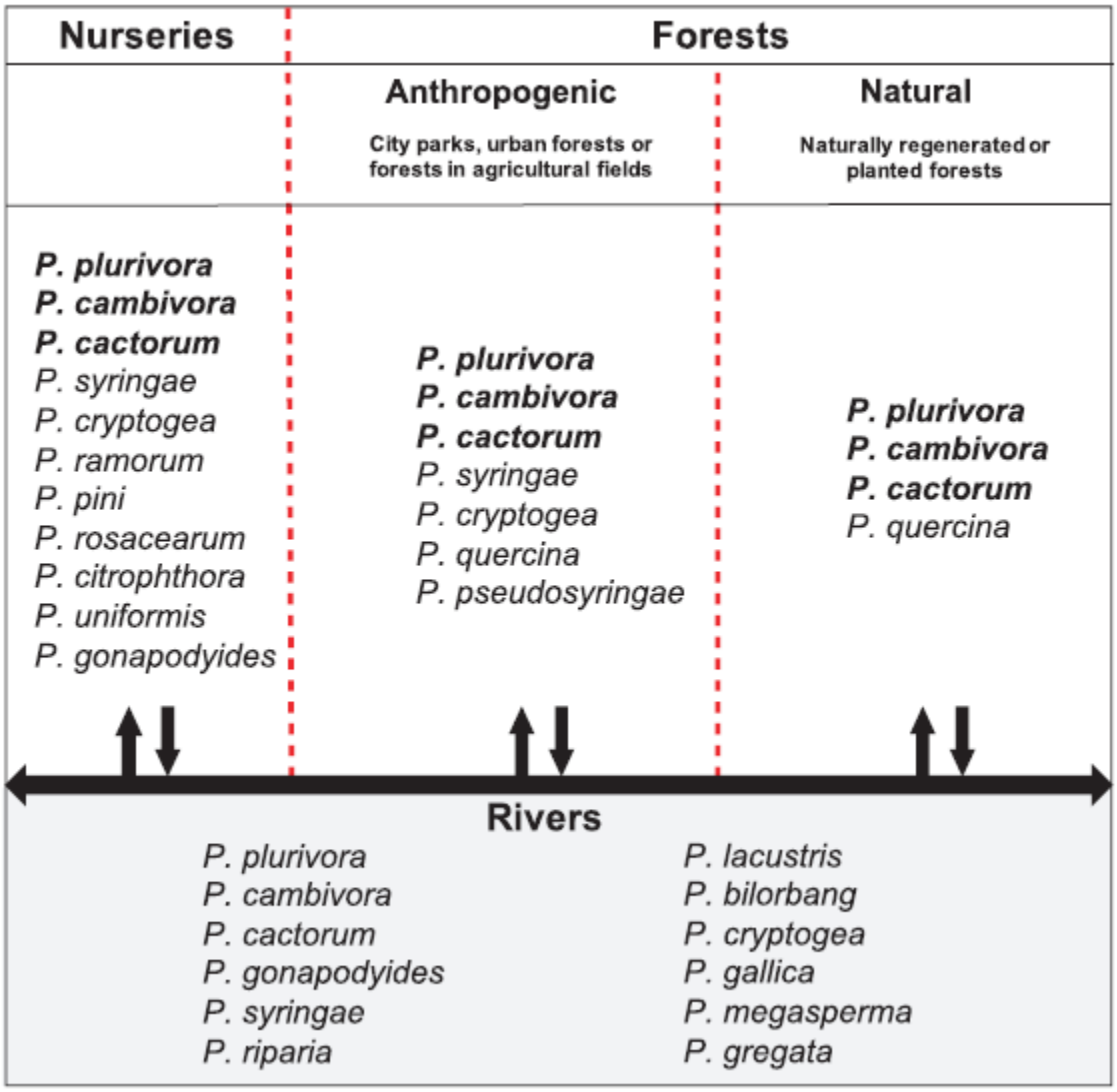


Figure 11: Liste des espèces de *Phytophthora* retrouvées dans chaque environnement (Source : Redondo et al., 2018)

P. x cambivora est la seule des deux espèces à être présente en Suède pour le moment et a été identifiée comme étant capable de s'établir dans les environnements naturels. Au vu de la large distribution de *P. cinnamomi* dans les écosystèmes mondiaux, il serait difficile de douter de sa capacité à s'établir dans un environnement exogène.

En Europe, la présence des agents causals de l'encre serait ancienne. Les premiers rapports officiels sur la maladie de l'encre sur châtaignier (*C. sativa*) date de 1838 au Portugal, mais son introduction serait beaucoup plus ancienne en Europe puisqu'elle daterait de 1726 (Grente, 1961). En France, la maladie aurait été introduite d'Espagne en 1860 dans le Sud-Ouest du territoire et se serait dispersée vers l'Est via le commerce et la plantation de châtaigniers

contaminés (Grente, 1961). Il existe également des mentions d'encre en Bretagne dès le début du XX^{ème} siècle (Ducomet, 1913).

2.3. L'établissement des espèces, grâce à leur capacité de survie et de reproductions

Un facteur clé à l'établissement des espèces invasives est leur capacité à former des structures de survie et de reproduction qui, chez les *Phytophthora*, sont intimement liées. Le type de reproduction conditionne la capacité d'un pathogène à s'établir et les espèces de *Phytophthora* possèdent à la fois des structures de reproduction sexuée (oospores) et asexuée (chlamydospores, sporanges, zoospores) (voir Figure 6A partie 1.3.). La reproduction asexuée permet une importante capacité de dissémination à moindre coût énergétique pour l'organisme. La reproduction sexuée quant à elle, permet une bonne capacité d'adaptation grâce aux mélanges des génotypes et les différentes recombinaisons qui en résultent (Ashu et Xu, 2015). Les champignons et oomycètes ont développé des systèmes génétiques limitant l'autofécondation, appelés systèmes d'incompatibilité sexuelles ou mating type. Etant des espèces hétérothalliques[3], *P. cinnamomi et P. x cambivora* ont besoin du mating type opposé pour effectuer une reproduction sexuelle. Seulement un des deux mating type de *P. cinnamomi* est présent en Europe, le A2 (Vitale *et al.*, 2017). Ce mating type est tellement fréquent qu'il est reconnu comme le plus agressif des deux (Kamoun *et al.*, 2015). Cependant, on observe des variations phénotypiques fortes qui pourraient s'expliquer par la capacité de compenser par des recombinaisons mitotiques (Hüberli *et al.*, 2001; Tommerup *et al.*, 2000). En revanche, les deux mating types de *P. x cambivora* seraient présents en Europe, mais en fréquence hétérogène (Vettraino *et al.*, 2005). Pour les espèces de *Phytophthora* qui nous intéressent, la reproduction asexuée est la principale source de survie. Les *Phytophthora* ont la capacité de se maintenir aussi bien dans le sol que sur des débris végétaux grâce à leur habilité à former des spores de dormance, *i.e.* chlamydospores (voir partie 1.3.). A l'heure actuelle, aucune preuve de l'existence des chlamydospores n'a été faite pour *P. x cambivora* (Vannini *et al.*, 2012) mais pour *P. cinnamomi* de telles structures ont été observées en milieu naturel (Crone *et al.*, 2013). Ainsi, le temps de persistance dans le sol de *P. cinnamomi* est de 6 mois contre 1.5 mois pour *P. x cambivora* (Hardam, 2005; Vannini *et al.*, 2012).

[3] Chez ces champignons/oomycetes, il existe deux principaux types de reproduction sexuée : l'homothallisme et l'hétérothallisme. Lorsque la reproduction est produite par la même souche, on parle d'homothallisme. L'hétérothallsime se caractérise par une reproduction sexuée produite par deux souches différentes qui possèdent des mating types différents.

2.4. La dissémination et colonisation des agents pathogènes, le franchissement des barrières écologiques

La dissémination à courte distance des *Phytophthora* est effectuée par le biais des zoospores qui infectent les racines les plus proches, ce qui se traduit par une progression des foyers d'arbres malades en tache d'huile autour des arbres infectés (Grente, 1961). La présence d'eau étant un facteur prépondérant dans leur cycle de vie, il a été démontré que les endroits favorisants, comme les voies de drainages naturelles, pouvaient être des focus pour les foyers d'encre (Vannini *et al.*, 2010). La compaction et l'humidité du sol sont également deux facteurs décisifs dans la distribution des espèces responsables de l'encre (Rhoades *et al.*, 2003; Portela *et al.*, 1998; Fonseca *et al.*, 2004). Cependant, l'occurrence de *P. cinnamomi* n'est pas uniquement corrélée à l'humidité du sol puisqu'il a été détecté dans des sols secs dans aux Etats-Unis (Sena *et al.*, 2019) mais également dans le bassin méditerranéen (Brasier *et al.*, 1993; Robin *et al.*,1998; Vettraino *et al.*, 2002). La dispersion à longue distance est effectuée par le transport de sol, des machines ou outils et les plantes (cf Partie 2.1.). Les porcs sauvages ont également été identifiés comme un moyen de transport de *P. cinnamomi* (Li *et al.*, 2014).

2.5. Les connaissances actuelles sur la distribution des espèces en France

Le Département de la Santé des Forêts (DSF) est en charge de surveiller l'état sanitaire des forêts dans les différentes régions françaises depuis sa création en 1989. Le DSF alimente une base de données qui contient les signalements de problèmes de santé observés en France par un réseau de forestiers formés au diagnostic de tous types d'affections sanitaires, qu'il s'agisse de dommages abiotiques, entomologiques ou pathologiques. Ces correspondants observateurs (CO) ne consacrent qu'une partie de leur temps aux activités du DSF, c'est-à-dire à l'observation et au signalement des problèmes de santé des forêts.

La carte de la distribution des signalements d'encre du châtaignier montre une augmentation des signalements de maladies d'encre dans les dix dernières années, particulièrement dans le Nord-Ouest (Figure 12). En revanche, dans le Sud- Est, là où le châtaignier est très implanté, le nombre de signalements d'encre se fait rare mais présent (Figure 12).

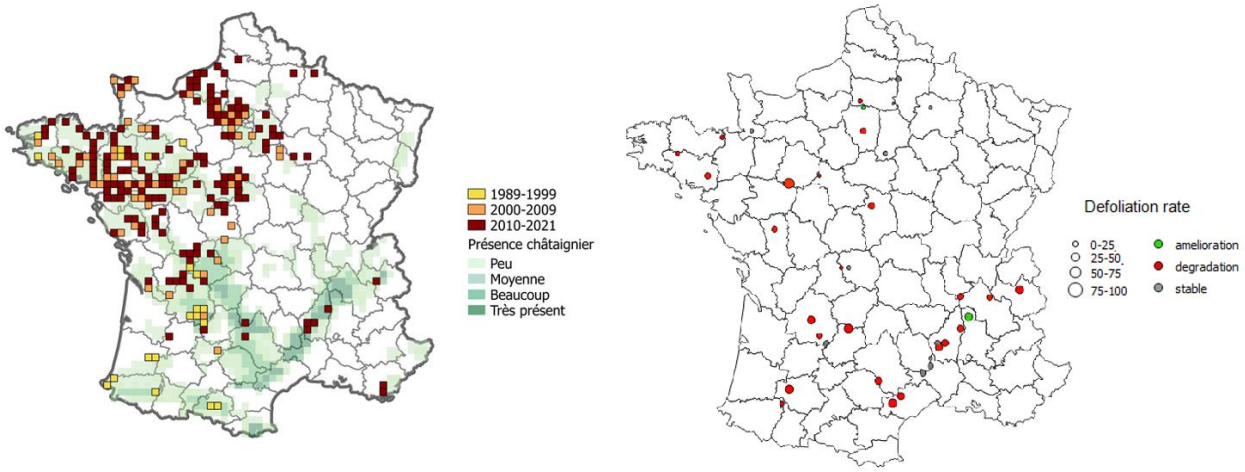


Figure 12: Evolution et distribution des signalements de la maladie de l'encre en France (gauche), évolution du déficit foliaire du châtaignier dans le réseau systématique (droite) (Source : DSF)

Comme il a été mentionné précédemment, l'infection du châtaignier par les agents de l'encre provoque un état de dépérissement qui se traduit par une défoliation progressive. Le DSF suit également le déficit foliaire (ou taux de défoliation) des arbres, chaque année sur un réseau systématique. Ce réseau de suivi des dégâts forestiers est composé au total de 542 placettes réparties sur l'ensemble du territoire dans lesquelles sont notés les taux de défoliation des 20 arbres dominants. Parmi ces 542 placettes, 38 sont composées d'au moins deux châtaigniers (Figure 12). Il est intéressant de noter que le taux de défoliation s'est accentué dans la région du Sud-Est au cours des dix dernières années mais que très peu de signalements de la maladie de l'encre ont été faits dans cette région (Figure 12). Dans ce réseau de placette la maladie de l'encre n'est jamais recherchée.

Il est possible que l'accentuation du dépérissement du châtaignier en France, et notamment dans le Sud-Est, soit liée à la présence de *P. cinnamomi*. La présence ancienne du pathogène suggère que la recrudescence d'encre observée récemment soit liée à des évènements climatiques favorisant son développement. D'après Blackburn *et al.* (2011), une espèce naturalisée est une espèce « dont les individus se dispersent, survivent et se reproduisent sur de multiples sites à travers un spectre plus ou moins large d'habitats ou d'étendue d'occurrence. ». Par la suite, nous considérons qu'une espèce se naturalise si elle franchit toutes les barrières et est donc au stade de dissémination (cf Figure 10). Ainsi, puisque *P. cinnamomi* semble avoir surmonté avec succès toutes les barrières jusqu'au stade de dissémination, la question de sa naturalisation se pose. Le chapitre 2 vise à étudier la répartition actuelle de *P. cinnamomi* en France pour y répondre.

RESUME

- *P. cinnamomi* a été transporté et introduit d'Asie en Europe au cours du XIX^{ème} siècle

- La présence de *P. cinnamomi* sur le territoire français est relativement ancienne

- L'agent pathogène a franchi avec succès les barrières géographiques et de captivité. Ses populations survivent et se reproduisent dans les zones d'introduction. Peut-on dire que l'espèce est naturalisée ?

3. Les hôtes et leur microbiote comme barrière écologique à la dissémination des agents de l'encre

Ici, nous définissons une communauté comme l'ensemble d'individus qui coexistent dans un même lieu et qui interagissent entre eux. Cette notion de communauté intervient à deux échelles spatiales et fonctionnelles, la première par la communauté végétale et la deuxième, par la communauté fongique.

3.1. La diversité de la communauté d'hôtes

L'idée que la diversité d'une communauté d'hôtes diminue la transmission des agents pathogènes est un concept faisant consensus et qui a été formalisé par l'écologiste Elton en 1958. Ce concept a été exploité depuis des siècles par les agricultures qui ont compris l'intérêt des rotations de cultures et de cultures associées sur la réduction des maladies. Cette perception de la relation diversité-maladie est fonctionnelle pour les maladies impliquant un hôte infecté spécifiquement par un pathogène. Cependant, les choses se complexifient pour les pathogènes multi-hôtes. Lorsque la diversité importante d'une communauté conduit à la diminution globale du risque de maladie, on parle d'effet de dilution. A l'opposé, lorsque l'augmentation de la diversité augmente le risque d'infection, on parle d'effet d'amplification (Keesing *et al.*, 2006). Si l'effet de dilution parait plutôt intuitif, son opposé ne l'est pas forcément. L'effet d'amplification peut se produire par exemple pour certains champignons qui ont besoin de deux hôtes différents pour compléter leur cycle de vie, *e.g.* la rouille du pin *Melampsora pinitorqua (*Mattila, 2002). Dans ce cas, une augmentation de la biodiversité pourrait faciliter leur développement et donc conduire à une amplification. La question de la généralisation de l'un ou l'autre effet fait l'objet d'un débat polarisant dans la communauté des écologues (Randolph et Dobson, 2012; Lafferty et Wood, 2013; Ostfeld et Keesing, 2011; Halsey, 2019). En effet, il existe des preuves de cas d'effet d'amplification (Halliday *et al.*, 2017; Nguyen *et al.*, 2016) mais également de nombreux cas d'effet de dilution (Haas *et al.*, 2011; Hantsch *et al.*, 2013; Roscher *et al.*, 2007; Rottstock *et al.*, 2014; Civitello *et al.*, 2015).

Historiquement, la maladie de Lyme (une bactérie pathogène vectorisée par une tique qui infecte un animal/humain) a été le premier modèle étudié pour explorer la relation entre diversité et maladie dans les années 1990s (Johnson *et al.*, 2015). La grande découverte à laquelle ont abouti ces recherches fut que l'hôte pouvait inhiber la transmission hôte-vecteur,

i.e. la tique, diminuant ainsi la prévalence du vecteur, et donc de la subséquente infection d'un autre hôte. Bien que l'effet de dilution ait été formalisé pour les maladies vectorisées, les recherches ont par la suite eu pour objectif de l'explorer sur d'autres pathosystèmes. C'est alors que la question de la compétence des hôtes a été mise en avant. Celle-ci se définit comme la capacité de l'hôte à transmettre l'inoculum (pour de plus amples détails sur cette notion, voir les chapitres 4 et 5). Une communauté ultra diversifiée composée d'espèces compétentes contribuera à amplifier la maladie alors qu'une communauté aussi diversifiée mais avec des espèces peu compétentes contribuera à diminuer la maladie. Cependant, les mécanismes qui conduisent à l'effet de dilution (et d'amplification) ne sont pas aussi simples (Figure 13). En effet, l'effet de la richesse de la communauté peut être indirect sur la maladie puisque cela peut passer par la suppression de l'abondance des hôtes réservoirs d'inoculum[4] plutôt que d'agir directement sur la transmission d'une maladie virale (Mitchell *et al.*, 2002).

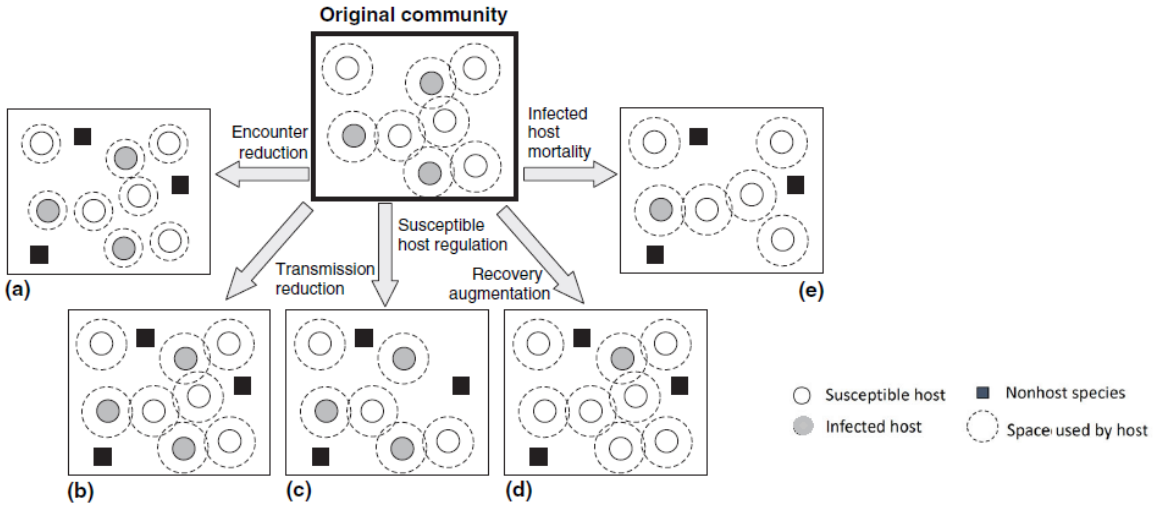


Figure 13: Modèle conceptuel des mécanismes qui conduisent à l'effet de dilution par la diversité végétale (Source : Keesing *et al.*, 2006)

La transmission de l'inoculum peut dépendre de la densité de l'hôte qui serait réduite dans les forêts mélangées (Mitchell *et al.*, 2002) même si ce n'est pas systématique (Hantsch *et al.*, 2014a, b). Le cas de *Phytophthora ramorum* est intriguant puisque c'est un pathogène généraliste qui infecte le système aérien de nombreux hôtes, mais qui ne cause de dommages que sur certains et sporulent sur ces hôtes de façon différentielle (Davidson *et al.*, 2008; Garbelotto *et al.*, 2021). Une étude terrain menée à large échelle sur plusieurs milliers

[4] Hôte asymptomatique mais qui possède une large capacité à transmettre l'inoculum, *i.e.* compétence

d'hectares a mis en évidence un potentiel effet de dilution de *P. ramorum* par ces hôtes (Haas *et al.*, 2011).

Le cas de *P. cinnamomi* est d'autant plus problématique que c'est une espèce particulièrement généraliste avec plus de 5 000 hôtes différents à son actif (Hardham, 2005; Blackwell *et al.*, 2020) qui démontrent des niveaux de sensibilités différents à l'infection (Shearer *et al.*, 2013). Dans des environnements naturels, l'étude de l'effet de la diversité sur *P. cinnamomi* infectant le châtaignier demeure complexe. En effet, pour étudier ces effets, il est d'usage d'étudier les symptômes et dommages visibles causés par l'agent infectieux or l'infection racinaire par les pathogènes sur le châtaignier ne résulte pas toujours en des symptômes facilement quantifiables en conditions naturelles (cf partie 1.4.). Néanmoins, nous avons exploré la possibilité d'un effet de la diversité de la communauté d'hôtes sur *P. cinnamomi* dans le chapitre 3, grâce à une méthode mise au point lors du chapitre 1.

3.2. Des hôtes de résistance, tolérance et compétence différentes

Lorsque l'agent pathogène est capable de se multiplier dans la plante, on dit alors que la plante est « sensible ». Le terme de « sensibilité » porte à confusion puisqu'il est employé pour décrire à la fois une absence de résistance et une absence de tolérance. La résistance se définit comme la capacité de l'hôte à limiter l'infection du pathogène et sa colonisation alors que la tolérance se définit comme la capacité de l'hôte à limiter les effets négatifs causés par l'infection et ce, pour une charge pathogène donnée (Baucom et De Roode, 2011). La résistance peut être classée de deux façons différentes : il y a la résistance totale (ou résistance qualitative), qui est à opposer à la résistance partielle (ou résistance quantitative). La résistance totale produit deux classes discrètes d'individus, les individus « résistants » et les individus « sensibles ». En revanche, la résistance partielle aboutit à des phénotypes graduels, de résistant à sensible. Il est reconnu que cette dernière gouverne la majorité des interactions plante-pathogène. D'un autre côté, la tolérance limite la réduction de la fitness de l'hôte et l'impact de la maladie sur l'hôte sans diminuer la quantité de pathogène et ce, en utilisant des mécanismes comme la régénération racinaires ou l'augmentation de l'absorption des nutriments (Kover et Schaal, 2002). Malgré des définitions qui semblent relativement claires, définir la réponse d'une plante comme étant de l'ordre de la résistance ou de la tolérance est une tâche délicate, surtout considérant que ces deux mécanismes ne sont pas nécessairement mutuellement exclusifs. En effet, la tolérance est parfois confondue avec de la résistance quantitative (Vale *et al.*, 2001; Paudel et Sanfaçon,

2018). Par exemple, Engelbrecht *et al.* (2013) ont montré grâce à un outil moléculaire de quantification de *P. cinnamomi* dans les tissus d'avocat que la quantité de pathogène était moindre dans un hôte qu'il qualifiait de « hautement tolérant », Dusa®, par rapport à un hôte « sensible », suggérant que Dusa® inhibait la colonisation du pathogène. Il a de ce fait été suggéré de classer cet hôte en tant que partiellement résistant plutôt que tolérant (Van Den Berg *et al.*, 2021).

Pour complexifier le tout, il apparait qu'un dernier composant de l'interaction hôte-pathogène soit largement sous-estimé : la compétence de l'hôte. Le Chapitre 4 est le produit d'une réflexion dont l'objectif est de définir le cadre conceptuel de la compétence de l'hôte et de montrer l'importance de la prise en compte de ce trait dans l'étude des pathosystèmes. Le chapitre 5 explore de façon empirique les relations entre la compétence, la résistance et la tolérance de différents hôtes de *P. cinnamomi*.

3.3. La diversité de la communauté fongique

Les arbres, et les plantes en général, possèdent un assemblage particulier d'organismes microbiens qui leur sont associés. En effet, les plantes sont colonisées par un large nombre de microorganismes, mais seulement une petite portion de ces interactions mène à une maladie. D'une façon assez similaire au fait que le bien-être des humains soit en lien avec la composition de la communauté des bactéries intestinales, le fonctionnement des plantes dépend aussi étroitement de la composition et des activités de leurs associés souterrains (et endophytes). Brader *et al.* (2017) présentent une alternative au triangle classique de la maladie qui incorpore la communauté microbienne dans le développement de la maladie (Figure 14).

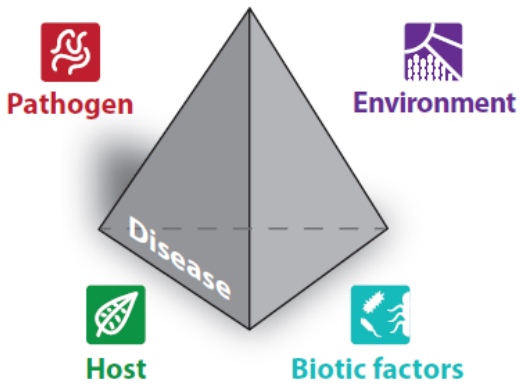


Figure 14: Tétraèdre des maladies des plantes (Source : Brader *et al.*, 2017)

Certains champignons sont inféodés à certaines espèces végétales (Zhou et Hyde, 2001; Ushio *et al.*, 2008), alors que d'autres dépendent de la composition chimique du sol (Allison *et al.*, 2009; Ayres *et al.*, 2009). Les écosystèmes multi-spécifiques offrent potentiellement un nombre

de niches écologiques[5] supérieur aux écosystèmes monospécifiques. Ainsi, cela augmenterait le nombre d'espèces microbiennes associées, qui interagissent entre elles de façon différente.

Les champignons présentent une incroyable diversité qui s'élèverait à presque 3 millions d'espèces différentes (Blackwell, 2011; Hawksworth, 2012). Parmi ceux-ci, seulement une infime partie est connue (~7%) et a été identifiée à un clade taxonomique (Figure 15).

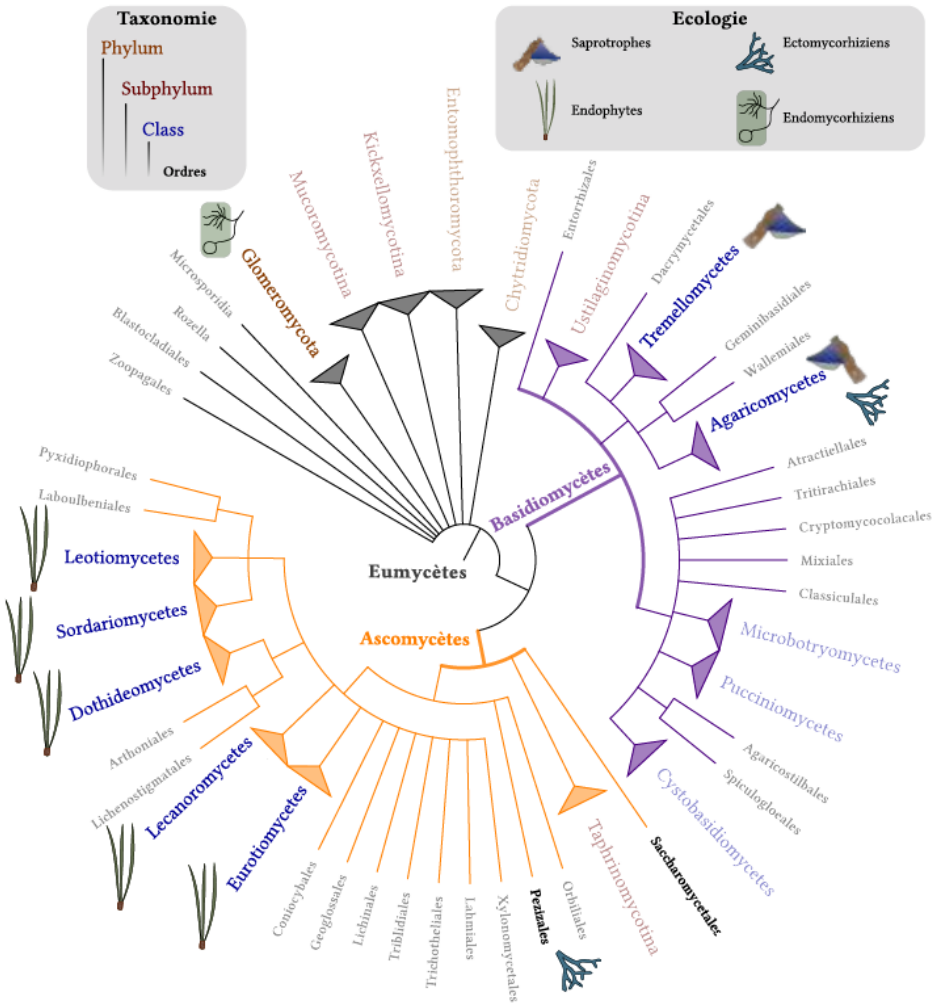


Figure 15: Classification phylogénétique des champignons (Source : Taudière, 2016)

Une autre manière de classifier ces microorganismes se base sur leur manière d'acquérir des nutriments, c'est-à-dire leur type trophique. Classiquement, on distingue trois types trophiques : les pathotrophes, qui acquièrent leurs nutriments au détriment d'un autre organisme vivant, *e.g.* les pathogènes des plantes comme les *Phytophthora*. Il y a les saprotrophes qui dégradent la matière organique morte. Pour finir, il y a les symbiotrophes qui échangent des nutriments avec un autre organisme. Certains groupes fongiques présentent une large diversité de modes

[5] Concept qui décrit la façon dont une espèce tolère son environnement abiotique et biotique (interactions avec les autres espèces)

trophiques, *e.g.* l'ordre des *Sebacinales* avec des espèces symbiotrophes et saprotrophes (Weiß *et al.*, 2016).

Chaque groupe trophique peut interagir avec les agents de l'encre d'une manière différente :

- Lorsqu'ils occupent la même niche écologique, deux espèces pathotrophes qui utilisent la même ressource (que ce soit une ressource spatiale ou nutritive) peuvent se trouver en compétition. Ainsi, la première sera éliminée au détriment de la deuxième. Il existe des cas documentés de compétition entre espèces d'un même genre qui ont abouti au remplacement de l'une par l'autre, à savoir *Cryphonectria radicalis* sur les châtaigniers ou *Ophiostoma ulmi* sur les ormes, a été complètement remplacée par son parent pathogène, respectivement *C. parasitica* (Hoegger *et al.*, 2002) et *O. novo-ulmi* (Brasier *et al.*, 2001). D'un autre côté, la présence de deux pathotrophes différents sur un même hôte peut conduire à un effet synergétique conduisant à l'accélération de sa mortalité, comme cela a été démontré pour *P. cinnamomi* et *Diplodia corticola* (Linaldeddu *et al.*, 2014).
- Les saprotrophes sont des décomposeurs, ils transforment la matière organique mortes. Leur composition peut dépendre de la présence d'autres pathotrophes racinaires qui rendent alors disponibles des tissus végétaux sur lesquels se nourrissent les saprotrophes.
- De nombreux arbres, comme les *Betulaceae* (Bouleau), les *Pinaceae*, les *Fagaceae* (Chênes, Châtaigniers) sont connus pour former des relations symbiotrophiques obligatoires (Smith et Read, 1997). Certains symbiotrophes peuvent offrir une protection racinaire contre les agents pathogènes fongiques (Maherali et Klironomos, 2007), la dessiccation (Smith et Read, 2008) ou des composés polluants (Egli et Brunner, 2002). Dans le cas des agents de l'encre, des châtaigniers d'abord myccorhizés et ensuite exposés à *P. cinnamomi* et *P. x cambivora* n'ont montré aucun signe d'infection par la suite (Branzanti *et al.*, 1999).

Loin d'être exhaustive, cette liste d'exemples illustre la manière dont les communautés fongiques, regroupant des types trophiques différents, pourraient directement influencer les interactions châtaignier-espèces de *Phytophthora* et être influencées par la présence des agents de l'encre.

3.4. Le métabarcoding pour étudier les communautés fongiques

L'étude de ces communautés fongiques peut s'avérer complexe en raison de leurs tailles microscopiques et de leurs milieux de vie difficiles à explorer. Récemment, l'étude de ces communautés se fait dans la grande majorité des cas via des méthodes indépendantes de la mise en culture, par le bais du métabarcoding. L'identification des espèces dans un échantillon environnemental (sol, eau, air) se fait grâce à l'amplification par PCR d'un barcode, c'est-à-dire d'un fragment d'ADN cible ou marqueur, qui est partagé par plusieurs taxons et qui est suffisamment variable pour distinguer les taxons entre eux. Le marqueur le plus utilisé chez les champignons provient de l'ADN ribosomique appelé Internal Transcribed Spacer 1 (ITS 1) et est amplifié grâce aux amorces ITS1F (Gardes et Bruns, 1993) et ITS 2 (White *et al.*, 1990) (Figure 16).

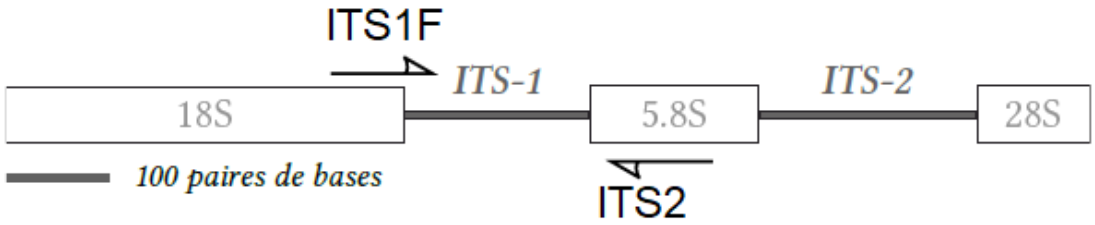


Figure 16: Schéma du marqueur utilisé pour l'étude des communautés fongiques

Le séquençage haut débit (ou HTS : High-Throughput Sequencing) permet d'obtenir un nombre de séquences très important comparé aux techniques de séquençage classique de type Sanger (Shokralla *et al.*, 2012; Lindahl *et al.*, 2013). Cette méthode permet d'obtenir à la fois une information sur la diversité et l'abondance des espèces fongiques présentes dans des échantillons. Lorsque cette donnée « abondance » est exploitée, il est d'usage de normaliser les données via des transformations qui visent à homogénéiser la variance à l'intérieur des échantillons (en forçant chaque échantillon à avoir une distribution de reads similaire) et cela pose problème dans l'étude des communautés. Prenons deux communautés avec le même nombre d'espèces mais en abondance différente (Figure 17).

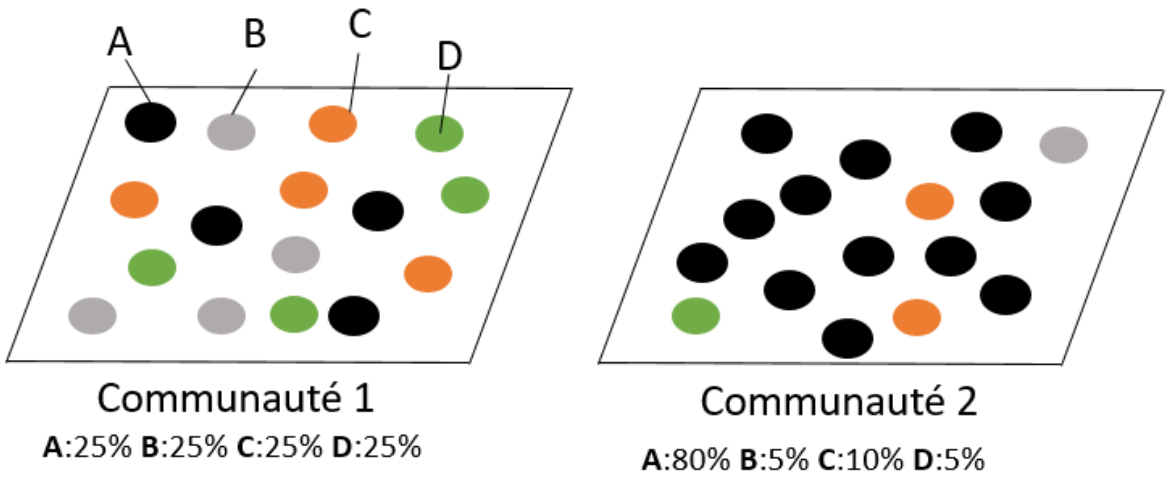


Figure 17: Différences entre diversité et richesse de deux communautés

Ici, les deux communautés ont une diversité similaire (4 espèces différentes), mais la communauté 2 est largement dominée par une espèce alors que la communauté 1 est plus homogène (Figure 17). La standardisation des données va nous amener à conclure de façon similaire sur ces deux communautés alors qu'elles auront, en réalité, des compositions largement différentes. L'intérêt de la standardisation est notamment de réduire l'effet des séquences particulièrement abondantes pour que l'effet des séquences rares puisse être visible. Cela reste cependant à débattre puisque si les membres rares ont un rôle important dans les communautés, les espèces dominantes ont tendance à piloter l'essentiel des fonctionnalités de la communauté (dans McKnight *et al.*, 2019).

Récemment, la façon de traiter les données de métabarcoding a connu un changement, avec le passage de l'utilisation d'Operational Taxonomical Units (OTU) à des Amplicon Sequence Variants (ASV). Avec les analyses OTU, les séquences ADN sont regroupées dans une OTU grâce à un seuil de similarité (généralement 97%) qui permet alors d'attribuer les séquences à un taxon. Bien que ce type de regroupement permette de réunir des séquences qui diffèrent à cause d'erreurs de séquençage ou d'amplification, il ne prend pas en compte les variations biologiques existantes. En revanche, les ASVs partitionnent les reads en fonction d'un modèle qui corrige les erreurs de séquençage tout en prenant en compte l'abondance et les similarités des séquences (Callahan *et al.*, 2016). Ainsi, un ASV peut détecter de petites variations biologiques et mettre de côté des erreurs techniques introduites par la préparation des librairies et de séquençage. Cela permettrait *in fine* d'améliorer la résolution taxonomique des résultats. La comparaison des deux méthodes a par ailleurs démontré la supériorité des ASVs par rapport aux OTU en terme richesse et diversité (McKnight *et al.*, 2019; Pauvert *et al.*, 2019).

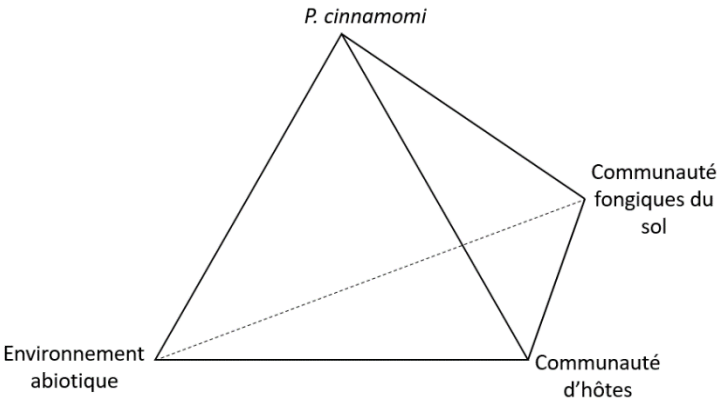
L’influence des microorganismes de différents types trophiques sur *P. cinnamomi* sera étudiée grâce au métabarcoding dans le Chapitre 3.

RESUME

- Le rôle de la diversité des hôtes dans la maladie de l’encre du châtaignier est encore à élucider
- La compétence des hôtes peut expliquer les effets de la diversité des hôtes sur *P. cinnamomi*
- Le rôle de la diversité du microbiote dans le pathosystème est également à déterminer

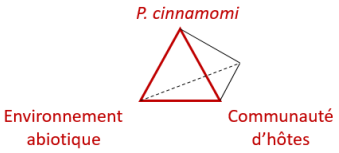
Organisation du manuscrit

Le but de la thèse est d'étudier les possibles barrières à la dissémination des agents de l'encre du châtaignier en France au travers des interactions entre les agents pathogènes (*P. cinnamomi* principalement et *P. x cambivora* dans une moindre mesure), la diversité d'hôtes et fongiques du sol, et l'environnement abiotique.

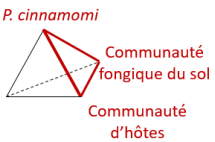


Le Chapitre 1 de cette thèse est dédié à la mise au point d'une méthode de détection et de quantification des agents pathogènes responsables de l'encre du châtaignier dans le sol, un prérequis pour les chapitres suivants.

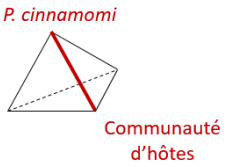
Le Chapitre 2 est consacré à l'étude de la distribution des pathogènes sur le territoire afin de déterminer la distribution réelle des espèces en France. Dans ce chapitre, sont également explorées les relations entre détection de *P. cinnamomi*, châtaignier, et conditions climatiques.



Le Chapitre 3 explore, par l'étude d'un dispositif expérimental en forêt, le rôle de la diversité des hôtes et de la communauté fongique du sol, sur l'abondance de l'inoculum des agents de l'encre.



Le chapitre 4 est consacré à la question de la compétence des hôtes et vise à poser un cadre conceptuel sur cette notion, qui serait un des mécanismes qui expliquerait les effets de la diversité sur l'inoculum. Le chapitre 5 démontre expérimentalement les différences de compétences entre les hôtes de *P. cinnamomi*.



Le chapitre 5 a été soumis à la revue *Plant Pathology*. Les autres chapitres ont été rédigés en anglais car ils sont destinés à être soumis pour une publication dans une revue scientifique.

En science, les résultats négatifs ou non significatifs sont mis de côté de façon quasi systématique. Seuls les résultats qui sont positifs de façon significative donnent lieu à une communication. Ce biais peut s'expliquer par plusieurs facteurs : rejet des résultats négatifs et/ou non-significatifs des revues concernées (moins de lecteurs et de citations), manque d'intérêt à la relecture, manque de motivation à exploiter de tels résultats, sensationnalisme … Les phrases telles que « différence non-significative » ont diminué en fréquence dans les abstracts pour être remplacées par des expressions « putaclics » comme « changements de paradigmes ». Dans sa revue, Fanelli (2011) montre qu'entre 1990 et 2007, 80% des papiers publiés portaient sur des résultats positifs, notamment 91% en écologie. Puisqu'un papier non publié conduit à une perte importante d'argent et de temps pour le chercheur·e, il est d'usage de ne pas tenter l'aventure. De cette pratique nait une véritable désinformation. Comme les seules données qui sont visibles dans la littérature sont positives, celles-ci sont amplifiées et reconnues comme seule vérité puisque aucune preuve du contraire n'est apportée. Ainsi dans ce manuscrit de thèse apparaitront les résultats négatifs, dans l'espoir que s'il est lu, les personnes concernées ne réitèreront pas les mêmes expériences que moi.

REFERENCES

Akili S., Serçe C. U., Katircioğlu Y. Z. and Maden S. (2012). Involvement of *Phytophthora* species in chestnut decline in Black Sea region of Turkey. *Forest Pathology*. 42: 377-386. doi: 10.1111/j.1439-0329.2012.00770.x

Akili Şimşek S., Katircioğlu Y. Z., Serçe C. U., Çakar D, Rigling D. and Maden S. (2019). *Phytophthora* species associated with dieback of sweet chestnut in Western Turkey. Forest Pathology. 49:e12533. https://doi.org/10.1111/efp.12533

Allison S. D., LeBauer D. S., Ofrecio M. R., Reyes R., Ta A.-M and Tran T. M. (2009). Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biology & Biochemistry 41: 393-302.

Anagnostakis, S. L. (2001). The effect of multiple importations of pests and pathogens on a native tree. *Biological Invasions*, *3*(3), 245–254. https://doi.org/10.1023/A:1015205005751

Arentz, F. (2017). *Phytophthora cinnamomi* A1: An ancient resident of New Guinea and Australia of Gondwanan origin? *Forest Pathology*, *47*(4), 1–8. https://doi.org/10.1111/efp.12342

Ashu, E. E., & Xu, J. (2015). The roles of sexual and asexual reproduction in the origin and dissemination of strains causing fungal infectious disease outbreaks. *Infection, Genetics and Evolution*, *36*, 199–209. https://doi.org/10.1016/j.meegid.2015.09.019

Ayres, E., Steltzer, H., Berg, S., & Wall, D. H. (2009). Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. *Journal of Ecology*, *97*(5), 901–912. https://doi.org/10.1111/j.1365-2745.2009.01539.x

Barwell, L. J., Perez-Sierra, A., Henricot, B., Harris, A., Burgess, T. I., Hardy, G., Purse, B. V. (2021). Evolutionary trait-based approaches for predicting future global impacts of plant pathogens in the genus *Phytophthora*. *Journal of Applied Ecology*, *58*(4), 718–730. https://doi.org/10.1111/1365-2664.13820

Baucom, R. S., & De Roode, J. C. (2011). Ecological immunology and tolerance in plants and animals. *Functional Ecology*, *25*(1), 18–28. https://doi.org/10.1111/j.1365-2435.2010.01742.x

Baveye, P. C., Berthelin, J., & Munch, J. C. (2016). Too much or not enough: Reflection on two contrasting perspectives on soil biodiversity. *Soil Biology and Biochemistry*, *103*, 320–326. https://doi.org/10.1016/j.soilbio.2016.09.008

Beakes, G. W., Glockling, S. L., & Sekimoto, S. (2012). The evolutionary phylogeny of the oomycete "fungi." *Protoplasma*, *249*(1), 3–19. https://doi.org/10.1007/s00709-011-0269-2

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in Ecology and Evolution*, *26*(7), 333–339. https://doi.org/10.1016/j.tree.2011.03.023

Blackwell, M. (2011). The fungi: 1, 2, 3 ... 5.1 million species? *American Journal of Botany*, *98*(3), 426–438. https://doi.org/10.3732/ajb.1000298

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L. J., & Sessitsch, A. (2017). Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology*, *55*, 61–83. https://doi.org/10.1146/annurev-phyto-080516-035641

Bradford, K. J., & Hsiao, T. C. (1982). Stomatal Behavior and Water Relations of Waterlogged Tomato Plants. *Plant Physiology*, *70*(5), 1508–1513. https://doi.org/10.1104/pp.70.5.1508

Branzanti, M. B., Rocca, E., & Pisi, A. (1999). Effect of ectomycorrhizal fungi on chestnut ink disease. *Mycorrhiza*, *9*(2), 103–109. https://doi.org/10.1007/s005720050007

Brasier, C. M. (2001). Rapid evolution of introduced plant pathogens via interspecific Hybridization. *BioScience*, *51*(2), 123–133. https://doi.org/10.1641/0006-3568(2001)051[0123:REOIPP]2.0.CO;2

Brasier, C. M., Robredo, F., & Ferraz, J. F. P. (1993). Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology*, *42*(1), 140–145. https://doi.org/10.1111/j.1365-3059.1993.tb01482.x

Breisch, H. (1999) *Châtaignes et marrons*. S.l. : Centre technique interprofessionnel des fruits et légumes. ISBN 978-2-87911-050-9

Bourgeois, C. (2004). *Le châtaignier: un arbre, un bois*. S.l. : Forêt privée française. ISBN 978-2-904740-90-9

Bourdu, R. (1999). *Le châtaignier*. Arles : Actes Sud. ISBN 978-2-7427-0705-8

Burdon, J. J., & Thrall, P. H. (2008). Pathogen evolution across the agro-ecological interface: implications for disease management. *Evolutionary Applications*, *1*(1), 57–65. https://doi.org/10.1111/j.1752-4571.2007.00005.x

Cahill, D. M., Cope, M., & Hardham, A. R. (1996). Thrust reversal by tubular mastigonemes: Immunological evidence for a role of mastigonemes in forward motion of zoospores of *Phytophthora cinnamomi*. *Protoplasma*, *194*(1–2), 18–28. https://doi.org/10.1007/bf01273164

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caudullo, G., Welk, E., & San-Miguel-Ayanz, J. (2017). Chorological maps for the main European woody species. *Data in Brief*, *12*, 662–666. https://doi.org/10.1016/j.dib.2017.05.007

Civitello, D. J., Cohen, J., Fatima, H., Halstead, N. T., Liriano, J., McMahon, T. A., Rohr, J. R. (2015). Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(28), 8667–8671. https://doi.org/10.1073/pnas.1506279112

Conedera M., Tinner W., Krebs P., de Rigo D., and Caudullo G. (2016). Castanea sativa in Europe: distribution, habitat, usage and threats. European Atlas of Forest Tree Species. *Publ. Off. EU, Luxembourg, pp. e0125e0+*

Cooke D. E. L., Schena L., and Cacciola S. O. (2007). Tools to detect, identify and monitor *Phytophthora* species in natural ecosystems. *Journal of Plant pathology*. 89(1):13-28. DOI 10.4454/jpp.v89i1.720.

Crandall B.S Gravatt, G.F. and Ryan, M.M. (1945). 'Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi'*, *Phytopathology* 35, 162–180

Crone, M., McComb, J. A., O'Brien, P. A., & Hardy, G. E. S. J. (2013). Survival of *Phytophthora cinnamomi* as oospores, Stromata, And thick-walled chlamydospores in roots of symptomatic and asymptomatic annual and herbaceous perennial plant species. *Fungal Biology*, *117*(2), 112–123. https://doi.org/10.1016/j.funbio.2012.12.004

Davidson, J. M., Patterson, H. A., & Rizzo, D. M. (2008). Sources of Inoculum for *Phytophthora* ramorum in a Redwood Forest . *Phytopathology*, *98*(8), 860–866. https://doi.org/10.1094/phyto-98-8-0860

Dajoz, R. (2000). Insect and Forests: The role and diversity of insects in Forest environment. Lavoisier, London, UK.

Decourcelle T. & Robin C. (2009). Détection de *Phytophthora cinnamomi* et P. *cambivora* sur plants de chêne et châtaignier élevés en pépinières forestières. Colloque des 20 ans du Département Santé des Forêts, Beaune, France

Desprez-Loustau, M. L., Robin, C., Reynaud, G., Déqué, M., Badeau, V., Piou, D., Marçais, B. (2007). Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. *Canadian Journal of Plant Pathology*, *29*(2), 101–120. https://doi.org/10.1080/07060660709507447

Dickie, I. A., Bufford, J. L., Cobb, R. C., Desprez-Loustau, M. L., Grelet, G., Hulme, P. E., Williams, N. M. (2017). The emerging science of linked plant–fungal invasions. *New Phytologist*, *215*(4), 1314–1332. https://doi.org/10.1111/nph.14657

Ducomet V (1913) Research on oak and chestnut diseases in Brittany (in French). Ann Epiphyt 1:87–105

Eden, M. A., Hill, R. A., & Galpoththage, M. (2000). An efficient baiting assay for quantification of *Phytophthora cinnamomi* in soil. *Plant Pathology*, *49*(4), 515–522. https://doi.org/10.1046/j.1365-3059.2000.00478.x

Egli, S. et I. Brunner (2002). ≪ Les mycorhizes ≫. Notice pour le praticien 35.8 (cite p. 29).

Elliot, M., Schlenzig, A., Harris, C. M., Meagher, T. R., & Green, S. (2015). An improved method for qPCR detection of three *Phytophthora* spp. in forest and woodland soils in northern Britain. *Forest Pathology*, *45*(6), 537–539. https://doi.org/10.1111/efp.12224

Engelbrecht, J., Duong, T. A., & van den Berg, N. (2013). Development of a nested quantitative real-time PCR for detecting *Phytophthora cinnamomi* in Persea americana rootstocks. *Plant Disease*, *97*(8), 1012–1017. https://doi.org/10.1094/PDIS-11-12-1007-RE

Erwin, D. C. et Ribeiro, O. K., (1996). *Phytophthora* diseases worldwide. *Phytophthora diseases worldwide*.. https://www.cabdirect.org/cabdirect/abstract/19971001256

Eshraghi, L., Aryamanesh, N., Anderson, J. P., Shearer, B., McComb, J. A., Hardy, G. E. S. J., & O'Brien, P. A. (2011). A quantitative PCR assay for accurate in planta quantification of the necrotrophic pathogen *Phytophthora cinnamomi*. *European Journal of Plant Pathology*, *131*(3), 419–430. https://doi.org/10.1007/s10658-011-9819-x

Fanelli, D. (2011). Negative results are disappearing from most disciplines and countries. Scientometrics. DOI: 10.1007/s11192-011-0494-7 ·

Fernandez-Conradi, P., Borowiec, N., Capdevielle, X., Castagneyrol, B., Maltoni, A., Robin, C., Jactel, H. (2018). Plant neighbour identity and invasive pathogen infection affect associational resistance

to an invasive gall wasp. *Biological Invasions*, *20*(6), 1459–1473. https://doi.org/10.1007/s10530-017-1637-4
Fonseca, T. F., Abreu, C. G., & Parresol, B. R. (2004). Soil compaction and chestnut ink disease. *Forest Pathology*, *34*(4), 273–283. https://doi.org/10.1111/j.1439-0329.2004.00371.x
Freitas, T. R., Santos, J. A., Silva, A. P., & Fraga, H. (2021). Influence of climate change on chestnut trees: A review. *Plants*, *10*(7), 1–17. https://doi.org/10.3390/plants10071463
Frisullo S., Lima G., Magnano di San Lio G., Camele I., Melissano L., Puglisi I., Pane A., Agosteo G. E., Prudente L. and Cacciola S. O. (2018). *Phytophthora cinnamomi* involved in the decline of Holm oak (*Quercus ilex*) stands in southern Italy. *For. Sci.* 00(00): 1-9
Garbelotto, M., Schmidt, D., & Popenuck, T. (2021). Pathogenicity and infectivity of *Phytophthora* ramorum vary depending on host species, infected plant part, inoculum potential, pathogen genotype, and temperature. *Plant Pathology*, *70*(2), 287–304. https://doi.org/10.1111/ppa.13297
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol.2, 113e118
Giglio, L., van der Werf, G. R., Randerson, J. T., Collatz, G. J., & Kasibhatla, P. (2006). Global estimation of burned area using MODIS active fire observations. *Atmospheric Chemistry and Physics*, *6*(4), 957–974. https://doi.org/10.5194/acp-6-957-2006
Grente, J., (1961). La maladie de l'encre du Châtaignier. In : *Annale Epiphyte*. 1961. 5-59.
Haas, S. E., Hooten, M. B., Rizzo, D. M., & Meentemeyer, R. K. (2011). Forest species diversity reduces disease risk in a generalist plant pathogen invasion. *Ecology Letters*, *14*(11), 1108–1116. https://doi.org/10.1111/j.1461-0248.2011.01679.x
Halliday, F. W., Heckman, R. W., Wilfahrt, P. A., & Mitchell, C. E. (2017). A multivariate test of disease risk reveals conditions leading to disease amplification. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1865). https://doi.org/10.1098/rspb.2017.1340
Halsey, S. (2019). Defuse the dilution effect debate. *Nature Ecology and Evolution*, *3*(2), 145–146. https://doi.org/10.1038/s41559-018-0764-3
Hantsch, L., Bien, S., Radatz, S., Braun, U., Auge, H., & Bruelheide, H. (2014). Tree diversity and the role of non-host neighbour tree species in reducing fungal pathogen infestation. *Journal of Ecology*, *102*(6), 1673–1687. https://doi.org/10.1111/1365-2745.12317
Hantsch, L., Braun, U., Haase, J., Purschke, O., Scherer-Lorenzen, M., & Bruelheide, H. (2014). No plant functional diversity effects on foliar fungal pathogens in experimental tree communities. *Fungal Diversity*, *66*(1), 139–151. https://doi.org/10.1007/s13225-013-0273-2
Hantsch, L., Braun, U., Scherer-Lorenzen, M., & Bruelheide, H. (2013). Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. *Ecosphere*, *4*(7), 1–12. https://doi.org/10.1890/ES13-00103.1
Hardham, A. R. (2005). *Phytophthora cinnamomi*. *Molecular Plant Pathology*, *6*(6), 589–604. https://doi.org/10.1111/j.1364-3703.2005.00308.x
Hawksworth, D. L. (2012). Global species numbers of fungi: Are tropical studies and molecular approaches contributing to a more robust estimate? *Biodiversity and Conservation*, *21*(9), 2425–2433. https://doi.org/10.1007/s10531-012-0335-x
Hoegger, P. J., Rigling, D., Holdenrieder, O., & Heiniger, U. (2002). Cryphonectria radicalis: Rediscovery of a Lost Fungus. *Mycologia*, *94*(1), 105. https://doi.org/10.2307/3761850
Hüberli, D., Tommerup, I. C., Dobrowolski, M. P., Calver, M. C., & Hardy, G. E. (2001). Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research*, *105*(9), 1053–1064. https://doi.org/10.1016/S0953-7562(08)61967-X
Jactel H., Bauhus J., Boberg J., Bonal D., Castagneyrol B., Gardiner B., Gonzalez-Olabarria J. R., Koricheva J., Meurisse N. and Brockerhoff E. (2017). Tree Diversity drives forest stand resistance to natural disturbances. *Curr Forestery Rep*. 3: 223-243.
Johnson, P. T. J., Ostfeld, R. S., & Keesing, F. (2015). Frontiers in research on biodiversity and disease. *Ecology Letters*, *18*(10), 1119–1133. https://doi.org/10.1111/ele.12479
Judelson, H. S. et Blancon, F. A. (2005). The spores of *Phytophthora*: weapons of the plant destroyer. *Nature Reviews. Microbiology*.3(1), p. 47-58. DOI 10.1038/nrmicro1064
Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A. G., Aguín Casal, O., Peréz-Sierra, A. (2016). Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural

and horticultural ecosystems at high risk of *Phytophthora* diseases. *Forest Pathology*, *46*(2), 134–163. https://doi.org/10.1111/efp.12239

Kamoun, S., Furzer, O., Jones, J. D. G., Judelson, H. S., Ali, G. S., Dalio, R. J. D., Govers, F. (2015). The Top 10 oomycete pathogens in molecular plant pathology. *Molecular Plant Pathology*, *16*(4), 413–434. https://doi.org/10.1111/mpp.12190

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, *17*(4), 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Keesing, F., Holt, R. D., & Ostfeld, R. S. (2006). Effects of species diversity on disease risk. *Ecology Letters*, *9*(4), 485–498. https://doi.org/10.1111/j.1461-0248.2006.00885.x

Kover, P. X., & Schaal, B. A. (2002). Genetic variation for disease resistance and tolerance among Arabidopsis thaliana accessions. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(17), 11270–11274. https://doi.org/10.1073/pnas.102288999

Krebs, P., Conedera, M., Pradella, M., Torriani, D., Felber, M., & Tinner, W. (2004). Quaternary refugia of the sweet chestnut (*Castanea sativa* Mill.): an extended palynological approach. Vegetation History and Archaeobotany, 13(3), 145-160.

Krebs, P., Pezzatt G.-B., Beffa G., Tinner W., & Conedera M. (2019). Revising the sweet chestnut (*Castanea sativa* Mill.) refugia history of the last glacial period with extended pollen and macrofossil evidence. Quaternary Science Reviews, 206, 111-128

Krehenwinkel, H., Wolf, M., Lim, J. Y., Rominger, A. J., Simison, W. B., & Gillespie, R. G. (2017). Estimating and mitigating amplification bias in qualitative and quantitative arthropod metabarcoding. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/s41598-017-17333-x

Lafferty, K. D., & Wood, C. L. (2013). It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. *Trends in Ecology and Evolution*, *28*(9), 503–504. https://doi.org/10.1016/j.tree.2013.06.012

Langrell, S. R., Morel O. and Robin C. (2011). Touchdown nested multiplex PCR detection of *Phytophthora cinnamomi* and *P. cambivora* from French and English chestnut grove soils. Fungal Biology. 115:672-682.

Lapin, D., & Van den Ackerveken, G. (2013). Susceptibility to plant disease: More than a failure of host immunity. *Trends in Plant Science*, *18*(10), 546–554. https://doi.org/10.1016/j.tplants.2013.05.005

Legeay, J., Husson, C., Cordier, T., Vacher, C., Marcais, B., & Buée, M. (2019). Comparison and validation of Oomycetes metabarcoding primers for *Phytophthora* high throughput sequencing. *Journal of Plant Pathology*, *101*(3), 743–748. https://doi.org/10.1007/s42161-019-00276-9

Li, A. Y., Williams, N., Fenwick, S. G., Hardy, G. E. S. J., & Adams, P. J. (2014). Potential for dissemination of *Phytophthora cinnamomi* by feral pigs via ingestion of infected plant material. *Biological Invasions*, *16*(4), 765–774. https://doi.org/10.1007/s10530-013-0535-7

Linaldeddu, B. T., Scanu, B., Maddau, L., & Franceschini, A. (2014). Diplodia corticola and *Phytophthora cinnamomi*: The main pathogens involved in holm oak decline on Caprera Island (Italy). *Forest Pathology*, *44*(3), 191–200. https://doi.org/10.1111/efp.12081

Lindahl, B. D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kauserud, H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers--a user's guide. *The New Phytologist*, *199*(1), 288–299. https://doi.org/10.1111/nph.12243

Maherali, H., & Klironomos, J. N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, *316*(5832), 1746–1748. https://doi.org/10.1126/science.1143082

Manion, P.l D., 1991. *Forest Decline Concepts*. First Edition edition. St. Paul, Minn : Amer Phytopathological Society. ISBN 978-0-89054-143-2

Marçais, B. (2018). Influence de facteurs de l'environnement sur le développement de l ' encre du chêne rouge *(Quercus rubra* L .), maladie provoquée par *Phytophthora cinnamomi HAL Id : tel-01754407*

Mattila, U. (2002). The risk of pine twisting rust damage in young scots pines: A multilevel logit model approach. *Forest Ecology and Management*, *165*(1–3), 151–161. https://doi.org/10.1016/S0378-1127(01)00657-0

Mattioni, C., Martin, M. A., Pollegioni, P., Cherubini, M., & Villani, F. (2013). Microsatellite markers reveal a strong geographical structure in European populations of *Castanea sativa* (Fagaceae): evidence for multiple glacial refugia. American journal of botany, 100(5), 951-961

Maurel, M., Robin, C., Capdevielle, X., Loustau, D., & Desprez-Loustau, M.-L. (2001). Effects of variable root damage caused by *Phytophthora cinnamomi* on water relations of chestnut saplings . *Annals of Forest Science*, *58*(6), 639–651. https://doi.org/10.1051/forest:2001151

McCarren K. L., McComb J. A., Shearer B. L. and Hardy G. E. St J. (2005). The role of chlamydospores of *Phytophthora cinnamomi* – a review. *Australian Plant Pathology*. 34:333-338

McGeoch, M. A., Butchart, S. H. M., Spear, D., Marais, E., Kleynhans, E. J., Symes, A., Hoffmann, M. (2010). Global indicators of biological invasion: Species numbers, biodiversity impact and policy responses. *Diversity and Distributions*, *16*(1), 95–108. https://doi.org/10.1111/j.1472-4642.2009.00633.x

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). Methods for normalizing microbiome data: An ecological perspective. *Methods in Ecology and Evolution*, *10*(3), 389–400. https://doi.org/10.1111/2041-210X.13115

McNeill, M., Phillips, C., Young, S., Shah, F., Aalders, L., Bell, N., Littlejohn, R. (2011). Transportation of nonindigenous species via soil on international aircraft passengers' footwear. *Biological Invasions*, *13*(12), 2799–2815. https://doi.org/10.1007/s10530-011-9964-3

Meurisse, N., Rassati, D., Hurley, B. P., Brockerhoff, E. G., & Haack, R. A. (2019). Common pathways by which non-native forest insects move internationally and domestically. *Journal of Pest Science*, *92*(1), 13–27. https://doi.org/10.1007/s10340-018-0990-0

Mitchell, C. E., Tilman, D., & Groth, J. V. (2002). Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology*, *83*(6), 1713–1726. https://doi.org/10.1890/0012-9658(2002)083[1713:EOGPSD]2.0.CO;2

Moore, C. (2003). *New Guinea: crossing boundaries and history*. Honolulu: Univ. Hawaii Press. pp. 274

Nguyen, D., Castagneyrol, B., Bruelheide, H., Bussotti, F., Guyot, V., Jactel, H., Boberg, J. (2016). Fungal disease incidence along tree diversity gradients depends on latitude in European forests. *Ecology and Evolution*, *6*(8), 2426–2438. https://doi.org/10.1002/ece3.2056

Niinemets, Ü. (2010). Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management*, *260*(10), 1623–1639. https://doi.org/10.1016/j.foreco.2010.07.054

O'Brien, P. A., Williams, N., & Stj Hardy, G. E. (2009). Detecting *Phytophthora*. *Critical Reviews in Microbiology*, *35*(3), 169–181. https://doi.org/10.1080/10408410902831518

O'Gara, E., Howard, K., Mccomb, J., Colquhoun, I. J., & Hardy, G. E. S. J. (2015). Penetration of suberized periderm of a woody host by *Phytophthora cinnamomi*. *Plant Pathology*, *64*(1), 207–215. https://doi.org/10.1111/ppa.12244

Oßwald, W., Fleischmann, F., Rigling, D., Coelho, A. C., Cravador, A., Diez, J., Werres, S. (2014). Strategies of attack and defence in woody plant-*Phytophthora* interactions. *Forest Pathology*, *44*(3), 169–190. https://doi.org/10.1111/efp.12096

Ostfeld, R. S., & Keesing, F. (2011). Effects of Host Diversity on Infectious Disease. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 157–182. https://doi.org/10.1146/annurev-ecolsys-102710-145022

Paudel, D. B., & Sanfaçon, H. (2018). Exploring the diversity of mechanisms associated with plant tolerance to virus infection. *Frontiers in Plant Science*, *871*(November), 1–20. https://doi.org/10.3389/fpls.2018.01575

Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., Vacher, C. (2019). Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology*, *41*, 23–33. https://doi.org/10.1016/j.funeco.2019.03.005

Portela, E., Aranha, J., Martins, A., & Pires, A. L. (1998). Soil factors, farmer's practices and Chestnut Ink Disease: some interactions. Acta Horticulturae, 494, 433–441

Rameau, J-C., Mansion, D. et Dumé, G., 2008. Flore forestière française: Plaines et collines. S.l. : *Forêt privée française*. ISBN 978-2-904740-16-9

Randolph, S. E., & Dobson, A. D. M. (2012). Pangloss revisited: A critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology*, *139*(7), 847–863. https://doi.org/10.1017/S0031182012000200

Redondo, M. A., Boberg, J., Stenlid, J., & Oliva, J. (2018). Functional traits associated with the establishment of introduced Phytophthora spp. In Swedish forests. *J. Appl. Ecol*. 1-18. DOI: 10.1111/1365-2664.13068

Rhoades, C. C., Brosi, S. L., Dattilo, A. J., & Vincelli, P. (2003). Effect of soil compaction and moisture on incidence of *Phytophthora* root rot on American chestnut (Castanea dentata) seedlings. *Forest Ecology and Management*, *184*(1–3), 47–54. https://doi.org/10.1016/S0378-1127(03)00147-6

Robin, C., Desprez-Loustau, M. L., Capron, G., & Delatour, C. (1998). First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. *Annales Des Sciences Forestieres*, *55*(8), 869–883. https://doi.org/10.1051/forest:19980801

Robin, C., & Husson, C. (2018). Pourquoi et comment contrôler les maladies des arbres forestiers en pépinières (Synthèse de l'atelier). *Revue Forestière Française*, (6), 683. https://doi.org/10.4267/2042/70323

Roscher, C., Schumacher, J., Foitzik, O., & Schulze, E. D. (2007). Resistance to rust fungi in Lolium perenne depends on within-species variation and performance of the host species in grasslands of different plant diversity. *Oecologia*, *153*(1), 173–183. https://doi.org/10.1007/s00442-007-0713-3

Rottstock, T., Joshi, J., Kummer, V., & Fischer, M. (2014). Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. *Ecology*, *95*(7), 1907–1917. https://doi.org/10.1890/13-2317.1

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications*, *8*, 1–9. https://doi.org/10.1038/ncomms14435

Sena, K. L., Yang, J., Kohlbrand, A. J., Dreaden, T. J., & Barton, C. D. (2019). Landscape variables influence *Phytophthora cinnamomi* distribution within a forested Kentucky watershed. *Forest Ecology and Management*, *436*(November 2018), 39–44. https://doi.org/10.1016/j.foreco.2019.01.008

Sena, K., Dreaden, T. J., Crocker, E., & Barton, C. (2018). Detection of *Phytophthora cinnamomi* in Forest Soils by PCR on DNA Extracted from Leaf Disc Baits. *Plant Health Progress*, *19*(3), 193–200. https://doi.org/10.1094/php-01-18-0004-rs

Sendall, B., & Drenth, A. (2001). Practical guide to detection and identification of *Phytophthora* Practical guide to detection and. *CRC for Tropical Plant Protection*, *1*, 1–41.

Sharpe, S. R. (2017). *Phytophthora* species Associated with American , Chinese , and Backcross Hybrid Chestnut Seedlings in Field Sites in the Southeastern United States. All Theses. 1–142.

Shearer, B. L., Crane, C. E., Cochrane, J. A., & Dunne, C. P. (2013). Variation in susceptibility of threatened flora to *Phytophthora cinnamomi*. *Australasian Plant Pathology*, *42*(4), 491–502. https://doi.org/10.1007/s13313-013-0215-1

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology*, *21*(8), 1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x

Sikes, B. A., Bufford, J. L., Hulme, P. E., Cooper, J. A., Johnston, P. R., & Duncan, R. P. (2018). Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. *PLoS Biology*, *16*(5), 1–16. https://doi.org/10.1371/journal.pbio.2006025

Simberloff, D., & Gibbons, L. (2004). Now you see them, now you don't! - Population crashes of established introduced species. *Biological Invasions*, *6*(2), 161–172. https://doi.org/10.1023/B:BINV.0000022133.49752.46

Smith S. E. & Read DJ. 1997. *Mycorrhizal symbiosis, 2nd edn*. London, UK:Academic Press.

Smith, S.E. & D. J. Read (2008). Mycorrhizal Symbiosis. 3rd. Academic Press. 800 p. (cite p. 17, 26, 29–34, 40)

Taudière, A. (2018). Déterminants de la structure des communautés fongiques dans les forêts de Corse : rôle des perturbations et de la composition forestière. *Bois & Forets Des Tropiques*, *334*, 75. https://doi.org/10.19182/bft2017.334.a31493

Thakur, M. P., van der Putten, W. H., Cobben, M. M. P., van Kleunen, M., & Geisen, S. (2019). Microbial invasions in terrestrial ecosystems. *Nature Reviews Microbiology*, *17*(10), 621–631. https://doi.org/10.1038/s41579-019-0236-z

Tomlinson, J. A., Dickinson, M., Hobden, E., Robinson, S., Giltrap, P. M., & Boonham, N. (2010). A five-minute DNA extraction method for expedited detection of *Phytophthora ramorum* following

prescreening using *Phytophthora* spp. lateral flow devices. *Journal of Microbiological Methods*, *81*(2), 116–120. https://doi.org/10.1016/j.mimet.2010.02.006

Tommerup, I. C., Dobrowolski, M. P., Hüberli D., O'Brien P. A., Hardy G. E. St. J. and Shearer, B. L. (1999). Risks and Threats Due To Genetic Variation in *Phytophthora cinnamomi* for disease management in natural vegetation ecosystems. In: First International Meeting on Phytophthoras in Forest and Wildland Ecosystems - *Phytophthora* Diseases of Forest Trees, IUFRO Working Party 7.02.09, August 30 – September 3, 1999, Grants Pass, Oregon USA pp. 55-63.

Trumbore, S., Prando P. and Hartmann H. (2015). Forest health and global change. Science. 349(6250): 814-818.

Ushio, M., Wagai, R., Balser, T. C., & Kitayama, K. (2008). Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? *Soil Biology and Biochemistry*, *40*(10), 2699–2702. https://doi.org/10.1016/j.soilbio.2008.06.023

Vale, F. X. R. Do, Parleviet J. E., & Zambolim, L. (2001). Concepts in plant disease resistance. *Fitopatologia Brasileira*, *26*(3), 577–589. https://doi.org/10.1590/s0100-41582001000300001

van den Berg, N., Swart, V., Backer, R., Fick, A., Wienk, R., Engelbrecht, J., & Prabhu, S. A. (2021). Advances in Understanding Defense Mechanisms in Persea americana Against *Phytophthora cinnamomi*. *Frontiers in Plant Science*, *12*(March). https://doi.org/10.3389/fpls.2021.636339

van Lierop, P., Lindquist, E., Sathyapala, S., & Franceschini, G. (2015). Global forest area disturbance from fire, insect pests, diseases and severe weather events. *Forest Ecology and Management*, *352*, 78–88. https://doi.org/10.1016/j.foreco.2015.06.010

Vannini, A., ., Brasier, C.M., Tomassini, A., Forlenza, V., Bruni, N., & Vettraino, A.M. (2014). Genetic variability of *Phytophthora cinnamomi* mating type A1 in native forests of Taiwan. Abstract, proceedings of 7th IUFRO Meeting Working Party 7-02-09, "*Phytophthora* in Forests and Natural Ecosystems"; Esquel, Chubut, Patagonia, Argentina, 98

Vannini, A., Breccia, M., Bruni, N., Tomassini, A., & Vettraino, A. M. (2012). Behaviour and survival of *Phytophthora cambivora* inoculum in soil-like substrate under different water regimes. *Forest Pathology*, *42*(5), 362–370. https://doi.org/10.1111/j.1439-0329.2012.00768.x

Vannini, A., Natili, G., Anselmi, N., Montaghi, A., & Vettraino, A. M. (2010). Distribution and gradient analysis of Ink disease in chestnut forests. *Forest Pathology*, *40*(2), 73–86. https://doi.org/10.1111/j.1439-0329.2009.00609.x

Vettraino, A. M., Barzanti, G. P., Bianco, M. C., Ragazzi, A., Capretti, P., Paoletti, E., Vannini, A. (2002). Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology*, *32*(1), 19–28. https://doi.org/10.1046/j.1439-0329.2002.00264.x

Vettraino, A. M., Morel, O., Perlerou, C., Robin, C., Diamandis, S., & Vannini, A. (2005). Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with Ink Disease and crown decline. *European Journal of Plant Pathology*, *111*(2), 169–180. https://doi.org/10.1007/s10658-004-1882-0

Vitale, S., Scotton, M., Vettraino, A. M., Vannini, A., Haegi, A., Luongo, L., Belisario, A. (2019). Characterization of *Phytophthora cinnamomi* from common walnut in Southern Europe environment. *Forest Pathology*, *49*(1), 1–10. https://doi.org/10.1111/efp.12477

Weiß, M., Waller, F., Zuccaro, A., & Selosse, M. A. (2016). Sebacinales - one thousand and one interactions with land plants. *New Phytologist*, *211*(1), 20–40. https://doi.org/10.1111/nph.13977

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc.: Guide Methods Appl. 315e322

Whyte, G., Howard, K., Hardy, G. E. S. J., & Burgess, T. I. (2016). The Tree Decline Recovery Seesaw; a conceptual model of the decline and recovery of drought stressed plantation trees. *Forest Ecology and Management*, *370*, 102–113. https://doi.org/10.1016/j.foreco.2016.03.041

Williamson, M. et Fitter, A. (1996). The varying success of invaders. Ecology, 77, 1661–1665.

Wilcox W. F. (1992). *Phytophthora* root and crown rots. *Tree Fruit crops IPM Disease identification Sheet No.7.*

Woodward S., Stenlid J, Karjalainen R, Hüttermann A. (1998) *Heterobasidion Annosum* Biol. Ecol. Impact Control. Wallingford: CABI. p. xi–xii

Yang, X., Tyler, B. M., & Hong, C. (2017). An expanded phylogeny for the genus *Phytophthora* . *IMA Fungus*, *8*(2), 355–384. https://doi.org/10.5598/imafungus.2017.08.02.09

Zentmyer, G. A. (1988). Origin and distribution of four species of *Phytophthora*. *Transactions of the British Mycological Society*, *91*(3), 367–378. https://doi.org/10.1016/s0007-1536(88)80111-6

Zhou, D., & Hyde, K. D. (2001). Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi* *Paper presented at the Asian Mycological Congress 2000 (AMC 2000) incorporating the 2nd Asia-Pacific Mycological Congress on Biodiversity and Biotechnology, and held at the Univer. *Mycological Research*, *105*(12), 1449–1457. Retrieved from http://www.sciencedirect.com/science/article/pii/S0953756208620304

CHAPTER 1

Toward a novel approach of the detection and quantification of *P. cinnamomi* and *P. x cambivora* in soil samples

Marylise Marchand[1], Emilie Chancerel[1], Marie Massot[1] and Cécile Robin[1]

[1]INRAE, University of Bordeaux, BIOGECO, F- 33 610 Cestas, France

INTRODUCTION

Surveillance and detection systems adapted to forest environment are needed to prevent establishment of invasive fungal pathogens in new areas or monitor the advances of such organisms across territories. Sentinel trees (Vettraino *et al.*, 2015), citizen sciences (Brown *et al.*, 2017), networks of spore trapping devices for aerial fungi like *Hymenoscyphus fraxinus* (Chandelier *et al.*, 2021) have been proven promising as monitoring techniques. On the other hand, due to the effects of the *Phytophthora cinnamomi* and *P. x cambivora* on the host physiology, some symptoms caused by the infection of these pathogens are not specific, *i.e.* chronic decline with transparency of the crown, making the presence of the pathogens uncertain. In adult chestnut trees, collar lesions are either not easy to excavate or inconspicuous. When such lesions are observed, isolations from infected tissues can be attempted to confirm the presence of suspected *Phytophthora* species. However, both *P. cinnamomi* and *P. x cambivora* are poor saprophytes (McCarren *et al.*, 2005), which renders isolation attempts invalid when cortical necrosis are old, despite the use of effective selective medium. Tests based on antibody-antigen affinity allow *in situ* confirmation of the presence of the pathogens on cortical tissues (O'Brien *et al.*, 2009). However, with these devices, there are risks of false positives with another genus of oomycete, the *Pythium* (Hardham, 2005). Isolations from infected roots proved to be the most effective method: when chestnut trees are naturally regenerating in infected sites, wilting seedlings showing root or collar lesions are frequent and fresh enough to provide *Phytophthora* isolates. However, when such plant material is not available and when the origin of the decline symptoms may be confusing, it is then necessary to attempt detection from the soil, the niche of the causal agents of the chestnut ink disease. While the link between the presence of the pathogens and the observed decline symptoms still need to be assessed, their detection remains a strong argument in favor of their involvement (Seddaiu *et al.*, 2020). However, the core problem remains that the distribution of microorganisms in soil is heterogeneous (Baveye *et al.*, 2016) and several soil compounds are known to inhibit subsequent detection methods. A widely used technique is the biological baiting, which enables the isolation of several telluric *Phytophthora* species from baits (Erwin and Ribeiro, 1996). Since sporulation production optima and timing vary among species, richness and diversity of *Phytophthora* species obtained by this method may vary with baiting parameters such as incubation time (Sarker *et al.*, 2021). Furthermore, the method requires sporulation events and thus only detects viable propagules, making the timing and location of sampling of the utmost importance. In addition, the release of zoospores in the soil suspension is not always sufficient for a positive result (O'Brien *et al.*, 2009). Finally, this method is time-

consuming and when morphological identification of isolates is ambiguous, it must be followed by molecular identification. Other more targeted approaches have been developed for *P. cinnamomi*, and to a lesser degree *P. x cambivora*, that are reviewed in O'Brien *et al.* (2009) or more recently in Kunadiya *et al.* (2017). These DNA-based methods use species-specific primers with conventional or nested Polymerase Chain Reaction (PCR) (Langrell *et al.*, 2011) or real-time PCR (qPCR) to determine the presence/absence of the species for the former or estimate the abundance for the latter. However, if there are efficient quantification methods *in planta* (Engelbrecht *et al.*, 2013; Eshragi *et al.*, 2011), there must yet be improved to enable an efficient specific detection and quantification in soil samples.

A first requirement to test my PhD hypotheses was to develop an efficient method for the detection and estimation of the inoculum quantity of the pathogens in soil samples. Refining detection methods would allow addressing the question of the extent of the invasion of the pathogen(s) on the French territory (cf Chapter 2) whereas their quantification in soil would allow the estimation of their abundance to study the possible dilution effect linked to host diversity (cf Chapter 3).

To meet these objectives, the droplet digital PCR (ddPCR) technology offers many promises in this area as it fractions a samples in 20 000 droplets where each droplet is a PCR reaction, making repetitions obsoletes (Zhao *et al.*, 2016). Thus, by counting the number of positive droplets, we can access to a quantification of the target DNA in the sample tested. Moreover, compared to real-time quantitative PCR (qPCR), which forms the foundation for many diagnostic tests, it exhibits a higher resilience to PCR inhibitors (Dingle *et al.*, 2013). Therefore, we developed a ddPCR method dedicated to the detection of *P. cinnamomi* or *P. x cambivora*. We compared the ddPCR assay we developed to the touchdown multiplex nested PCR developed by Langrell *et al.* (2011) as it was proven to be a sensitive detection method of the pathogens in soil samples.

By essence, the metabarcoding is not a species-targeted approach as it employs conserved primers, *i.e.* targets biding sites that are shared across multiple taxa, and flank a region that is variable enough to discriminate these taxa among themselves. In recent years, the Illumina MiSeq Next Generation-Sequencing technology has emerged as the most relevant way to survey microbial diversity (Caporaso *et al.*, 2012) as it allows high coverage levels of sequences for a relative low cost (Ficetola *et al.*, 2015). However, some issues remain with this approach

because the complexity of such an extensive data renders challenging the disentanglement of sequencing errors from biological reality (Callahan *et al.*, 2016). Each step of the procedure may be a source of error possibly leading to misinterpretation (Aguayo *et al.*, 2018). For example, the construction of a *Phytophthora* library from environmental DNA typically requires three successive PCRs, possibly amplifying biases at each round PCR. Moreover, the DNA of different species within a sample may be in competition for binding with the metabarcoding primers during the library construction. Therefore, high abundance species may mask low abundance species (Brandon-Mong *et al.*, 2015). Over the past years, several bioinformatics algorithms processing sequencing data have been elaborated to address those issues among those, DADA2 (Divisive Amplicon Denoising Algorithm, version 2), a R package introduced by Callahan *et al.* (2016). This approach was identified as the most accurate for metabarcoding analyses (Pauvert *et al.*, 2019). The challenging step of this work was to adapt a whole fungal diversity pipeline for the investigation of the closely related *Phytophthora* taxa, finely tuning the balance between the detection of taxa and the removal of error, *e.g.* PCR and sequencing errors (Alberdi *et al.*, 2017). Here we developed a *Phytophthora* dedicated pipeline and used it to assess its sensitivity in the detection of *P. cinnamomi/P. x cambivora*. Secondly, we evaluated its relevance as a tool for the estimation of a single species abundance across several soil samples. To summarize, in the present chapter, I compared three target DNA-based methods (nested PCR, ddPCR and metabarcoding methods), i) to detect *P. cinnamomi* and/or *P. x cambivora* and ii) to quantify them in soil samples.

***Preamble**: The ddPCR method is fully described in Chapter 5 since the quantification of P. cinnamomi in root tissues is an important part of this chapter that was submitted to a journal. To avoid repetitions, I have not copied the description of the method in extendo. In this chapter 1, I present and focus on ddPCR results for quantification in soil.*

MATERIAL AND METHODS

Soil sampling and *Phytophthora* baiting

In July 2019, soils were sampled in a study area in the forest of Montmorency (Val d'Oise, France) known to be infected by *P. cinnamomi* (FX Saintonges and Cécile Robin, personal communication). Briefly, we collected at a 25cm depth, four soil samples at 1m of chestnut trees, showing various levels of chronic decline (more details in the Chapter 3). These samples were pooled together to form one focal soil sample (FSS). Thirty focal trees were sampled and served as centres of plots, in which subplots soil samples (SPSS) were collected (at 5m from

the focal trees and at 25 cm depth, two or three samples per plots). A total of 120 SPSS were collected.

A biological baiting was performed in summer 2019 to detect *Phytophthora* species in FSS. Soils (50mL) were placed into a 10x8x4cm container and were pre-wetted by adding 50mL of osmosed water for the night. The next day, 100mL osmosed water was added, all excess organic matter is removed from the surface of the water, and five oak (*Quercus robur*) leaf discs (1 cm diameter) were placed and incubated for 3 days at 22-23°C in the light. The leaf discs were then placed onto a PARBHy selective media (malt 1.5%, agar 1.8%, pimaricin 10 ppm, ampicillin 250 ppm, rifampicin 10 ppm, benomyl 15 ppm, hymexazol 50 ppm). After two days, culture implants from isolates growing from the discs were plated on Malt Agar (MA) (1.5% malt, 1.8% agar). Identification was achieved by microscopic observation and confirmed by Sanger sequencing. With this method, a total of 13 FSS were found to be positive to *P. cinnamomi*, and none to *P. x cambivora*. No other *Phytophthora* species were isolated.

Soil DNA extraction

Out of the 120 SPSS, we selected 86 soil samples distributed across the 30 plots. The DNA from 0.2g of these 86 soils was extracted using the FASTDNA ™ SPIN kit for soil (MP Biomedicals, Inc, Eschwege, Germany) in two replicates that were then pooled together. One water sample was use as a negative control for each round of DNA extraction (for a four negative controls at the end).

Mock community

To test the accuracy of the metabarcoding, mock communities were composed by mixing DNA from different species known to be associated with *P. cinnamomi* in the soil substrate (Vannini *et al.*, 2013; Tewoldemedhin *et al.*, 2011; Akili *et al.*, 2012; Li *et al.*, 2010 and Bose *et al.*, 2018). Details of all oomycetes isolates (and one fungus) used in this study are documented in Supplementary Table S1. Mocks were composed of the following species (at 0.1ng/µL) : *Phytophthora cinnamomi, P. x cambivora, P. syringea, P. nicotinae, P. plurivora, P. sojae, P. gonapodyides, P. castanea, P. pseudosyringea, P. x alni, P. taxon forestsoil, P. quercina, P. x multiformis, P. megasperma, Pythium irregulare, Py. ultimum, Py. intermedium, Py. sylvaticum and Fusarium solani* (Table S1). Five different mock communities were implemented in which DNA from the different species were mixed together but *P. cinnamomi* was added in decreasing concentrations at 0.1ng/µL, 10 pg/µL, 1 pg/µL, 100 fg/µL and 10 fg/µL.

For DNA extraction from mycelium, the isolates were grown on vegetables juice agar (V8A) plates (100mL/L filtered vegetable juice (Campbell Grocery products Ltd. Norfolk, UK), 900 mL/L distilled water, 0.1 g/L CaCO3, pH adjusted to 7 and 18g grade A Agar (Becton, Dickenson and Company, Sparks, MD, USA) overlaid with cellophane. Total DNA was extracted from lyophilized mycelium (20 mg) obtained from those cultures and ground to a fine powder, using the Invisorb® Spin Plant mini kit (Stratec molecular, Berlin). Identification of each species was confirmed either by Ypt1 or ITS sequencing (Table S1).

Detection by touchdown nested multiplex PCR

The soil samples, mock communities and negative controls were studied by amplifying the internal transcribed spacer 1 (ITS 1) and ITS2 region fragment using touchdown multiplex nested PCR with primer pairs ITS4 and ITS5 in the first round and PciF2/PciR2 and PcaFshort/PcaR in the second round according to the protocol of Langrell *et al.* (2011). The reaction mix consisted in 1.5µL 10X Tampon, 0.8 µL MgCl2, 0.6 µL dNTP, 0.3 µL of each primer, 0.1 µL Taq Polymerase (Eurogentec all), 9.9 µL mQ H2O and 1.5µL DNA or mQ water in the negative control for a final reaction of 15µL. Amplification conditions comprised an initial denaturation (3min at 94°C) followed by 30 cycles of 1min at 94°C, 1 min at 55°C and 1 min at 72°C with a final post extension of 72°C for 10 min. 1,5 µL of the first round amplicons diluted to the 1/50 were used as DNA template for the second round PCR. The second PCR was performed following the same PCR conditions as the first one at the exception that cycling conditions comprised a 0.7°C decrease per cycle (from 71°C to 64°C) for the first 10 cycles, followed a constant for the remaining 20 cycles.

Post-amplification, PCR products were assessed by electrophoresis (100V, 20 min) and revealed on a 2% agarose gel (GelRed).

P. cinnamomi detection by the ddPCR assay

The soil samples, mock communities and negative controls were analyzed with the ddPCR developed by Marchand *et al.* (submitted) to detect *P. cinnamomi* and/or *P. cambivora*. The ddPCR reactions were carried out in 22 µl using the ddPCR™ Supermix for Probes - No dUTP (Bio-Rad, USA). The reaction mix consisted of 1X Probe Supermix, 500 nM of each primer and probe, and 2 µl DNA or ultrapure water in the negative control. Droplets containing mix/sample were generated using the QX200 droplet generator (Bio-Rad, Munich, Germany) according to manufacturer's instructions to transfer to a 96-well PCR plate. PCR amplification

were performed in a thermal cycler (Biorad, Göttingen, Germany) using the following conditions : 10 min of initial denaturation at 95°C followed by 40 cycles of 10 s denaturation at 94°C, 1 min annealing at 60°C and a final step of inactivation of 10 min at 98°C. Results were analyzed on the QuantaSoft software v. 1.7.4 (Bio-Rad, Munich, Germany). The threshold for detection of positive droplets was set manually at a fluorescence amplitude of 2 000 for *P. cinnamomi* and 4 000 for *P. x cambivora*. Additionally, measurement of single PCR wells were excluded whether the total number of droplet was <10 000 and if only a single droplet was detected above the amplitude threshold. The dilution of the DNA extract in the reaction mixture (2 µl in 20 µl) and the final 100µL elution volume for soil samples was taken into account to adjust the final concentration in copies/µl of DNA extract.

Phytophthora library construction and sequencing

The *Phytophthora* community in the soil samples, the mock communities and negative controls were studied by amplifying a ~250 pb sequence of the internal transcribed spacer 1 (ITS 1) and 18S region fragment using nested PCR with primer pairs 18Ph2F and 5.8S-1R in the first round and ITS6 and 5.8S-1R in the second round according to the protocol of Català *et al.* (2015). A negative water control for 96-well plate sample preparation further referenced as « metabarcoding control » was included. The PCR mix was composed of 1 µL Bovine Serum Albumine (BSA) at 10 ng/µL concentration, 12.5 µl 2X KAPA HiFi HotStart Ready Mix enzyme (KAPA Biosystems, Wilmington, MA, USA), 4µL H_2O mQ and 2.5 µL of primers at 2 µM for a final reaction of 25 µL. The PCR temperature cycling conditions were as follows : initial denaturation at 95°C for 3 min followed by 40 cycles of 95°C for 30s, 60°C for 30 s, 72°C for 30 s and a final extension of 72°C for 10 min. Amplicons were purified using homemade SPRI beads. 2,5 µL of the first round purified amplicons were used as DNA template for the second round PCR. In addition, Illumina barcoded overhang adapters were added directly to the primers of the second PCR (ITS6 and 5.8S-1R) as mentioned by Laforest-Lapointe *et al.*, 2017. The second PCR was performed following the same PCR conditions as the first one at the exception that the number of cycles was 35. Barcoded amplicons were purified, quantified with Quant-iT™ dsDNA High Sensitivity Assay Kit (Invitrogen™) and equimolarly pooled. Average size fragment was checked using Tapestation instrument (Agilent Technologies).

The *Phytophthora* library was then sequenced on the MiSeq Instrument (Illumina, San Diego, CA, USA) with the reagent kit v2 (500-cycles) on the PGTB facility (Plateforme Génome

Transcriptome de Bordeaux). Sequence demultiplexing (with exact index search) was performed using DoubleTagDemultiplexer. After quality control, the FASTQ files for each sample were exported for bioinformatics analysis.

Bioinformatic analysis of sequencing data

To analyse the *Phytophthora* community, we used the DADA2 and FilterAndTrim option (Callahan *et al.*, 2016) with the following parameters: minimal read length = 100 bp, sequences with more than maximum number of N allowed in the reads = 0, maximal expected errors = 2, discard reads that match the phiX genome (viral genome used to test contamination) = TRUE. Sequences were dereplicated and Amplified Sequence Variants (ASVs) were constructed using the dada option after error rate estimation. Chimeric sequences were filtered out and the final ASVs were taxonomically assigned using MegaBLAST against a homemade database (16 080 sequences retrieved from *Phytophthora*db.org, *Phytophthora*-ITS-ID.org and all NCBI sequences assigned to *Phytophthora, Peronospora and Pythium* species with the « ITS 1 » keyword in their GenBank definition.

To test for the accuracy of the ASV construction we used the mock communities, composed of known species, to determine which ASV construction method was the most accurate and allowed the retrieval of the maximum of species present in these positive controls. The Illumina MiSeq technology produces paired-end sequences, *i.e.* the target gene is sequenced forward (R1) and reverse (R2). The basic ASV construction correspond to the merger of the R1 and R2 (ASV construction method 1). However, it is possible to only used the R1 (ASV construction method 2) or the R2 (ASV construction method 3).

RESULTS

Nested PCR and ddPCR for the detection of *P. cinnamomi*

In the mock communities, *i.e.* samples with a known *P. cinnamomi* DNA concentration, from 100 pg/µL to 10 fg/µL, the nested PCR was the most efficient for the detection of *P. cinnamomi* as it allowed the detection at 10 fg/µL whereas the pathogen was detected at 1 pg/µL through the ddPCR (Table 1). Moreover, the nested PCR was positive for *P. cinnamomi* in 15 soil samples and in none with the ddPCR (Table 1).

Table 1: Detection of *P. cinnamomi* using the three methods on the five mock communities (+ detection, - no detection)

P. cinnamomi **concentration in the mock communities**	**Nested PCR**	**ddPCR** (copies/µL)	**Metabarcoding** (ASV number/read)
0	-	0	0/0
10 fg/µL	+	0	0/0
100 fg/µL	+	0	0/0
1 pg/µL	+	600	4/314
10 pg/µL	+	3600	7/1 642
0,1 ng/µL	+	36 000	9/11 790
Detection in soil samples (/86)	15	0	73

No comparison of the methods was presented for *P. x cambivora* since the species was not detected in soils samples with either method.

Evaluation of the targeted metabarcoding approach

The sequencing run generated 3 945 628 quality sequences that could be considered for analyses. From these, 462 280 reads passed the filter and were grouped in 476 ASVs (Figure 1). 57 ASVs in 2 831 reads gave no hit after blast against the homemade database nor the NCBI nucleotide collection (Figure 1).

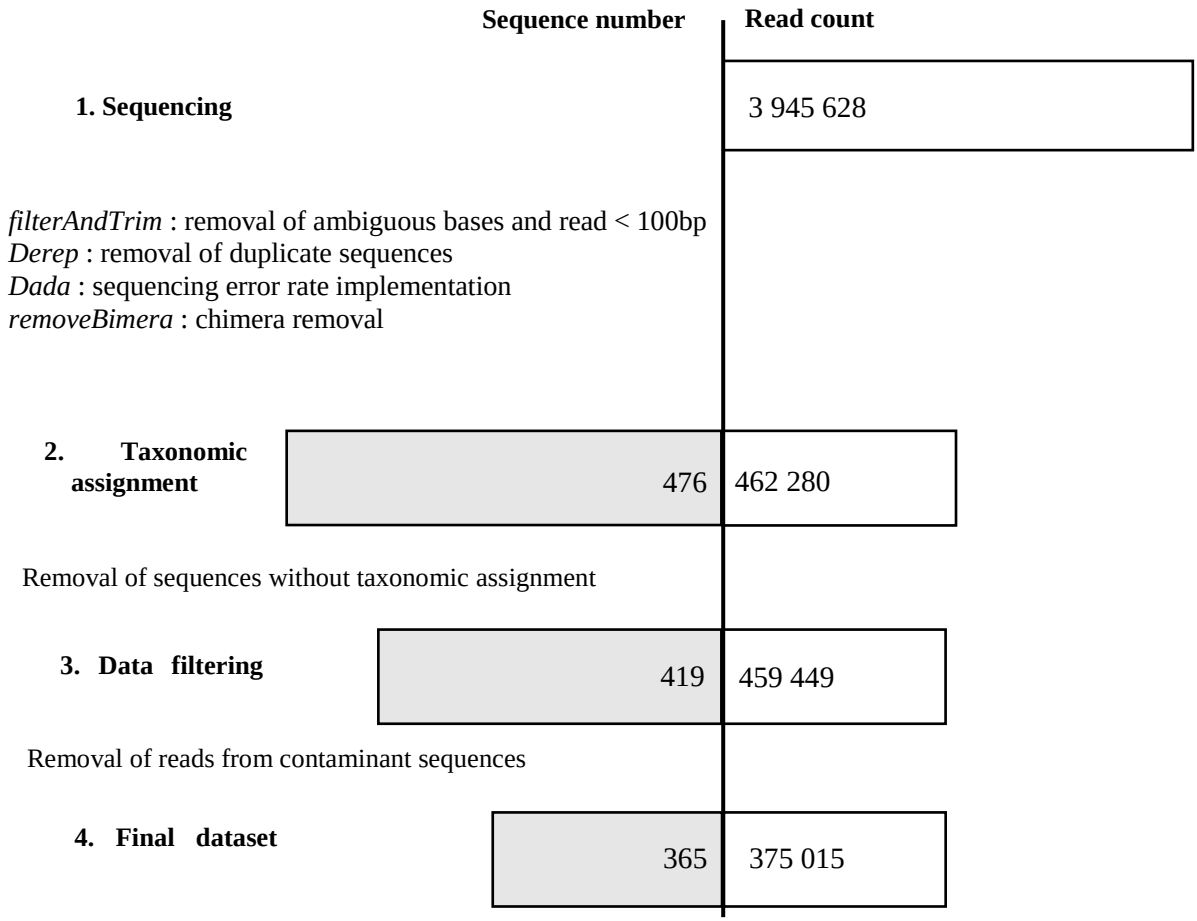


Figure 1: *Phytophthora* metabarcoding pipeline

We evaluated the ASV construction methods using the mock communities by testing the accuracy of the taxonomic diversity found through the different construction methods. Ten different *Phytophthora* species were accurately detected in the mock communities whatever the construction method used (Table 2). Three *Phytophthora* species of the mock community were not detected through any of the method; two other were detected by only one or two methods. Conversely, ASVs were assigned to two *Phytophthora* species (*P. cactorum* and *P. heveae*) which were not present in the mock community. *Pythium* species and *Fusarium solani* were never detected (Table 2). For further analysis, we used the ASVs constructed using the method 1.

Table 2: Summary of species present in the mock communities (at 0.1 DNA ng/µL per species) and species retrieved through ASV construction method 1 (merged R1 and R2), 2 (R1 only) and 3 (R2 only).
In green: Species detection consistent with mock composition and primers, in red: inconsistent results.

Species	**Mock communities**	ASV construction		
		method 1	**method 2**	**method 3**
P. alni	+	+	-	+
P. x cambivora	+	+	+	+
P. castanea	+	+	+	+
P. cinnamomi	+	+	+	+
P. gonapodyides	+	+	+	+
P. megasperma	+	+	+	+
P. nicotinae	+	+	+	+
P. pseudosyringea	+	+	+	+
P. quercina	+	+	+	+
P. sojae	+	+	+	+
P. syringea	+	-	-	-
P. plurivora	+	-	-	-
P. taxon forestsoil	+	-	+	-
P. x multiformis	+	-	-	-
P. heveae	-	-	+	+
P. cactorum	-	+	+	+
Pythium irregulare	+	-	-	-
Py. ultimum	+	-	-	-
Py. sylvaticum	+	-	-	-
Py. intermedium	+	-	-	-
Fusarium solani	+	-	-	-

Seven *P. cinnamomi* ASVs were present in the mocks, the negative controls and the soil samples (Figure 2). These core *P. cinnamomi* ASVs (representing more than 40% of soil samples) accounted for 90% of the total read count of the dataset. We implemented two types of negative controls: a metabarcoding water control and four DNA water controls. In the metabarcoding water control, reflecting the contaminations occurring during the library construction, one ASV was detected (27 reads). We removed from all soil samples the 27 read of the ASV. In the four DNA water controls, reflecting the contaminations occurring during each DNA extraction round, the other six core ASVs were detected with number of reads varying from 31 and 734. For each extraction round, the read count of ASVs obtained for the negative control of this round was removed from each soil sample processed during the round. Eight *P. cinnamomi* sequences were present in the negative controls only and were removed from further analyses.

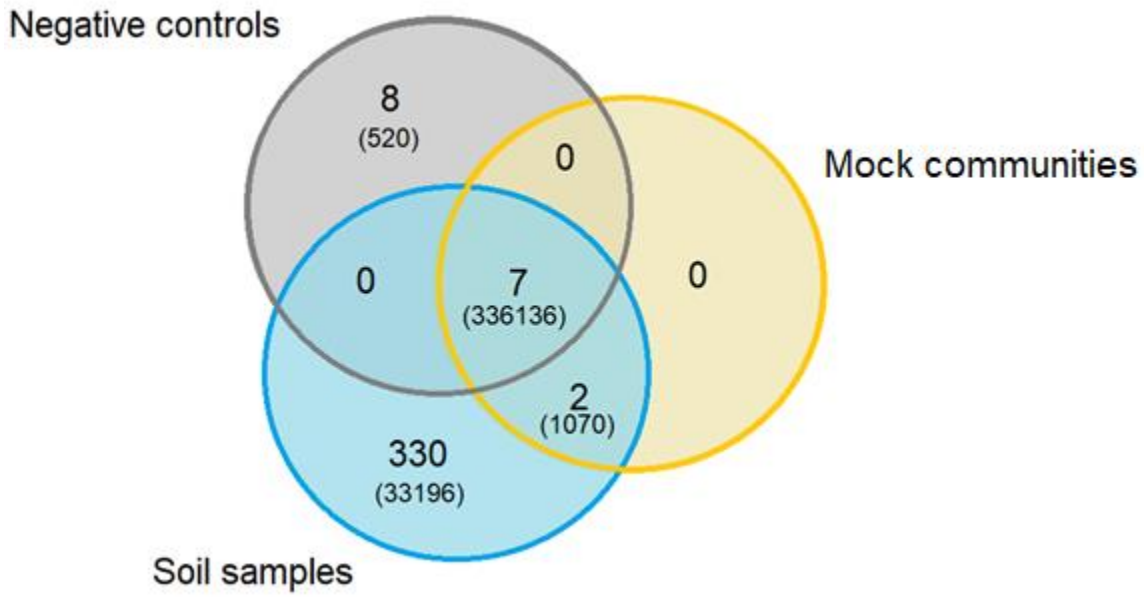


Figure 2: *P. cinnamomi* ASVs present in negative controls (grey), mock communities (yellow) and soil samples (blue) and their read count in parenthesis

We operated on the same way on the other *Phytophthora* species found in the negative controls and the soil samples and obtained a final dataset of 365 ASVs among which 347 were assigned to *P. cinnamomi* (Table 3). There was a surprisingly lack of *Phytophthora* diversity in our samples with only one sample displayed other species than *P. cinnamomi*.

Table 3: *Phytophthora* taxonomic diversity observed in the 86 soil samples after analysis through metabarcoding (* detection in the same soil sample)

Taxa	**Total ASV number**	**Total read count**	**Number of samples in which the taxa is found**
Phytophthora UI	3	474	1*
P. cactorum	2	306	1*
P. cambivora	2	411	1*
P. castanea	5	1701	1*
P. gonapodyides	2	438	1*
P. nicotinea	1	24	1*
P. pseudosyringea	3	739	1*
P. cinnamomi	347	370 922	73

Comparison of the three DNA-based detection methods

In the mock communities, the nested PCR was the most efficient in the detection of *P. cinnamomi* compared to the ddPCR and the metabarcoding (Table 1). Both the number of

copies/µL (ddPCR) and the read count (metabarcoding) appeared to reflect the initial *P. cinnamomi* DNA concentration found in the mock communities (Table 1). On the other hand, *P. x cambivora* was detected in the five mocks using the nested PCR but was only detected once through the metabarcoding approach (data not shown).

In the soil samples, *P. cinnamomi* was detected in 73 samples through metabarcoding, in 15 samples using the nested PCR and in no samples with the ddPCR (Table 1). On the other hand, *P. x cambivora* was detected only in one sample and with the metabarcoding approach only (Table 3). These soil samples were retrieved from plots for which we used the biological baiting approach to detect *P. cinnamomi*. By considering that if one of the soil from the same plot was positive through either DNA-based method then the plot was positive, 33.3% and 100% of plots proved to be positive with respectively the nested PCR and metabarcoding approach. Hence, the nested PCR allowed the detection of *P. cinnamomi* in only two plots, which were negative by biological baiting (Table 4).

Table 4: Detection of *P. cinnamomi* through nested PCR and metabarcoding in subplot soil samples (SPSS) collected in plots, which were assessed positive or negative after biological baiting with soil sampled in rhizosphere of focal tree (FSS)

Number of plots with biological baiting		Number of plots with at least one *P. cinnamomi* SPSS positive with	
		Nested PCR	Metabarcoding
P. cin positive FSS	13	8	13
P. cin negative FSS	17	2	17

No results for the ddPCR were displayed since all soil samples were negative to *P. cinnamomi* with this method

With the metabarcoding method, *P. cinnamomi* was detected in all the plots, whatever the infection status obtained by biological baiting. On the other hand, the ddPCR approach proved to be completely inefficient on soil samples, yet we still required a quantification method of the pathogen in soil samples.

Quantification method using the metabarcoding approach

We used the five different mock communities to study the recovered *P. cinnamomi* read abundance and ASV number. In the mock community with 100 pg/µL *P. cinnamomi* initial DNA concentration, there was nine different ASVs in 11 790 reads whereas in the mock community with 10 pg/µL, there was seven ASVs in 1 642 read (Table 1). Hence, the read

count reflected more accurately the input *P. cinnamomi* DNA concentrations than the ASV number. Moreover, soils that were sampled from plots positive to *P. cinnamomi* through baiting displayed a significantly higher read abundance but not ASVs number (Figure 3), confirming that the read count is a better proxy for the *P. cinnamomi* abundance than the ASV number.

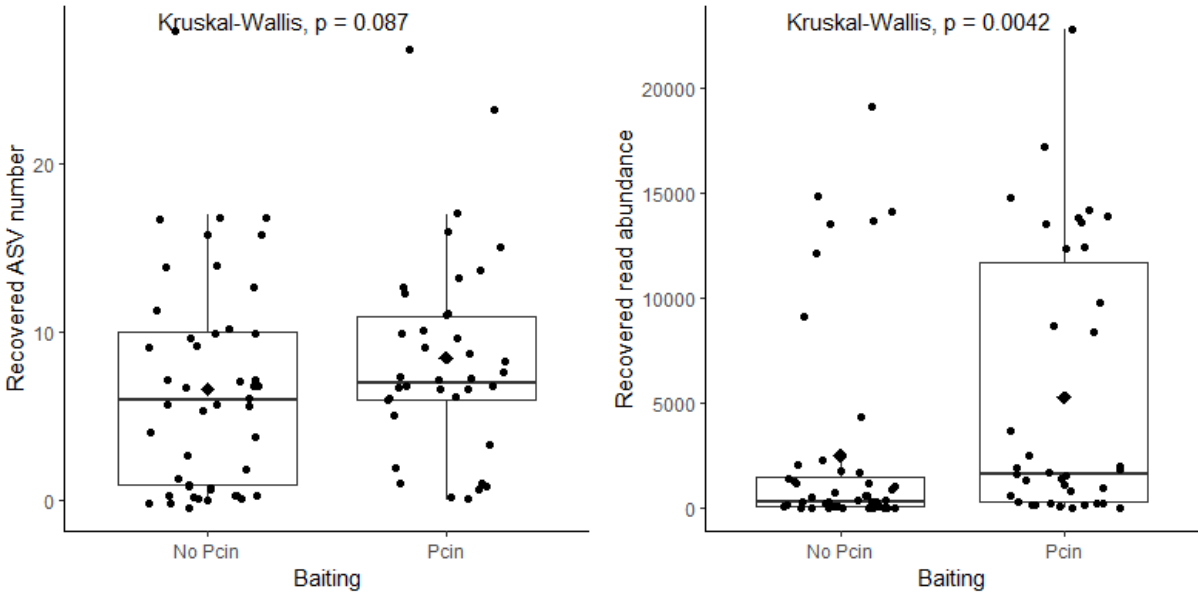


Figure 3: Recovered *P. cinnamomi* ASV number (left) and read abundance (left) in samples according to whether they were taken from plot positive to *P. cinnamomi* with biological baiting or not

DISCUSSION

The first aim of the study was to find an efficient detection method for the pathogens that was applicable to soil samples. Thus, we compared three DNA-based methods that is a nested PCR, the ddPCR and the metabarcoding approach.

The nested PCR approach proved to be the most efficient in the detection of *P. cinnamomi* at lower DNA concentration in the mock communities than the metabarcoding approach and the ddPCR assay. Even though the ddPCR method was proven as efficient as the metabarcoding approach for the detection of mycelial DNA, the detection in environmental soil samples was not feasible. We tried to improve the detection through ddPCR by modifying the extraction step (by using 10g of soil samples for the DNA extraction as recommended by Langrell *et al.* (2011), purification after extraction, serial dilution) but it was fruitless (data not shown). This low detection sensitivity could be explained by the fact that the target gene used for the ddPCR assay, the Ypt1, is in a single copy in the organism DNA (Chen and Roxby, 1996). Therefore, the amplification of this gene in a complex matrix like the soil, which is extracted in a very

small amount 0.4g (0.2g repeated twice) through the commercial kit, could have been prevented. Thus, despite the ddPCR being a very sensitive method (Zhao *et al.*, 2016), the choice of primers made it an inefficient method for the detection of the pathogens in soil samples. With the nested PCR, the pathogen was detected in 17% of soil samples whereas it was detected in 84.8% in soil samples with the metabarcoding. The nested PCR approach was initially designed to be used 10g soil for the DNA extraction step (Langrell *et al.*, 2011) and we only used 0.4g possibly explaining the method lack of sensitivity in such case. This result also suggests that in the soil samples, the *P. cinnamomi* concentration was at least consistently higher than 1 pg/µL.

For the metabarcoding approach, we used the mock communities in two different ways; first, to decipher which ASVs construction method allowed to retrieve the most species actually present in the mocks. We chose the method 1, *i.e.* merged R1 and R2, because it was the ASVs construction method with the least false-positive and false-negative compared to the two other methods. Secondly, by making mock communities with different *P. cinnamomi* DNA concentration, we showed that the presence of other species did not prevent the detection of *P. cinnamomi* except at low concentration (under 1 pg/µL).

A major problem with the metabarcoding approach is the samples displaying low abundances, because it is a challenge to decipher whether the reads are present from contaminations during the procedure (false-positive) or reflect an actual low DNA concentration in the sample. While this is a complicated problem to solve, to first address this issue, we used the DADA2 metabarcoding data processing, which generates lower false-positive rates than comparable methods (Callahan *et al.*, 2016). Secondly, we used the technical negative controls we implemented along the metabarcoding procedure, from the DNA extraction to the library construction, to remove these potential false-positives. We also propose that to use a 10 reads threshold as a minimal detection limit for subsequent analyses, as using a minimum read count is a broadly employed strategy to remove artefactual sequences (Giguet-Covex *et al.*, 2014; Leray and Knowlton, 2017). Furthermore, the use of mock communities should only be restricted to studies aiming to evaluate the taxonomic diversity and richness, as they are a potential source of contaminations for the samples. On the contrary, a better calibration of abundance would require increasing the number of positive controls with different concentrations of the target species while being careful to take into consideration the putative contaminations generated from such a set up.

From a pure methodological standpoint, we remain aware that using the metabarcoding approach as a quantification method may be criticized since amplification artefacts of targeted DNA may result in read abundances, which do not correlate closely enough with the exact species abundance. However, some studies showed a positive relationship between species abundance and sequencing read abundance for other species (Evans *et al.*, 2016) and this is not the first time that sequence abundance obtained through a metabarcoding approach was used as a proxy for pathogen abundance (Liang *et al.*, 2016). Here, we reduced these artefacts by modifying the library construction process from three to two successive PCRs. Moreover, these amplification artefacts are problematic when comparing different taxa as the ITS is a multicopy gene that can be present in different repetition number in the different organisms. Furthermore, there could be differential matches between primers and target DNA of certain taxa possibly leading to misrepresentation of these taxa (Brandon-Mong *et al.*, 2015). Here, we may consider these non-issues for the estimation of the quantity of *P. cinnamomi* because we used metabarcoding approach to single out one species specifically and we can reasonably assume that the PCR biases were the same for all the samples.

The analysis of soil samples showed an incredible lack of *Phytophthora* diversity. Indeed, only one out of the 86 soil samples contained other species than *P. cinnamomi*. Depending on the biotope sampled, such a low diversity is not unheard of (Legeay *et al.*, 2020). Indeed, Zeng *et al.* (2009) found 14 different *Phytophthora* species in a tropical island, but only five solely associated with forest settings. Similarly, Burgess *et al.* (2017) found a mean of five different *Phytophthora* species per sampling location in an extensive survey across Australia. Here, we found one dominant *Phytophthora* taxa, *P. cinnamomi*, which could be accounted for the fact we only investigated one forest massif.

The mock communities with different initial *P. cinnamomi* mycelial concentration showed that read count reflected more accurately the initial abundance of the pathogens than the ASV number. Furthermore, the biological baiting is a method for which the probability of a positive result was shown to be correlated to the initial inoculum abundance (Sarker *et al.*, 2021), *i.e.* a higher inoculum quantity leads to a higher probability to detect the pathogen. Here we showed that the plots positive to *P. cinnamomi* through baiting displayed a significantly higher read abundance than negative plots. Altogether, this suggests that the read count is an accurate proxy to estimate the abundance of *P. cinnamomi* in the soil samples.

AKNOWLEDGEMENTS

Marylise Marchand was financially supported by the Région Nouvelle-Aquitaine (Grant Number 2018-1R20204 project DIGIE "chestnut DIeback: vulnerability and GenetIc determinism of ink disease resistance) and by the French Ministry of Agriculture and Forestry (project VISTA). We thank Claude Husson (DSF, French Ministry of Agriculture and Forestry, France) and Jonathan Gaudin (INRAE UMR SAVE, Bordeaux, France) for providing fungal and oomycete isolates. ddPCR and metabarcoding assays were conducted at the Genome Transcriptome Facility of Bordeaux.

REFERENCES

Aguayo, J., Fourrier-Jeandel, C., Husson, C., & Loos, R. (2018). Assessment of Passive Traps Combined with High-Throughput. Applied and Environmental Microbiology, 84(11), 1–17.

Akili S., Serce C. U., Katırcıoglu Y. Z. and Maden S. (2012). Involvement of *Phytophthora* spp. in chestnut decline in the Black Sea region of Turkey. For. Path. 42 377–386

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2017). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, *9*(1), 134–147. https://doi.org/10.1111/2041-210X.12 849

Baveye, P. C., Berthelin, J., & Munch, J. C. (2016). Too much or not enough: Reflection on two contrasting perspectives on soil biodiversity. *Soil Biology and Biochemistry*, *103*, 320–326. https://doi.org/10.1016/j.soilbio.2016.09.008

Bose T., Wingfield M. J., Roux J. Vivas M. and Burgess T. I. (2018). Community composition and distribution of *Phytophthora* species across adjacent native and non-native forests of South Africa. Fungal Ecology 36 (2018) 17e25

Brandon-Mong, G.-J., Gan, H.-M., Sing, K.-W., Lee, P.-S., Lim, P.-E., & Wilson, J.-J. (2015). DNA metabarcoding of insects and allies: An evaluation of primers and pipelines *Bulletin of Entomological Research*, *105*, 717–727. https://doi.org/10.1017/S0007485315000681

Brown N., van den Bosch F., Parnell S. and Denman S. (2017). Integrating regulatory surveys and citizen science to map outbreaks of forest diseases: acute oak decline in England and Wales. Proc. R. Soc. B. 284:2017547.

Burgess, T. I., White, D., McDougall, K. M., Garnas, J., Dunstan, W. A., Català, S., Hardy, G. E. S. J. (2017). Distribution and diversity of *Phytophthora* across Australia. *Pacific Conservation Biology*, *23*(2), 150. https://doi.org/10.1071/pc16032

Callahan B., McMurdie P. J., Rosen M. J., Han A. W., Johnson A; J.O. and Holmes S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 13(7): 581-586

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal*, *6*(8), 1621–1624. https://doi.org/10.1038/ismej.2012.8

Català, S., Pérez-Sierra, A., & Abad-Campos, P. (2015). The use of genus-specific amplicon pyrosequencing to assess *Phytophthora* species diversity using eDNA from soil and water in northern spain. *PLoS ONE*, *10*(3), 1–14. https://doi.org/10.1371/journal.pone.0119311

McCarren K. L., McComb J. A., Shearer B. L. and Hardy G. E. St J. (2005). The role of chlamydospores of *Phytophthora cinnamomi* – a review. *Australian Plant Pathology*. 34:333-338.

Chandelier, A., Hulin, J., Martin, G. S., Debode, F., & Massart, S. (2021). Comparison of qPCR and metabarcoding methods as tools for the detection of airborne inoculum of forest fungal pathogens. *Phytopathology*, *111*(3), 570–581. https://doi.org/10.1094/PHYTO-02-20-0034-R

Chen Y., and Roxby R. (1996). Characterization of a *Phytophthora infestans* gene involved in the vesicle transport. Gene. 181: 89-94.

Dingle T. C., Sedlak R. H., Cook L. and Jerome K. R. (2013). Tolerance of droplet-digital PCR versus real-time quantitative PCR to inhibitory substances. *Clin Chem*. 59(11): 1670–1672.

Eden, M. A., Hill, R. A., & Galpoththage, M. (2000). An efficient baiting assay for quantification of *Phytophthora cinnamomi* in soil. *Plant Pathology*, *49*(4), 515–522. https://doi.org/10.1046/j.1365-3059.2000.00478.x

Engelbrecht J., Duong T. A. and Berg N.v.d. (2013). Development of a nested quantitative real time PCR for detection Phytohthora *cinnamomi* in Persea americana rootstocks. Plant Disease. 97(8). 1011-1017

Erwin, D. C. and Ribeiro, O. K. (1996). *Phytophthora* diseases worldwide. *Phytophthora diseases worldwide.*

Eshraghi, L., Aryamanesh, N., Anderson, J. P., Shearer, B., McComb, J. A., Hardy, G. E. S. J., & O'Brien, P. A. (2011). A quantitative PCR assay for accurate in planta quantification of the necrotrophic pathogen *Phytophthora cinnamomi*. *European Journal of Plant Pathology*, *131*(3), 419–430. https://doi.org/10.1007/s10658-011-9819-x

Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y., Jerde, C. L., … Lodge, D. M. (2016). Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 29–41. https://doi.org/10.1111/1755-0998.12433

Ficelota G. F., Pansu J., Bonin A., Coissac E., Giguet-Covex C., De Barba M., Gielly L., Lopes C. M., Boyer F., Gilles P. G. F. and Taberlet P. (2015). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. Molecular Ecology Resources 15, 543–556

Giguet-Covex C., Pansu J., Arnaud F., Rey P-J., Griggo C., Gielly L., Demaizon I., Coissac E., David F., Choler P., Poulenard J. and Taberlet P. (2014). Long livestock farming history and human landscape shaping revealed by lake sediment DNA. Nature Communications. 5:3211. DOI: 10.1038/ncomms4211 |www.nature.com/naturecommunications

Hardham, A. R. (2005). *Phytophthora cinnamomi*. *Molecular Plant Pathology*, *6*(6), 589–604. https://doi.org/10.1111/j.1364-3703.2005.00308.x

Kunadiya, M., White, D., Dunstan, W. A., Hardy, G. E. S. J., Andjic, V., & Burgess, T. I. (2017). Pathways to false-positive diagnoses using molecular genetic detection methods; *Phytophthora cinnamomi* a case study. *FEMS Microbiology Letters*, *364*(7), 1–8. https://doi.org/10.1093/femsle/fnx009

Laforest-Lapointe I., Messier C. and Kembel S. W. (2017). Tree leaf bacterial community structure and diversity along a gradient of urban density. Ecological and Evolutionary Science. 2(6).

Langrell, S. R. H., Morel, O., & Robin, C. (2011). Touchdown nested multiplex PCR detection of *Phytophthora cinnamomi* and P. *cambivora* from French and English chestnut grove soils. *Fungal Biology*, *115*(7), 672–682. https://doi.org/10.1016/j.funbio.2011.04.009

Legeay, J., Husson, C., Boudier, B., Louisanna, E., Baraloto, C., Schimann, H. Buée, M. (2020). Surprising low diversity of the plant pathogen *Phytophthora* in Amazonian forests. *Environmental Microbiology*, *22*(12), 5019–5032. https://doi.org/10.1111/1462-2920.15099

Leray M. and Knowlton N. (2017). Random sampling causes the low reproducibility of rare eukaryotic OTUs in Illumina COI metabarcoding. PeerJ 5:e3006; DOI 10.7717/peerj.3006

Liang M., Liu X., Gilbert G. S., Zheng Y., Lyo S., Huang F. and Yu S. (2016). Adult trees cause density-dependent mortality in conspecific seedlings by regulating the frequency of pathogenic soil fungi. Ecology Letters. 19: 1448-1456.

Li M., Senda M., Komatsu T. Suga H. and Kageyama K. (2010). Development of real-time PCR technique for the estimation of population density of *Pythium* intermedium in forestsoils. MicrobiologicalResearch 165. 695—705

Marchand M., Allery T., Massot M., Capdevielle X. and Robin C. –submitted. Resistance, Tolerance and competence for a root pathogen in six woody species.

Mccarren, K. L. (2005). Saprophytic ability and the contribution of chlamydospores and oospores to survival of *Phytophthora cinnamomi* Saprophytic ability and the contribution of chlamydospores and oospores to the survival of *Phytophthora cinnamomi* by School of Biological Science.

O'Brien, P. A., Williams, N., & Stj Hardy, G. E. (2009). Detecting *Phytophthora*. *Critical Reviews in Microbiology*, *35*(3), 169–181. https://doi.org/10.1080/10408410902831518

Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., … Vacher, C. (2019). Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology*, *41*, 23–33. https://doi.org/10.1016/j.funeco.2019.03.005

Sarker, S. R., McComb, J., Burgess, T. I., & Hardy, G. E. S. J. (2021). Timing and abundance of sporangia production and zoospore release influences the recovery of different *Phytophthora* species by baiting. *Fungal Biology*, *125*(6), 477–484. https://doi.org/10.1016/j.funbio.2021.01.009

Seddaiu S., Brandano A., Ruiu P. A., Sechi C. and Scanu B. (2020). An overview of *Phytophthora* species inhabiting declining Quercus suber stands in Sardinia (Italy). Forests, 11, 971;

Sena, K., Dreaden, T. J., Crocker, E., & Barton, C. (2018). Detection of *Phytophthora cinnamomi* in Forest Soils by PCR on DNA Extracted from Leaf Disc Baits . *Plant Health Progress*, *19*(3), 193–200. https://doi.org/10.1094/php-01-18-0004-rs

Tewoldemedhin Y. T., Mazzola M., Botha W. J., Spies C. F. J. and McLeod A. (2011). Characterization of fungi (Fusarium and Rhizoctonia) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. Eur J Plant Pathol. 130:215–229

Vannini A., Bruni N., Tomassini A. Franceschini S. and Vettrain A. M. (2013). Pyrosequencing of environmental soil samples reveals biodiversity of the *Phytophthora* resident community in chestnut forests. FEMS Microbiol Ecol 85. 433–442

Vettraino, A. M., 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 ONE*, *10*(3), 1–15. https://doi.org/10.1371/journal.pone.0120571

Zeng, H. C., Ho, H. H., & Zheng, F. C. (2009). A survey of *Phytophthora* species on Hainan Island of South China. *Journal of Phytopathology*, *157*(1), 33–39. https://doi.org/10.1111/j.1439-0434.2008.01441.x

Zhao, Y., Xia, Q., Yin, Y., & Wang, Z. (2016). Comparison of Droplet Digital PCR and Quantitative PCR Assays for Quantitative Detection of *Xanthomonas citri* Subsp. *Citri*. *PLoS ONE*, *11*(7), 1–18. https://doi.org/10.1371/journal.pone.0159004

Table S1

Summary of isolates used in the study

Genus	Species	ID	Geographical origin	Sampling year	Host	Source	Identification *
Phytophthora	*cambivora*	Phy85	Switzerland	N. A.	*C. sativa*	Inra, UMR BioGeCo	Multiplex PCR
Phytophthora	*cambivora*	Phy117	Tenereo-Contra, (Switzerland)	2020	*C. sativa*	WSL, Switzerland	M
Phytophthora	*castanea*	1121-NEB	Kourou, Guyane (France)	2015	*Bombacaceae*	INRA, UMR IAM	Ypt1
Phytophthora	*cinnamomi*	Phy16	Dordogne, (France)	2018	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy18	Dordogne (France)	2018	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy24	Montmorency, Val d'Oise (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy31	Montmorency, Val d'Oise (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy20	Montmorency, Val d'Oise (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy33	Montmorency, Val d'Oise (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy35	Montmorency, Val d'Oise (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy38	Haute-Vienne (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy41	Dordogne (France)	2019	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy42	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy43	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy44	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy46	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy47	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy49	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy51	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy52	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy55	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy60	Var (France)	2017	*C. sativa*	Inra, UMR BioGeCo	ITS
Phytophthora	*cinnamomi*	Phy71	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1

Phytophthora	*cinnamomi*	Phy79	Gard (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy93	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy94	Dordogne (France)	2017	*C. sativa*	Inra, UMR BioGeCo	Ypt1
Phytophthora	*cinnamomi*	Phy118	Lorcano-Verigana (Switzerland)	2020	*C. sativa*	WSL, Switzerland	M
Phytophthora	*cinnamomi*	Phy9	Pyrénées-Atlantiques (France)	1988	*Q. rubra*	INRA, UMR BioGeCo	ITS
Phytophthora	*gonapodyides*	AB4	Haut-Rhin (France)	1998	*Quercus sp.*	INRA, UMR IAM	Ypt1
Pythium	*intermedium*	Py. INT 476	N. A.	N. A.	*N. A.*	INRA, UMR SAVE	M
Pythium	*irregulare*	Py. IRR 33	Germany	N. A.	*N. A.*	INRA, UMR SAVE	M
Phytophthora	*megasperma*	MEG1	Charente (France)	2003	*Alnus glutinosa*	ANSES	-
Phytophthora	*nicotianae var nicotianae*	NICN1	N.A.	N.A.	*Skimmia sp.*	INRA, UMR IAM	Ypt1
Phytophthora	*plurivora*	2KF1	Haute-Saône (France)	2000	*Quercus sp.*	INRA, UMR IAM	-
Phytophthora	*pseudosyringae*	RES128	Meurthe-et-Moselle (France)	2014	*Carpinus sp.*	INRA, UMR IAM	Ypt1
Phytophthora	*quercina*	2KP7	Haute-Saône (France)	2000	*Quercus sp.*	INRA, UMR IAM	Ypt1
Phytophthora	*sojae*	SOJ1	N.A.	N.A.	*N.A.*	INRA, Institut Sophia Agrobiotech	Ypt1
Fusarium	*solani*	Fsol L348	N. A.	N. A.	*N.A.*	INRA, UMR SAVE	M
Pythium	*sylvaticum*	Py. Syl 91	Alsace (France)	N. A.	*N.A.*	INRA, UMR SAVE	M
Phytophthora	*syringae*	2KC3	Haute-Saône (France)	2000	*Quercus sp.*	INRA, UMR IAM	-
Phytophthora	*taxon forestsoil*	MEG 5	Haut-Rhin (France)	1998	*Quercus sp.*	INRA, UMR IAM	-
Pythium	*ultimum*	Py. Ult 92	Alsace (France)	N. A.	*N.A.*	INRA, UMR SAVE	M
Phytophthora	*x alni*	PAA115	Moselle (France)	2002	*Alnus glutinosa*	INRA, UMR IAM	-

Phytophthora	*x cambivora*	PcGA1	Haute-Saône (France)	1999	*Quercus sp.*	INRA, UMR IAM	-
Phytophthora	*x multiformis*	PAM392	Deux-Sèvres (France)	2009	*Alnus glutinosa*	INRA, UMR IAM	-

* **M** : Morphological identification, **Ypt1** : sequencing of the related protein Ypt1 DNA fragment, **ITS** : Sequencing of the Internal Transcribed Spacer 1 rDNA fragment, **PCR multiplex** : Identification through touchdown nested multiplex PCR (Langrell *et al.*, 2011) and **« - »** : Identification method not supplied by the provider of the isolate

CHAPTER 2

The distribution of the causal agents of the chestnut ink disease in French chestnut stands

Marylise Marchand[1], Emilie Chancerel[1], Xavier Capdevielle [1] and Cécile Robin[1]

[1]INRAE, University of Bordeaux, BIOGECO, F- 33 610 Cestas, France

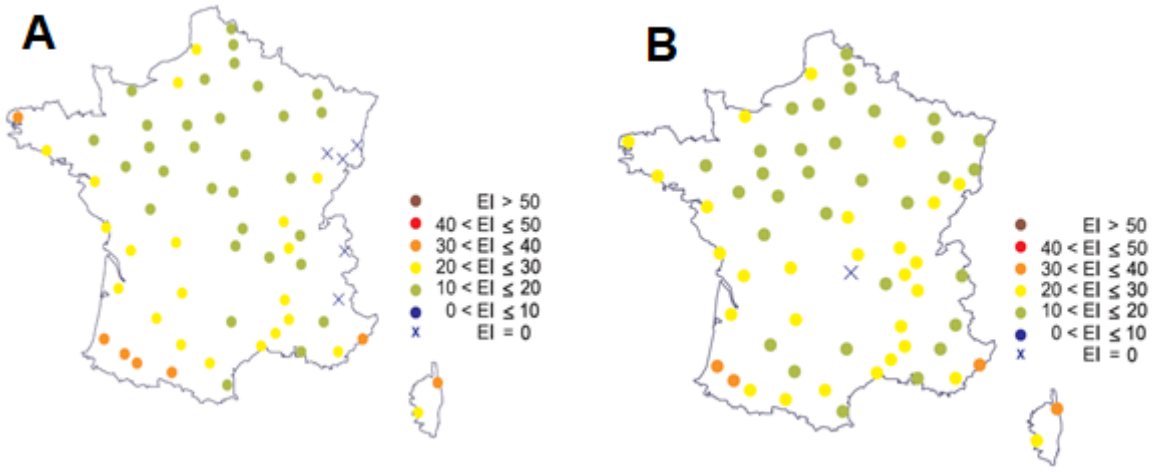
INTRODUCTION

Chestnut ink disease (CID) is caused by invasive pathogens, *P. cinnamomi* and *P. x cambivora*, which have been introduced in Europe during one of the first known tree pandemics. It was reported in France at least in the XIXth century in the Pays-Basque (Barnouin and Sache, 2010). CID has an important impact in chestnut orchards in the XXth century (Grente, 1961; Schad *et al.*, 1952). In forest, since 1989 CID has been steadily reported across the whole territory by the Forest Health Service (DSF for Département de la Santé des Forêts, Robin and Gaudry, 2019). An accurate knowledge of the distribution of *P. cinnamomi* and *P. x cambivora* is required in order to establish relevant preventive and monitoring measures. Above all, it is necessary to forecast accurately CID dynamics in the context of climate change and then to assess the threat to current and future stands (Desprez-Loustau *et al.*, 2007). Causal pathogens of CID have reached the different invasion steps proposed by Blackburn *et al.* in their framework (2011), so they are likely to be widely spread all over chestnut stands in France.

The DSF database is composed of reports from observers who are foresters highly trained in forest pathology and entomology. DSF observers do not systematically send samples for analysis in a laboratory. However, since 2014, they frequently use antibody-antigen LFD devices. Despite the fact that these devices are not specific to *P. cinnamomi* nor *P. x cambivora*, they allow the confirmation that the observed symptoms are likely due to either *P. cinnamomi* or *P. x cambivora*. Since 1989, CID reports have multiplied, and this especially in western France (Figure 12 in Introduction 2.5.). Very few reports were made in south-eastern France, despite old reports of the disease in this part of France and recent records in orchards (C. Robin, personal communication). This discrepancy is certainly stemming from the difficulties in making a field diagnosis of the CID (cf introduction 1.4.). *P. cinnamomi* and *P. x cambivora* are soil-borne pathogens, which infect and progress in chestnut root systems. When they do not kill their hosts, they develop in the cortical tissues of the collar and lower part of the trunk. These bleeding cortical necrosis are the symptoms, which gave name of the disease but are not always observed. However, highly susceptible trees die before expressing aerial oozing symptoms just because of root infections. These primary symptoms result in the wilting and defoliation of trees, which may be more or less severe and frequent depending on climatic conditions and the causal *Phytophthora* species involved. Finally, the infection may lead to the tree death. The physiological response to a *Phytophthora* infection is similar to that caused by extreme climatic events such as summer drought or/and spring or fall waterlogging. Indeed, the chestnut tree is a rather demanding tree species in term of climatic factors in order to flourish

properly, *e.g.* requires a minimum 700 mm/year and maximum 1500 mm/year rainfall (Freitas *et al.*, 2021). The DSF also monitors the foliar deficit (or defoliation rate) of tree every year on a systematic network (Bilan 2020 du Réseau systématique de suivi des dommages forestiers). Interestingly, the defoliation rate accentuated in the Southeast region over the ten past years (cf Figure 12 in Introduction 2.5). However, the defoliation rate might not be directly related to the active inoculum of the causal agents of the CID since resilient trees may recover from decline (Whyte *et al.*, 2016).

Another reason why CID distribution may be underestimated in the DSF database is that bleeding cankers are inconspicuous, sometimes are healed and recovered by callusing tissues. Moreover, survival of *P. cinnamomi* in trunk cortical tissues is known to be highly impaired on temperature below 0°C (Benson, 1982) which favours the healing of cortical lesions by trees (Marçais *et al.*, 2004). Thus, cold winters may result in the mitigation of ink diseases symptoms in collars and trunk of chestnut trees, rendering more difficult to detect the presence of *P. cinnamomi* in the field. A simulation of potential geographical range of *P. cinnamomi* were carried through the assessment of an ecoclimatic index (EI) (Desprez-Loustau *et al.*, 2007). The higher this index is, the higher the survival probability of the pathogen, and thus the higher the probability of observing symptoms. This index was calculated for survival in roots for CID and in trunks for *Quercus rubra* disease. Results showed the potential distribution area of *P. cinnamomi* under the average climate of the years 1961-1990 (Figure 1A) and under the average climate of the years 2070-2099 modelled according to the IPCC B2 scenario (Figure 1B). This IPCC scenario is a moderate climate change scenario that predicts an important increase in the CO_2 concentration with a slow population growth (IPCC, 2001). Root chestnut EI indices were inferior to trunk ones, and predicted ranges for CID, under actual or future climate, were wider than reported distribution map.



Under average climate of the years 1961-90 Under average climate of the years 2070-2099

Figure 1: Predicted *P. cinnamomi* range estimated by CLIMEX model for root rot (Desprez-Loustau *et al.*, 2007)

The first objective of the study was to i) improve our knowledge of *P. cinnamomi* and *P. x cambivora* distribution in France. To do so, we used the DSF systematic network to detect and quantify the pathogens in the different regions. The second aim of the objective was to ii) decipher if there was a link between the increased defoliation rate and the pathogen presence. Therefore, we explored the link between the climate, the pathogen presence and the defoliation rate.

MATERIAL AND METHODS

Soil sampling, biological baiting and DNA extraction

Soil was sampled in 29 plots of the systematic network, in which at least two chestnut trees were scored during the 2018-2021, from June to November. The plots were classed in three geographical regions: North-west (eight plots), South-east (seven plots) and South-west (14 plots) (Figure 2).

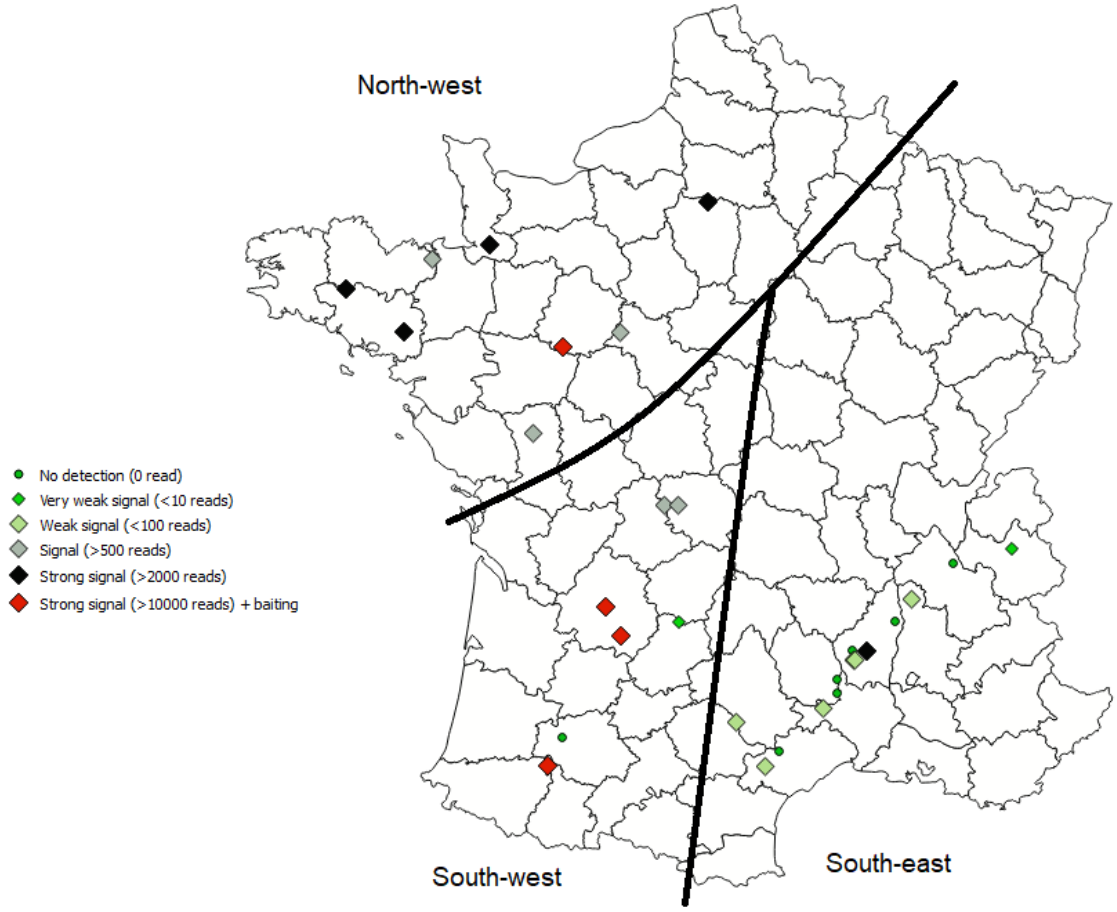


Figure 2: Systematic network sites investigated for *P. cinnamomi* presence through both biological baiting and metabarcoding

At each sampling site, the three (or five in two plots) chestnuts trees showing the most important defoliation symptoms were surveyed. The soil of these trees was collected at the four cardinal points around the tree at a depth of 25 cm and mixed together. An aliquot of 50mL soil was placed into a 10x8x4cm container and were pre-wetted by adding 50mL of osmosed water for the night. The next day, 100mL osmosed water was added, all excess organic matter is removed from the surface of the water, and five oak (*Quercus robur*) leaf discs (d=1 cm) were placed and incubated for 3 days at 22-23°C in the light. The leaf discs were then placed onto a PARBHy selective media (malt 1.5%, agar 1.8%, pimaricin 10 ppm, ampicillin 250 ppm, rifampicin 10 ppm, benomyl 15 ppm, hymexazol 50 ppm). After two days, a culture implant containing mycelium was plated on Malt Agar (MA) (1.5% malt, 1.8% agar). Identification was achieved by microscopic observation.

Thereafter, the DNA from 0.2g of the 90 soils was extracted using the FASTDNA ™ SPIN kit for soil (MP Biomedicals, Inc, Eschwege, Germany) in two replicates that were then pooled together. Four water samples were used as negative controls for each round of DNA extraction.

Phytophthora library construction, sequencing and bioinformatics analysis

The *Phytophthora* community in the soil samples and the positive (*P. cinnamomi* mycelial DNA at 0.1 ng/µL) and negative controls (four extraction controls and one water metabarcoding control) were prepared according to the protocol presented in Chapter 1. The difference is that when building the library, we did not purified the amplicons between the first round PCR and the second round PCR but diluted them to the fiftieth.

The analysis of the *Phytophthora* community was performed with the bioinformatic pipeline described in Chapter 1.

Analysis of *P. cinnamomi* distribution in relation with climate

To collect the climate data on our plots, we used the SAFRAN (Système d'Analyse Fournissant des Renseignements atmosphériques à la Neige) available at https://donneespubliques.meteofrance.fr/?fond=produit&id_produit=122&id_rubrique=40. It is a dataset producing an analysis at the hourly step time using ground data observations that cover the French territory with a spatial resolution of 8km since 1958. We extracted the climatic data from the projected point at our plot coordinates.

Statistical analyses

Statistical analyses were performed using the R Studio interface of the R software (version 3.6.3) and lme4 (Bates *et al.*, 2015), MuMIn (Barton, 2018) multcomp (Hothorn *et al.*, 2008), ggplot2 (Wickham, 2016), lmerTest (Kuznetsova *et al.*, 2017), plyr (Wickam, 2011), dplyr (Wickam *et al.*, 2018), MASS (Venables and Riplay, 2002), ggpubr (Wickham, 2016) and Hmisc packages (Harrell *et al.*, 2008). Assumptions of the normality of distribution and homoscedasticity of the variables were not satisfied therefore non-parametric tests were used in alternative (Kruskal-Wallis and Dunn tests).

To analyse the climatic data, we used the means daily temperatures and precipitations over the 1989 and 2020 period of the plots based on the SAFRAN data. They were classified into four seasons : winter (December-February), spring (March-May), summer (June-August) and fall

(September-November). We performed with generalized linear models (GLM) following the procedure recommended by Zuur *et al.* (2009) testing the influence of plots average overall precipitations (mm/year), average temperatures (°C) and plot composition (chestnut frequency) on the presence/absence of *P. cinnamomi* in the different plots (binomial family and logit function). We used a first linear model (LM) to test the influence of the same parameters on the average defoliation rate of chestnut trees (tdefo) at the plot level. We used a second LM to test the influence of seasonal precipitations on the tdefo. For all models, significance of parameters was assessed using χ^2 tests by comparing models with and without the term to be tested. To estimate model fit, R^2 were calculated for GLM and LM models.

RESULTS

The metabarcoding approach

MiSeq sequencing of the *Phytophthora* amplicons from the 90 environmental samples and six controls produced 3 948 402 reads. From these, one environmental sample did not pass the filter which resulted in 977 639 reads were grouped into 2 484 ASVs (Figure 3). After blast against the homemade database, 2 396 sequences in 353 722 reads gave no hit and no additional blast results were found against the GenBank collection (Figure 3).

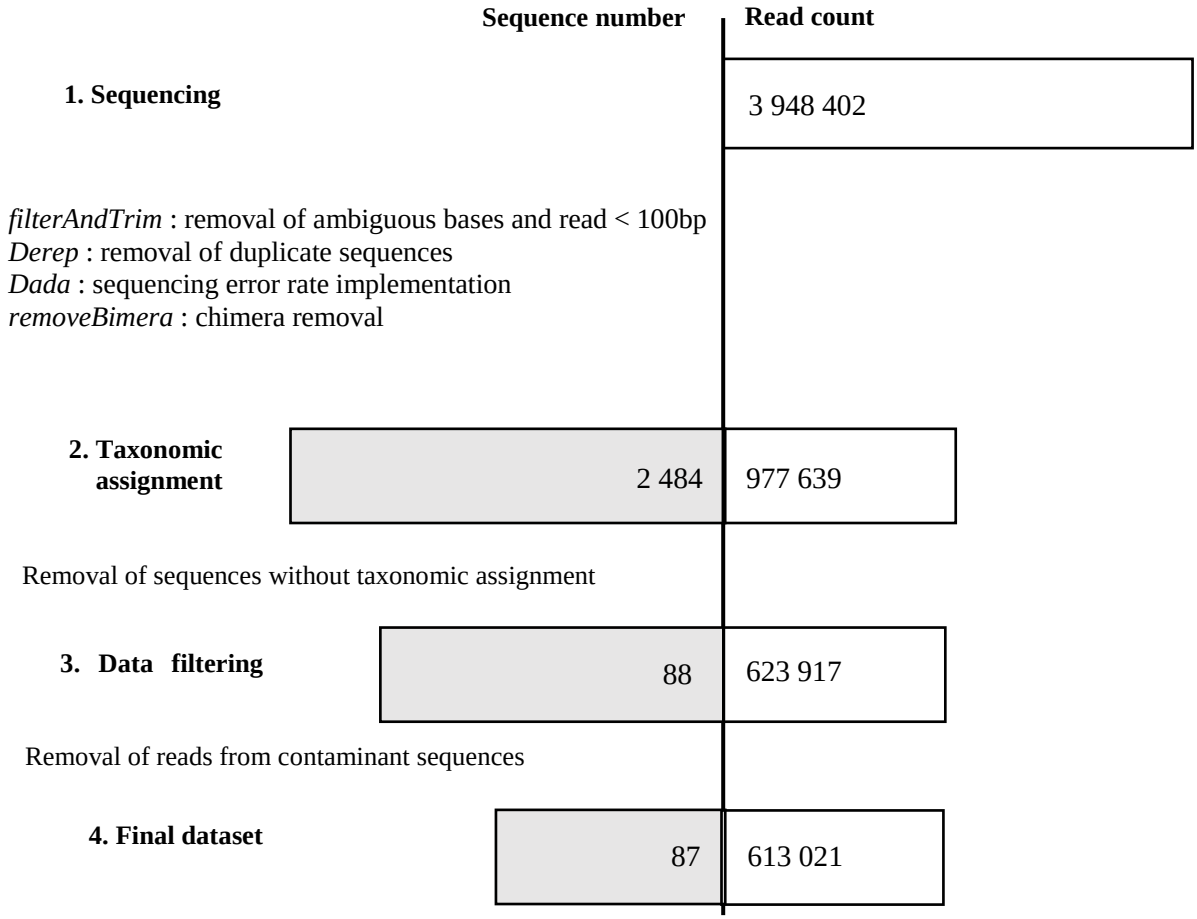


Figure 3: *Phytophthora* metabarcoding pipeline

We removed the contaminant sequence present solely in the positive control (Figure 4). We removed the read/ASV that were present in negative controls from the soil samples as done in the Chapter 1. We obtained a final dataset of 87 ASVs in 613 021 reads. The taxa found in the soil samples are presented Table 1.

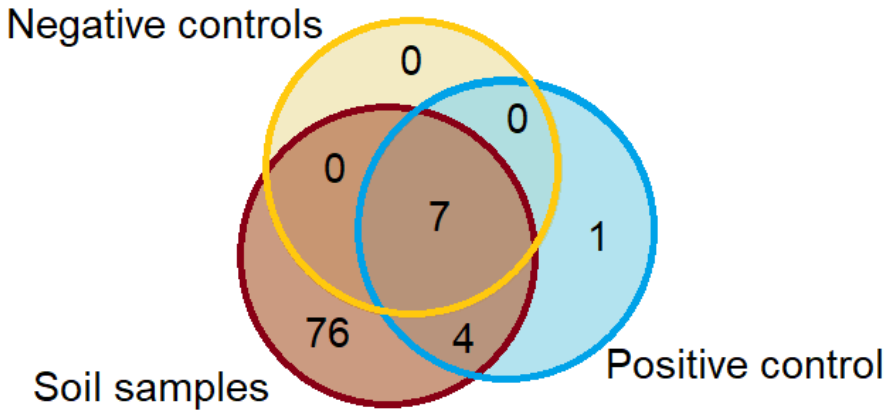


Figure 4: Number of ASV present in the positive control (blue), negative controls (yellow) and soil samples (brown)

Table 1: Taxa to which ASV detected in soil samples were assigned, with their respective ASV number, read count and number of soil samples in which they were observed

Taxa	ASV number	Read count	Number of positive soil samples
P. cactorum	4	2 081	1
P. castanea	1	21	1
P. cinnamomi	61	536 505	49
P. sojae	19	41 928	2
Pythium aphanidermatum	1	19	1
Uncultured Pythium	1	28	1
Total	87	580 582	

P. cinnamomi distribution in France in chestnut stands

Through biological baiting of soils, we detected *P. cinnamomi* in four plots in the west of France (Figure 2, Table 2). *P. cinnamomi* was the only species ever isolated through biological baiting. After DNA analysis and read count correction, plots were classified according to the number of cumulated *P. cinnamomi* reads that were found (Table 2, Figure 2).

Table 2: *Phytophthora* detection in soil in the three studied regions in France, through soil DNA analysis and biological baiting

Metabarcoding signal classified according to the number of *P. cinnamomi* reads detected	**North-west**	**South-west**	**South-east**
No detection (0 read)	-	1	6
Very weak signal (<10 reads)	-	1	1
Weak signal (<100 reads)	-	-	6
Signal (>500 reads)	3	2	-
Strong signal (>2000 reads)	4	-	1
Strong signal (>10000 reads) + baiting	1	3	-

As such, *P. cinnamomi* was detected in all the regions investigated (Figure 2, Table 2). In the north-west region, the eight plots were positive with signals over 500 reads. In the south-west, five plots were positive to *P. cinnamomi* with a signal > 500 reads, one was negative and one displayed a very weak signal. Finally, in the southeast, only one plot displayed a strong signal to *P. cinnamomi*, six plots were negative to the pathogen and the remaining seven displayed a very weak to weak signal (Figure 2). For the following analyses, we only considered as positive the plots the one with a signal superior to 10 *P. cinnamomi* reads as a precaution.

Chestnut frequency in monitored plots was evaluated by the ratio of the number of chestnut trees on the total number of trees observed (20 trees in most of the plots). This frequency did not vary with the region (Kruskal-Wallis chi-squared = 1.9914, df = 2, p-value = 0.3695). The average frequency of chestnut trees in positive plots was 74% and not significantly different to the frequency in negative sites (Table 3). Neither the overall precipitations nor the average temperatures were significant in explaining the presence of *P. cinnamomi* (Table 3).

Table 3: summary of generalized linear model (binomial distribution) explaining variations in presence/absence of *P. cinnamomi* in the plots according to the plot tree composition and average temperature (AT) and overall precipitations (OP) (The model fit R^2 was 0.27)

Predictors	**Estimate**	**Standard error**	**z value**	**Pr(>|z|)**
Chestnut frequency	0.014548	0.016531	0.880	0.379
AT (°C)	0.492523	0.369805	1.332	0.183
OP (mm)	-0.002661	0.001881	-1.415	0.157

Defoliation rates of chestnut trees monitored in the systematic network

In all plots, the average defoliation was increasing, with the highest rate observed in the south-eastern region (Figure 5).

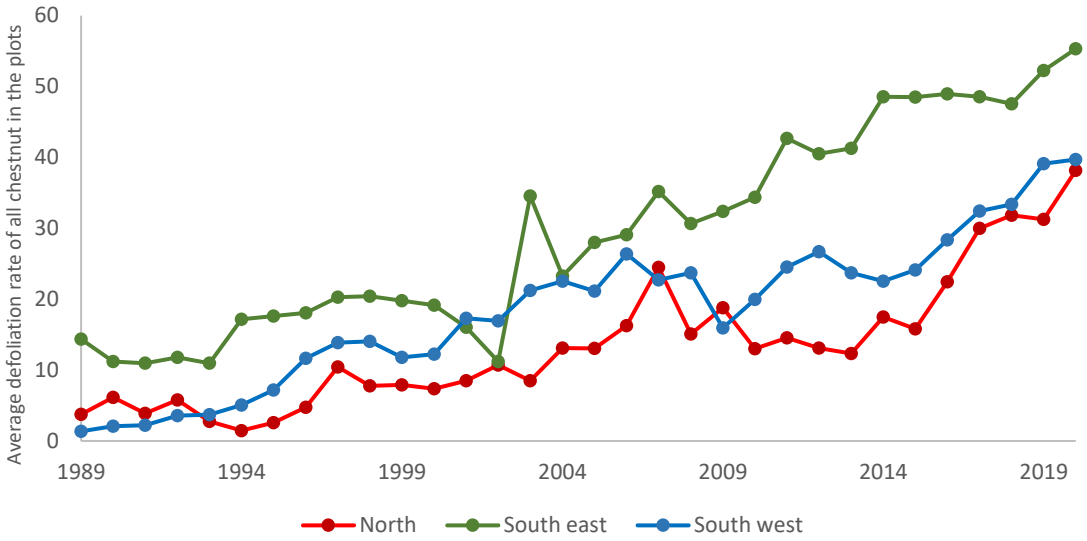


Figure 5: Evolution of the average defoliation rate of chestnut trees since 1989

Chestnut sites in the western part of France displayed a lower defoliation rate than sites in the south-eastern part of France (Figure 6). The average annual rainfall was superior to 700 mm for almost all plots (Figure 6), which is considered as the minimal annual rainfall for chestnut trees. In contrast, in plots with the higher defoliation rate (higher than 30%), the average annual rainfall was superior to 900 mm (Figure 6), which is considered as the optimum for chestnut trees.

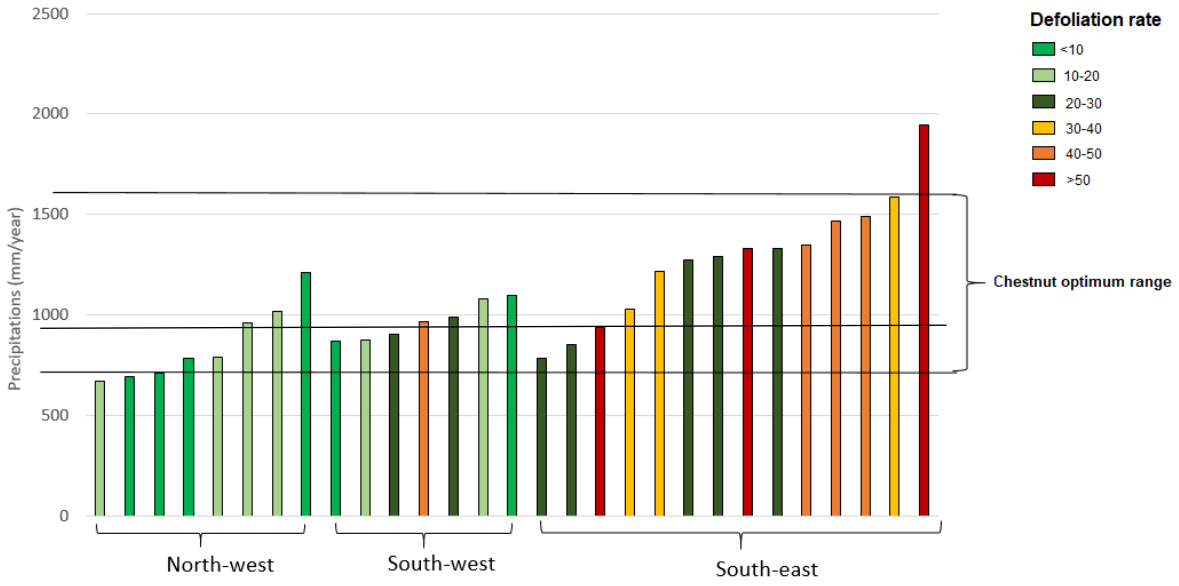


Figure 6: Average defoliation rate of chestnut trees according to the rainfall regimens in plots

Modelling showed that the overall precipitations significantly explained variations in average defoliation rate of chestnut trees in plots (Table 4). More specifically, a second LMM showed that winter rainfall regimens was the significant parameter explaining the variations in defoliation rate (Table 4).

Table 4: Summary of linear models (LM) used to test the influence the of average temperatures (AT) and overall precipitations (OP) and season precipitations (FP : Fall, WP : Winter, SuP: summer and Sp : Spring precipitations) on the average defoliation rate (tdefo) of chestnut trees in plots. In bold are the significant *p*-values. The R^2 correspond to the fit of the model.

Model expression	Parameters tested	Estimate	SE	z value	Pr(>|z|)
LM : tdefo ~AT + OP (R^2=0.22)	AT (°C)	3.01822	1.94479	1.552	0.1328
	OP (mm)	0.02658	0.01018	2.611	**0.0148**
LM : tdefo ~ FP + WP + SuP + SpP (R^2=0.43)	FP (mm)	0.03479	0.02717	1.281	0.21259
	WP (mm)	-0.14307	0.04948	-2.892	**0.00801**
	SuP(mm)	-0.08910	0.04761	-1.871	0.07353
	SpP (mm)	0.13527	0.08860	1.527	0.13990

Plots with an average defoliation rate (30-40%) were subjected to the highest winter precipitations over the years (Figure 7). Otherwise, the plots were subjected to similar winter precipitations variations no matter the average defoliation rate of chestnut trees.

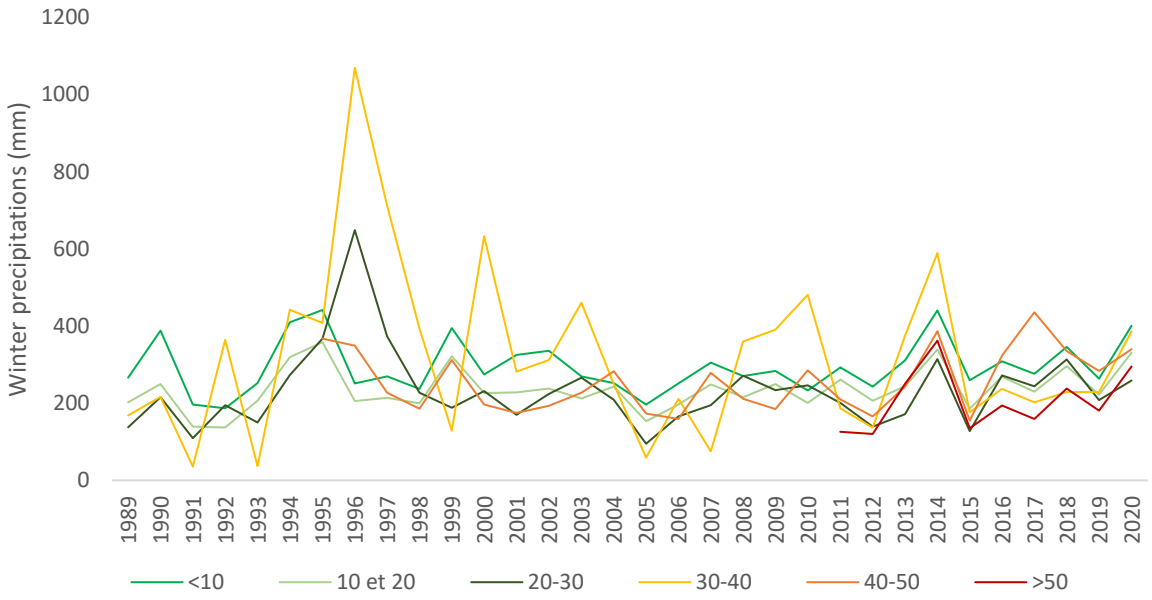


Figure 7: Evolution of winter precipitations in plots (mm) according to the intensity of average defoliation rate (%).

Chestnut trees under which a high pressure of *P. cinnamomi* inoculum was detected (more than 10k *P. cinnamomi* reads) displayed an abrupt increase in defoliation rate in 2015 (Figure 8).

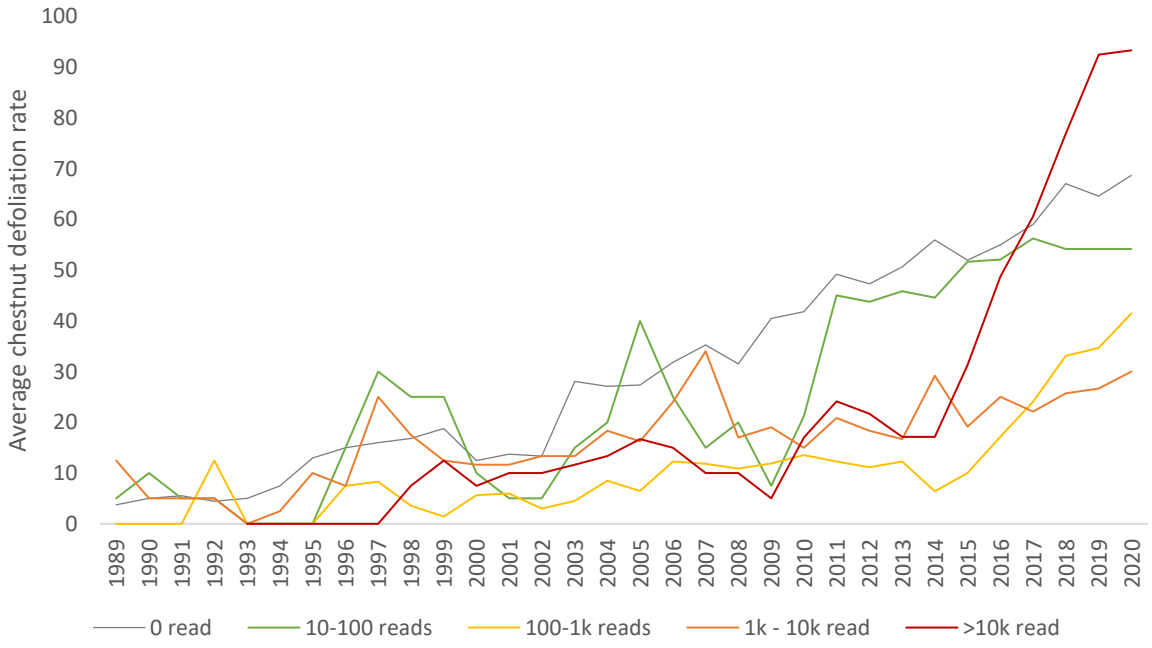


Figure 8: Evolution of the foliar deficit of chestnut trees according to the *P. cinnamomi* read abundance found in their rhizosphere

The other chestnut trees sampled within the same plots displayed a similar increase in defoliation in 2015 but to a lesser degree as shown for one plot (Figure 9). A similar tendency was found for the other plots with chestnut trees with high *P. cinnamomi* inoculum (data not shown). These plots experienced a sharp increase in winter precipitations during this period (Figure 7).

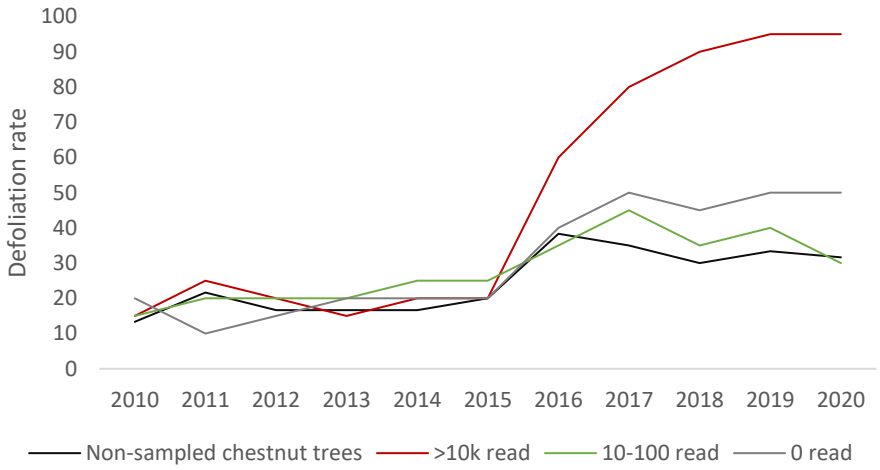


Figure 9: Defoliation rate of chestnut trees under which *P. cinnamomi* was found in high inoculum (10k read) compared to the defoliation rate of other trees within the same plot (Plot n°1570 located in the south-western region)

The defoliation rate of chestnut trees under which *P. cinnamomi* was not detected increased steadily over the years (Figure 8). Interestingly, these chestnut trees displayed a higher defoliation rate that chestnut trees under which *P. cinnamomi* was found in strong (1k-10k read) and medium signal (100-1k read) signal (Figure 8).

DISCUSSION

Our results confirmed that *P. cinnamomi* is widespread in French chestnut forest stands, more than what could be estimated from ink disease reports available in the DSF database, and this especially in the southeastern region. In half of the studied plots in south-eastern France, a *P. cinnamomi* DNA signal was detected in soil (six weak and one strong). This is consistent with ancient reports of the pathogen in the region (Grente, 1961) and some more recent and obtained by isolations from soil (Vettraino *et al.*, 2005 and C. Robin personal communication). This is also consistent with simulations of geographical range of *P. cinnamomi* under different climates (Desprez-Loustau *et al.*, 2007), which suggested that climatic conditions were favorable for survival of *P. cinnamomi* in roots. Moreover, 20 plots among the 29 studied proved to be positive for *P. cinnamomi*, whatever the average defoliation rate. The northwestern region has been the object of an overwhelming number of reports and this, ever since the beginning of the

monitoring of the chestnut trees health by the DSF agents. In our survey, 100% and 86% of plots proved to be positive for *P. cinnamomi* in the northern and southern region respectively. This is consistent with an ancient introduction in France of the invasive species, which has spread over the years. We may be witnessing a reemergence of the disease. Indeed, the pathogen stayed undetected most likely because of the low level of root infection it caused partly due to the tree ability to regenerate and partly due to unfavorable climatic conditions. The pathogen may cause disease outbreaks in some sites that could be in relation with climatic events, which could favor the pathogen multiplication and/or increase tree vulnerability (Maurel *et al.*, 2001). Nurseries has been identified as the prime mean of inoculum transportation (Jung *et al.*, 2016) and the wide spread of *P. cinnamomi* may be the result of human activities, but the hypothesis of dispersal by feral animals cannot be discarded without further considerations (Li *et al.*, 2014).

Once introduced in a new site, the establishment of *P. cinnamomi* is dependent upon local conditions and the host availability and susceptibility (Prospero and Cleary, 2017). Interestingly, we did not observe any effect of the plots tree composition on *P. cinnamomi* detection, suggesting that there were no lessening effect provided by the mixture of chestnut trees with other species. However, since the sampling was not designed to answer this question, this has yet to be confirmed (but see Chapter 3).

When considering environmental conditions, plots with *P. cinnamomi* DNA signal were not characterized by different climatic indicators. The soil having a high buffering capacity to temperatures, the low temperatures may not be a relevant parameter for the pathogen survival. Because high levels of soil moisture are essential for the germination and dispersal of *P. cinnamomi* spores, like most pathogenic soil-borne organisms necessitate (Lacey and Harper, 1986; Desprez-Loustau *et al.*, 2006), we expected for high precipitations to be correlated to the strength of *P. cinnamomi* signals and found no such effect. Soil compaction and pH are notably recognized as determinant in *P. cinnamomi* distribution (Rhoades *et al.*, 2003; Hernandes-Lambraño *et al.*, 2018), two parameters we did not assess.

The average defoliation rate of chestnut trees with high inoculum pressure showed a more important canopy damage than negative trees, but this only after heavy winter rain events. As a rule, we did not find a straightforward relationship between pathogen presence and intensity of defoliation, which confirms previous studies on the matter (Ruiz-Gomez *et al.*, 2019; Corcobado *et al.*, 2014). A chestnut dieback-monitoring network in Dordogne (Nouvelle

Aquitaine, France) confirmed that *P. cinnamomi* could be found in plots where decline was low or non-existent (C. Robin, personal communication). Detecting *P. cinnamomi* in chestnut forest sites without any sign of chestnut ink disease was already reported, for example in England (Vettraino *et al.*, 2005). The decline and recovery model proposed by Whyte *et al.* (2016) could also explain this result (see Introduction section 1.4.). Trees with the highest inoculum and the lowest defoliation rate could be in the first stage of the decline, as the presence of *P. cinnamomi* is initiating the decline. On the other hand, trees with the lowest inoculum and the highest defoliation rate could be in the latter stages of decline, as the presence of *P. cinnamomi* was more ancient and the defoliation rate was increased by climatic events. Here, we showed that winter precipitations were incremental in explaining the defoliation rate of chestnut trees, especially in the southeast. In this region, plots were subjected to high precipitations that could suggest waterlogging. However, soil moisture conditions are the result of the interactions between precipitations and edaphic factors such as soil depth, compaction and drainage, which are parameters we did not assess. High precipitations in fine textured soils do not have the same impact on the available water than high precipitations in coarse textured soils.

Because of the weakness of the DNA signal detected in some plots, resampling and additional isolation attempts must be carried out in all plots. Indeed, the metabarcoding method being DNA-based, it does not detect an ongoing ink infection contrary to the biological baiting. To confirm our findings, we emphasis the need to reiterate the sampling at different dates (we sampled some plots during the winter season, possibly at times where the inoculum was at its lowest). However, soil DNA analysis by metabarcoding revealed as an adequate method for the detection of *P. cinnamomi* in various soil samples (see Chapter 1). *P. cinnamomi* read abundances obtained after close analysis of negative and positive controls, provided DNA signals, which appeared consistent with *P. cinnamomi* DNA content (Chapter 1). This DNA signal is a fair proxy for the pathogen inoculum potential in soil samples.

Surprisingly, we detected *P. sojae* in our soil samples, which is soil-borne species mostly associated with soybeans. This could be explained by a mismatch of taxonomic assignment through BLAST. Some *Phytophthora* species display little variation across the ITS region used for identification, possibly explaining ambiguous species assignment (Yang and Hong, 2018). However, no other species belonging to the *Phytophthora* subclade as this species (subclade 7b, Yang *et al.*, 2017) are likely to be present in natural soil of chestnut groves in France. On the other hand, *P. sojae* subclade is relatively close to *P. cinnamomi* subclade (7c), *P. x*

cambivora and *P. x alni* (7a) (Yang *et al.*, 2017), three taxa that could be conceivably found in such settings. Most significantly, the metabarcoding approach revealed a lack of *Phytophthora* diversity. A result that was confirmed by the biological baiting as we only ever recovered *P. cinnamomi* through both methods despite that several other *Phytophthora* species are recognized to be associated in chestnut ink disease, among which *P. x cambivora* (Vettraino *et al.*, 2005). The lack of saprophytic species like *P. gonapodyides* might suggest that our sampling was inadequate to encompass the whole diversity of *Phytophthora* species. However, it remains difficult to assess whether the species were absent or present in a quantity undetectable with our methods.

The sweet chestnut tree has been identified as a tree species that is not as future-proof as previously estimated (Conadera *et al.*, 2021). The models highlighted a high sensitivity of the chestnut to water stresses as a combination of lack of precipitation and high temperatures during vegetation period, which are tendencies that climate change models seem to agree on. The complex interplays between climatic conditions, tree health and *P. cinnamomi* presence are yet to be understood, especially considering that the biotic environment of the trees, *i.e.* associated microbiota, might be involved in the tree's health (Brader *et al.*, 2017). Finally, in the present study we solely focused on the infection of *P. cinnamomi* on chestnut trees, but the pathogen is known to infect may infect over 5 000 different hosts (Barwell *et al.*, 2020; Hardham, 2005), among which oak species present in France, *e.g. Q. ilex, Q. suber* and *Q. rubra.* These specific hosts do not display a similar reaction to the pathogen as chestnut trees (Marchand *et al.*, submitted) and could contribute differentially to the overall spread dynamics of the pathogen on the territory.

REFERENCES

Barnouin J. and Sache I. (2010). Les maladies emergentes: Epidemiologie chez le végétal, l'animal et l'homme. Edition QUae. Collection Synthèses.

Barton, K. 2018. MuMIn: Multi-Model Inference. R Package Version 1.42.1.https://cran.r-project.org/web/packages/MuMIn/index.html

Barwell, LJ, Perez-Sierra, A, Henricot, B, *et al.* (2021). Evolutionary trait-based approaches for predicting future global impacts of plant pathogens in the genus *Phytophthora*. J Appl Ecol. 58: 718–730. https://doi.org/10.1111/1365-2664.13820

Bates, D., Machler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1-48

Benson D. M. (1982). Cold inactivation of *Phytophthora cinnamomi*. Phytopathology. 72:650-653.

Blackburn T. M., Pysek P., Bacher S., Carlton J. T., Duncan R. P., Jarosik V., Wilson J. R. U. and Richardson D. M. (2011). A proposed unified framework for biological invasions. Trends in Ecology and Evolution. 26(7).

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L. J., & Sessitsch, A. (2017). Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology*, *55*, 61–83. https://doi.org/10.1146/annurev-phyto-080516-035641

Conedera, M., Krebs, P., Gehring, E., Wunder, J., Hülsmann, L., Abegg, M., & Maringer, J. (2021). How future-proof is Sweet chestnut (Castanea sativa) in a global change context? *Forest Ecology and Management*, *494*(May), 119320. https://doi.org/10.1016/j.foreco.2021.119320

Corcobado, T., Vivas, M., Moreno, G., & Solla, A. (2014). Ectomycorrhizal symbiosis in declining and non-declining Quercus ilex trees infected with or free of *Phytophthora cinnamomi*. *Forest Ecology and Management*, *324*, 72–80. https://doi.org/10.1016/j.foreco.2014.03.040

Desprez-Loustau, M.L., Robin C., Reynaud G., Déqué M., Badeau V., Piou D. Husson C. and Marçais B. (2007). Stimulating the effects of climate-change scenario on the geographical range and activity of forest-pathogenic fungi. Canadian Journal of Plant Pathology, Taylor & Francis, 29 (2), pp.101-120.

Desprez-Loustau, M.L., Marcais, B., Nageleisen, L.M., Piou, D., Vannini, A., 2006. Interactive effects of drought and pathogens in forest trees. Ann. For. Sci. 63,597–612. https://doi.org/10.1051/forest:2006040.

Freitas T. R., Santo J. A., Silva A. P. and Fraga H. (2021). Influence of climate change on chestnut trees: a review. Plants. 10, 1463.https://doi.org/10.3390/plants10071463

Grente, J., (1961). La maladie de l'encre du Châtaignier. *Annale Epiphyte*. 1961. 5-59.

Hansen, E., & Delatour, C. (1999). *Phytophthora* species in oak forests of north-east France. *Annals of Forest Science*, *56*(7), 539–547. https://doi.org/10.1051/forest:19990702

Hardham, A. R. (2005). *Phytophthora cinnamomi*. *Molecular Plant Pathology*, *6*(6), 589–604. https://doi.org/10.1111/j.1364-3703.2005.00308.x

Harrell Jr. F.E., Dupont C., *et al.* (2008) ''Hmisc: Harrell miscellaneous.'' R. package version 3.2.

Hernández-Lambraño, R. E., González-Moreno, P., & Sánchez-Agudo, J. Á. (2018). Environmental factors associated with the spatial distribution of invasive plant pathogens in the Iberian Peninsula: The case of *Phytophthora cinnamomi* Rands. *Forest Ecology and Management*, *419–420*(March), 101–109. https://doi.org/10.1016/j.foreco.2018.03.026

Horton, T. R., & Bruns, T. D. (2001). The molecular revolution in ectomycorrhizal ecology: Peeking into the black-box. *Molecular Ecology*, *10*(8), 1855–1871. https://doi.org/10.1046/j.0962-1083.2001.01333.x

IPPC (2001). Climate change 2001. The scientific basis. Contribution of Working group I to the third assessment of the IPCC. Eds Houghton JT., Ding Y., Griggs D. J. et al. *CambridgeUniversity press*, Cambridge UK.

Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A. G., Aguín Casal, O., Peréz-Sierra, A. (2016). Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of *Phytophthora* diseases. *Forest Pathology*, *46*(2), 134–163. https://doi.org/10.1111/efp.12239

Kuznetsova A., Brockhoff P.B., Christensen R.H.B. (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software*, **82**(13), 1–26. doi: 10.18637/jss.v082.i13

Lacey, L.A., Harper, J.D., 1986. Microbial control and integrated pest management. J. Entomol. Sci. 21, 206–213. https://doi.org/10.18474/0749-8004-21.3.206

Li A. Y., Williams N. Fenwick S. G., Hardy G. E. St. J., and Adams P. J. (2014). Potential for dissemination of *Phytophthora cinnamomi* by feral pigs via ingestion of infected plant material. Biol Invasions. 16:765–774

Marcais B., Bergot M., Pérarnaud V., Levy A. and Desprez-Loustau M.L. (2004). Prediction and mapping of the impact of winter temperature on the development of *Phytophthora cinnamomi*-induced cankers on red and pedunculateoak in France. Phytopathology 94:826-831.

Maurel M., Robin C. Capdevielle X., Loustau D. and Desprez-Loustau M.L. (2001). Effects of variable root damage caused by *Phytophthora cinnamomi* on water relations of chestnut saplings. Annals of Forest Science, Springer Verlag/EDP Sciences, 58 (6), pp.639-651.

Prospero, S., & Cleary, M. (2017). Effects of host variability on the spread of invasive forest diseases. *Forests*, *8*(3), 1–21. https://doi.org/10.3390/f8030080

Rhoades, C. C., Brosi, S. L., Dattilo, A. J., & Vincelli, P. (2003). Effect of soil compaction and moisture on incidence of *Phytophthora* root rot on American chestnut (Castanea dentata) seedlings. *Forest Ecology and Management*, *184*(1–3), 47–54. https://doi.org/10.1016/S0378-1127(03)00147-6

Robin C. and Gaudry J. (2019). La maladie de l'encre complexifie la sylviculture du châtaignier. Forêt-entreprise - N° 247

Ruiz Gómez, F. J., Navarro-Cerrillo, R. M., Pérez-de-Luque, A., Oβwald, W., Vannini, A., & Morales-Rodríguez, C. (2019). Assessment of functional and structural changes of soil fungal and oomycete communities in holm oak declined dehesas through metabarcoding analysis. *Scientific Reports*, *9*(1), 1–16. https://doi.org/10.1038/s41598-019-41804-y

Schad C., Solignat G., Grente J., Venot P. (1952). Recherches sur le châtaignier à la station de Brive. Annales de l'amélioration des plantes III, 376–458

Venables W.N., Ripley B.D. (2002). *Modern Applied Statistics with S*, Fourth edition. Springer, New York. ISBN 0-387-95457-0, https://www.stats.ox.ac.uk/pub/MASS4/

Vettraino, A. M., Morel, O., Perlerou, C., Robin, C., Diamandis, S., & Vannini, A. (2005). Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with Ink Disease and crown decline. *European Journal of Plant Pathology*, *111*(2), 169–180. https://doi.org/10.1007/s10658-004-1882-0

Whyte, G., Howard, K., Hardy, G. E. S. J., & Burgess, T. I. (2016). The Tree Decline Recovery Seesaw; a conceptual model of the decline and recovery of drought stressed plantation trees. *Forest Ecology and Management*, *370*, 102–113. https://doi.org/10.1016/j.foreco.2016.03.041

Wickham, H. François, R. Henry, L. and Müller K. (2018). dplyr: A Grammar of Data Manipulation. R package version 0.7.6. https://CRAN.R-project.org/package=dplyr

Wickham H. (2011). "The Split-Apply-Combine Strategy for Data Analysis." *Journal of Statistical Software*, 40(1), 1–29. http://www.jstatsoft.org/v40/i01/

Wickham H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org

Wickham, H. (2020). Modelr : modelling functions that work with the pipe. R package version 0.1.8. https://CRAN.R-project.org/package=modelr

Yang, X., Tyler, B. M., & Hong, C. (2017). An expanded phylogeny for the genus *Phytophthora* . *IMA Fungus*, *8*(2), 355–384. https://doi.org/10.5598/imafungus.2017.08.02.09

Yang X. and Hong C. (2018*).* Differential Usefulness of Nine Commonly Used Genetic Markers for Identifying *Phytophthora* Species. *Front. Microbiol. 9:2334. doi: 10.3389/fmicb.2018.02334*

Zuur AF, Ieno EN, Walker N et al (2009) Mixed effects models and extensions in ecology with R. Springer, New York

CHAPTER 3

Evidence for the dilution effect in the chestnut ink disease pathosystem

Marylise Marchand[1], Emilie Chancerel[1], Xavier Capdevielle [1] and Cécile Robin[1]

[1]INRAE, University of Bordeaux, BIOGECO, F- 33 610 Cestas, France

INTRODUCTION

Host diversity and susceptibility have been identified as driving factors of invasive forest diseases (Prospero and Cleary, 2017). However, the impact of host diversity on epidemiological dynamics of pathogens is highly dependent upon the pathosystem studied. In some cases, a high host diversity may decrease the disease risk, an effect that is called the dilution effect (Keesing *et al.*, 2006). The dilution effect is expected for pathogens with a direct transmission rate dependent on the frequency of infected hosts (Cortez and Duffy, 2020). On the opposite side, an amplification effect may be observed in some ecosystems with less susceptible species that may act as reservoir hosts (Keesing *et al.*, 2006). There are evidences of both patterns existing for *Phytophthora* driven diseases in forest settings (Jules *et al.*, 2002; Haas *et al.*, 2011; Davidson *et al.*, 2008; Cardillo *et al.*, 2021). In the case of the chestnut ink disease (CID), caused by *P. cinnamomi* and/or *P. x cambivora*, no strong evidence has been provided for either type of effect. The infection by CID pathogens induces root rots, causing crown wilting and defoliation resulting in a gradual decline of the host health (Vannini and Vettraino, 2001). Root lesions may also spread to the trunk, causing then typical ink diseases symptoms, but these are not so frequent, especially after a *P. cinnamomi* infection. Chestnut tree decline, as other tree declines, may also be the expression of a complex pathological state that involves both biotic and abiotic factors (See Introduction section 1.4.). Thus, CID diagnosis is difficult and may be flawed when collar or trunk lesions are not frequent and root excavation not possible. The intensity and frequency of the decline may be related to other factors than the abundance of *P. cinnamomi*, as it has been observed for *Quercus ilex* (Ruiz-Gomez *et al.*, 2019; Corcobado *et al.*, 2014).

To avoid this risk of misdiagnosis and erroneous assessments of *Phytophthora* infected trees, to study the effects of the host diversity on CID it is necessary to assess soil inoculum rather than looking for symptoms. *P. cinnamomi* being transmitted between hosts following an environmental transmission mode, *i.e.* infection via contacts between motile zoospores and fine roots of hosts, an amplification effect may be observed (Cortez and Duffy, 2020). However, since zoospores are released not only from chlamydospores germinating in soils, but also on infected roots, the frequency of non-competent hosts (*i.e.* not able to support and transmit pathogen sporulation) may result in a dilution effect. Moreover, zoospores being dispersed in short distances by free water in soil, pathogen transmission may be dependent on the density of infected chestnut trees. Chestnut trees can sometimes be found mixed with sessile oaks, which are reported as ink disease resistant (Marchand *et al.*, submitted, see Chapter 5). The effect of

such a tree composition on the CID are yet to be understood. Predicting a dilution or an amplification effect for CID also requires a thorough study of soil communities. In recent years, with the endorsement of High Throughput Sequencing (HTS) technologies allowing the description of whole microbial communities in soil, the predominant role of the microbiome in plant health and pathogen suppression has become evident (Berendsen *et al.*, 2012; Brader *et al.*, 2017). Soil and root associated microbiotas may gather different species with a known antagonistic effect on *Phytophthora* species, *e.g. Trichoderma* species on *P. cinnamomi* (Chambers and Scott, 1995). In forests, the composition of symbiotrophs community was related to the CID level caused by *P. x cambivora* (Blom *et al.*, 2009; Scattolin *et al.*, 2012) and *P. cinnamomi* (Venice *et al.*, 2021). The presence of some symbiotrophs species may indirectly drive the dilution effect. Plants in general, produces characteristic components such as root exsudates and leaf litter that impact differently the soil biota (Kardol and De Long, 2018). For example, various species of oak produces various root exudates that are known to attract differently *P. cinnamomi* (Gomez *et al.*, 2020). It is thus expected that in polyspecific forests, offering a larger number of ecological niches, trees harbour a higher microbiota diversity than monospecific stands.

The objective of our work was to investigate a possible effect of tree diversity on CID in a forest composed of chestnut monocultures and mixtures with other tree species. For this purpose, the soil-borne inoculum of CID agents was quantified in an experimental set-up consisting of plots with different levels of decline and forest diversity. The second objective was to assess and describe the diversity and structure of soil fungal communities in this *Phytophthora*-affected environment. To this end, we used a metabarcoding approach.

MATERIAL AND METHODS

Study area

The 1972 ha study site, known as `Massif de Montmonrency' is a forest managed by the Office National des Forêts (ONF) that is located in the Val d'Oise department (95) at 15km North of Paris. Its geographic coordinates are 49° 01' 48" North, 2° 17' 33' East with an altitude going from 96m to 192m. The forest is composed at 70% of chestnut (*Castanea sativa*) and to a lesser degree, there are mixed-stands with other species such as sessile oaks (*Quercus petraea*), beech trees (*Fagus sylvatica*), birch trees (*Betula pendula*) and other tree species in less than 1% abundance. The forest massif lies on two plateaus oriented roughly east-west, intersected by a talweg (Supplementary data 1). On these plateaus and at the bottom of the valley, the soils are

frequently hydromorphic but rich, which explains the good vigour of chestnut trees and their dominant height.

In 2015, the chestnut trees in the massif first started to show signs of decline. In 2016, a year marked by a rainy spring and a dry summer, the decline of chestnut trees has since increased sharply, to such an extent that the massif was classified as a "health crisis" by the French Ministry of Agriculture (Supplementary data 2). *P. cinnamomi* was isolated from chestnut seedlings in different stand since 2016 (F. X Saintonges and C. Robin, personal communications). In order to better characterize the extent of the phenomenon and to adapt silvicultural management, high spatial resolution images from ESA Sentinel-2 were analysed, and after pre-processing (geo-referencing, corrections for atmospheric and topographic effects), used to perform a supervised classification of the health status of chestnut tree stands (Belouard *et al.*, 2020). Three stand classes were discriminated: (1) healthy or slightly dying (stands with less than 50% dying trees); (2) dying (at least 50% dying trees and less than 90% very dying trees) and (3) dead or dying (more than 90% very dying trees) (Figure 1).

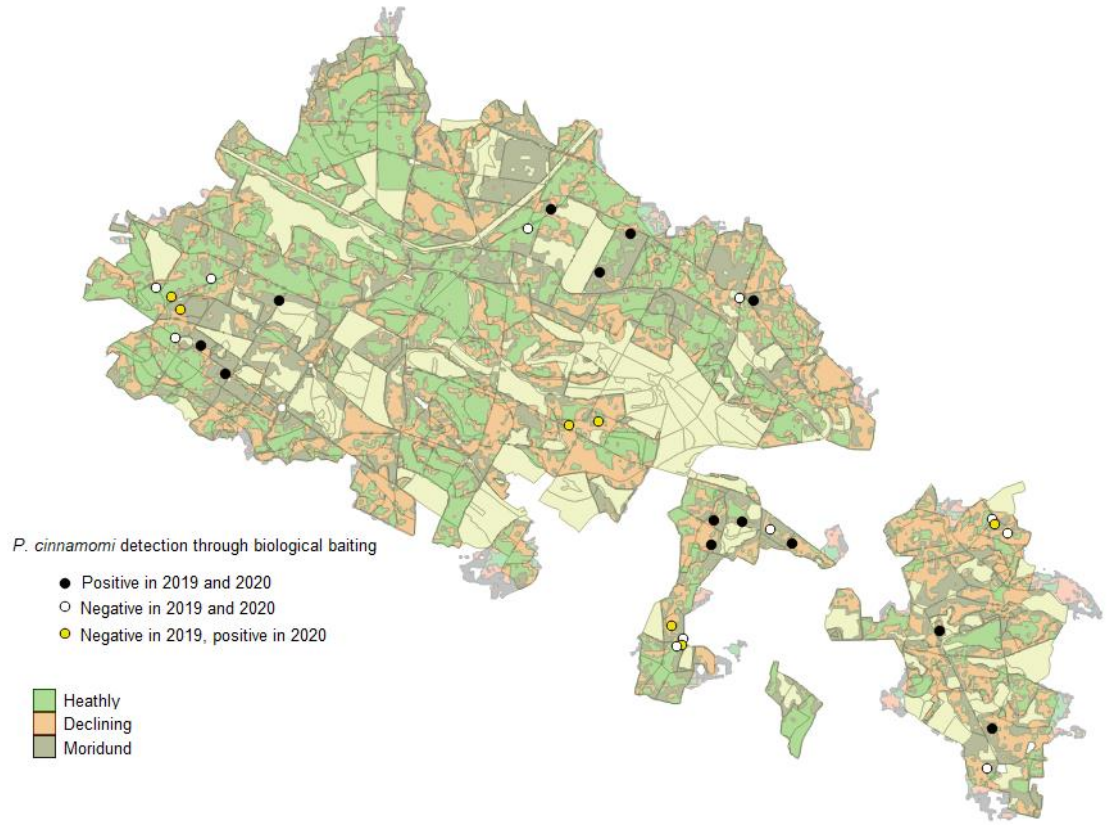


Figure 1: Map of the 30 focal trees samples in the Montmorency forest
(Background map provided by Thierry Belouard, DSF)

Experimental design

In July 2019, an experimental design was set up consisting of 30 plots (20m radius). Plots were selected in order to have a design well-balanced for tree composition (Figure 2) and to reduce as much as possible confusing factors such as type of soil, acidity and topography (Table 1). They were centered on a focal chestnut tree which spatial coordinates were recorded using a Trimble® Geo 7X (Figure 1).

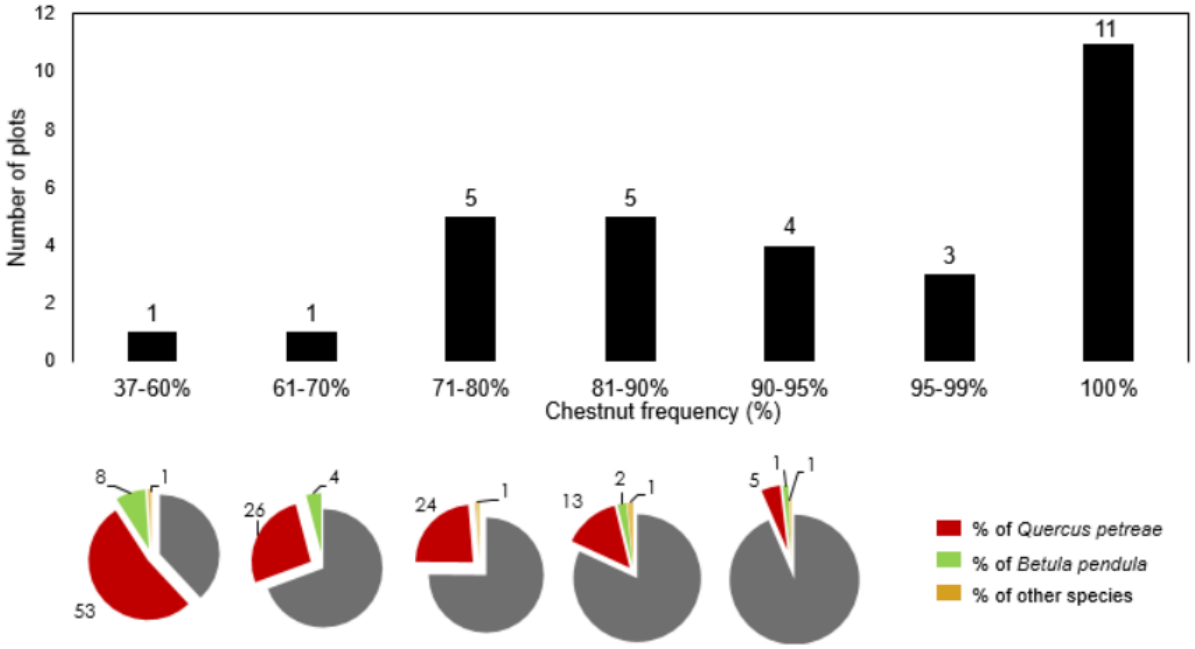


Figure 2: Distribution of plots according to their chestnut frequency

In May and June 2019, September 2020 and July 2021, the soil under the focal trees was sampled (at 30cm depth, using an auger) from four points at 1m around the tree, and mixed together to make one soil sample per focal tree, referred to as the focal soil samples (FSS). In 2019, plot soil compaction was classified as “clay” or “sandy-clay” (Table 1). We measured the basal area of all trees with a trunk diameter superior to 7.5 cm (Table 1). The understory vegetation was characterized (number and height of chestnut seedlings and other plants species) within four 50 x 50 cm square (randomly located in each plot) in order to calculate the average chestnut and other plant understory density.

Table 1: Plot composition according to their edaphic factors (station type, soil type, slope, altitude) and total basal area.

Tree composition	Number of plots in station with average acidity hydromorphic	Number of plots in station with low acidity	Number of plots with clay soil	Number of plots with sandy-clay soil	Mean slope	Mean altitude	Total basal area (cm^2/m^2)
Monospecific (n=14)	5	9	3	11	24	157	588 ± 169
Polyspecific (n=16)	5	11	5	11	21	158	668 ± 252

In 2020 and 2021, the crown health of the 20 dominant chestnut trees of each plot was assessed by using the DEPERIS protocol elaborated by the DSF (https://agriculture.gouv.fr/la-methode-deperis-pour-quantifier-letat-de-sante-de-la-foret). The functional crown of the tree (part of the crown out of tree competition) was rated from A to F according to its percentage of dead branches and lack of branching (Table 2). The intensity of decline in the plots was assessed by the proportion of chestnut trees scored E and F and will be further referenced as EF20.

Table 2: DEPERIS protocol for the estimation of functional crown of the tree (Source : DSF).

		Lack of ramification					
		0 to 5%	6 to 25%	26 to 50%	51 to 75%	76 to 95%	96 to 100%
Lack of branches	0 to 5%	A	B	C	D	E	F
	6 to 25%	B	B	C	D	E	F
	26 to 50%	C	C	D	D	E	F
	51 to 75%	D	D	D	E	F	F
	76 to 95%	E	E	E	F	F	F
	96 to 100%	F	F	F	F	F	F

Within each plot, four subplots were identified, located at 5m of the focal chestnut tree at the four cardinal points (Figure 3) for a total of 120 subplots.

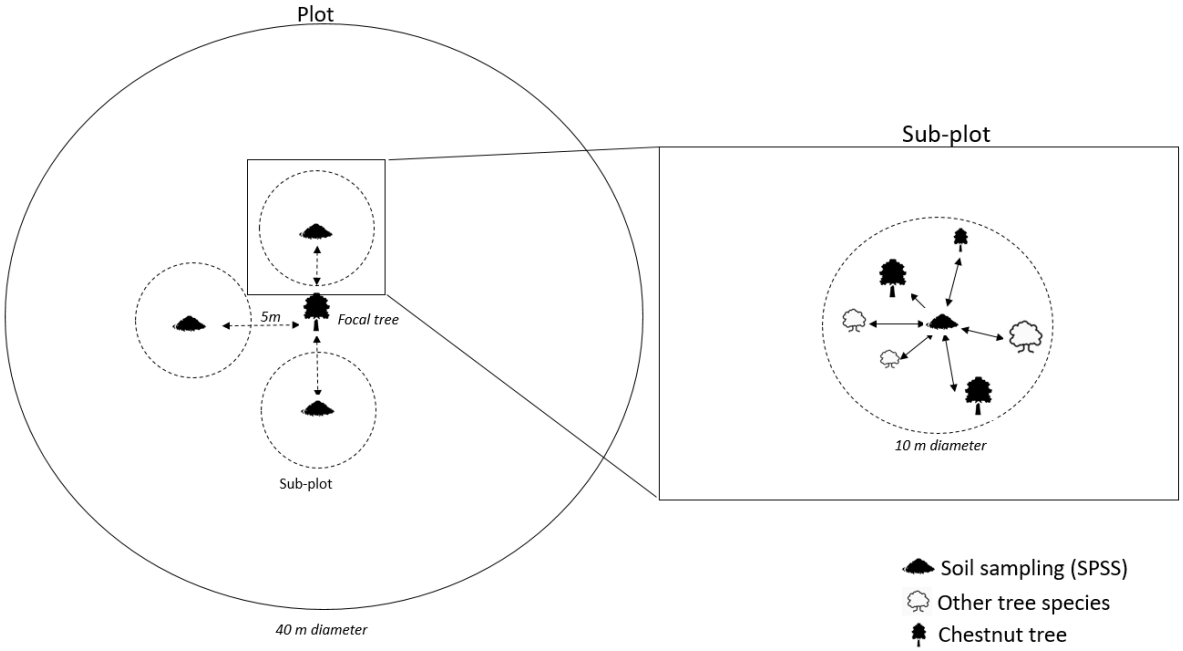


Figure 3: Schematic of plot and subplot settings

In each subplot, soil subplot samples (SPSS) were collected in 2019 at a 25 cm depth using an auger. Within each subplot, the species composition and forest structure was assessed. We measured the distance of the closest trees to the SPSS was sampled, the basal area and crown health status of these trees using the DEPERIS protocol.

Biological baiting

Aliquots of FSS (50mL) were placed into 10x8x4cm containers and were pre-wetted by adding 50mL of osmosed water for the night. after one day incubation, 100mL osmosed water was added, all excess organic matter was removed from the surface of the water, and five oak (*Quercus robur*) leaf discs (1 cm diameter) were placed and incubated for three days at 22-23°C in the light. The leaf discs were then placed onto a PARBHy selective media (malt 1.5%, agar 1.8%, pimaricin 10 ppm, ampicillin 250 ppm, rifampicin 10 ppm, benomyl 15 ppm, hymexazol 50 ppm). After two days, a mycelial plug was sampled from each *Phytophthora* alike colony growing from the leaf discs and plated on Malt Agar (MA) (1.5% malt, 1.8% agar). Identification of *Phytophthora* species was achieved by microscopic observation and confirmed by Sanger sequencing.

DNA extraction and library constructions

Out of the 120 SPSS collected in 2019, we selected 86 of them (distributed in the 30 plots), with three SPSS per plot, except for four plots in which only two SPSS were kept. These samples were selected as to maximize the number of subplots which were polyspecific chestnut subplots. Total DNA from 0.2g of 86 soils was extracted using the FASTDNA ™ SPIN kit for soil (MP Biomedicals, Inc, Eschwege, Germany) following the manufacturer's instructions. The samples were extracted in two replicates that were then pooled thereafter. Four water samples were used as negative controls for each round of DNA extraction. One water metabarcoding control was implemented.

The ***Phytophthora*** **community** in these samples was studied according to the protocol developed in the Chapter 1.

The **fungal community** was studied by amplifying a partial sequence of the ITS 1 using the fungal specific ITS1-F (Gardes and Bruns, 1993) and ITS2 primers (White *et al.*, 1990) modified with a 12-base pairs barcode and Illumina adaptor sequence as illustrated in Laforest-Lapointe *et al.* 2017. Amplicon libraries were produced in a one-step PCR amplification. The PCR mix was composed of 1 µL Bovine Serum Albumine (BSA) at 10 ng/µL concentration, 10 µl 2X Qiagen, 4.5 µL H_2O mQ and 1µL of primers at 2 µM for a final reaction of 20 µL. PCR reactive solution following the cycling conditions : 95°C for 15 min followed by 35 cycles of 95°C for 30s, 53°C for 45 s, 72°C for 30 s and a final extension of 72°C for 10 min. Barcoded amplicons were thereafter purified with homemade SPRI beads, quantified with Quant-iT™ dsDNA High Sensitivity Assay Kit (Invitrogen™) and equimolarly pooled.

The *Phytophthora* and fungal community libraries were then pooled together and sequenced on the MiSeq Instrument (Illumina, San Diego, CA, USA) with the reagent kit v2 (500-cycles) on the PGTB facility (Plateforme Génome Transcriptome de Bordeaux). Sequence demultiplexing (with exact index search) was performed using DoubleTagDemultiplexer. After quality control, the FASTQ files for each sample were exported for bioinformatics analysis.

Bioinformatic analyses

Sequences resulting from the *Phytophthora* library were processed using the pipeline presented Chapter 1. Briefly, the reads were treated using DADA2 (Callahan *et al.*, 2016). The final sequences (ASV) were taxonomically assigned using MegaBLAST against a homemade

database (16 080 sequences retrieved from Phytophthoradb.org, Phytophthora-ITS-ID.org and all NCBI sequences assigned to *Phytophthora, Peronospora and Pythium* species with the « ITS 1 » keyword in their GenBank definition).

To analyze the fungal diversity in SPSS we used the Si5 bioinformatic strategy as proposed by Pauvert *et al.* (2019). Only the forward reads (R1) produced by the sequencer were used and filtered using DADA2 and the filterAndTrim option (Callahan *et al.* 2016) with the following parameters: minimal read length=100 nucleotides, sequences with more than maximum number of N allowed in the reads = 0, maximal expected errors = 2, discard reads that match the phiX genome (viral genome used to test contamination) = TRUE. Sequences were dereplicated and Amplified Sequence Variants (ASVs) were constructed using the dada option after error rate estimation. Chimeric sequences were filtered out and the remaining ASVs were blasted against the UNITE fungi database (General FASTA release v.8) using megaBLAST. For each ASV, the blast result retained were >95 % identical coverage and >95% query coverage and ASV without taxonomic assignment were removed. Taxonomic assignment was kept at the genus level and up when possible.

We used the online version of FUNGuild (Nguyen *et al.*, 2016a at http://www.funguild.org/query.php?qText=&qDB=funguild_db&qField=taxon) to determine the ecological guild and trophic modes of the ASVs identified after ITS amplification. Trophic modes for ASVs were accepted if the match confidence was « highly probable » or « probable ».

Statistical analysis

Statistical analyses were performed using the R Studio interface of the R software (version 3.6.3) and lme4 (Bates *et al.*, 2015), MuMIn (Barton, 2018) multcomp (Hothorn *et al.*, 2008), ggplot2 (Wickham, 2016) and corrplot (Wei, 2021), lmerTest (Kuznetsova *et al.*, 2017), plyr (Wickam, 2011), dplyr (Wickam *et al.*, 2018), modelr (Wickham, 2020), MASS (Venables and Riplay, 2002), ggpubr (Wickham, 2016), Hmisc (Harrell *et al.*, 2008), FactoMineR (Lê *et al.*, 2008), phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen, 2020) packages.

First, we analyzed the 30 plots as they displayed differential soil compaction, forest tree composition, decline intensity and presence of *P. cinnamomi* (analyzed through biological baiting) by using a Factor Analysis of Mixed Data (FAMD). This FAMD was then used to

classify the plots using a hierarchical clustering on principal components (HCPC). The plot categorization was then used as a fixed factor in the following model analyses.

Then, the subplots were characterized with individual plot category, tree composition, distances, density and *P. cinnamomi* read abundance. We used FAMD to visualize the subplots organization according to these variables.

We analyzed the factors influencing the *P. cinnamomi* abundance using a Generalized Linear Mixed Models (GLMM) with a poisson error distribution. Plot identity was included as a random factor (1|Plot.ID in *R* syntax) to account for the correlated data structure arising from the three (or two) replicate soil sampled per plot (Nakagawa and Schielzeth, 2013). Significance of parameters was assessed using χ^2 tests by comparing models with and without the term to be tested. We applied model simplification by starting with the highest order interaction and sequentially removing non-significant predictors. We tested i) the effect of surrounding tree composition a categorical fixed-effect factor with two levels (monospecific subplot or polyspecific subplot) on *P. cinnamomi* abundance. Secondly, to assess mechanisms that might explain the effect of surrounding tree composition, we ran another model with a general structure described as above, but with different predictors. This model included two types of predictors referring to nearby tree composition described by four continuous variables: chestnut density (chestnut trees basal area), chestnut frequency (relative proportion of chestnut basal area to basal area of all trees species in the plot), average chestnut distances and average non-host distances. Despite the correlation between chestnut density and frequency (Pearson's r = 0.39), both were introduced in the same model (Dormann *et al.*, 2013).

Finally, we analyzed the factor influencing the fungal community composition in SPSS using the Montmorency ITS metabarcoding dataset. Alpha-diversity was measured through the Shannon-wiener index (H) and evenness. Evenness was calculated as the H/number of species. Differences in alpha-diversity indices were analyzed using a linear mixed model approach with the plot as a random factor. Differences in fungal community between samples (beta-diversity) were tested using ANOSIM analyses (9999 permutations) on Bray-Curtis dissimilarities matrices. To visualize the taxa present in the different sample, a heat tree of taxa was computed using the metacoder package (Foster *et al.*, 2017).

RESULTS

CID prevalence and forest composition

Only one *Phytophthora* species was identified among the isolates baited in FSS. All recovered isolates had a similar morphotype on V8. Sanger sequencing was carried out to confirm it was *P. cinnamomi* (data not shown). Among the 30 focal chestnut trees sampled, 13 plot were positive through biological baiting in 2019 against 19 in 2020 (Figure 4) and no additional positive plots were found in 2021. The prevalence of CID in polyspecific stands was lower than in monospecific chestnut stands across all the years (5 polyspecific vs 8 monospecific in 2019 and 9 polyspecific vs 10 monospecific in 2020). However, this difference was not significant and decreased in 2020 (χ^2= 1.1205, df = 1, *p*-value = 0.2898 for 2019 and χ^2= 0.23133, df = 1, *p*-value = 0.6305 for 2020). The CID prevalence did not vary with neither the slope nor the altitude (χ^2 test, non-significant).

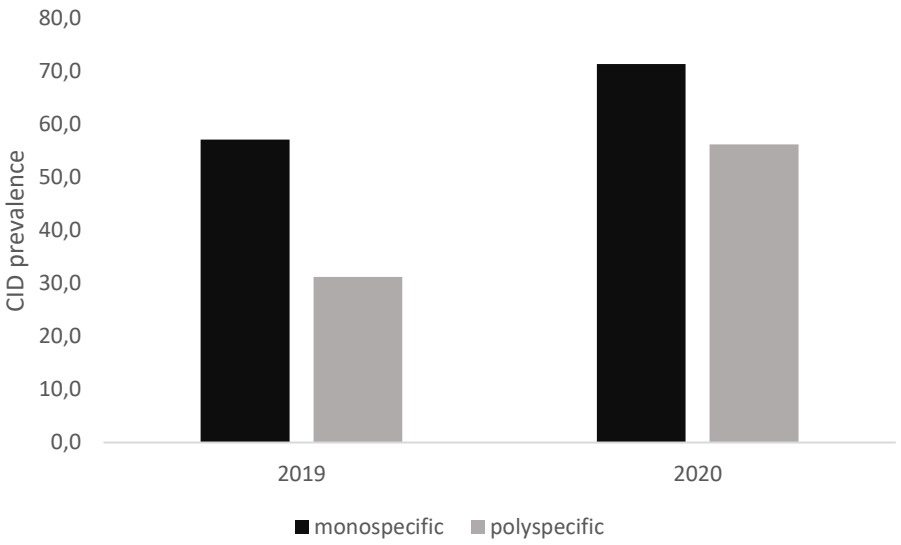


Figure 4: CID prevalence in monospecific (n=14) and polyspecific (n=16) plots.

In 2020, the severity of crown deterioration was similar in monospecific and polyspecific plots, respectively 77% and 75% EF. During 2021, 12 plots of the experimental design were clear-cut. For the remaining 18 plots, the average EF decreased from 69.4% in 2020 to 42.5% in 2021. This reflected the improvement of health status of the functional crown of chestnut trees observed in 2021 and confirmed by forest managers (Denis Hemmer, ONF personal communications). However, in our experimental set-up, there was a bias since plots that were severely declining were preferentially cut.

A Hierarchical Clustering on principal component (HCPC) based on a Factor Analysis of Mixed Data (FAMD) was used to classify the plots with the tree composition descriptors (chestnut

frequency, density, chestnut seedlings heights, % chestnut seedlings, % other species in the understory), station descriptors (type, soil) and CID (*Phytophthora* detection, decline severity). The first three axis of the FAMD explained 64% of the variation in the dataset (Table 3). The CID descriptors did not contribute for more than 20% of these axes, whereas seedlings heights, chestnut frequency and station types were some descriptors that mainly represented on these axes (Table 3).

Table 3: Contribution of variables (%) to the three dimensions resulting from the FAMD

Descriptors	Dim.1 (27,3%)	Dim.2 (20%)	Dim.3 (16,7%)
Chestnut density	19,4	4,2	15,6
Chestnut frequency	7,3	0	43,1
Seedlings proportion	12,8	2,2	1,4
Seedlings height	3,6	22,1	4,6
Understory proportion	15,5	9,9	7,2
Station type	6,4	31,4	0,3
Soil type	6,1	11,4	18,2
Phytophthora detection	12	18,7	0
EF20	17	0,3	9,5

The HCPC allowed the classification of the plots into three distinct categories (Figure 5).

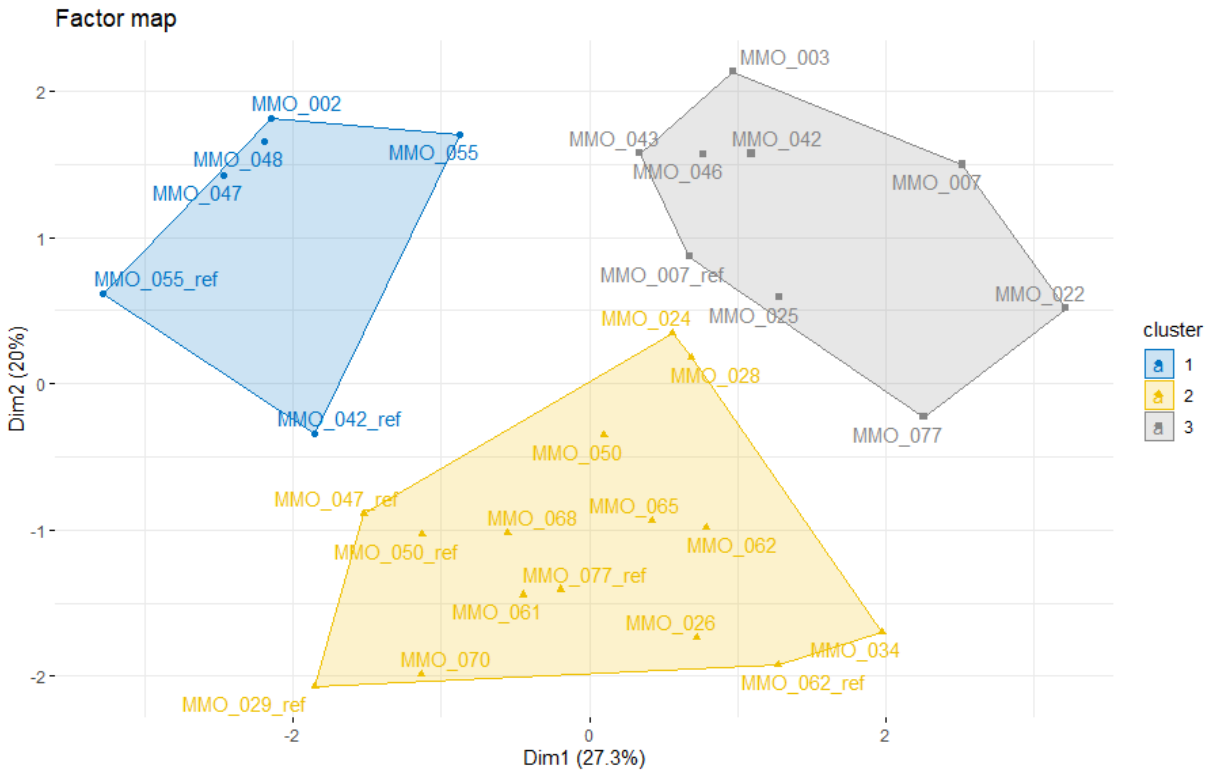


Figure 5: Categorization of plots according to HCPC classification

The intensity of decline in plots was independent from the plot category (χ^2, *p*-value > 0.05). The intensity of decline in plots of diverse categories seemed unrelated to the detection of *P. cinnamomi* (Table 4).

Table 4: Summary of average for each descriptors for plots in each HCPC category (n=Number of plot in the category)

Descriptors	**Plot category**		
	Cat1 (n=6)	**Cat2 (n=15)**	**Cat3 (n=9)**
Chestnut density	369	596	651
Frequency chestnut (%)	82	87	97
Seedlings proportion (%)	10,3	25,5	58,0
Seedlings height (cm)	55	69	130
Understory proportion (%)	68,9	18,9	16,4
Number of plots with average acidity (hydromorphic)	6	0	4
Number of plots with low acidity	0	15	5
Number of plots with soil (clay)	1	1	6
Number of plots with soil (sandy-clay)	5	14	3
EF (%)	53,3	81,0	83,3
Number of plots *P. cinnamomi* positive	2	3	8

The *P. cinnamomi* detection in plots was dependent of plot HCPC category (χ^2= 11.176, df = 2, p-value = 0.003742) being higher in plots of the third category. Chestnut seedlings in higher proportion and height also characterized these plots (Table 4).

Analysis of *Phytophthora* community

The processing of *Phytophthora* metabarcoding data proceeded as shown in Figure 6. After processing, *P. cinnamomi* was the only taxa found in 347 ASV distributed in 73 different soil samples (see Chapter 1).

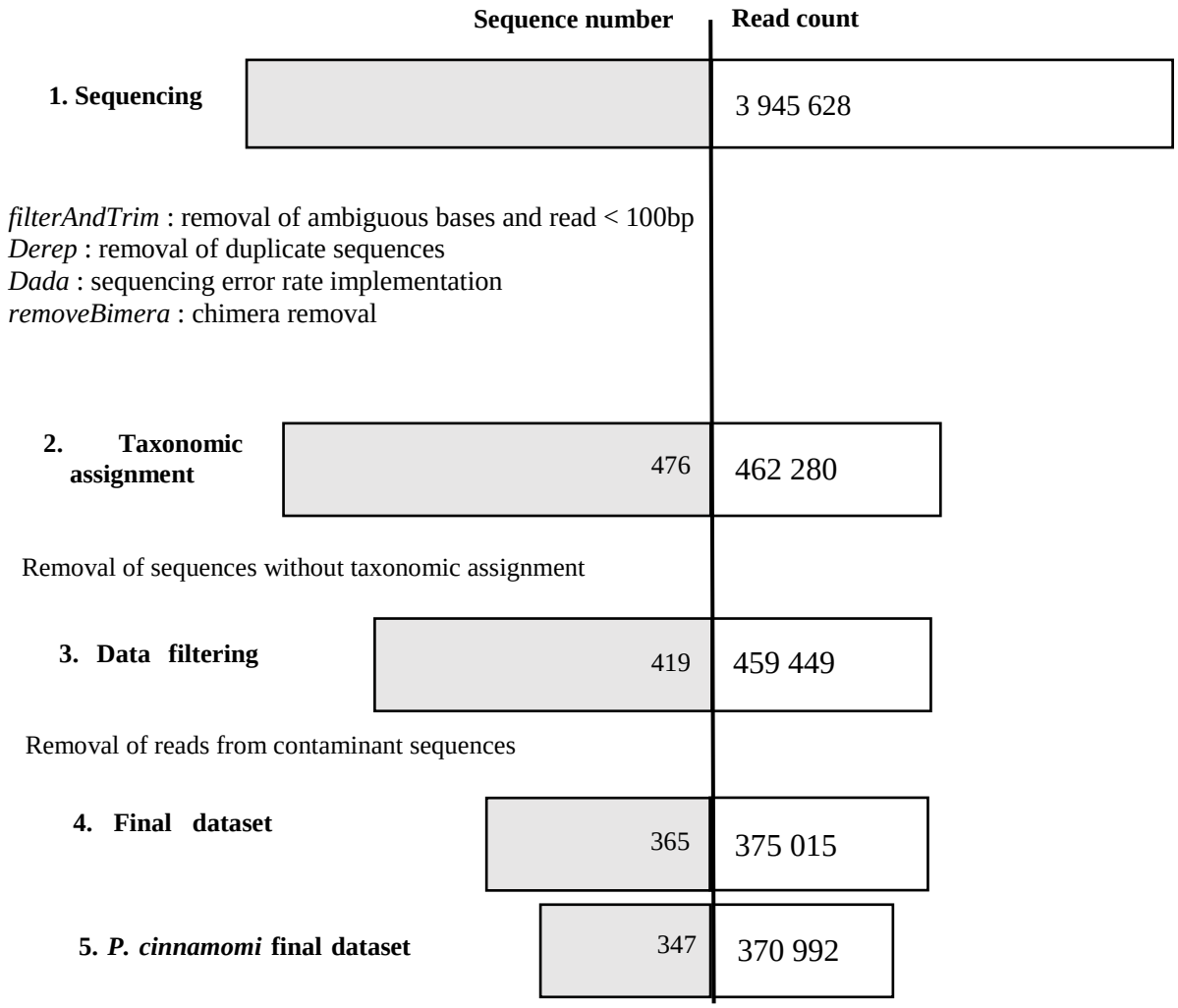


Figure 6: Pipeline of the *Phytophthora* metabarcoding

Effect of the host diversity on *P. cinnamomi* abundance

We used a FAMD to study how the *P. cinnamomi* abundance was related with the subplot composition (chestnut density, chestnut frequency, average distance chestnut and other species) and decline severity (% EF). Cumulated, the first two axis explained 45.3% of the variation in the dataset (Figure 7A). The first axis was mostly explained by the distance of non-chestnut species and chestnut density whereas the second axis by the chestnut frequency and the chestnut distance (Figure 7A). The *P. cinnamomi* read abundance in subplots was organized in a gradient along the second dimension (Figure 7B). *P. cinnamomi* abundance increased with chestnut frequency and decreased with chestnut distance (Figure 7A and 7B). Subplots did not gather according to their plot category nor according to the CID prevalence assessed through biological baiting (results not highlighted in Figure 7). This was confirmed by a χ^2 test, which showed that plot categorization did not significantly account for the variation in *P. cinnamomi* read abundance (χ^2, *p*-value > 0.05). The decline severity did not contribute as much as other

variables to the first axis. A Kruskal-Wallis test confirmed that EF did not vary the subplot tree diversity (*p*-value > 0.05). There was a weak positive correlation between the EF and the *P. cinnamomi* read abundance (Spearman *p*-value = 0.04833, rho = 0.21).

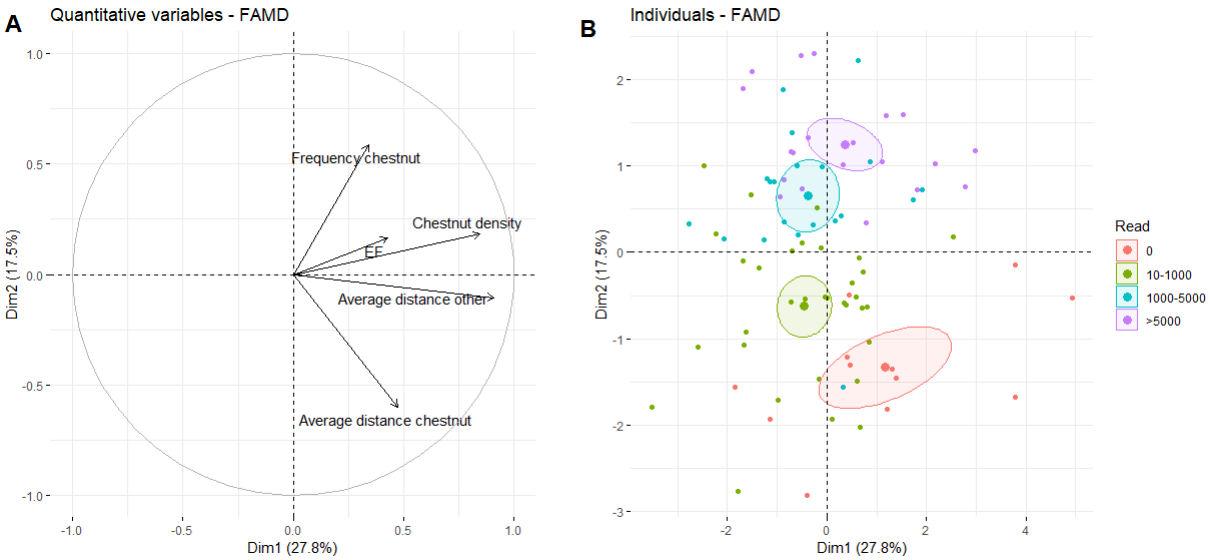


Figure 7: **A**: Graph of quantitative variables of the FAMD, **B**: Graph of individual subplots according to the FAMD analysis showing the first and second dimension. The subplots are colored according to their *P. cinnamomi* read abundance.

P. cinnamomi was found in higher abundance in monospecific chestnut subplots than in polyspecific subplots (both >80% and <80 of chestnut) (Figure 8).

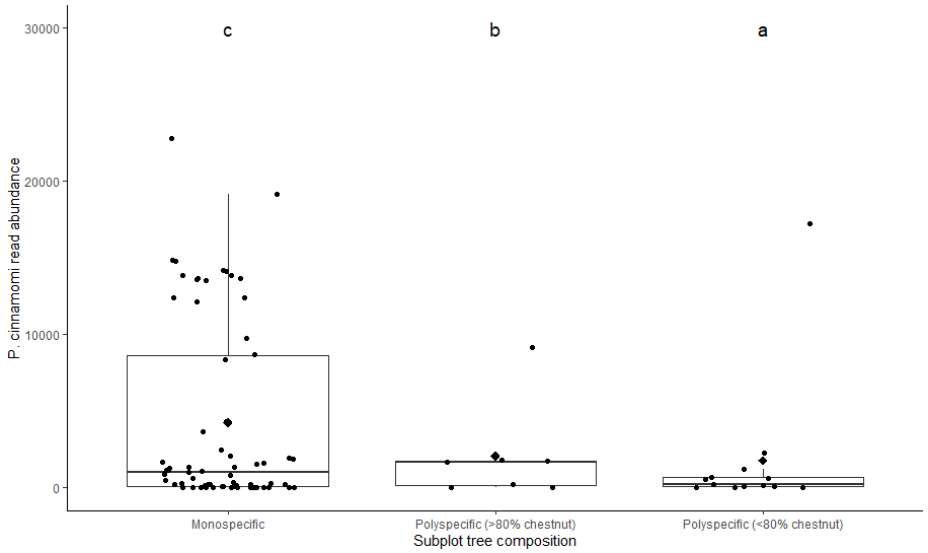


Figure 8: Boxplots of *P. cinnamomi* read abundance per soil samples as a function of the subplots tree composition (differences were calculated with a post hoc tukey test)

There was a significant effect of the average distances, density and frequency of chestnut trees and the other tree species on the *P. cinnamomi* abundance (Table 5).

Table 5: Summary of generalized linear mixed effect model evaluating the effects of subplot structure and composition on *P. cinnamomi* read abundance (Poisson distribution).

Predictors	Estimate	SE	Z value	Pr(>|z|)
Average distance chestnut	-0.436763	0.009097	-48.01	**< 0,001**
Frequency chestnut	1.342847	0.011790	113.90	**< 0,001**
Density chestnut	0.080278	0.019159	4.19	**< 0,001**
Average distance other species	0.133350	0.012658	10.54	**< 0,001**

Marginal $R_{m\ 2}$ represents the variance explained by fixed factors, while conditional $R_{c\ 2}$ is interpreted as variance explained by both fixed and random factors. For the final model (retained after model selection), they equaled 0.43 and 0.99, respectively Explanatory variables in bold characters had a significant effect for chi² values (at P<0.05).

Fungi identified from the metabarcoding sequencing

MiSeq sequencing of the fungal amplicons from the 96 environmental samples, five negative controls and five mocks produced 2 526 839 reads. From these, 2 285 155 fungal reads successfully passed the quality filter and were grouped into 3 594 ASVs (Table 6).

Table 6: Data processing for fungal sequencing

Sequencing		DADA2		Database and 95%coverage+ID
read	**ASV**	**read**	**ASV**	**read**
2 526 839	3 594	2 285 155	685	787 380

After blast against the UNITE fungal database, 2 909 fungi sequences did not pass the filter of >95% coverage and % identical sites. The remaining 685 fungi ASVs represented 787 380 reads. Contaminants sequences were removed from further analysis. Thus, the final dataset for the fungi amplicon sequencing is composed of 675 ASVs for 784 372 reads. These 675 ASVs were successfully assigned to a taxonomic rank as displayed Table 7.

Table 7: Fungal taxa found in the samples

Taxonomic rank	ASV number	Number of taxa
Phylum	29	4
Order	96	10
Class	29	16
Family	360	48
Genus	161	59
Total	675	137

Soil samples were dominated by *Ascomycota* (55.6%), *Basidiomycota* (30.5%) and *Mortierellomycota* (10.8%). The class *Agaromycetes* was the most abundant in ASV (24.1%)

and together, *Leotiomycetes*, *Sordariomycetes*, *Eurotiomycetes*, *Mortierellomycetes* and *Dothideomycetes* represented the five dominant fungal classes (Figure 9).

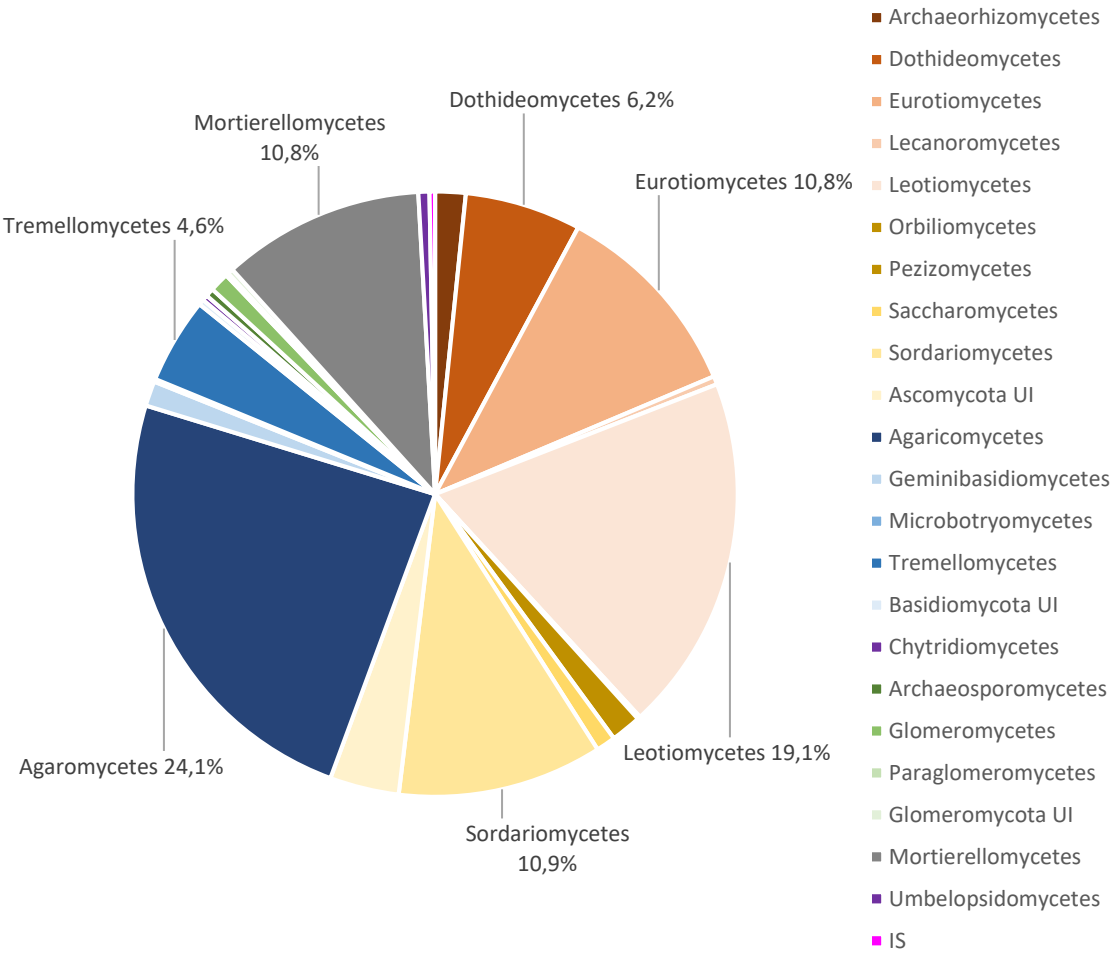


Figure 9: Fungal relative ASVs classifed by « class » taxonomic level
(UI : Unidentified, for those ASVs which classification at the chosen taxonomic level was impossible, IS : *Incertideae sedis*).

Most rarefaction curves for each samples approached the saturation plateau (Figure 10), which indicated that the sampling effort had sufficient sequence coverage to describe accurately the fungal composition of each plot category.

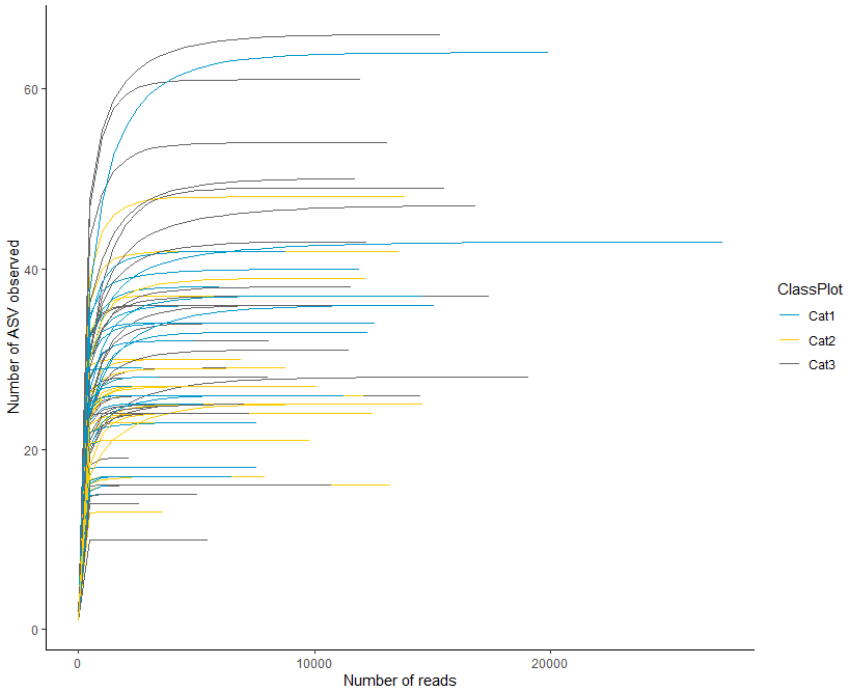


Figure 10: Rarefaction curves (number of ASV as a function of number of reads)

Trophic modes were successuflly assigned to 55.7% of fungal ASVs. After trophic modes assigned with « possible » confidence were removed (41.7% ASVs total remaining ASVs), we obtained four dominant trophic modes (each mode > 10% of the remaning ASVs), which included 29.1% saprotrophs, 25.9% symbiotrophs, 17.0% symbiotroph/saprotrophs and 14.2% pathotroph/saprotrophs (Figure 11).

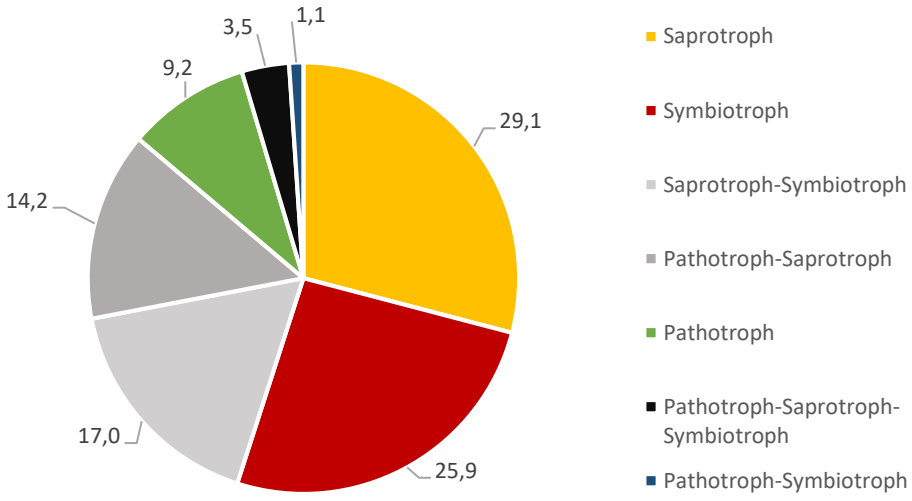


Figure 11: Percentage of ASV assigned to fungi of different trophic modes in the soil samples

The trophic modes were distributed across the taxa as displayed in Figure 12.

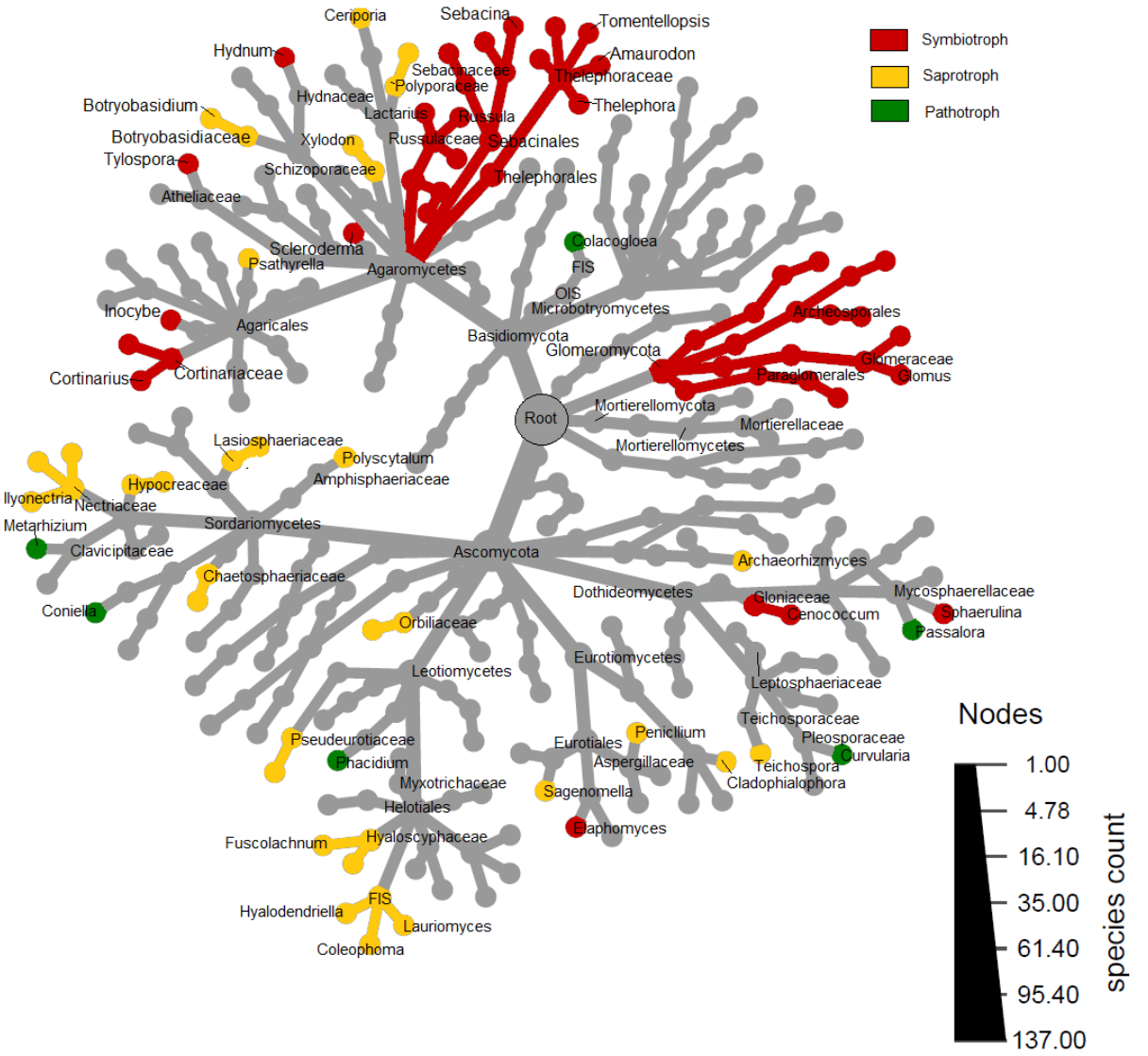


Figure 12: Tree of taxa found in the soil samples and their assigned trophic modes (FIS : *Family Incertae sedis, OIS : Order Incertae sedis*)

Interactions between *P. cinnamomi*, the fungal community and the host diversity

Subplots were grouped in four types according to their tree composition (chestnut monospecific versus polyspecific) and the detection of *P. cinnamomi* DNA signal (positive versus negative).

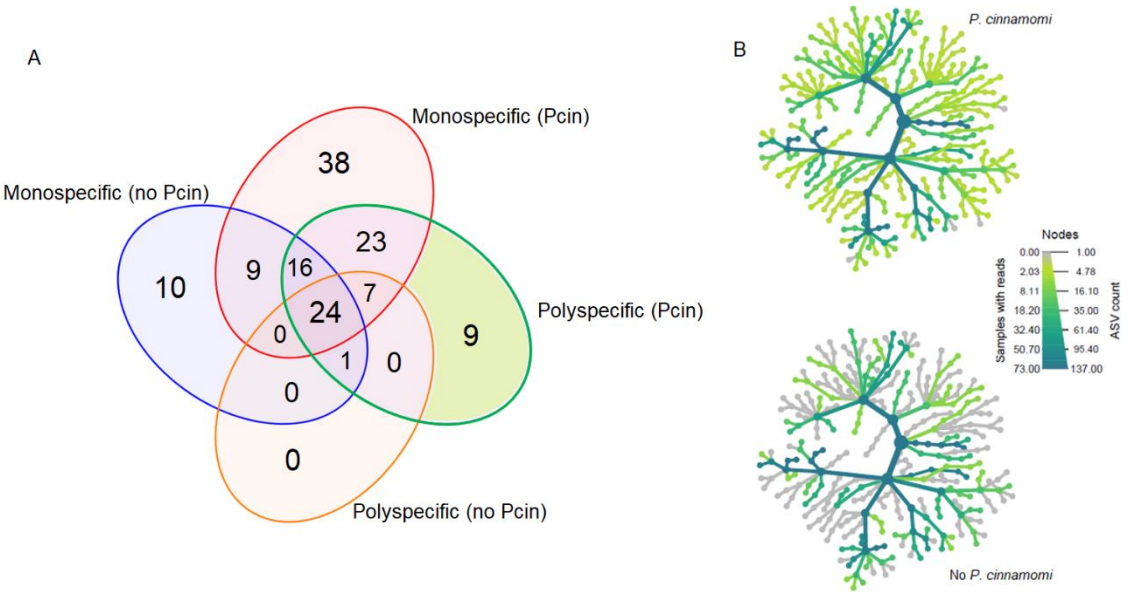


Figure 13: **A**: Venn diagram of taxa found in monospecific subplots negative to *P. cinnamomi* (blue), positive to *P. cinnamomi* (red) and polyspecific subplots negative to *P. cinnamomi* (orange) and positive to *P. cinnamomi* (green).
B: Tree of taxa present in subplots positive to *P. cinnamomi* or negative to the pathogen

Twenty-four different taxa found in common to all type of samples (Figure 13A) but only six of them could be considered as core taxa (>0.01% relative abundance and found in more than 60% of samples) (Figure 14). The samples were dominated by either saprotrophs, *i.e. Lasiosphaeriaceae*, *Hypocreaceae*, or saprotrophs/pathotrohs, *i.e. Mortierellaceae*, *Aspergillaceae* (Figure 14). The relative abundance of these 24 taxa did not change according to the intensity of decline of the subplots (data not shown).

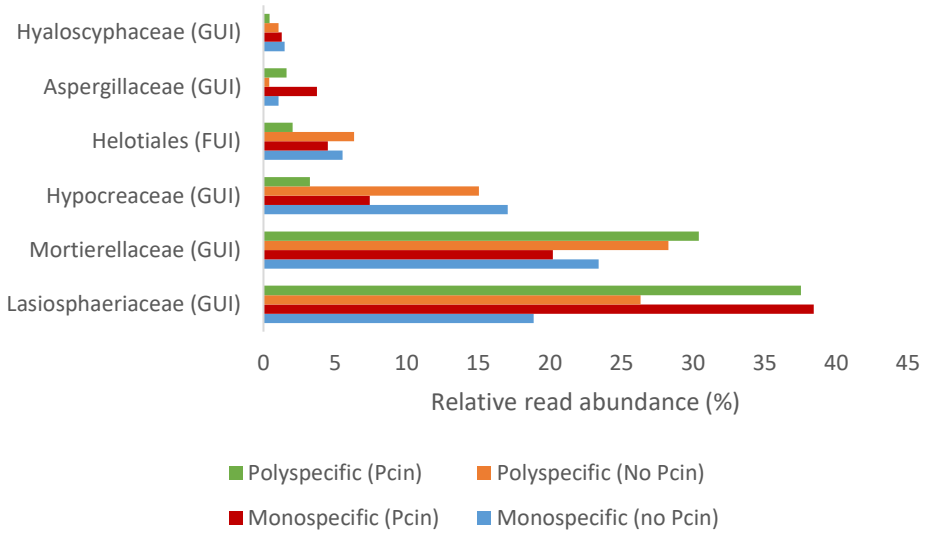


Figure 14: Relative abundance of reads of the most abundant taxa present in the soil samples (GUI : Genus unidentified, FUI: Family unidentified).

We found no difference in Evenness nor Shannon's diversity between subplots according to their tree composition (χ^2, *p*-values>>0.05, Figure 15). ANOSIM analyses revealed no differences in Bray-curtis nor Jaccard distances of the chestnut frequency, density or distances on the samples (p-values >>0.05). Principal Coordinates Analysis (PCoA) were not displayed, as they showed no grouping of the subplots (with Bray-Curtis, Jaccard and UniFrac distances).

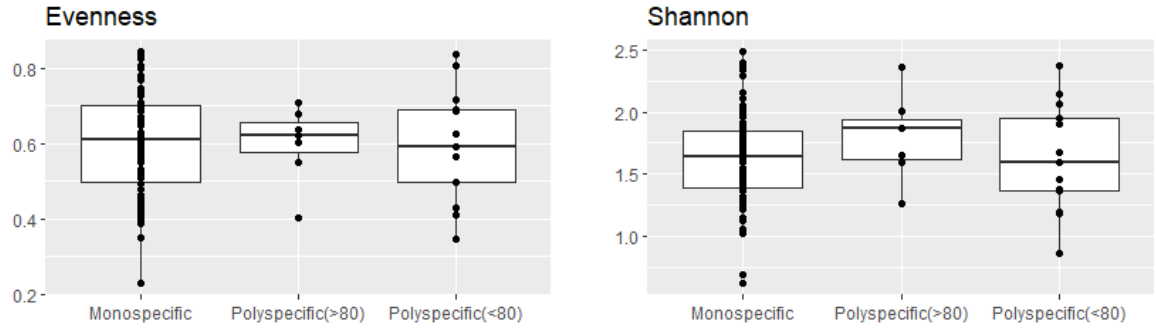


Figure 15: Eveness and Shannon's diversity of subplots according to their tree composition

Overall, a greater fungal diversity could be found in subplots positive to *P. cinnamomi* as compared to subplots negative to the pathogen (Figure 13B). A total of 70 different taxa (with 11 found with a relative abundance > 0.01% in several subplots) could be found in monospecific and polyspecific subplots infected by *P. cinnamomi* whereas only 10 different taxa could be found in non-infected subplots (Figure 13A). Out of these 10 taxa, only one taxa belonging to *Tremellales* order of unknown family was found at 0.04% relative abundance in more than one subplot. Altogether, subplots infected with *P. cinnamomi* displayed a higher symbiotroph diversity and richness (Figure 16) and saprotroph diversity (Figure 17) than subplots negative to the pathogen. Symbiotrophs were mostly abundant when *P. cinnamomi* was present between 10 and 1000 read (Figure 16D). On the other hand, the saprotrophs were mostly abundant in subplots with the highest *P. cinnamomi* signal (Figure 17D). However, some taxa such as *Hypocreaceae* and *Ilyonectria* were most abundant in subplots without *P. cinnamomi* (Figure 17B). Finally, pathotrophs were in low abundance and never found in more than one subplot.

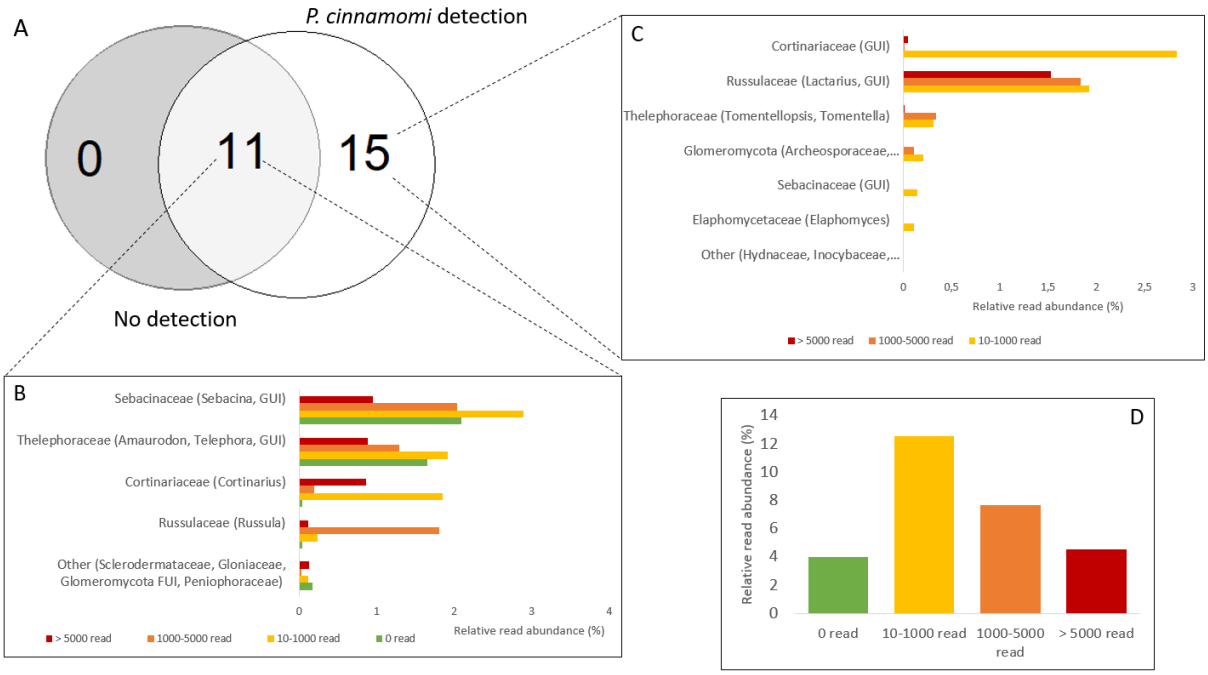


Figure 16: **A** : Venn diagram of symbiotrophic taxa in subplots according to the presence/absence of *P. cinnamomi*. **B** and **C**: relative abundance of symbiotrophs found in subplots according to the intensity of *P. cinnamomi* DNA signal. **D** : total symbiotrophs relative abundance according to the intensity of *P. cinnamomi* DNA signal

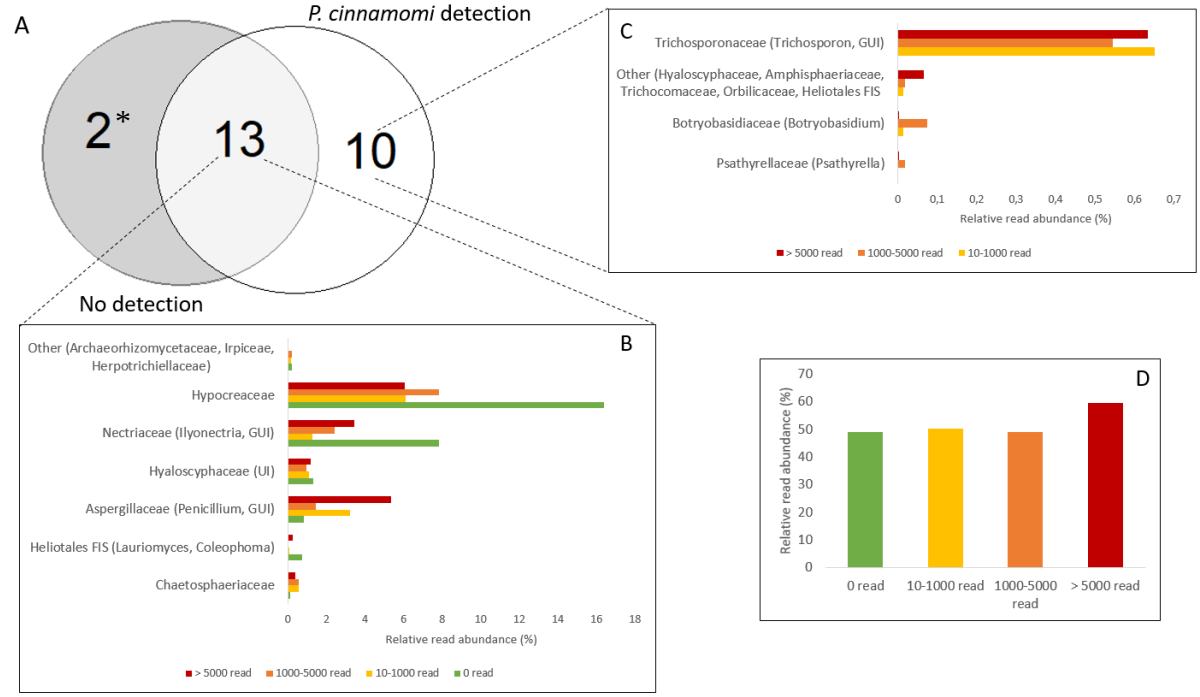


Figure 17: **A** : Venn diagram of saprotrophic taxa in subplots according to the presence/absence of *P. cinnamomi*. **B** and **C**: relative abundance of saprotrophs found in subplots according to the intensity of *P. cinnamomi* DNA signal. **D**: total saprotrophs relative abundance according to the intensity of *P. cinnamomi* DNA signal. (* *Dactylonectria* genus of the *Nectriaceae* family and *Teichospora* of the *Teichosporaceae* family in less than 0.01% relative abundance)

DISCUSSION

The objective of the study was to study the role of biodiversity on the agent of CID by investigating the influence of both the tree diversity and the soil fungal diversity on *P. cinnamomi* abundance.

In the studied forest, chestnut tree decline, assessed through the deterioration of the functionanl crown, did not vary with plot or subplot tree diversity: the percentage of moribund trees (E and F DEPERIS scores) was similar whatever the proportion of chestnut trees. This may be accounted for the origin(s) of the decline as it may be caused by other factors than CID agents. Two observations were in agreement with this assumption: i) plots characterized with average acidity demonstrated trees in better health than plots with low acidity, ii) chestnut trees showed signs of partial recovery during the study time, suggesting that decline was not irreversible and climatic events might be involved. This is consistent with the decline model as interpretated by Whyte *et al.* (2016) (see Chapter 2).The same decline model may also explain the weak correlation found, at the subplot level, between the intensity of decline and *P. cinnamomi* DNA signal. These results and observations confirmed that studying the dilution or amplification effect of CID by scoring decline severity may be not relevant unless precisely identifying the causes of the decline. Rather than studying the eventual dilution of the disease, we focused on the dilution of the soil inoculum by the mixture of host susceptible trees (chestnut trees) with resistant species (mainly sessile oaks).

For this end, we used the detection of *P. cinanmomi* DNA (*i.e.* the count of *P. cinnamomi* reads obtained through a metabarcoding soil analysis) as a proxy for its abundance (see Chapter 1). First, we found that *P. cinnamomi* was in higher abundance in monospecific chestnut trees subplots compared to polyspecific subplots, suggesting there was a dilution of the inoculum by the non-chestnut tree species. We also observed that the chestnut density, frequency and the distance of host (*i.e.* chestnut trees) and other species influenced the pathogen abundance. Interestingly, the pathogen abundance seemed to correlate negatively with the distance of chestnut trees and positively with the chestnut frequency. The “encounter reduction” may be occurring in our system (Keesing *et al.*, 2006), where the addition of less competent hosts may interfer with *P. cinnamomi* transmission pathways. In pure stands, the dispersal and survival of the pathogen is facilitated compared to mixed stands, where the other tree species may act as physical barrier to the dissemination of the pathogen by intercepting the inoculum or by increasing the distance the inoculum must get around to reach another competent host. Such

pattern depends on the tree species and its ability to support and transmit the pathogen, *i.e.* its competence. This phenomenon were already reported for *P. ramorum* on other hosts (Haas *et al.*, 2011). By contrast, an amplification of *P. cinnamomi* was suggested to exist in mixed stands of the Dehesas forest composed of *Q. ilex* and *Q. suber* compared to pure stands (Cardillo *et al.*, 2021). Interestingly, we found that chestnut seedlings proportion and height was higher in plots positive to *P. cinnamomi*. Since adult trees may be reservoirs for pathogens that are then transmitted to understory seedlings, overstory tree density may contribute to disease of conspecific seedlings in the understory (Schweizer *et al.*, 2013). However, we did not design our experiment to answer this question and this would deserve further investigations.

A second objective of the study was to explore the soil fungal diversity, since one mechanism explaining dilution effect by the host diversity lies in the species associated to them (Collins *et al.*, 2020). To describe these fungal communities we used a metabarcoding approach. First and foremost, the taxa were mostly assigned to the family level or up, and only a handful could be assigned to the genus level. This low taxonomic resolution could be on the account of the lack of specific data associated to the rhizosphere that leads to a scarcity of the fungal references available in the database used for taxonomic assignment. This idea is conforted by the fact that less than half of the ASV were assigned confidently to a trophic mode and only a small portion of these were assigned to exploitable trophic modes, *i.e.* pathotrophs, symbiotrophs and saprotrophs.

Overall, our soils sampled in monopspecific and polyspecific chestnut plots were dominated with *Sordariomycetes, Letiomycetes* (both Ascomycota) and *Agaricomycetes* (Basidiomycota), in accordance with previous works (Baptista *et al.*, 2015; Venice *et al.*, 2021; Bani *et al.*, 2019). We expected that local hosts would affect the composition and structure of the soil fungal community. Our polyspecific plots (and subplots) were only composed of angiosperms belonging to the same *Fagaceae* family (chestnut, oak) and *Betulaceae* family (birch). Typically, the symbiotroph species richness was demonstrated to be constrated between stands composed of gymnosperms compared to angiosperms but not within stands composed of angiosperms (Nguyen *et al.*, 2016b). The polyspecific plots in our experimental design might be composed of tree species too close phylogenetically for a marked difference of whole fungal composition to be evident. It may also be explained by the sampling strategy we used. Indeed, we found an overwhelming number of taxa solely associated to single subplots making it impossible to conclude on their distribution, a recurring problem in studies of soil communities

(Richard *et al.*, 2005; Horton and Bruns, 2001; Taylor, 2002). The divergence between subplots emphasizes the importance of selecting the appropriate soil sampling because edaphic parameters, *e.g*. soil physico-chemical parameters or plant community features (Cubera *et al.*, 2009; Venice *et al.*, 2021; Manter *et al.*, 2010), greatly influence the description of the soil microbial community. These are parameters that we did not measure in our study, which could differ locally within plots. Here, with three (or two) subplots taken from the same plots we expected to minimize variability and to characterize better fungal communities.

In any case, the samples were dominated with core families of potential pathotrophs and saprotrophs that were not assigned to a genus. These families (and putative genus) may be found associated with *Fagaceae* forest settings, *e.g. Aspergicallaceae* (Marjanovic *et al.*, 2020) or naturally occurring in soils, *e.g. Mortierellaceae* (Li *et al.*, 2018). The *Lasiosphaeriaceae* is one of the most diverse family *Ascomycota* and there are yet to be data relating to either chestnut stands or the presence of *P. cinnamomi*. However, their distribution was shown to be associated with decreased soil pH (Yin *et al.*, 2021) suggesting their presence could be driven by the soil acidity that is characteristic of the forest massif of Montmorency.

The other main objective of the study was to describe the fungal community in the relationship with *P. cinnamomi* to decipher whether the presence of the pathogen changed (or was changed by) the structure and composition of the community. We found higher saprotrophic taxa diversity in subplots infected by the pathogen than those without. This could be explained by the fact that under infected trees, a higher availability of plant debris, *i.e.* dead roots, favor the development of the saprotrophs (Corcobado *et al.*, 2020; Sánchez-Cuesta *et al.*, 2020). On the other hand, a *Hypocreaceae* taxon was found in stands without *P. cinnamomi.* Species in this family with known antagonistic effect on *P. cinnamomi*, *e.g. Trichoderma* species, were reported to occur naturally in oak ecosystems (Ruiz-Gomez *et al.*, 2021). Similarly, in subplots without *P. cinnamomi*, we found that *Nectriaceae* (*Ilyonectria* and *Dactylonectria*) genus were in higher abundance than suplots with the pathogen. These taxa are known root rot agents on different hosts (El-Agamy Farh *et al.*, 2018; Vitale *et al.*, 2012) and this suggests that a possible competition occurred between the *Nectriaceae* taxa and *P. cinnamomi*. In addition, the presence of the pathogen shifted the community of symbiotrophs in a marked way. Symbiotrophs were the most frequently associated with *P. cinnamomi*-infected samples as compared to non-infected samples, particularly at low *P. cinnamomi* abundance. Species of the *Russulaceae* family, *Thelephoraceae, Cortinaceae* and *Sebacinaceae* were found higher abundance in *P.*

cinnamomi infected subplots. Similar taxa that were found to be associated with *P. x cambivora* infected chestnut stands (Blom *et al.*, 2009). Either the symbiotrophs are facilitators for *P. cinnamomi*, either the pathogen facilitates the symbiotrophs community. The latter could be explained by the fact that a differential root turn-over caused by intensive root necrosis, linked to *P. cinnamomi*, may result in change of symbiotroph communities (Dahlberg, 2001). Since *P. cinnamomi* affects the root system architecture, it also might result in potential indirect changes in soil properties that would exert a selective pressure on the nearby fungal community. However, inferring whether the presence of the pathogen changed the fungal community or if it was the reverse remains difficult from a descriptive study such as the present one. Finally, here, we only took into account the fungal community, or there are evidences that the soil bacterial community is heavily affected by *P. cinnamomi* (Yang *et al.*, 2001) which in turn may affect the fungal community.

The dilution effect highlighted in this study, which is reported for the first time for CID, should be confirmed in other environmental conditions and mixtures with other tree species, *e.g.* with gymnosperms. Most specifically, the underlying mechanisms involving the underground fungal structure and composition need further investigations.

AKNOWLEDGMENTS

We thank the ONF agency in charge of the Montmorency forest massif and especially Denis Hemmer for their precious help in the implementation of the experimental design. We thank Clothide Carteron, Thierry Bélouard, Claude Husson (DSF, French Ministry of Agriculture and Forestry, France) for their help in the field trips. A special acknowledgment is given to Marc Buée for his expertise and advices in the metabarcoding analysis.

REFERENCES

Bani, A., Borruso, L., Matthews Nicholass, K. J., Bardelli, T., Polo, A., Pioli, S., … Brusetti, L. (2019). Site-specific microbial decomposer communities do not imply faster decomposition: Results from a litter transplantation experiment. *Microorganisms*, *7*(9). https://doi.org/10.3390/microorganisms7090349

Baptista, P., Reis, F., Pereira, E., Tavares, R. M., Santos, P. M., Richard, F., … Lino-Neto, T. (2015). Soil DNA pyrosequencing and fruitbody surveys reveal contrasting diversity for various fungal ecological guilds in chestnut orchards. *Environmental Microbiology Reports*, *7*(6), 946–954. https://doi.org/10.1111/1758-2229.12336

Barton, K. 2018. MuMIn: Multi-Model Inference. R Package Version 1.42.1.https://cran.r-project.org/web/packages/MuMIn/index.html

Bates, D., Machler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1-48

Belouard T., Husson C., Gaudry J., Le-Mesle V., Nowak C., Witkowiski S., Marchand M. and Robin C. (2020). Devant l'augmentation de dépérissements du châtaignier en Ile-de-France, les forestiers de la forêt privée et publique s'associent avec le DSF et INRAE pour évaluer l'ampleur du problème.

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17*(8), 478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Blom, J. M., Vannini, A., Vettraino, A. M., Hale, M. D., & Godbold, D. L. (2009). Ectomycorrhizal community structure in a healthy and a *Phytophthora*-infected chestnut (Castanea sativa Mill.) stand in central Italy. *Mycorrhiza*, *20*(1), 25–38. https://doi.org/10.1007/s00572-009-0256-z

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L. J., & Sessitsch, A. (2017). Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology*, *55*, 61–83. https://doi.org/10.1146/annurev-phyto-080516-035641

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cardillo, E., Abad, E., & Meyer, S. (2021). Iberian oak decline caused by *Phytophthora cinnamomi*: A spatiotemporal analysis incorporating the effect of host heterogeneities at landscape scale. *Forest Pathology*, *51*(2), 1–16. https://doi.org/10.1111/efp.12667

Chambers, S. M., & Scott, E. S. (1995). In vitro Antagonism of *Phytophthora cinnamomi* and P. citricola by Isolates of Trichoderma spp. and Gliocladium virens. *Journal of Phytopathology*, *143*(8), 471–477. https://doi.org/10.1111/j.1439-0434.1995.tb04557.x

Collins, C. D., Bever, J. D., & Hersh, M. H. (2020). Community context for mechanisms of disease dilution: insights from linking epidemiology and plant–soil feedback theory. *Annals of the New York Academy of Sciences*, *1469*(1), 65–85. https://doi.org/10.1111/nyas.14325

Corcobado, T., Cech, T. L., Brandstetter, M., Daxer, A., Hüttler, C., Kudláček, T., … Jung, T. (2020). Decline of European beech in Austria: Involvement of *Phytophthora* spp. and contributing biotic and abiotic factors. *Forests*, *11*(8). https://doi.org/10.3390/F11080895

Corcobado, T., Vivas, M., Moreno, G., & Solla, A. (2014). Ectomycorrhizal symbiosis in declining and non-declining Quercus ilex trees infected with or free of *Phytophthora cinnamomi*. *Forest Ecology and Management*, *324*, 72–80. https://doi.org/10.1016/j.foreco.2014.03.040

Cortez, M. H., & Duffy, M. A. (2020). Toward a unified dilution effect theory: How competence, competition, and the transmission mechanism influence disease prevalence. *BioRxiv*. https://doi.org/10.1101/2020.01.31.928820

Cubera, E., Moreno, G., & Solla, A. (2009). Quercus ilex root growth in response to heterogeneous conditions of soil bulk density and soil NH4-N content. *Soil and Tillage Research*, *103*(1), 16–22. https://doi.org/10.1016/j.still.2008.09.002

Dahlberg, A. (2001). Community ecology of ectomycorrhizal fungi: An advancing interdisciplinary field. *New Phytologist*, *150*(3), 555–562. https://doi.org/10.1046/j.1469-8137.2001.00142.x

Davidson, J. M., Patterson, H. A., & Rizzo, D. M. (2008). Sources of Inoculum for *Phytophthora ramorum* in a Redwood Forest . *Phytopathology*, *98*(8), 860–866. https://doi.org/10.1094/phyto-98-8-0860

Dormann C.F., Elith J., Bacher S. et al. (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Farh, M. E. A., Kim, Y. J., Kim, Y. J., & Yang, D. C. (2018). Cylindrocarpon destructans/Ilyonectria radicicola-species complex: Causative agent of ginseng root-rot disease and rusty symptoms. *Journal of Ginseng Research*, *42*(1), 9–15. https://doi.org/10.1016/j.jgr.2017.01.004

Foster Z. Sharpton T., Grünwald N. (2017). "Metacoder: An R package for visualization and manipulation of community taxonomic diversity data." *PLOS Computational Biology*, **13**(2), 1-15. doi: 10.1371/journal.pcbi.1005404,
https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005404

Gómez, M. C., González, M., Gómez-Aparicio, L., & Serrano, M. S. (2020). Coexistent Mediterranean woody species as a driving factor of *Phytophthora cinnamomi* infectivity and survival. *Annals of Applied Biology*, *177*(1), 41–50. https://doi.org/10.1111/aab.12599

Haas, S. E., Hooten, M. B., Rizzo, D. M., & Meentemeyer, R. K. (2011). Forest species diversity reduces disease risk in a generalist plant pathogen invasion. *Ecology Letters*, *14*(11), 1108–1116. https://doi.org/10.1111/j.1461-0248.2011.01679.x

Harrell Jr. F.E., Dupont C., *et al.* (2008) ''Hmisc: Harrell miscellaneous.'' R. package version 3.2.

Horton, T. R., & Bruns, T. D. (2001). The molecular revolution in ectomycorrhizal ecology: Peeking into the black-box. *Molecular Ecology*, *10*(8), 1855–1871. https://doi.org/10.1046/j.0962-1083.2001.01333.x

Hothorn, T., Bretz, F., Westfall, P., and Heiberger, R. M. 2008. multcomp:Simultaneous inference in general parametric models. R package version 1.0-0. http://CRAN.R-project.org

Jules, E. S., Kauffman, M. J., Ritts, W. D., & Carroll, A. L. (2002). Spread of an invasive pathogen over a variable landscape: A nonnative root rot on Port Orford cedar. *Ecology*, *83*(11), 3167–3181. https://doi.org/10.1890/0012-9658(2002)083[3167:SOAIPO]2.0.CO;2

Kardol, P., & De Long, J. R. (2018). How anthropogenic shifts in plant community composition alter soil food webs. *F1000Research*, *7*(0), 1–12. https://doi.org/10.12688/f1000research.13008.1

Keesing, F., Holt, R. D., & Ostfeld, R. S. (2006). Effects of species diversity on disease risk. *Ecology Letters*, *9*(4), 485–498. https://doi.org/10.1111/j.1461-0248.2006.00885.x

Kuznetsova A., Brockhoff P.B., Christensen R.H.B. (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software*, **82**(13), 1–26. doi: 10.18637/jss.v082.i13

Lê, S., Josse, J. & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*. **25(1)**. pp. 1-18.

Li, F., Peng, Y., Fang, W., Altermatt, F., Xie, Y., Yang, J., & Zhang, X. (2018). Application of Environmental DNA Metabarcoding for Predicting Anthropogenic Pollution in Rivers. *Environmental Science and Technology*, *52*(20), 11708–11719. https://doi.org/10.1021/acs.est.8b03869

Manter, D. K., Delgado, J. A., Holm, D. G., & Stong, R. A. (2010). Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. *Microbial Ecology*, *60*(1), 157–166. https://doi.org/10.1007/s00248-010-9658-x

Marjanović, Ž., Nawaz, A., Stevanović, K., Saljnikov, E., Maček, I., Oehl, F., & Wubet, T. (2020). Root-associated mycobiome differentiate between habitats supporting production of different truffle species in serbian riparian forests. *Microorganisms*, *8*(9), 1–27. https://doi.org/10.3390/microorganisms8091331

McMurdie and Holmes (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 8(4):e61217

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., … Kennedy, P. G. (2016a). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nguyen, N. H., Williams, L. J., Vincent, J. B., Stefanski, A., Cavender-Bares, J., Messier, C., … Kennedy, P. G. (2016b). Ectomycorrhizal fungal diversity and saprotrophic fungal diversity are linked to different tree community attributes in a field-based tree experiment. *Molecular Ecology*, *25*(16), 4032–4046. https://doi.org/10.1111/mec.13719

Oksanen J. (2020). Venga: Community Ecology Package. R package version 1.8-5

Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., … Vacher, C. (2019). Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology*, *41*, 23–33. https://doi.org/10.1016/j.funeco.2019.03.005

Prospero, S., & Cleary, M. (2017). Effects of host variability on the spread of invasive forest diseases. *Forests*, *8*(3), 1–21. https://doi.org/10.3390/f8030080

Richard, F., Millot, S., Gardes, M., & Selosse, M. A. (2005). Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by Quercus ilex. *New Phytologist*, *166*(3), 1011–1023. https://doi.org/10.1111/j.1469-8137.2005.01382.x

Ruiz Gómez, F. J., Navarro-Cerrillo, R. M., Pérez-de-Luque, A., Oβwald, W., Vannini, A., & Morales-Rodríguez, C. (2019). Assessment of functional and structural changes of soil fungal and oomycete communities in holm oak declined dehesas through metabarcoding analysis. *Scientific Reports*, *9*(1), 1–16. https://doi.org/10.1038/s41598-019-41804-y

Ruiz-Gómez, F. J., & Miguel-Rojas, C. (2021). Antagonistic potential of native trichoderma spp. Against *Phytophthora cinnamomi* in the control of holm oak decline in dehesas ecosystems. *Forests*, *12*(7). https://doi.org/10.3390/f12070945

Sánchez-Cuesta, R., Navarro-Cerrillo, R. M., Quero, J. L., & Ruiz-Gómez, F. J. (2020). Small-scale abiotic factors influencing the spatial distribution of *Phytophthora cinnamomi* under declining Quercus ilex trees. *Forests*, *11*(4). https://doi.org/10.3390/F11040375

Scattolin, L., Dal Maso, E., Mutto Accordi, S., Sella, L., & Montecchio, L. (2012). Detecting asymptomatic ink-diseased chestnut trees by the composition of the ectomycorrhizal community. *Forest Pathology*, *42*(6), 501–509. https://doi.org/10.1111/j.1439-0329.2012.00784.x

Schweizer, D., Gilbert, G. S., & Holl, K. D. (2013). Phylogenetic ecology applied to enrichment planting of tropical native tree species. *Forest Ecology and Management*, *297*, 57–66. https://doi.org/10.1016/j.foreco.2013.02.012

Taylor, A. F. S. (2002). Fungal diversity in ectomycorrhizal communities: Sampling effort and species detection. *Plant and Soil*, *244*(1–2), 19–28. https://doi.org/10.1023/A:1020279815472

Vannini, A., & Vettraino, A. M. (2001). Ink disease in chestnuts: impact on the European chestnut. *Forest Snow and Landscape Research*, *76*(3), 345–350.

Venables W.N., Ripley B.D. (2002). *Modern Applied Statistics with S*, Fourth edition. Springer, New York. ISBN 0-387-95457-0, https://www.stats.ox.ac.uk/pub/MASS4/.

Venice, F., Vizzini, A., Frascella, A., Emiliani, G., Danti, R., Della Rocca, G., & Mello, A. (2021). Localized reshaping of the fungal community in response to a forest fungal pathogen reveals resilience of Mediterranean mycobiota. *Science of the Total Environment*, *800*, 149582. https://doi.org/10.1016/j.scitotenv.2021.149582

Vitale, A., Aiello, D., Guarnaccia, V., Perrone, G., Stea, G., & Polizzi, G. (2012). First Report of Root Rot Caused by Ilyonectria (=Neonectria) macrodidyma on Avocado (Persea americana) in Italy. *Journal of Phytopathology*, *160*(3), 156–159. https://doi.org/10.1111/j.1439-0434.2011.01869.x

Wei T., Simko V. (2021). *R package 'corrplot': Visualization of a Correlation Matrix*. (Version 0.91), https://github.com/taiyun/corrplot

Whyte, G., Howard, K., Hardy, G. E. S. J., & Burgess, T. I. (2016). The Tree Decline Recovery Seesaw; a conceptual model of the decline and recovery of drought stressed plantation trees. *Forest Ecology and Management*, *370*, 102–113. https://doi.org/10.1016/j.foreco.2016.03.041

Wickham, H. (2020). Modelr : modelling functions that work with the pipe. R package version 0.1.8. https://CRAN.R-project.org/package=modelr

Wickham, H. François, R. Henry, L. and Müller K. (2018). dplyr: A Grammar of Data Manipulation. R package version 0.7.6. https://CRAN.R-project.org/package=dplyr

Wickham H. (2011). "The Split-Apply-Combine Strategy for Data Analysis." *Journal of Statistical Software*, 40(1), 1–29. http://www.jstatsoft.org/v40/i01/

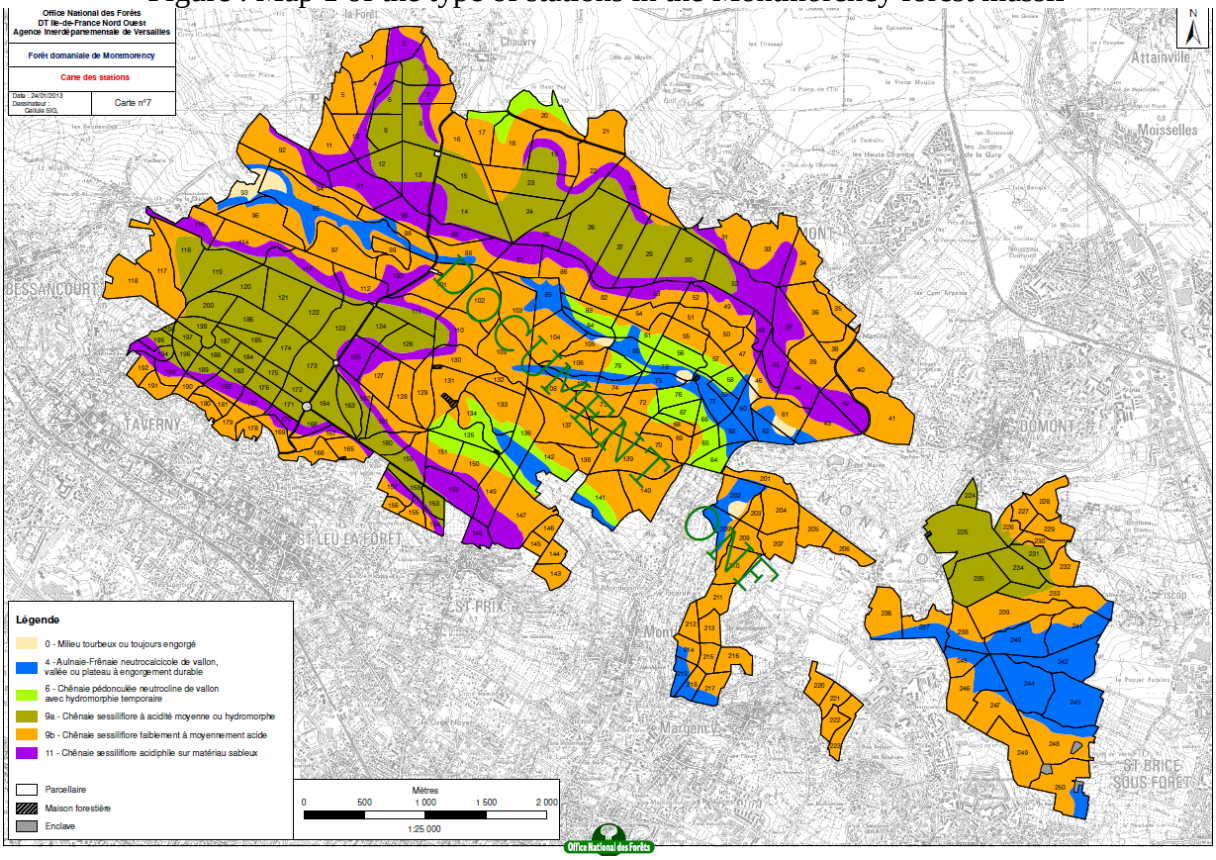
Wickham H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Yang, C. H., Crowley, D. E., & Menge, J. A. (2001). 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. *FEMS Microbiology Ecology*, *35*(2), 129–136. https://doi.org/10.1016/S0168-6496(00)00120-3

Yin, C., Schlatter, D. C., Kroese, D. R., Paulitz, T. C., & Hagerty, C. H. (2021). Responses of Soil Fungal Communities to Lime Application in Wheat Fields in the Pacific Northwest. *Frontiers in Microbiology*, *12*(May). https://doi.org/10.3389/fmicb.2021.576763

Supplementary data 1

Figure : Map 1 of the type of stations in the Montmorency forest massif



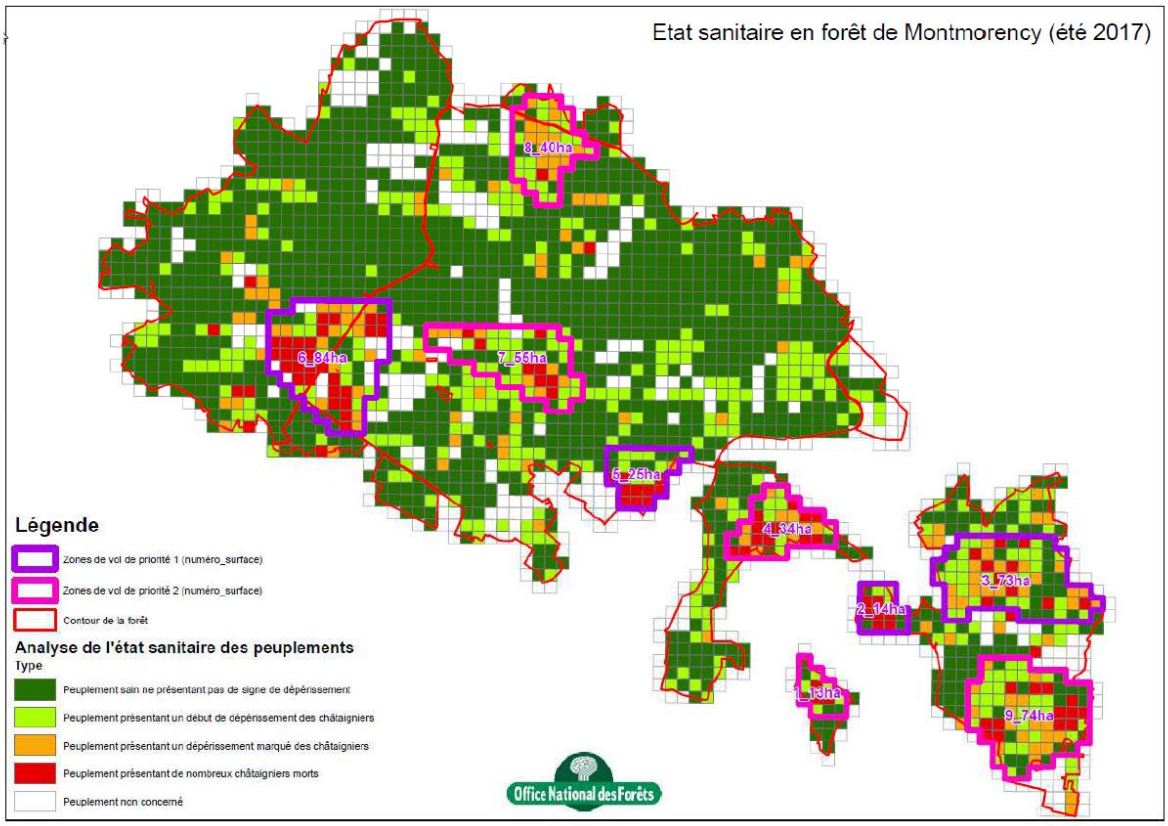
Légende

- 0 - Milieu tourbeux ou toujours engorgé
- 4 - Aulnaie-Frênaie neutrocalcicole de vallon, vallée ou plateau à engorgement durable
- 6 - Chênaie pédonculée neutrocline de vallon avec hydromorphie temporaire
- 9a - Chênaie sessiliflore à acidité moyenne ou hydromorphe
- 9b - Chênaie sessiliflore faiblement à moyennement acide
- 11 - Chênaie sessiliflore acidiphile sur matériau sableux

- Parcellaire
- Maison forestière
- Enclave

Supplementary data 2

Figure : Map 2 of the health status of the Montmorency forest massif classified by the ONF managing the forest



CHAPTER 4

Toward a more inclusive definition of host competence in plant pathosystems

Paper in progress, elaborated with Cécile Robin[1] and Matteo Garbelotto[2]

[1]UMR BioGeCo, INRAE, Cestas, France

[2]Department of Environmental Science Policy and Management, University of California, Berkeley, CA, USA

INTRODUCTION

Plant epidemiologists aimed at understanding the disease spatial and temporal spread and the biotic and abiotic drivers of this dynamic, in order to forecast disease risk at different places (site, country or whole ecosystem) and times (days, weeks, seasons, years…). To infer evolutionary dynamics of the host and pathogen, they pass the baton to evolutionary biologists, who by incorporating the concepts of quantitative and neo-darwinian genetics into epidemiological models, aimed at predicting the evolution of specific traits of the host or of the parasite. The key mechanisms underlying disease dynamics rely on different steps of the disease cycle: exposure, infection, disease, transmission, host recovery or mortality. These steps depend on parasite and host traits. For pathogens, phytopathologists have long developed a different body of theory from that of animal epidemiologists, and decomposed the pathogenicity in different components (Lannou, 2012). Here, with the ambition to unify plant epidemiology and evolutionary biology, we will refer to virulence as the harm a plant pathogen does to its host, and transmission as its ability to disperse and infect a new host. From the host side, resistance and tolerance are the two different mechanisms plants have to defend themselves against pathogens. The concept of resistance is much more consensual and widespread among plant epidemiologists than the tolerance one (Pagan and Garcia-Arenal, 2020). These pathogen and host traits are key parameters for plant disease dynamics, at the different spatio-temporal scales. Another plant component decisive at the individual plant level, but also at the community level, is the host competence. This trait is complementary to the pathogen transmission to explain disease transmission from one host to another. It can be seen as another linking trait between host and pathogen interactions, which was missing to explain pathogen transmission and plant epidemic trajectory (Figure 1).

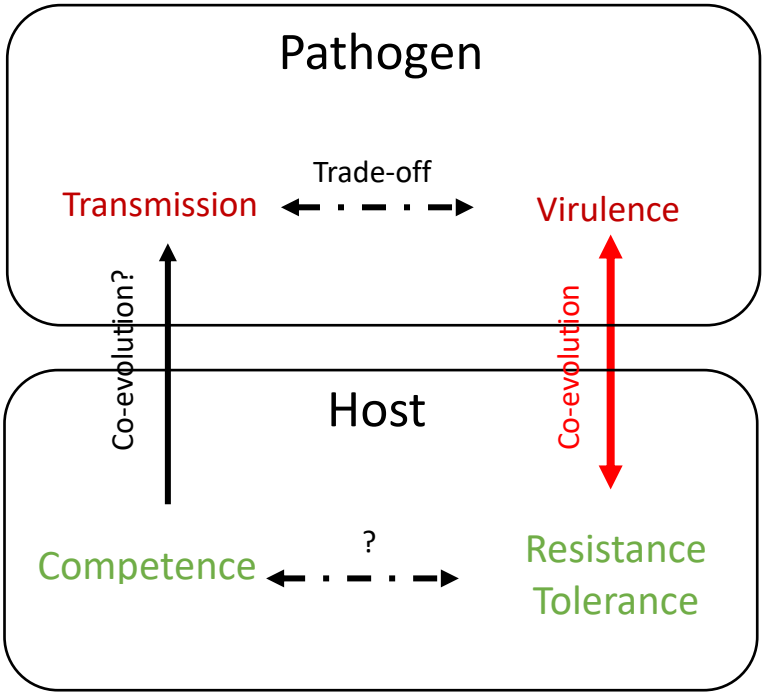


Figure 1: Host and pathogen traits interaction

The concept of host competence has been largely overlooked by many plant epidemiologists until now and has been mostly studied through the animals' disease prism (Martin *et al.*, 2016, 2019). Since animals can move, they respond to biotic stresses through physiological responses but they can also modify their behaviour whereas plants cannot (Baucom and de Roode., 2011). Behaviours can affect the encounter between the host and the parasite while genetic, molecular and cellular processes generally regulate the host responses (Gervasi *et al.*, 2015), responses that include the competence to transmit the infection or not. The concept of competence has gained recognition in plant disease field recently because of emerging diseases. The Sudden Oak Death, devastating epidemics which first emerged in California in the beginning of this century caused by *Phytophthora ramorum*, reminded plant epidemiologists that host plants differ in their ability to transmit a given pathogen to other plants (Rizzo and Garbelotto, 2003). In this chapter, I will : i) define the host competence as a plant trait, ii) suggest methods and proxies to characterize and quantify host competence and iii) propose some avenues for future research for this host trait.

The notion of competence is intimately linked to the different types of transmission, which can be environmental (via some infectious propagules) or/and by host-host contact (Downs *et al.* 2019; Cortez and Duffy, 2020). Although competence can be defined for vectors, as well as for host, we will here focus on host competence, keeping in mind that some plant pathogens have

obligatory multiple hosts to perform their whole life cycle and acknowledging the important role of vectors in dispersal and infection steps for pathogens. Here, the specific question of host competence for vectored pathogens will not be addressed.

How can we define plant host competence as an individual host trait?

Different definitions were proposed in disease ecology field. Some are rather restrictive (Gervasi *et al.*, 2015) whereas others encompass different components which contribute to the ability of a host to transmit the pathogen but not only (Martin *et al.* 2019; Downs *et al.* 2019). How can these different definitions match with plant disease specificities? Shall we adapt them for plant-pathogen interactions? Our objective is to suggest a non-ambiguous definition of an individual host trait, which would not encompass different components of plant-pathogen interactions (not composite) and can be quantified (continuous).

Should host competence definition be limited to transmission and should it encompass the whole host infection process?

VanderWaal and Ezenwa (2016) define the individual competence as the combined outcome of infectivity, infectiousness and rate of contact with hosts. These latter depend on pathogen multiplication, the vigor of the host (its resilience to stress and health state previous to infection), its extended phenotype, *e.g. Xyllela fastidiosa* transmission was shown to be dependent on bacterial populations within plants (Hill and Purcell, 1997) and host phenology. A fairly close conception of competence is proposed by Downs *et al.* (2019) who defined host competence as "the ability of a host to acquire and transmit parasites to another host either directly or through shedding into the environment". In plant pathology, acquisition by the host of the pathogen is usually considered as dependent on its immunity system. If this step of pathogen acquisition were included as a component in plant host competence, it would be not easy to disentangle the different processes and physiological mechanisms that occur after the host –pathogen encounter and during the whole span of time of their interaction. So the answer would be "no".

Should host competence definition be limited to transmissibility or to transmission?

Martin *et al.* (2019) proposed a different definition "competence is the propensity of one host to cause an infection in another host or vector". Transmission is effective when the receptor host is really infected; this requires that the pathogen propagules released by the donor are infectious and compatible with this receptor host. In this sense, this component does appear as a composite one. The same authors defined transmissibility as "the propensity of a host to transfer parasites to another susceptible host or vector, including the sensitivity of said host to manipulative effects of parasites". Such host trait, the transmissibility, is not dependent on the second host neither on pathogen propagules. Although little studied and less frequent than in animal systems, the manipulation of plants by their pathogens has been reported. Plant pathogens may influence the host ability to allow their transmission, by changing for example plant host stomatal aperture which is required for the release of their infectious propagules (*e.g.* Yang *et al.*, 2021). We suggest not to include the infection step, which is the final one of the transmission step, in host competence component but to keep host susceptibility to manipulative and infectious pathogens.

Should host competence definition be limited to transmissibility to another susceptible host?

Host competence is defined by Gervasi *et al.* (2015) as « the proficiency with which a host transmits a parasite to another susceptible host or vector». In phytopathology the term "another" echoes to the notion of alloinfection, first defined by Robinson as "infection in which the donor host individual is different from the receptor host individual" and autoinfection, as "infection in which the donor host individual is the same as the receptor host individual (in Mundt, 2009). This definition has been adapted to different spatial scales, and the host individual was sometimes considered as a sub-unit of the plant host, or at the contrary as a field or population (Laloi *et al.*, 2016). If we consider host competence as a host trait, the outcome of the infectious propagules released in the environment, whatever the distance to the donor host at which it is spread, should not influence its competence assessment. Within plant-infection is a key process to understand spatio-temporal development of plant pathogens (Lannou *et al.*, 2008). Host competence is interfering within this process. We thus suggest not restricting competence to the transmissibility to another host, but to another host unit, which allows autoinfection to be considered as a part of the epidemiological process.

What is a non-competent host ?

In light to all that was previously stated, a non-competent host would be a host which either dies before they can transmit the pathogen or that cannot transmit the disease but may be infected (Martin *et al.*, 2016). Moreover, a host that produces a low number of spores for hundreds of years may not be competent if the number of spores produced never reach the threshold necessary for infection of another host. The very definition of competence needs to take into account the various threshold levels necessary to infect other hosts (different hosts may have different thresholds). Lastly, a host that produces spores but at the wrong time, reduces its competence, *e.g.* Tanoaks produce *P. ramorum* spores when it is colder, hence reducing their ability to transmit (Davidson *et al.*, 2008). This phenological synchrony was demonstrated as determinant in the interaction between oak species and an obligate fungal parasite (Desprez-Loustau *et al.*, 2010). Thus, competence is intrinsically connected to the synchronicity between infection potential of the propagules and host susceptibility.

Methods and proxies to characterize plant host competence

The sporulation supported by the host

Host competence for many pathogens is quantified by the number of spores emitted by the host upon infection. That is the most intuitive proxy for this host trait. This was done to measure the competence for *P. ramorum* of various plant species (Garbelotto *et al.*, 2021, Davidson *et al.*, 2008). However, the amount of produced spores is sometimes measured as a component of host resistance especially when pathogen colonization of the host tissue does not result in clear lesions (*e.g.* infection by biotrophic pathogens as rusts or powdery mildews). In these pathosystems, sporulation area is confounded with lesion size, and spore amount can be assessed as a resistance trait. The quantity of emitted propagules, if not assessed for the host side (competence), may be assessed for the pathogen side (as a component of virulence). For example, the total weight of spores produced on one wheat leaf infected by rust, expressed in µg of spores x mm^{-2} of sporulating area x sporulating day^{-1}), was defined as the spore production capacity of one pathogen genotype infecting one host genotype (Pariaud *et al.*, 2012). It could also be interpreted as the competence of this host genotype for this pathogen genotype. Thus, many data characterizing host competence could be retrieved from the literature, especially for biotrophic and leaf pathogens for which the amount of spores is a frequent trait measured to assess the virulence and to describe trade-offs among pathogen traits. However, as other traits

of host-pathogen interaction, these estimates of competence may be controlled by assessing the pathogen x host interaction.

Sporulation is also dependent on pathogen load

Nevertheless, there are several limits in using this intuitive and rather easy-to-measure proxy. In the same way as host tolerance is defined for a given pathogen burden (point tolerance), or as a reaction norm between host fitness and pathogen burden (position tolerance, Pagan and Garcia-Arenal, 2020), host competence should be estimated for a given pathogen within-host colonization or as a reaction norm between sporulation supported by the host and pathogen load (Figure 2). Measuring host competence should integrate a measure of spore washes or air catches and a measure of pathogen load.

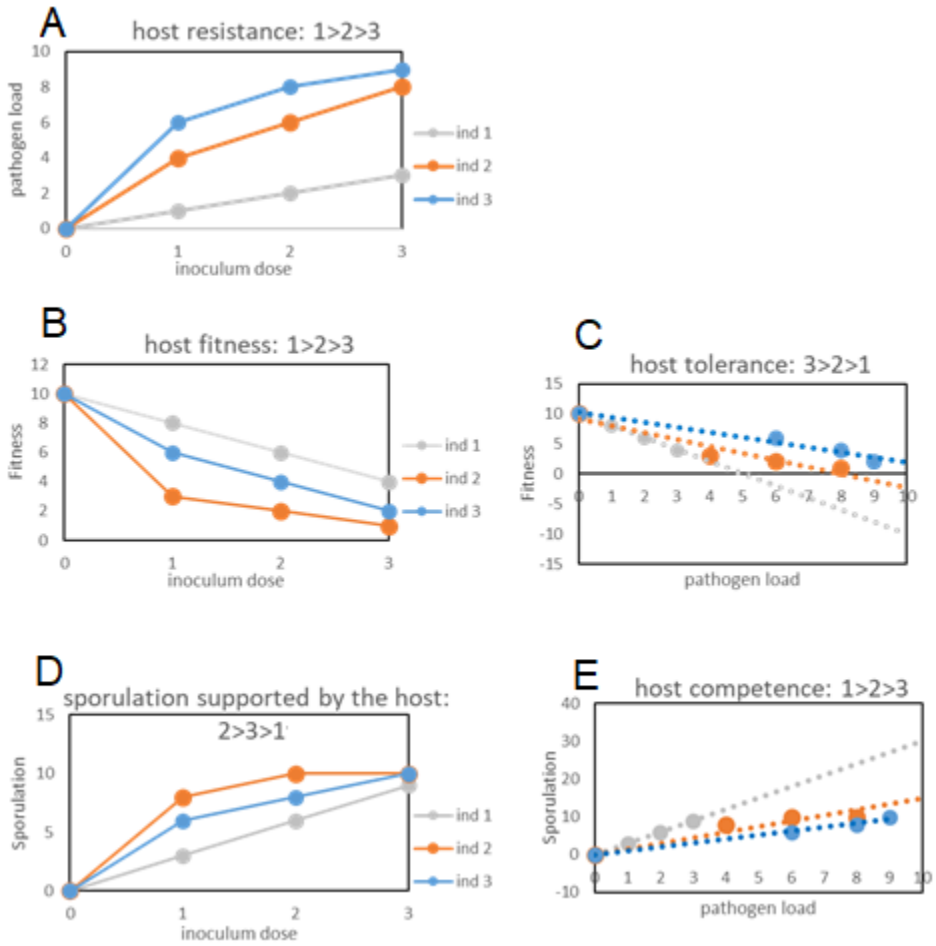


Figure 2: Relationship between different measures of resistance, tolerance and competence. Ind 1, 2, 3 : host genotype 1, 2 and 3. Host genotype resistance is ranked from 1>2>3 (A). When evaluating the host reaction to the infection through a loss of fitness (B), the genotype 1 displays the lowest loss in fitness. However, when the pathogen load is taken into account with the fitness (which is the measure of tolerance, in C), this same genotype displays the lowest tolerance (illustrated by a steeper slope). Similarly, the genotype 1 displays the lowest ability to support the inoculum compared to the two other genotypes (D). However, when the pathogen load is taken into account (which is the measure of competence) this genotype displays the highest competence (E).

Moreover, lesion size and sporulation area may not be good indicators of pathogen colonization. Quantifying pathogen load with molecular methods is a measure complementary of spore quantification and necessary to really disentangle host resistance and host competence.

Sporulation vary with infections and time

Although factors enabling and increasing the sporulation of the pathogen are major components of host competence, factors related to the number of sporulating sites may be taken into account for assessing the whole plant competence for a pathogen. Compared to animal hosts which can move within their environment to increase their resources and escape their parasites and enemies, plants are sessile and have developed different strategies to exploit their environment and defend themselves. One of them is to reduce the possibility of autoinfections. In addition, the availability of infection site may be reduced by the degree of competition between microorganisms present (Wilson and Lindow, 2014).

The amount of emitted propagules is much more a proxy of the transmissibility ability of one host whereas amount standardized with pathogen load should be a proxy of host competence.

Epidemiological perspectives

Epidemiological models

To better understand and predict epidemics, models of disease dynamics and transmission have been elaborated. Most classically, SIR models took into account three types of host individuals Susceptible (S), Infected (I) and Recovered (R). These models have known refinement over time with the addition of exposed individuals not yet infectious (SEIR model) for example. However in these models, all individuals as equally competent. To model how a pathogen is spreading in a population and at what speed, the standard procedure is to calculate the basic reproduction number (R_0) (Anderson and May, 1991). It represents the mean number of secondary cases produced by a single initial case in a naive population (Anderson and May, 1991). Depending on how it is calculated, the R_0 may not be an accurate measure of the severity of an outbreak (Breban *et al.*, 2007). This R_0 parameter serves as a threshold to decipher whether an outbreak will end up as a epidemic ($R_0>1$) or become extinct ($R_0<1$). The R_0 is considered as a pathogen trait. However, R_0 is intimately dependent on the host capacity to support propagule production and release. Without properly naming it, it takes into account host

competence. This problematic was addressed by Llyod-Smith *et al.* (2005) who noticed that the R_0 used in models was a pathogen-dependent constant that did not take into account the number of secondary cases produced by an individual host. To address this issue, they devised an individual's reproduction number V that reflected the importance of key individuals in outbreaks of disease. This shades a new light that help understanding the heterogeneities in disease transmission in host communities. However, the reproduction number does not take into account the entire epidemic dynamic. Indeed, transmission from a competent host to a competent host will cause an exponential increase of disease spread, as long as hosts are not a limiting factor. On the other hand, transmission from a competent host to a non-competent host will stop the spread from these. In both cases, the reproduction number will be over 1.

Disease dynamics and host diversity

In disease ecology, variation in disease transmission is acknowledged and recognized as the 20/80 rule (*i.e.* 20% of individuals in a species contribute to 80% of new infections, Woolhouse *et al.*, 1997). Martin *et al.* (2019) more explicitly argued that significant variations in competence occur within populations and that individuals characterized by extreme competences are "keystone hosts of infection". Diversity plays a crucial role in both the emergence of diseases and the regulation of pathogen transmission. However, there are many arguments as to how biodiversity affects disease transmission (Randolph and Dobson 2012; Ostfeld 2013; Lafferty and Wood 2013; Ostfeld and Keesing 2012; Hasley 2019) and biodiversity has, arguably, a confusing role. There are arguments leaning on the one hand, toward a dilution effect, *i.e.* host species diversity acts as a buffer to pathogen spread (Hantsch *et al.*, 2013; Roscher *et al.*, 2007; Rottsctock *et al.*, 2014; Civitello *et al.*, 2015; Johnson *et al.*, 2015) and, on the other hand, toward an amplification effect (Nguyen *et al.*, 2016; Halliday *et al.*, 2017). In any case, there are striking evidences that not all hosts play an equal part in the disease spread as some species appear to dominate the transmission dynamics of multi-host pathosystems (Streicker *et al.*, 2013; Hantsch *et al.*, 2013; Haydon *et al.*, 2002). For example, some individuals are qualified to be superspreaders that because they are responsible for a large portion of new pathogen transmissions compared to other individuals (Llyod-Smith *et al.*, 2005). This variation of host transmission potential is partly due to between-host processes that influence the potential targets but also to dissimilar within-host processes. Hence, considering host competence in the pathosystem would help decipher these complex epidemiological dynamics.

REFERENCES

Anderson R.M. and May R. M. 1986. The invasion, persistence and spread of infectious diseases within animal and plant communities. Phil. Trans. R. Soc. Lond. B314533–570. http://doi.org/10.1098/rstb.1986.0072.

Baucom, R. S., & De Roode, J. C. (2011). Ecological immunology and tolerance in plants and animals. *Functional Ecology*, *25*(1), 18–28. https://doi.org/10.1111/j.1365-2435.2010.01742.x

Breban, R., Vardavas, R., & Blower, S. (2007). Theory versus data: How to calculate R0? *PLoS ONE*, *2*(3), 0–3. https://doi.org/10.1371/journal.pone.0000282

Civitello, D. J., Cohen, J., Fatima, H., Halstead, N. T., Liriano, J., McMahon, T. A., … Rohr, J. R. (2015). Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(28), 8667–8671. https://doi.org/10.1073/pnas.1506279112

Cortez, M. H., & Duffy, M. A. (2020). Toward a unified dilution effect theory: How competence, competition, and the transmission mechanism influence disease prevalence. *BioRxiv*. https://doi.org/10.1101/2020.01.31.928820

Davidson, J. M., Patterson, H. A., & Rizzo, D. M. (2008). Sources of Inoculum for Phytophthora ramorum in a Redwood Forest . *Phytopathology*, *98*(8), 860–866. https://doi.org/10.1094/phyto-98-8-0860

Desprez-Loustau, M. L., Vitasse, Y., Delzon, S., Capdevielle, X., Marçais, B., & Kremer, A. (2010). Are plant pathogen populations adapted for encounter with their host? A case study of phenological synchrony between oak and an obligate fungal parasite along an altitudinal gradient. *Journal of Evolutionary Biology*, *23*(1), 87–97. https://doi.org/10.1111/j.1420-9101.2009.01881.x

Downs, C. J., Schoenle, L. A., Han, B. A., Harrison, J. F., & Martin, L. B. (2019). Scaling of Host Competence. *Trends in Parasitology*, *35*(3), 182–192. https://doi.org/10.1016/j.pt.2018.12.002

Garbelotto, M., Schmidt, D., & Popenuck, T. (2021). Pathogenicity and infectivity of Phytophthora ramorum vary depending on host species, infected plant part, inoculum potential, pathogen genotype, and temperature. *Plant Pathology*, *70*(2), 287–304. https://doi.org/10.1111/ppa.13297

Gervasi, S. S., Civitello, D. J., Kilvitis, H. J., & Martin, L. B. (2016). *Parasite Dynamics*. *31*(9), 419–425. https://doi.org/10.1016/j.pt.2015.05.002.The

Halliday, F. W., Heckman, R. W., Wilfahrt, P. A., & Mitchell, C. E. (2017). A multivariate test of disease risk reveals conditions leading to disease amplification. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1865). https://doi.org/10.1098/rspb.2017.1340

Halsey, S. (2019). Defuse the dilution effect debate. *Nature Ecology and Evolution*, *3*(2), 145–146. https://doi.org/10.1038/s41559-018-0764-3

Hantsch, L., Braun, U., Scherer-Lorenzen, M., & Bruelheide, H. (2013). Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. *Ecosphere*, *4*(7), 1–12. https://doi.org/10.1890/ES13-00103.1

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: A conceptual and practical challenge. *Emerging Infectious Diseases*, *8*(12), 1468–1473. https://doi.org/10.3201/eid0812.010317

Hill, B. L., & Purcell, A. M. (1997). Populations of Xylella fastidiosa in plants required for transmission by an efficient vector. *Phytopathology*, *87*(12), 1197–1201. https://doi.org/10.1094/PHYTO.1997.87.12.1197

Johnson, P. T. J., Ostfeld, R. S., & Keesing, F. (2015). Frontiers in research on biodiversity and disease. *Ecology Letters*, *18*(10), 1119–1133. https://doi.org/10.1111/ele.12479

Lafferty, K. D., & Wood, C. L. (2013). It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. *Trends in Ecology and Evolution*, *28*(9), 503–504. https://doi.org/10.1016/j.tree.2013.06.012

Laloi, G., Montarry, J., Guibert, M., Andrivon, D., Michot, D., & Le May, C. (2016). Aggressiveness changes in populations of Didymella pinodes over winter and spring pea cropping seasons. *Applied and Environmental Microbiology*, *82*(14), 4330–4339. https://doi.org/10.1128/AEM.00480-16

Lannou, C. (2012). Variation and selection of quantitative traits in plant pathogens. *Annual Review of Phytopathology*, *50*, 319–338. https://doi.org/10.1146/annurev-phyto-081211-173031

Lannou, C., Soubeyrand, S., Frezal, L., & Chadœuf, J. (2008). Autoinfection in wheat leaf rust epidemics. *New Phytologist*, *177*(4), 1001–1011. https://doi.org/10.1111/j.1469-8137.2007.02337.x

Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., & Getz, W. M. (2005). Superspreading and the effect of individual variation on disease emergence. *Nature*, *438*(7066), 355–359. https://doi.org/10.1038/nature04153

Martin, L. B., Addison, B. A., Bean, A. G. D., Buchanan, K. L., Crino, O. L., Eastwood, J. R., … Grogan, L. F. (2019). Extreme Competence: Keystone Hosts of Infections. *Trends in Ecology and Evolution*, *34*(4), 303–314. https://doi.org/10.1016/j.tree.2018.12.009

Martin, L. B., Burgan, S. C., Adelman, J. S., & Gervasi, S. S. (2016). Host competence: An organismal trait to integrate immunology and epidemiology. *Integrative and Comparative Biology*, *56*(6), 1225–1237. https://doi.org/10.1093/icb/icw064

Mundt, C. C. (2009). Importance of autoinfection to the epidemiology of polycyclic foliar disease. *Phytopathology*, *99*(10), 1116–1120. https://doi.org/10.1094/PHYTO-99-10-1116

Nguyen, D., Castagneyrol, B., Bruelheide, H., Bussotti, F., Guyot, V., Jactel, H., … Boberg, J. (2016). Fungal disease incidence along tree diversity gradients depends on latitude in European forests. *Ecology and Evolution*, *6*(8), 2426–2438. https://doi.org/10.1002/ece3.2056

Ostfeld, R. S. (2013). A Candide response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. *Parasitology*, *140*(10), 1196–1198. https://doi.org/10.1017/S0031182013000541

Ostfeld, R. S., & Keesing, F. (2011). Effects of Host Diversity on Infectious Disease. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 157–182. https://doi.org/10.1146/annurev-ecolsys-102710-145022

Pagan, I., & Garcia-Arenal, F. (2020). Tolerance of Plants to Pathogens: A Unifying View. *Annual Review of Phytopathology*, *58*, 77–96. https://doi.org/10.1146/annurev-phyto-010820-012749

Pariaud, B., Van den Berg, F., Van den Bosch, F., Powers, S. J., Kaltz, O., & Lannou, C. (2013). Shared influence of pathogen and host genetics on a trade-off between latent period and spore production capacity in the wheat pathogen, Puccinia triticina. *Evolutionary Applications*, *6*(2), 303–312. https://doi.org/10.1111/eva.12000

Randolph, S. E., & Dobson, A. D. M. (2012). Pangloss revisited: A critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology*, *139*(7), 847–863. https://doi.org/10.1017/S0031182012000200

Rizzo, D. M., & Garbelotto, M. (2003). Sudden Oak Death: Endangering California and Oregon Forest Ecosystems. *Frontiers in Ecology and the Environment*, *1*(4), 197. https://doi.org/10.2307/3868064

Roscher, C., Schumacher, J., Foitzik, O., & Schulze, E. D. (2007). Resistance to rust fungi in Lolium perenne depends on within-species variation and performance of the host species in grasslands of different plant diversity. *Oecologia*, *153*(1), 173–183. https://doi.org/10.1007/s00442-007-0713-3

Rottstock, T., Joshi, J., Kummer, V., & Fischer, M. (2014). Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. *Ecology*, *95*(7), 1907–1917. https://doi.org/10.1890/13-2317.1

Streicker, D. G., Fenton, A., & Pedersen, A. B. (2013). Differential sources of host species heterogeneity influence the transmission and control of multihost parasites. *Ecology Letters*, *16*(8), 975–984. https://doi.org/10.1111/ele.12122

VanderWaal, K. L., & Ezenwa, V. O. (2016). Heterogeneity in pathogen transmission: mechanisms and methodology. *Functional Ecology*, *30*(10), 1606–1622. https://doi.org/10.1111/1365-2435.12645

Wilson, M., & Lindow, S. E. (1994). Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. *Applied and Environmental Microbiology*, *60*(12), 4468–4477. https://doi.org/10.1128/aem.60.12.4468-4477.1994

Woolhouse, MEJ., Dye, C., Etard, J-F., Smith, T., Charlwood, JD., Garnett, P., Hagan, P., Hii, JLK., Ndhlovu, PD., Quinnell, RJ., Watts, CH., Chandiwana, SK., Anderson, R., Woolhouse, M. E. J., Dye, C., Etard, J. F., Smith, T., Charlwood, J. D., … Anderson, R. M. (1997). Heterogeneities in the transmission of infectious agents :implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(January), 338–342. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC19338/pdf/pq000338.pdf

Yang, L. N., Liu, H., Wang, Y. P., Seematti, J., Grenville-Briggs, L. J., Wang, Z., & Zhan, J. (2021). Pathogen-Mediated Stomatal Opening: A Previously Overlooked Pathogenicity Strategy in the Oomycete Pathogen Phytophthora infestans. *Frontiers in Plant Science*, *12*(July), 1–14. https://doi.org/10.3389/fpls.2021.668797

CHAPTER 5

Resistance, tolerance and competence for a root pathogen in six woody species

Received: 31 January 2022 | Accepted: 10 June 2022

DOI: 10.1111/ppa.13608

Plant Pathology WILEY

ORIGINAL ARTICLE

Resistance, tolerance and competence for a root pathogen in six woody species

Marylise Marchand | Tatiana Allery | Marie Massot | Xavier Capdevielle | Cécile Robin

INRAE, Univ. Bordeaux, BIOGECO, Cestas, France

Correspondence
Marylise Marchand, INRAE, Univ. Bordeaux, BIOGECO, F-33610 Cestas, France.
Email: marylisemarchand0@gmail.com

Abstract

The outcome of host–pathogen interactions depends on host defence and pathogen infection strategies. On the host side, two strategies, resistance and tolerance, have long been identified. They differ in their underlying mechanisms and their effects on pathogens. However, the dynamics of disease transmission also rely on host competence. This trait, defined as the ability of the host to allow pathogen transmission, is rarely studied for pathogen-supporting plants. We assessed the competence for *Phytophthora cinnamomi* of seedlings of five *Quercus* and one *Castanea* species and studied how this trait is correlated with resistance and tolerance. *P. cinnamomi* is responsible for the chestnut ink disease, alongside with *Phytophthora* × *cambivora*, which has a much less generalist behaviour. Each seedling was inoculated in minirhizotrons by root–mycelium contact and phenotyped for its resistance, tolerance and competence. The amount of necrosis (lesion length), the effect of infection on plant performance (root growth) and the sporangia number were measured. Moreover, we developed a droplet digital (dd) PCR assay to quantify the pathogen within host tissues near the inoculation point. This measure was used as a proxy of pathogen load. We were thus able to relate individual host competence to the pathogen content measured in the same root fragment. No significant correlation between the phenotyped traits was found at the individual level. However, at the species level, the least competent species (*Q. robur*, *Q. petraea*) proved to be the most resistant. These results may have important implications for management of chestnut and oak ecosystems threatened by ink disease.

KEYWORDS
Castanea sativa, host–pathogen interaction, *Phytophthora* × *cambivora*, *Phytophthora cinnamomi*, *Quercus* species

1 | INTRODUCTION

The outcome of the interaction between a host plant and a pathogen depends on the environment, in all its abiotic and biotic components, and on the plant and pathogen traits. On the host side, defence strategies to a given pathogen are often assumed to involve two different components, resistance and tolerance. These two plant traits both limit the damage caused by the pathogen on the host, but they operate through different physiological pathways (Baucom & de Roode, 2011). Resistance, in a strict definition, is the plant's ability to prevent or to reduce host colonization by the pathogen. Resistance may be total, in the case of incompatible interactions, or partial if some development of the pathogen occurs. In this case, it is assessed by the quantification of damaged tissues, for example, lesion length or by the degree of other symptoms, for example, defoliation. However, the strict definition of resistance would rather suggest a quantification of within-host

pathogen development through, for example, ergosterol (Gunnarsson et al., 1996) or DNA (Engelbrecht et al., 2013).

Multiple definitions and implied meanings of plant tolerance have been developed over time and in different contexts (Burgan et al., 2018; Pagan & Garcia-Arenal, 2020). Tolerance is sometimes confused with a quantitative aspect of resistance and its quantification remains a challenge (Vale et al., 2001). However, disentangling resistance from tolerance mechanisms has paramount epidemiological implications as resistance has a direct negative effect on the pathogen by reducing infection success and development, while tolerance does not. Selective pressures exerted by the host on pathogen fitness may also have evolutionary consequences (Roy & Kirchner, 2000). We define tolerance as the host's ability to reduce the effect of infection on its fitness, regardless of the level of pathogen proliferation (Pagan & Garcia-Arenal, 2018). Tolerance can be assessed by establishing the reaction norm, which describes how the fitness of an individual host varies when faced with an increasing range of pathogen loads (Pagan & Garcia-Arenal, 2018). When the norm is linear (for nonlinear relationships see Simms, 2000), individual tolerance is quantified by its slope and is defined as "range tolerance" (Pagan & Garcia-Arenal, 2020). Assessing range tolerance at the species level through a sufficiently extensive range of pathogen loads and for a large number of genotypes is also often difficult to carry out. Due to these limitations, comparing different genotypes or varieties for their tolerance is often performed by comparing their fitness at a given pathogen load. This tolerance is then referred to as "point tolerance" (Pagan & Garcia-Arenal, 2020).

A key step in the host–pathogen interaction is the transmission of the pathogen. This is often analysed from the pathogen's side and less often, at least in the field of plant pathology, from the host's side. Host competence was first defined in animal ecology and parasitology as the ability of one host to enable pathogen transmission (Gervasi et al., 2016). This host trait is a key parameter to understand disease cycle and epidemiological dynamics, especially for multihost pathogens (Martin et al., 2016). Few studies have specifically addressed the issue of plant competence for pathogens (but see Garbelotto et al., 2021; Liu et al., 2017; Lopes et al., 2010; Rosenthal, Fajardo, & Rizzo, 2021). It is a functional and complex trait, which depends on the host, the pathogen and the environment. We need proxies that can be measured at the host level, even if the competence has a meaning at the community level (Rosenthal, Simler-Williamson, & Rizzo, 2021). Thus, host competence can be assessed through the sporulation potential, or quantity of inoculum, that an individual plant transmits to infect another one. The relationships between individual resistance, tolerance and competence of plants, as well as the intraspecific variation in the latter trait, are poorly documented in the plant pathology literature. One key question to understand epidemiological dynamics of plant pathogens is whether resistant and/or tolerant individuals are also low inoculum producers, that is, not competent. Intuitively, one might think that resistance and competence are negatively correlated, while some tolerant hosts can contribute efficiently to the inoculum production. *Phytophthora ramorum* interactions with various plants showed that different scenarios may arise. Thus, *P. ramorum* epidemics are driven by the frequency of some host plants allowing high sporulation without being significantly affected, whereas some dead-end hosts of the pathogen do not allow its sporulation while being susceptible (Davidson et al., 2008; Garbelotto et al., 2021; Harris & Webber, 2016).

Here, we focus on woody plant defence strategies against a generalist plant pathogen, *Phytophthora cinnamomi*. This oomycete causes significant damage in crops and forests worldwide and is associated with the degradation of ecosystem services and biodiversity in several ecosystems. Originating from Asia, *P. cinnamomi* was introduced all over the world and is listed among the 100 worst invasive alien species of the Global Invasive Species database (Invasive Species Specialist Group, 2011). It is able to infect over 5000 species in 266 genera (Barwell et al., 2020; Hardham, 2005) and among them several tree species such as *Castanea* and *Quercus* species. *P. cinnamomi* is responsible for the chestnut ink disease, alongside *Phytophthora* × *cambivora*, which has a much less generalist behaviour (Barwell et al., 2020). These two hemibiotrophic species infect and kill the root system of their hosts, and depending on the level of resistance, can colonize trunk tissues (Hardham, 2005). Few effective mitigation methods are available against the disease in a forest environment. The most impacted stands are clear-cut and replaced by reforestation with other species, for example, oak species. Although several of them are known to host *P. cinnamomi*, oak species are believed to be more resistant than chestnut trees (Marçais et al., 1996; Maurel et al., 2001; Robin et al., 2001).

Our objective was to evaluate how the competence for *P. cinnamomi* of different oak species and European chestnut is linked to resistance and tolerance. Using an experimental design and seedlings, we measured quantitative variables to assess each of these three phenotypic components of the plant–pathogen interaction. Because host individual competence also depends on the pathogen strain, chestnut inoculation with *P.* × *cambivora* was also carried out to assess the extent to which competence can vary with the pathogen.

2 | MATERIALS AND METHODS

2.1 | Oomycete isolates and culture conditions

Details of all oomycete isolates (and one fungus) used in this study are documented in Table S1. For DNA extraction, the isolates were grown on vegetable juice agar (V8A) plates (100 ml/L filtered vegetable juice [Campbell Grocery products Ltd], 900 ml/L distilled water, 0.1 g/L $CaCO_3$, pH adjusted to 7 and 18 g grade A agar [Becton, Dickinson & Co.]) overlaid with cellophane. For inoculation, we used the most recent isolates in our collection, that is, isolate Phy118 for *P. cinnamomi* and isolate Phy117 for *P.* × *cambivora* (Table S1).

2.2 | DNA extraction

Total fungal DNA was extracted from lyophilized mycelium (20 mg) obtained from those cultures and ground to a fine powder, using

the Invisorb Spin Plant mini kit (Stratec Molecular). For seedlings, several protocols were tested to extract the DNA from the roots and the DNeasy plant mini extraction kit (Qiagen) was selected as it allows a higher final yield (data not shown). DNA concentrations were fluorometrically determined using Quant-it dsDNA assay kit (Life Technologies).

2.3 | Development of a droplet digital PCR assay

To quantify the infection of *P. cinnamomi* and *P.* × *cambivora* in tissues we developed a molecular tool through droplet digital PCR (ddPCR). The ddPCR is a sensitive method that allows fine detection of the pathogen in infected tissues (Zhao et al., 2016). This method performs an absolute quantification of the target DNA, measured in number of copies. As in conventional PCR, target DNA is amplified in the sample to be analysed. This sample is split into 20,000 droplets, within each of which a PCR is performed, making repetitions obsolete and quantification of the target DNA possible (Zhao et al., 2016).

To design the primers and probe for *P. cinnamomi*, we modified the primers developed by Bi et al. (2019) and Liao et al. (2018), targeting the RAS-related protein Ypt1 gene sequence (Table 1). For this aim, sequences of the *Ypt1* genes of *Phytophthora* species were retrieved from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and aligned using Geneious Pro v. 9 (Biomatters Ltd). Melting temperatures, hairpins, self-dimer formation and GC content were assessed with the same software. *P.* × *cambivora* primers and probe were selected from the ddPCR transposition of a quantitative PCR assay designed by Liao et al. (2018) and targeting *Ypt1*. Both primer pair and probe combinations yielded fragments of 90–100 bp.

The ddPCRs were carried out in 22 µl using the ddPCR Supermix for Probes - No dUTP (Bio-Rad). The reaction mix consisted of 1× Probe Supermix, 500 nM of each primer and probe, and 2 µl DNA or ultrapure water in the negative control. The optimal conditions were determined to test the specificity of primer–probes by doing an annealing temperature gradient (59–62°C degree by degree) and a probe and primer concentration gradient. Droplets containing mix/sample were generated using the QX200 droplet generator (Bio-Rad) according to the manufacturer's instructions and transferred to a 96-well PCR plate. PCR amplifications were performed in a thermal cycler (Bio-Rad) using the following conditions: 10 min of initial denaturation at 95°C; followed by 40 cycles of 10 s denaturation at 94°C, 1 min annealing at 60°C; and a final step of inactivation of 10 min at 98°C. Results were analysed on the QuantaSoft software v. 1.7.4 (Bio-Rad). The threshold for detection of positive droplets was set manually at a fluorescence amplitude of 2000 for *P. cinnamomi* and 4000 for *P.* × *cambivora*. Additionally, measurements of single PCR wells were excluded when the total number of droplets was <10,000 and if only a single droplet was detected above the amplitude threshold. The dilution of the DNA extract in the reaction mixture (2 µl in 20 µl) and the final 100 µl elution volume for root samples was taken into account to adjust the final concentration in copies/µl of DNA extract.

2.4 | Tests of specificity and sensitivity of ddPCR assay

Primers and probe specificity was checked by conducting a BLAST analysis against the GenBank database. *P. cinnamomi* primers showed no similarity with sequences of other fungal/oomycete species but a 100% sequence identity with the *Ypt1* region of *P. cinnamomi* species. The assay specificity was also tested using DNA from 13 nontarget *Phytophthora* species, four closely related *Pythium* species and one fungus species (Table S1). We also tested 23 different *P. cinnamomi* isolates and two *P.* × *cambivora* isolates to confirm the specificity of the assay (Table S1).

TABLE 1 Primers and probes tested for the droplet digital PCR assay targeting *Phytophthora cinnamomi* and *P.* × *cambivora*

Type	Name	Sequence	Length (bp)	Source	Target species	Specificity
Forward	PC-F	GGTCCACCATGGCTAAGTT	19	Liao et al. (2018)	*P.* × *cambivora*	–
Reverse	PC-R	CCAGCTCGATCGTGCGAAT	19	Liao et al. (2018)	*P.* × *cambivora*	–
Probe	PC-Pr	CTCCAGGCTGACGTTATCGTGCTCG	25	Liao et al. (2018)	*P.* × *cambivora*	HEX fluorochrome simple dark quencher
Forward	Pcin_F	ATTCGCACGATCGAGCTGG	19	Liao et al. (2018)	*P. cinnamomi*	Modification of the reverse primer Liao et al. (2018) to forward
Reverse	Pcin_R	GTTCAGTCAGCTCCACGAACA	21	Designed for the assay	*P. cinnamomi*	–
Probe	Pcin_Pr	CGTCCTTCGCGGTCCTATTCGCCT	21	Bi et al. (2019)	*P. cinnamomi*	Modified from Bi et al. (2019) reverse, 5′-FAM/ZEN/3′-IBFQ double-quenched probe

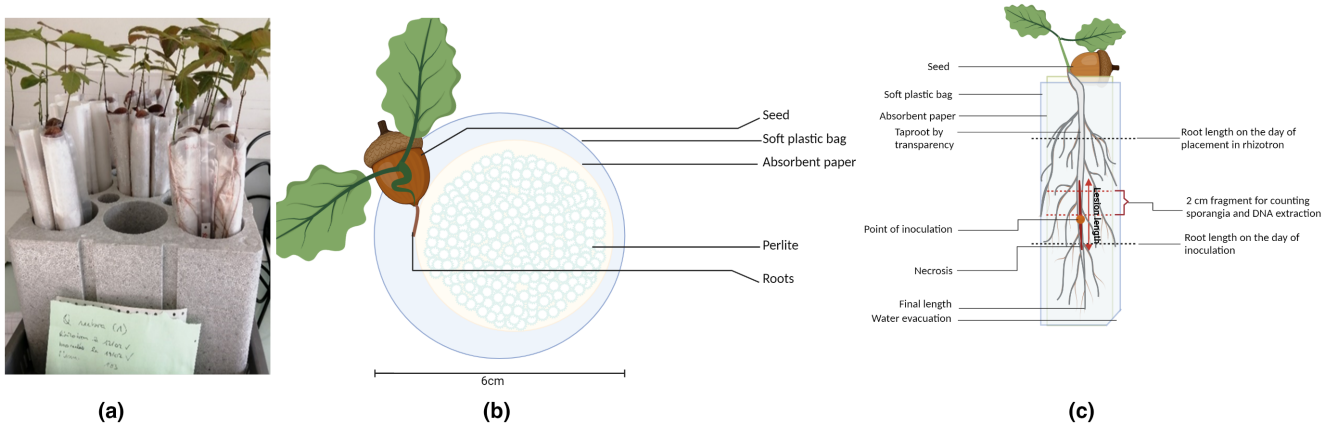


FIGURE 1 Experimental design: (a) rhizotrons placed in one 12-bottle rack; schematic view of a rhizotron, (b) top view, (c) side view.

The sensitivity of the method was tested on serial dilutions of the target mycelium DNA from 1 ng/µl to 1 fg/µl in sterile water and with four replicates for each concentration. The limit of quantification was determined as the lowest dilution showing a coefficient of variation (CV) below 25, which is the usual threshold for acceptance criteria of quantitative methods (Anonymous, 2015). Serial dilutions of target mycelium DNA from 1 ng/µl to 1 fg/µl were also performed in DNA extracts from *Quercus robur*, *Q. petraea*, *Q. ilex*, *Q. suber*, *Q. rubra* and *Castanea sativa* roots, in order to assess the sensitivity of the ddPCR assay for *Phytophthora* quantification in plant tissues.

2.5 | Plant material and seedling inoculation

Seeds of *Q. ilex* (provenance France, coastal dunes), *Q. rubra* (France, east), *Q. robur* (France, south-west), *Q. petraea* (France, east), *Q. suber* (France, south-west) and one sweet chestnut species *C. sativa* (France, north-west) were soaked in water for 2 h then incubated in moist perlite for about 14 days to allow them to germinate. Each germinated seed was placed at the top of a minirhizotron. These rhizotrons were plastic tubes measuring 36 cm in length by 6 cm in diameter, lined with filter paper and filled with perlite. The rhizotrons were inserted in polystyrene 12 bottle racks (Figure 1), which were angled at 40°, with the seeds on the side facing down to encourage the root growth between the plastic and paper and keep them visible and free of perlite (Figure 1). Seedlings were watered daily in order to keep the rhizotrons moist. An incision at the bottom of the tubes was done to prevent water stagnation. The whole experiment was conducted in a climatic chamber with constant conditions (23°C and 70% relative humidity, with a 16/8 h day/night period).

2.6 | Inoculation assay and trait measurements

Seedlings were inoculated after a 7-day growth period in the rhizotrons. The rhizotron plastic was cut and lifted to enable an inoculum plug to be lodged on the root at 1 cm from the apex without any wounding. Thereafter, the rhizotron tube was put back in place and taped. Inoculum plugs (2 mm diameter) were cut from a 7-day-old *Phytophthora* culture grown on V8A or in a sterile V8A dish (for mock inoculations). Sixty plants per species were inoculated with *P. cinnamomi* and 60 additional *C. sativa* seedlings were inoculated with *P.* × *cambivora*. Negative controls were mock inoculated with a sterile V8A plug. Two different species per bottle rack were used and in each individual bottle rack, four inoculated seedlings of the same species were placed as well as one mock-inoculated seedling of the same species. Thus, 60 seedlings were monitored per rack.

The length of the main root of each seedling was measured at three dates: when seedlings were inserted in the rhizotron, 7 days after insertion in the rhizotron (i.e., the day before inoculation) and 10 days after inoculation. Root growth was calculated over the period before inoculation and at the end of experimentation, that is, at 10 days. We evaluated seedling resistance by measuring lesion length (LL in cm) on the feeder root at the end of the experiment. Seedling point tolerance to *Phytophthora* was estimated by a divided difference, called delta (Δ), between pre- and postinfection root growth by lesion length. This trait was not computed for host species with individuals displaying no changes in Δ. To assess sporulation potential, we quantified the number of *Phytophthora* sporangia at the surface of a 2-cm root fragment cut just above the inoculation point (Figure 1). Care was given to remove mycelium and inoculum plugs adhering to the root surface. Each root fragment was placed on a microscope slide, one drop of sterile water was added, and the number of sporangia at the surface was counted using a BH-2 microscope (Olympus). Thereafter, the root fragment was lyophilized before DNA extraction. The DNA content of *P. cinnamomi* and *P.* × *cambivora* was measured (in copies/µl) in this root sample using the ddPCR. Individual host competence was estimated for inoculated seedlings by the ratio of sporulation potential by the pathogen load. This trait was not computed for host species with individuals displaying no sporulation potential.

2.7 | Statistical analysis

Assumptions of the normality of distribution and homoscedasticity of the variables were not satisfied, therefore nonparametric

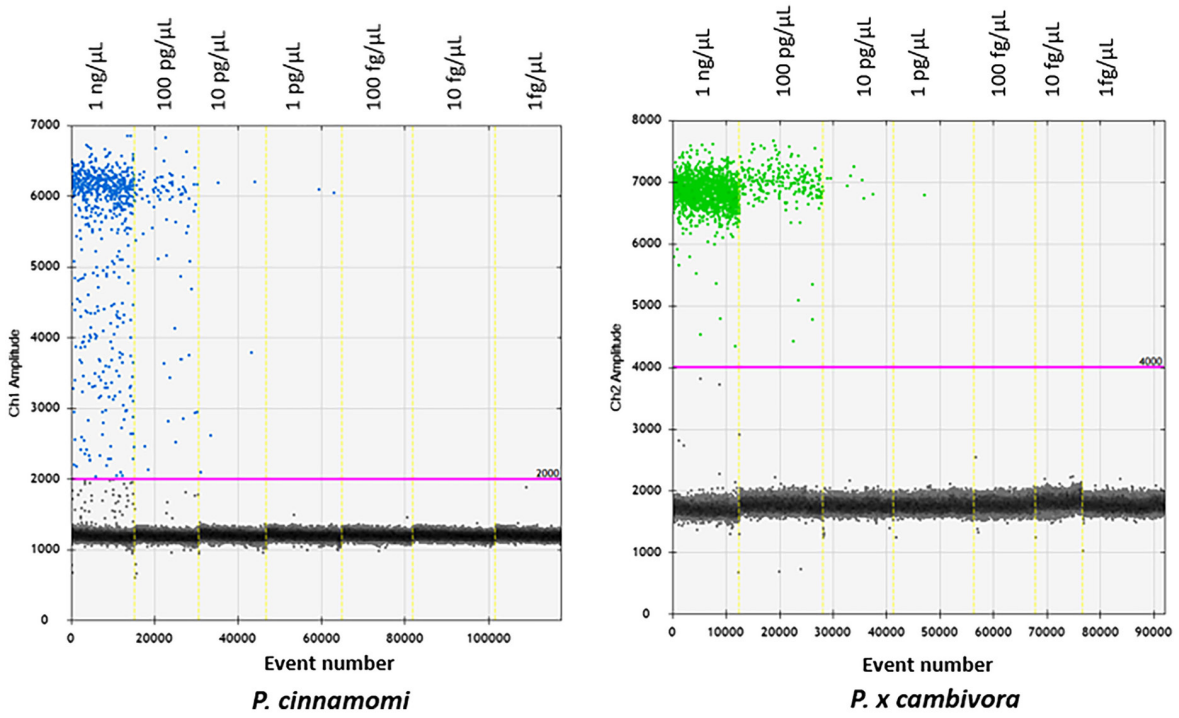


FIGURE 2 Droplet digital PCR (ddPCR) mycelial target DNA of *Phytophthora cinnamomi* (left) and *P.* × *cambivora* (right) diluted from 1 ng/µl to 1 fg/µl. Each point represents a reading from a single droplet indicative positive (above threshold line) or negative (below threshold line) reaction. The threshold for detection of positive results was set at an amplitude of 2000 for *P. cinnamomi* and 4000 for *P.* × *cambivora*.

TABLE 2 Number of *Phytophthora* target copies per reaction as determined by droplet digital PCR (mean and coefficient of variation [CV]) for *Phytophthora cinnamomi* and *P.* × *cambivora* on 6-fold serial dilutions of genomic DNA (four replicates)

	P. cinnamomi		*P.* × *cambivora*	
DNA concentration	Mean (copies/µl)	CV (%)	Mean (copies/µl)	CV (%)
1 ng/µl	1,075,500	8	317,000	7
0.1 ng/µl	98,750	5	30,850	18
10 pg/µl	11,300	15	2050	32
1 pg/µl	1140	28	80	200
100 fg/µl	350	84	0	–
10 fg/µl	0	–	0	–
1 fg/µl	0	–	0	–
0	0	–	0	–

Kruskal–Wallis and Dunn tests were used as alternatives. Correlations were tested with the Spearman correlation coefficient between each pair of traits. Statistical analyses were performed using the R Studio interface of the R software (v. 3.6.3) and ggplot2 (Wickham, 2016), dplyr (Wickham et al., 2018), ggpubr (Wickham, 2016) and Hmisc (Harrell Jr. & Dupont, 2008) packages.

3 | RESULTS

3.1 | Validation of the ddPCR assay

The specificity of *P.* × *cambivora* and *P. cinnamomi* primers and probe was confirmed by the failure to amplify the DNA of all other species tested. With *P. cinnamomi*-specific primers and probe, there was a light amplification signal for *P. megasperma*, but in only a single copy. In further analyses, we considered as positive only samples that displayed more than one copy.

For all runs performed for the sensitivity analysis, there was no amplification in the negative controls. The amplification of *P. cinnamomi* DNA extracted from pure mycelial cultures and diluted in pure water was obtained at concentrations as low as 100 fg/µl (Figure 2) but with high inconsistency (CV > 25%) for concentrations less than 1 pg/µl (Table 2). When *P. cinnamomi* was diluted in the DNA extracts of the different tree species, the detection threshold was still 100 fg/µl. Pairwise comparisons showed that there were almost no differences in *P. cinnamomi* copy number when diluted in water compared to when it was diluted in tree matrices, with the exception of

TABLE 3 Comparison of host interaction traits among *Quercus* host species root inoculated with *Phytophthora cinnamomi* and between *Castanea sativa* inoculated with *P. cinnamomi* or *P.* × *cambivora* (mean values and percentages were calculated for each interaction)

	P. cinnamomi							*P.* × *Cambivora*	
	Q. petraea	*Q. robur*	*Q. suber*	*Q. ilex*	*Q. rubra*	*C. sativa*	Significance[a]	*C. sativa*	Significance[b]
No. of inoculated seedlings	26	31	24	37	41	55		19	
Unsuccessful inoculations (%)[c]	40.0	31.1	21.9	2.6	6.0	4.5	***	21.0	
Symptomless plants (%)	77.4	69.2	0	0	0	0	***	13.3	
Resistance (lesion length, in cm)	1.23	0.92	7.83	9.23	8.78	10.98	***	7.13	
Pathogen load (DNA copies $\times 10^3$/µl)	52.39	63.48	106.20	140.41	140.92	145.74	***	50.80	
Root growth loss (Δ, cm/day)[d]	−0.31	−0.68	−1.34	−0.79	−1.50	−1.35	***	−1.36	ns
Tolerance (Δ/lesion length in cm)	–	–	−0.21	−0.10	−0.18	−0.13	***	−0.16	*
Competent plants (%)	15.4	19.4	75.0	64.9	82.9	92.7	***	6.7	
Sporulation potential[e]	0.92	0.48	4.50	5.19	6.00	13.93	***	0.27	
Competence (sporulation potential/pathogen load)	na	na	0.057	0.048	0.067	0.182	**	na	

Abbreviation: ns, not significant.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

[a]Results of statistical tests performed for *P. cinnamomi* inoculations; χ^2 tests were carried out for comparisons of percentages, Kruskal–Wallis for other variables.

[b]Results of Kruskal–Wallis tests to compare *C. sativa* defence strategies during interaction with *P. cinnamomi* or *P.* × *cambivora*.

[c]Plants unsuccessfully inoculated were discarded for resistance, tolerance and competence assessments.

[d]Difference between pre- and postinfection root growth.

[e]Number of sporangia counted in a 2 cm root fragment proximal to the inoculation point.

Q. suber, which showed significant differences at 1 ng/µl and 100 pg/µl from *C. sativa* and water, respectively (Figure S1).

In water, the amplification of *P.* × *cambivora* was less efficient than for *P. cinnamomi*, as shown by the higher detection threshold (1 pg/µl; Figure 2) and the number of copies (Table 2). However, in the *C. sativa* matrix, amplification was observed at a lower concentration than in water (100 fg/µl; Figure S2). The number of *P. cinnamomi* and *P.* × *cambivora* DNA copies generated by ddPCR for each of the two species was different at each dilution (Dunn tests, $p < 0.05$).

3.2 | Lesion length and quantification of *P. cinnamomi* in root tissues

Ten days after inoculation, lesions, which appeared as discoloured tissues developing from the point of inoculation in both directions, could be observed in 141 of 269 inoculated seedlings, whereas in 88 inoculated and in 92 mock-inoculated seedlings, no root damage could be distinguished after careful inspection. To assess the infection status of these symptomless plants, we used the results of ddPCR analyses run on the 2-cm root fragment cut from each seedling. *P. cinnamomi* DNA was detected in 43 of the 88 inoculated symptomless plants, which confirmed infection success in these seedlings. However, in 12 of 92 mock-inoculated seedlings, *P. cinnamomi* was also detected at a concentration ranging from 300 to 5600 copies/µl. Detection of *P. cinnamomi* in these seedlings might arise through cross-contaminations within the racks where rhizotrons were incubated. Consequently, symptomless inoculated seedlings in which the pathogen content was less than or equal to the maximum detected in mock-inoculated plants of the same species were considered as inoculation failures. The rate of unsuccessful inoculations per species varied from 2.6% (*Q. ilex*) to 40% (*Q. petraea*) (Table 3). Only results of successful inoculations were considered in the following: 77.4% of *Q. robur* and 69.2% of *Q. petraea* seedlings that were inoculated and actually infected were symptomless, that is, they exhibited no root lesion. For these two species, mean lesion lengths were less than 2 cm (1.2 and 0.9 cm, respectively; Table 3) and they displayed significantly lower lesion lengths than any other species (Figure 3a). The mean pathogen content measured in the 2-cm root fragment was <65,000 copies/µl and lower than densities measured in other species (Figure 3b). These two species were considered as resistant to *P. cinnamomi*. In other species, all inoculated seedlings showed symptoms. In *Q. suber*, *Q. rubra* and *Q. ilex* mean infected seedling lesions were 7.8, 8.8 and 9.2 cm, respectively (Table 3). *C. sativa* displayed the greatest lesion lengths with a mean value of 10.9 cm (Table 3). In these four species, *P. cinnamomi* DNA content was similar (Figure 3b) but was not correlated with the lesion length (Figure 4).

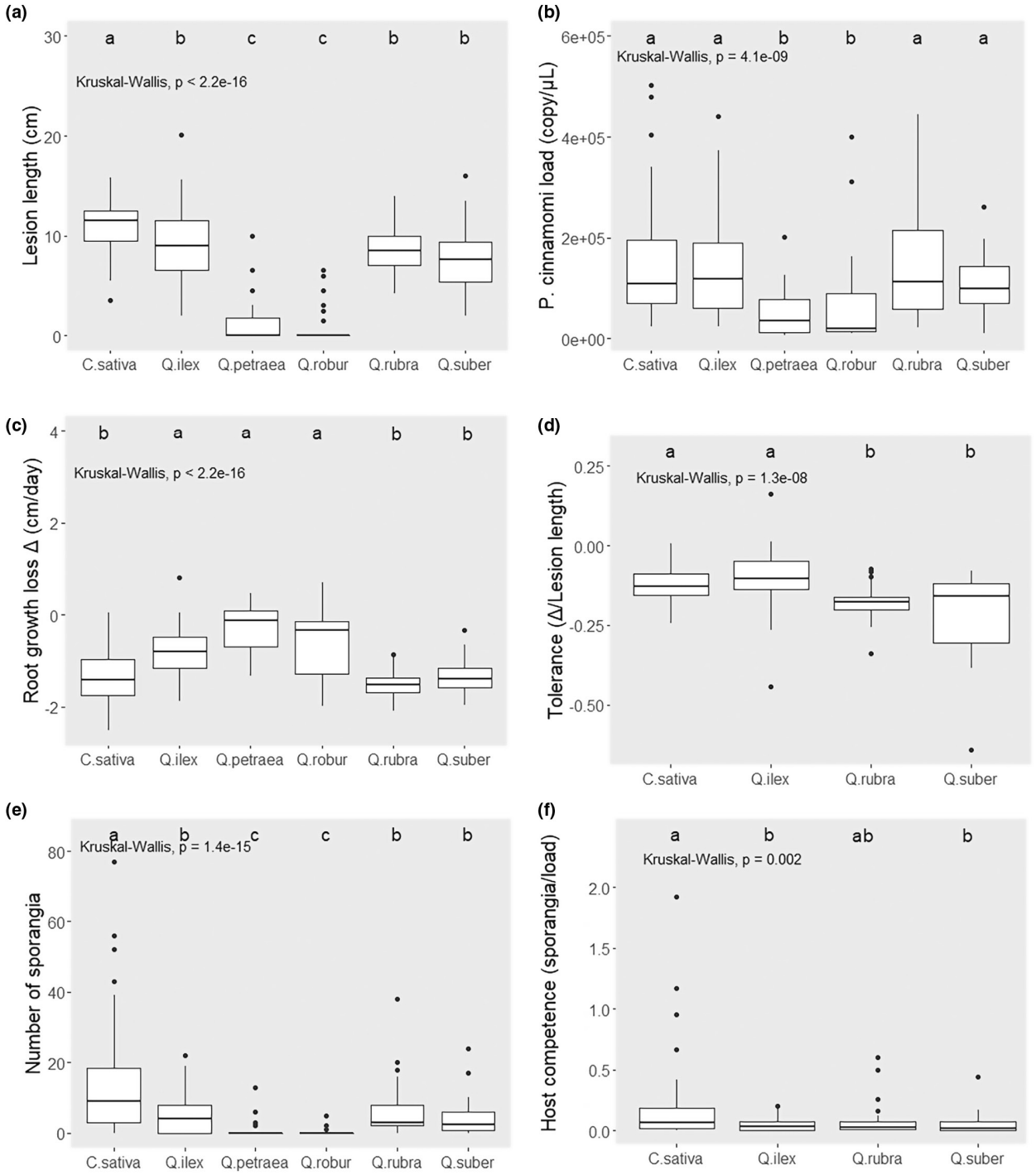


FIGURE 3 Boxplot of lesion length (a), pathogen load (b), root growth loss Δ (c), tolerance (d), number of sporangia (e) and competence (f) for *Castanea sativa* and *Quercus* species inoculated with *Phytophthora cinnamomi* (boxplots display median).

3.3 | Root growth and tolerance to *P. cinnamomi*

Before inoculation, there was no significant difference in root growth between mock-inoculated and inoculated seedlings for each species (Welch comparison, $p > 0.05$). In mock-inoculated plants of all species excepted *Q. suber*, root growth was similar when measured before and at the end of the experiment (Kruskal–Wallis, $p > 0.05$), suggesting that roots of seedlings grew at a constant rate during the experiment. Postinoculation growth of inoculated seedlings was significantly lower than that of mock-inoculated plants (Welch comparisons, $p < 0.05$), except in *Q. petraea* and *Q. robur*, which displayed similar root growth to mock-inoculated plants. These two resistant species showed no reduced fitness due to *P. cinnamomi* infection. Thus, they were highly tolerant to the pathogen. For *C. sativa*, *Q. ilex*, *Q. suber* and *Q. rubra*, the difference between

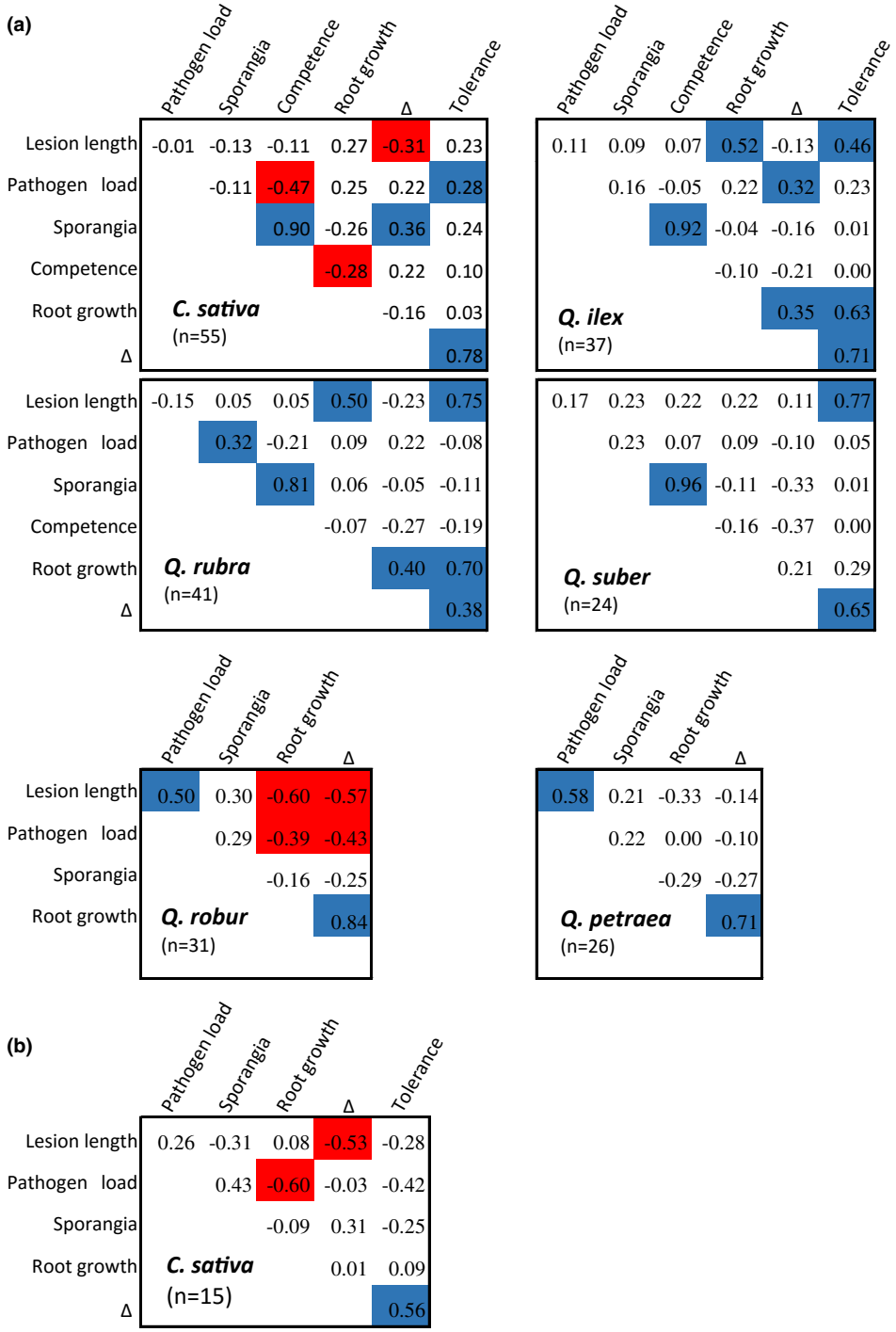


FIGURE 4 Spearman correlation matrix of traits of *Castanea sativa* and *Quercus* spp. in interaction with *Phytophthora cinnamomi* (a) or *P.* × *cambivora* (b). Significant negative correlations ($p < 0.05$) in red and positive in blue. Δ = root growth loss.

pre- and postinfection growth, Δ, was significantly different between mock-inoculated and inoculated seedlings (Welch comparisons, $p > 0.05$). *Q. ilex* displayed a lower root growth loss Δ than the three remaining species (Figure 3c). Point tolerance was then estimated for each inoculated seedlings by the ratio Δ/LL in order to take into account the pathogen load in the root. Both *Q. ilex* and *C. sativa* displayed a similar tolerance significantly higher than that of *Q. rubra* and *Q. suber* (Figure 3d, Table 3). Tolerance was correlated with the resistance estimated through the lesion length for *Q. ilex*, *Q. rubra* and *Q. suber* (Figure 4).

3.4 | Evaluation of host competence for *P. cinnamomi*

Sporangia of *P. cinnamomi* were observed in 137 of the 214 root fragments cut from the inoculated seedlings, in frequencies ranging

from 1 to 77. Only six *Q. robur* and two *Q. petraea* seedlings supported some sporulation, which resulted in percentages of competent seedlings lower than 20% (Table 3). Moreover, fewer than five sporangia were observed in *Q. robur* whereas in *Q. petraea* up to 13 sporangia could be observed in one seedling. Interestingly enough, although symptomless, five of these *Q. robur* and *Q. petraea* seedlings supported sporangia. In *Q. ilex*, *Q. suber* and *Q. rubra*, the percentages of competent seedlings were greater than or equal to 64.9% and the mean number of sporangia was higher than four (Table 3). Fifty-one of 55 inoculated *C. sativa* seedlings supported *P. cinnamomi* sporulation. Out of these, 26 supported more than 10 sporangia. Sporulation potential was higher for *C. sativa* than for any other species and was intermediate for *Q. suber*, *Q. rubra* and *Q. ilex* (Figure 3e). *C. sativa* displayed a higher competence for *P. cinnamomi* than *Q. ilex* and *Q. suber* but not different from *Q. rubra* (Figure 3f). Finally, *Q. rubra* had an intermediate competence for the pathogen to the three other species (Figure 3f).

Within species, sporangia number and competence were not correlated with resistance or tolerance (Figure 4). However, ranking species by the sporulation potential was similar to their ranking by resistance (Figure 3a,e). On the other hand, no such pattern could be deciphered for sporangia number or competence with Δ and tolerance (Figure 3c,d).

3.5 | *C. sativa* defence strategies against *P. × cambivora*

Inoculation of *C. sativa* roots with *P. × cambivora* was unsuccessful for four plants and induced significantly smaller lesions than with *P. cinnamomi* (Kruskal–Wallis, $p = 6.9e^{-5}$, Table 3). The effect of infection on root growth of *C. sativa* seedlings was similar between *Phytophthora* species (Kruskal–Wallis, $p = 0.96$, Table 3). On the other hand, *C. sativa* displayed a higher tolerance to *P. cinnamomi* than to *P. × cambivora* (Table 3, Kruskal–Wallis, $p = 0.034$). Tolerance and resistance were not correlated with each other but pathogen content was negatively correlated with the Δ root growth loss (Figure 4). Only one among the 15 inoculated seedlings with *P. × cambivora* supported sporangia and the average sporulation potential (0.27) was more than 10 times lower than that of *P. cinnamomi*.

4 | DISCUSSION

Our initial aim was to estimate host competence for *P. cinnamomi* in different species, and to study how this trait was related to resistance and tolerance. These three traits either act on the within-host pathogen proliferation (resistance) or may depend on it (tolerance and competence). Thus, quantifying *P. cinnamomi* within-host was required, so we developed a quantification assay based on ddPCR technology. First, we confirmed the specificity of the primers and probes to target *P. cinnamomi* and *P. × cambivora*. Second, the sensitivity of the assay was assessed. Primers and probes allowed detection at 100 fg for *P. cinnamomi* and at 1 pg for *P. × cambivora*. These primers detected *P. cinnamomi* at 0.25 pg in conventional PCR (Bi et al., 2019). This is consistent with the fact that ddPCR is a particularly sensitive method as a similar threshold was found for *P. infestans* using a targeted ddPCR approach (Ristaino et al., 2020). Interestingly, because the *P. cinnamomi* genome is 109.7 Mb (Engelbrecht et al., 2021), the weight of a single copy can be estimated at about 120 fg. Therefore, the ddPCR we developed would be able to detect a single cell of *P. cinnamomi* in extracts of pure DNA. Moreover, the sensitivity of the assay was not altered by the different plant matrices. This confirmed that the ddPCR technology efficiently counteracts inhibitors present in samples (Zhao et al., 2016) and can be used for quantification of *P. cinnamomi* DNA in plant extracts. For *P. × cambivora*, we transposed primers and probes designed for real-time applications to ddPCR (Liao et al., 2018). However, we were not able to obtain the same sensitivity as in the original study (detection threshold of 1 pg in our tests but 20 fg in the original study). This may account for the lower sensitivity of *P. × cambivora* detection than for *P. cinnamomi* observed in our tests.

Each seedling was assessed for its resistance, tolerance and competence through the lesion length, the effect of infection on plant performance (root growth) and the number of sporangia, respectively. Moreover, the within-host content of *P. cinnamomi* DNA near the inoculation point was estimated and used as a proxy for local pathogen load.

Quantification of *P. cinnamomi* DNA in inoculated seedlings showed that its mycelium was present in symptomless roots and thus can grow inside root tissues of *Q. robur* and *Q. petraea* without causing lesions. This endophytic behaviour of *P. cinnamomi* was observed in other studies (Crone et al., 2013). Hüberli et al. (2000) showed that the assessment of disease severity caused by *P. cinnamomi* through underbark lesions measurement was inaccurate for certain host species. Thus, measuring the lesion length may result in a misestimation of seedling resistance. In our assay, the lesion length (10 days after inoculation) was not significantly correlated to DNA content measured in the 2-cm fragment proximal to the inoculation point, whatever the species tested. This may be accounted for by the endophytic behaviour of *P. cinnamomi* as well as its hemibiotrophic lifestyle. The necrotrophic step, which is associated with probable degradation of *P. cinnamomi* DNA, is likely to start at different times after inoculation depending on seedling resistance. Thus, *P. cinnamomi* load in the fragment proximal to the inoculation point is not a good predictor of final lesion length, and then resistance, at the individual level. However, quantifying the pathogen DNA in the whole root with the ddPCR tool we developed might allow an estimation of the individual host resistance, as has been shown for two avocado cultivars (Engelbrecht et al., 2013).

In our trial, *Q. petraea* and *Q. robur* appeared to be the most resistant species to *P. cinnamomi*, both by symptom (percentage of symptomless plants and lesion length) and by pathogen content assessments. Moreover, a high percentage of *Q. robur* and *Q. petraea* seedlings, after exposure to *P. cinnamomi* mycelium, were not

infected by the pathogen. Because it was not possible to discard the possibility of experimental artefacts, we removed these individuals from further analyses as a precaution. At the other end of the spectrum, *C. sativa* showed the lowest resistance and an average pathogen load similar to that of *Q. rubra*, *Q. suber* and *Q. ilex*. These results are in good agreement with previous studies, even if the ranking of the different oak species for their resistance to *P. cinnamomi* differed slightly depending on the inoculation trial and the field observations. *Q. rubra* and *Q. robur* were reported moderately resistant to *P. cinnamomi*, and *Q. ilex* appeared as highly susceptible (Marçais et al., 1996; Maurel et al., 2001; Robin et al., 2001). There are yet to be any reports of *Q. petraea* infection by *P. cinnamomi* in French forests, even in *P. cinnamomi*-infested sites. However, it should be kept in mind that we have deliberately reduced our tests to one *P. cinnamomi* isolate and one provenance per species, although host × *P. cinnamomi* isolate interactions may be significant (Robin & Desprez-Loustau, 1998).

Tolerance was estimated for each seedling as the ratio between the root growth loss Δ, a proxy for plant performance, and lesion length, a proxy for pathogen load. With this indicator, *Q. ilex* and *C. sativa* displayed better tolerance and ability to respond to *P. cinnamomi* than *Q. rubra* and *Q. suber*, while reduction in root growth was lower for *Q. ilex* than for *C. sativa*, *Q. suber* and *Q. rubra*. This is not in line with previous work showing that *Q. suber* was more tolerant than *Q. ilex* due to its ability to compensate for *P. cinnamomi* infection via secondary root development (León et al., 2017). This compensation may not have had an effect on primary root growth during the time of our experiment.

This study is the first to focus on sporulation potential and host competence for *P. cinnamomi* for *C. sativa* and these five *Quercus* species. In our experimental conditions, *Q. robur* and *Q. petraea* exhibited the lowest abilities to support *P. cinnamomi* sporulation. In contrast, *Q. ilex*, *Q. suber* and *Q. rubra* displayed a similar sporulation potential while *C. sativa* had the highest frequency. However, in the same way that tolerance must be studied across a range of pathogen loads, the competence of a host should be characterized at a constant amount of within-host pathogen, or by adjusting the estimate to that amount. Consequently, we assessed host competence as the ability of the host to produce inoculum, that is, sporulation potential, when infected by a given *Phytophthora* load. Using this proxy, *C. sativa* showed a similar competence as *Q. rubra*, but appeared more competent than the two evergreen oak species studied. These results must be confirmed under different experimental protocols. Differences in latency period, that is, lag time between inoculation and first observation of sporangia, were observed for *P. cinnamomi* in different host species (Cahill et al., 1989). Thus, the timing for assessment of sporangial production is certainly a key parameter in the measurement of host competence.

Our results confirm that host competence is a phenotypical trait that, similar to resistance and tolerance, is the outcome of the interaction between the host genotype and the pathogen genotype. In our assay *C. sativa* seedlings appeared less resistant but more tolerant to *P. cinnamomi* than to *P.* × *cambivora*. For resistance, this is in agreement with previous results (Akilli Şimşek et al., 2019). Sporulation potential for *P.* × *cambivora* was lower than for *P. cinnamomi*. Competence for *P.* × *cambivora* was not assessed for the single plant that supported sporulation. Indeed, the pathogen load was hardly comparable between both pathogen species; chestnut seedlings appeared to be much more competent in transmitting *P. cinnamomi* inoculum than *P.* × *cambivora*. Our preliminary results must be confirmed by supplementary studies, which should take into account the timing of host competence assessments as sporangium production varies among *Phytophthora* species (Sarker et al., 2021). Such host differences for competence between both *Phytophthora* species, as well as in differences in pathogen functional traits, are likely to contribute to different epidemiological dynamics observed in chestnut ecosystems.

One main objective of our study was to understand how host competence relates to other traits. Within species, we did not observe a clear pattern between resistance and competence related to a given pathogen load, nor between tolerance and competence. Correlations between host × pathogen interaction traits at the individual level might be nonsignificant due to intraspecific variation of these traits. In particular, the level of *P. cinnamomi* sporulation supported by seedlings was highly variable and was not related with the extent of lesions. A similarly high level of intraspecific variability was also reported for competence for *P. ramorum* (Harris & Webber, 2016). Although the most resistant or tolerant individuals were not always the least competent ones, at the species level, high resistance was significantly associated with low competence. *Q. robur* and *Q. petraea*, the most resistant species, supported on average little sporulation. In contrast, *C. sativa* appeared as the species with the lowest resistance, a moderate tolerance and the highest competence. These results suggest that *Q. robur* and *Q. petraea* would be viable alternatives as replacement species for reforestation of sites invaded by *P. cinnamomi*. Both species, although hosts of *P. cinnamomi*, are resistant enough to establish in sites with residual *P. cinnamomi* inoculum in the soil. Moreover, even if in our experiment *P. cinnamomi* sporulated on a few symptomless *Q. robur* and *Q. petraea* plants, it is highly likely that oak plants mixed with chestnut trees in a forest stand may behave as diluters because they do not transmit a high quantity of *P. cinnamomi* zoospores. However, the risk that some individuals within these species behave as spreaders requires assessment by additional experiments. By contrast, *C. sativa* should be avoided in oak forests. Even though *Q. ilex* is less able to handle *P. cinnamomi* infection than *Q. suber*, mixing both species, which is quite common, has no significant consequence in terms of inoculum dilution. Disease dynamics may also diverge in mixed compared with monospecific forests due to interspecific variation in chemotaxis (Gómez et al., 2020). Identifying which individual host plants may behave as *P. cinnamomi* spreaders or diluters in forest ecosystems is of foremost importance to understand patterns of ink disease transmission. Analysing host and pathogen functional traits appears to be a promising approach to reach this objective.

ACKNOWLEDGEMENTS

Marylise Marchand was financially supported by the Région Nouvelle-Aquitaine (grant number 2018-1R20204 project DIGIE "Chestnut DIeback: vulnerability and GenetIc determinism of ink disease resistance") and by the French Ministry of Agriculture and Forestry (project VISTA). We thank Claude Husson (DSF, French Ministry of Agriculture and Forestry, France) and Jonathan Gaudin (INRAE UMR SAVE, Bordeaux, France) for providing fungal and oomycete isolates and ML Desprez-Loustau for fruitful discussions. ddPCR assays were conducted at the Genome Transcriptome Facility of Bordeaux.

DATA AVAILABILITY STATEMENT

Data used for the article are available in a public repository after revision of the article using the INRAE portal data at @data{6KE0UO_2022, author = {Robin, Cécile}, publisher = {Portail Data INRAE}, title = {{Resistance, tolerance and competence for Phytophthora cinnamomi}}, UNF = {UNF:6:EA0uxOgnZcvoXCZQrC0EgQ==}, year = {2022}, version = {V1}, doi = {10.57745/6KE0UO}, url = {https://doi.org/10.57745/6KE0UO}. The R code is available in File S1.

ORCID

Marylise Marchand https://orcid.org/0000-0001-7717-6205

REFERENCES

Akilli Şimşek, S., Katircioğlu, Y.Z., Ulubaş Serçe, Ç., Çakar, D., Rigling, D. & Maden, S. (2019) *Phytophthora* species associated with dieback of sweet chestnut in western Turkey. *Forest Pathology*, *49*, e12533.

Anonymous, R. (2015). JRC technical report - definition of minimum performance requirements for analytical methods of GMO testing. ISPRA: European Commission, Joint Research Centre; Institute for Health and Consumer Protection.

Barwell, L.J., Perez-Sierra, A., Henricot, B., Harris, A., Burgess, T.I., Hardy, G.E.S.J. et al. (2020) Evolutionary trait-based approaches for predicting future global impacts of plant pathogens in the genus *Phytophthora*. *Journal of Applied Ecology*, *58*, 718–730.

Baucom, R.S. & De Roode, J.C. (2011) Ecological immunology and tolerance in plants and animals. *Functional Ecology*, *25*, 18–28.

Bi, X., Hieno, A., Otsubo, K., Kageyama, K., Liu, G. & Li, M. (2019) A multiplex PCR assay for three pathogenic *Phytophthora* species related to kiwifruit diseases in China. *Journal of General Plant Pathology*, *85*, 12–22.

Burgan, S.C., Gervasi, S.S. & Martin, L.B. (2018) Parasite tolerance and host competence in avian host defense to West Nile virus. *EcoHealth*, *15*, 360–371.

Cahill, D., Legge, N., Grant, B. & Weste, G. (1989) Cellular and histological changes induced by *Phytophthora cinnamomi* in a group of plant species ranging from fully susceptible to fully resistant. *Phytopathology*, *79*, 417–424.

Crone, M., McComb, J.A., O'Brien, P.A., & Hardy, G.E.S.J. (2013) Survival of *Phytophthora cinnamomi* as oospores, stromata and thick-walled chlamydospores in roots of symptomatic and asymptomatic annual and herbaceous perennial plant species. *Fungal Biology*, *117*, 112–123.

Davidson, J.M., Patterson, H.A. & Rizzo, D.M. (2008) Sources of inoculum for *Phytophthora ramorum* in a redwood forest. *Phytopathology*, *98*, 860–866.

Engelbrecht, J., Duong, T.A., Prabhu, S.A., Seedat, M. & van den Berg, N. (2021) Genome of the destructive oomycete *Phytophthora cinnamomi* provides insights into its pathogenicity and adaptive potential. *BMC Genomics*, *22*, 302.

Engelbrecht, J., Duong, T.A. & van den Berg, N. (2013) Development of a nested quantitative real time PCR for detecting *Phytophthora cinnamomi* in *Persea americana* roostocks. *Plant Disease*, *97*, 1012–1017.

Garbelotto, M., Schmidt, D. & Popenuck, T. (2021) Pathogenicity and infectivity of *Phytophthora ramorum* vary depending on host species, infected plant part, inoculum potential, pathogen genotype, and temperature. *Plant Pathology*, *70*, 287–304.

Gervasi, S.S., Civitello, D.J., Kilvitis, H.J. & Martin, L.B. (2016) The context of host competence: a role for plasticity in host–parasite dynamics. *Parasite Dynamics*, *31*, 419–425.

Gómez, M.C., González, M., Gómez-Aparicio, L. & Serrano, M.S. (2020) Coexistent Mediterranean woody species as a driving factor of *Phytophthora cinnamomi* infectivity and survival. *Annals of Applied Biology*, *177*, 41–50.

Gunnarsson, T., Almgren, I., Lyden, P., Ekesson, H., Jansson, H.B., Odham, G. et al. (1996) The use of ergosterol in the pathogenic fungus *Biploaris sorokiniana* for resistance rating of barley cultivars. *European Journal of Plant Pathology*, *20*, 883–889.

Hardham, A.R. (2005) *Phytophthora cinnamomi*. *Molecular Plant Pathology*, *6*, 589–604.

Harrell, Jr. F. E. & Dupont C (2008) Hmisc: Harrell miscellaneous. R package version *3.2*. Available at: https://hbiostat.org/R/Hmisc/ [Accessed 14 June 2022].

Harris, A.R. & Webber, J.F. (2016) Sporulation potential, symptom expression and detection of *Phytophthora ramorum* on larch needles and other foliar hosts. *Plant Pathology*, *65*, 1441–1451.

Hüberli, D., Tommerup, I.C. & Hardy, G.E.S.J. (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology*, *29*, 164–169.

Invasive Species Specialist Group ISSG (2011). *Global Invasive Species Database*. https://doi.org/10.15468/aaobov.

León, I., García, J.J., Fernández, M., Vázquez-Piqué, J. & Tapias, R. (2017) Differences in root growth of *Quercus ilex* and *Quercus suber* seedlings infected with *Phytophthora cinnamomi*. *Silva Fennica*, *51*, 6991.

Liao, F., Zhang, Y., Zhu, L.H., Cao, B., Lv, D., Luo, J.F. et al. (2018) Triplex real-time PCR detection of three quarantine *Phytophthora* pathogens infecting *Malus* Miller. *Journal of Plant Diseases and Protection*, *125*, 325–330.

Liu, X., Lyu, S., Sun, D., Bradshaw, C.J.A. & Zhou, S. (2017) Species decline under nitrogen fertilization increases community-level competence of fungal diseases. *Proceedings of Royal Society B*, *284*, 20162621.

Lopes, J.R.S., Daugherty, M.P. & Almeida, R.P.P. (2010) Strain origin drives virulence and persistence of *Xylella fastidiosa* in alfalfa. *Plant Pathology*, *59*, 963–971.

Marçais, B., Dupuis, F. & Desprez-Loustau, M.L. (1996) Susceptibility of the *Quercus rubra* root system to *Phytophthora cinnamomi*; comparison with chestnut and other oak species. *European Journal of Forest Pathology*, *26*, 133–143.

Martin, L.B., Burgan, S.C., Adelman, J.S. & Gervasi, S.S. (2016) Host competence: an organismal trait to integrate immunology and epidemiology. *Integrative and Comparative Biology*, *56*, 1225–1237.

Maurel, M., Robin, C., Capdevielle, X., Loustau, D. & Desprez-Loustau, M.-L. (2001) Effects of variable root damage caused by *Phytophthora cinnamomi* on water relations of chestnut saplings. *Annals of Forest Science*, *58*, 639–651.

Pagan, I. & Garcia-Arenal, F. (2018) Tolerance to plant pathogens: theory and experimental evidence. *International Journal of Molecular Sciences*, *19*, 810.

Pagan, I. & Garcia-Arenal, F. (2020) Tolerance of plants to pathogens: a unifying view. *Annual Review of Phytopathology*, *58*, 77–96.

Ristaino, J.B., Saville, A.C., Paul, R., Cooper, D.C. & Wei, Q. (2020) Detection of *Phytophthora infestans* by loop-mediated isothermal

amplification, real-time LAMP, and droplet digital PCR. *Plant Disease*, *104*, 708–716.

Robin, C., Capron, G. & Desprez-Loustau, M.L. (2001) Root infection by *Phytophthora cinnamomi* in seedlings of three oak species. *Plant Pathology*, *50*, 708–716.

Robin, C. & Desprez-Loustau, M.-L. (1998) Testing variability in pathogenicity of *Phytophthora cinnamomi*. *European Journal of Plant Pathology*, *104*, 465–475.

Rosenthal, L.M., Fajardo, S.N. & Rizzo, D. (2021) Sporulation potential of *Phytophthora ramorum* differs among common California plant species in the Big Sur region. *Plant Disease*, *105*, 2209–2216.

Rosenthal, L.M., Simler-Williamson, A.B. & Rizzo, D.M. (2021) Community-level prevalence of a forest pathogen, not individual-level disease risk, declines with tree diversity. *Ecology Letters*, *24*, 2477–2489.

Roy, B.A. & Kirchner, J.W. (2000) Evolutionary dynamics of pathogen resistance and tolerance. *Evolution*, *54*, 51–63.

Sarker, S.R., McComb, J., Burgess, T.I. & Hardy, G.E.S.J. (2021) Timing and abundance of sporangia production and zoospore release influences the recovery of different *Phytophthora* species by baiting. *Fungal Biology*, *125*, 477–484.

Simms, E.L. (2000) Defining tolerance as a norm of reaction. *Evolutionary Ecology*, *14*, 563–570.

Vale, F.X.R.D., Parlevliet, J.E. & Zambolim, L. (2001) Concepts in plant disease resistance. *Fitopatologia Brasileira*, *26*, 577–589.

Wickham, H. (2016) *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.

Wickham, H., François, R., Henry, L. & Müller, K. (2018). *Dplyr: A grammar of data manipulation*. R package version 0.7.6. Available at: https://dplyr.tidyverse.org/ [Accessed 14 June 2022].

Zhao, Y., Xia, Q., Yin, Y. & Wang, Z. (2016) Comparison of droplet digital PCR and quantitative PCR assays for quantitative detection of *Xanthomonas citri* subsp. *citri*. *PLoS One*, *11*, e0159004.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Marchand, M., Allery, T., Massot, M., Capdevielle, X. & Robin, C. (2022). Resistance, tolerance and competence for a root pathogen in six woody species. *Plant Pathology*, *00*, 1–12. https://doi.org/10.1111/ppa.13608

DISCUSSION

1. Le caractère invasif de *P. cinnamomi*

L'étude de la distribution des agents pathogènes de l'encre du châtaignier à l'aide du réseau systématique suggère que *P. cinnamomi* est bien établi sur le territoire. De plus, l'espèce était, quasiment systématiquement, la seule détectée dans les échantillons de sol, quel que soit le dispositif. Bien que *P. x cambivora* soit également reconnue comme étant responsable de la maladie de l'encre, elle n'a jamais été identifiée dans nos échantillons. Ceci confirme un phénomène qui se déroule sur l'ensemble du territoire français (C. Robin, communication personnelle). Récemment, il semblerait que dans plusieurs pays européens, *e.g.* en France, en Italie ou en Suisse, les signalements de *P. x cambivora* se fassent de plus en plus rares alors que ceux de *P. cinnamomi* ne cessent de croitre (Simone Prospero et Andrea Vannini, communications personnelles). Ceci serait le signe qu'un phénomène de " sur-invasion " est localement en cours. La sur-invasion est un concept épidémiologique qui décrit le déplacement d'une espèce invasive par une autre partageant des identités fonctionnelles similaires (Russell *et al.*, 2014). Il existe de nombreux cas documentés de ce phénomène, pour des mammifères (Russell *et al.*, 2014) ou les plantes (Rauschert et Shea, 2017) mais aussi les espèces fongiques (Brasier, 2001). L'espèce non-pathogène, à savoir *Ophiostoma ulmi* sur les ormes, a été complètement remplacée par son parent pathogène, *O. novo-ulmi* (Brasier *et al.*, 2001). Lors de cette thèse, j'ai entrepris, en collaboration avec Simone Prospero (WSL, Suisse), d'étudier cette question de la compétition possible de *P. cinnamomi* avec *P. x cambivora* au travers d'essais en serre qui pourrait expliquer ce phénomène de remplacement. Les résultats de cette étude n'ont pas été présentés dans ce manuscrit puisqu'ils sont toujours en cours d'acquisition. Cependant, grâce au chapitre 5, nous avons montré que le châtaignier était moins compétent pour à *P. x cambivora* que *P. cinnamomi*. Ces mécanismes pourraient éventuellement expliquer le fait que nous n'avons quasiment jamais détecté, quantifié ou isolé *P. x cambivora.*

De plus, cette sur-représentation de *P. cinnamomi* confirme son caractère particulièrement invasif. La capacité d'invasion des espèces pathogènes serait notamment déterminée par certains traits qu'elles possèdent, *i.e.* la morphologie des spores qu'elles produisent, leurs optimums de température de croissance, la capacité à faire de la reproduction sexuée et asexuée, leurs résistances au froid (Barwell *et al.*, 2020; McDonald and Linde, 2002; Redondo *et al.*, 2018). Le cycle de vie de *P. cinnamomi* est dominé par la reproduction asexuée, cependant, cette espèce est capable de faire des « selfed » oospores (Crone *et al.*, 2013), des structures qui sont normalement issues de la reproduction sexuée. Ces oospores lui confèrent une grande

capacité de résistance à la dessiccation (Jung *et al.*, 2013) qui permet à l'espèce de rester viable dans le sol sur le long terme ou de façon asymptotique dans les tissus et augmenterait le taux de rencontre et le succès de transmission entre un nouvel hôte et l'agent pathogène (Barwell *et al.*, 2020). Bien que les isolats n'aient pas été typés, il est probable qu'ils soient de mating type A2 qui est présent actuellement en France puisqu'il a été précédemment identifié (Robin et Desprez-Loustau, 1998) et est le plus fréquent des deux mating types à l'échelle mondiale (Kamoun *et al.*, 2015). Bien qu'étant majoritairement clonal (Hüberli *et al.*, 2000; 2001; Dobrowski *et al.*, 2003; Tommerup *et al.*, 2000), *P. cinnamomi* est une espèce qui est parvenue à s'établir avec succès dans de nombreux environnements. La clonalité est une caractéristique assez commune des espèces qui ont connu un succès d'invasion (Caron *et al.*, 2014), on parle du paradoxe de l'invasion biologique. En effet, si la diversité génétique découlant de la reproduction sexuée apporte l'avantage à l'espèce de produire des isolats potentiellement adaptés à un large panel de nouvelles conditions, la clonalité permettrait de faciliter l'établissement des espèces dans de nouveaux environnements par une reproduction rapide à partir d'isolats adaptés (McDonald and Linde, 2002). De façon intéressante, même quand les deux mating types de *P. cinnamomi* sont présents à proximité, il n'y a aucune évidence de reproduction sexuée (Dobrowski *et al.*, 2003). Il est possible que ce type de reproduction ait eu lieu mais que les oospores ne germent pas ou bien que la descendance ne survive pas. Une étude sur l'évolution de populations de levures, a mise en évidence que la reproduction sexuée serait utile pour l'élimination de gènes délétères dans des environnements stables, mais que si l'organisme est en train de s'adapter à de nouveaux environnements, il n'y aurait aucun avantage à la reproduction sexuée (Zeyl and Bell, 1997). Cependant, bien qu'issus de lignée clonale, une diversité génétique peut s'observer chez certains isolats de *P. cinnamomi*, qui aurait pour origine des évènements de recombinaisons mitotiques (Dobrowski *et al.*, 2003).

Ma thèse s'inscrit dans un projet plus large qui vise à collecter des isolats de *P. cinnamomi* sur le territoire français afin d'effectuer une étude de populations. Il a été démontré pour une autre espèce de *Phytophthora, P. plurivora*, que les isolats responsables de lésions seraient plus homogènes génétiquement que ceux strictement présents dans le sol (Beaulieu *et al.*, 2017). Ce travail suggère que les isolats qui provoquent des lésions seraient des clones spécifiquement adaptés et que les populations telluriques pourraient servir de réservoir génétique, permettant à l'espèce de s'adapter aux changements environnementaux et changements d'hôtes (Brasier, 1992). Ainsi, de tels phénomènes observés pour d'autres espèces pourraient être étudié pour *P. cinnamomi*. Une étude de populations de *P. cinnamomi* en Espagne a permis de mettre en

évidence l'existence de deux populations distinctes ayant chacune colonisées deux régions différentes et possédant des optimums de croissances dissimilaires (Caetano *et al.*, 2009). Ainsi, étude des populations de *P. cinnamomi* sur châtaigniers dans les différentes régions de France permettrait de déterminer si une telle adaptation locale aurait pu avoir lieu. De surcroit, puisque *P. cinnamomi* est capable d'infecter d'autres essences en France, *e.g. Quercus rubra*, cette étude de population permettrait de déterminer si une adaptation locale à l'hôte pourrait exister.

2. La dilution de l'inoculum par les hôtes

Une des premières questions de recherche que j'ai voulu aborder est la question de la mesure de l'effet de dilution dans notre pathosystème. Il est d'usage d'étudier soit directement l'effet de la diversité sur les hôtes par le biais de jeunes plants (Gerlach *et al.*, 1997), soit de quantifier cet effet au travers de la charge pathogène sur l'hôte, *e.g.* le nombre de larves (Levi *et al.*, 2016). Dans notre cas, l'effet de la diversité a toujours été quantifié par l'inoculum présent dans le sol et non sur l'hôte (dans les tissus) et ce, en utilisant le métabarcoding. Ce n'est pas la première fois que l'abondance des séquences obtenues par métabarcoding est utilisée comme proxy de l'abondance des pathogènes dans le sol (Liang *et al.*, 2016). Une hypothèse sous-jacente à cette mesure de l'effet de dilution est que l'inoculum dans le sol est responsable de la maladie puisque la quantification de la charge pathogène sur l'hôte est difficile à entreprendre. En effet, il existe un seuil minimal de charge d'inoculum qui conduit à des symptômes racinaires suffisamment important pour provoquer des dommages sur arbres, ici *Q. suber* (Serrano *et al.*, 2015). Ainsi, le pathogène peut être présent sans que sa présence ne soit évidente (Whyte *et al.*, 2016). C'est la raison pour laquelle nous avons étudié l'effet de dilution sur la dissémination du pathogène et non sur les dommages provoqués sur l'hôte. Lors du Chapitre 3, nous avons montré que l'inoculum de *P. cinnamomi* serait dilué lorsque le châtaignier est en mélange avec d'autres essences et notamment le chêne sessile (*Quercus petraea*).

La question du type de transmission de *P. cinnamomi* se pose alors puisqu'une transmission directe fréquence-dépendante (via contact hôte-hôte dépendant de la fréquence de l'hôte infecté), densité-dépendante (via contact hôte-hôte dépendant de la densité de l'hôte infecté) et environnementale (via contact avec une propagule infectieuse) n'ont pas les mêmes conséquences. L'effet de dilution est souvent prédit pour les transmissions fréquence-dépendante (Rudolf et Antonovics, 2005) alors que les transmissions densité-dépendante et environnementale peuvent conduire à une amplification (Begon *et al.*, 1992; Begon et Bowers

1994; Dobson, 2004). Un effet de dilution est attendu lorsque les changements de densité d'hôte sont substituants (la densité totale d'hôtes ne change pas, mais l'identité des hôtes change) et l'amplification est attendue lorsque les changements de densité sont additifs (la densité totale d'hôtes augmente lorsque de nouveaux hôtes sont ajoutés) (Searle *et al.*, 2016). Cependant, certaines études montrent des changements qualitatifs sur ces prédictions lorsque soit les effets de compétitions interspécifiques des hôtes, soit la compétence sont pris en compte (Rudolf et Antonovics, 2005; O'Regan *et al.*, 2015; Roberts et Heesterbeek, 2018). *P. cinnamomi* est capable de transmission hôte-hôte via le contact racine-racine dans des peuplements denses, mais également de transmission environnementale via ses zoospores. Quant à savoir si la transmission hôte-hôte est fréquence-dépendante ou densité-dépendante… Sur le terrain, il semblerait que des paramètres comme la fréquence des hôtes et leurs distances soient déterminants pour la quantité d'inoculum trouvé localement suggérant une transmission plutôt fréquence-dépendante qui pourrait expliquer l'effet de dilution observé. De plus, nous avons démontré expérimentalement que *Q. petreae* n'est pas compétent à *P. cinnamomi* (Chapitre 5) ce qui pourrait expliquer que lorsque le châtaignier est mélangé à cette essence, la quantité globale d'inoculum soit moins importante. La question de la compétence en phytopathologie a été abordée lors du chapitre 4, où nous avons tout d'abord entrepris de poser une définition claire et précise de ce trait. Ce chapitre reste toutefois en cours de réflexion entre les différentes parties prenantes du projet. Nous avons comme objectif *in fine* de réfléchir à l'étude de ce trait comme trait adaptatif, mais également à la façon de l'incorporer dans les modèles épidémiologiques. De plus, lors du chapitre 5, une première réflexion a été initiée quant à la possibilité que la compétence soit un trait différencié de la résistance et la tolérance, mais cet aspect reste encore largement à approfondir.

Il semble important de souligner l'importance du sous-étage dans la transmission du pathogène. C'est une question qui a été brièvement évoquée lors du Chapitre 3 mais qui mériterait de plus amples investigations. En effet, une plus grande mortalité des arbres (chênes) peut être observée dans des peuplements avec sous-bois que sans (Costa *et al.*, 2010), et Moreira et Martins (2005) ont montré que plus de 50% des plantes composant le sous-bois pouvaient être infectées de façon asymptomatique par *P. cinnamomi*. Les sols sous les arbres qui poussent avec une plus grande abondance d'individus co-spécifiques accumulent un plus grand taux de pathogène du sol (Liang *et al.*, 2016). Certaines de ces plantes du sous-bois peuvent être compétentes et d'autres au contraire, non compétentes. La régénération peut jouer un rôle sur la transmission du pathogène, en facilitant la transmission de proche en proche jusqu'à un nouvel arbre adulte

par exemple. Dans le sens inverse, les pathogènes sont connus pour réguler l'établissement des semis. D'un côté, des jeunes semis *Q. suber* traités avec du fongicide (avec du métalaxyl couramment utilisé contre les oomycetes) puis exposés à *P. cinnamomi* présentaient un meilleur taux de survie que des semis non traités (Domininguez-Begines *et al.*, 2020). Ces derniers auraient une biomasse de racines fines bien inférieure à des semis non infectés (Gomez-Aparicio *et al.*, 2017; Homet *et al.*, 2019), ce qui expliquerait ces résultats. D'un autre côté, le taux de survie de semis issus d'arbres infectés (*Q. ilex*) par *P. cinnamomi* a été démontré comme étant plus important que des semis issus d'arbres non infectés par le pathogène (Vivas *et al.*, 2021). De plus, les descendants de châtaigniers infectés ont démontré une meilleure tolérance au pathogène mais impliquerait un tradeoff sur la croissance du semis (Camisón *et al.*, 2019). Les effets de *P. cinnamomi* sur la régénération (et les plantes du sous-bois) et dans le sens inverse, du sous-étage sur la transmission du pathogène encore à explorer.

3. Perspectives

La rapidité et la magnitude des changements climatiques prédits par les scénarios du GIEC (Groupement Intergouvernemental d'experts sur l'Evolution du Climat) sont telles qu'il est probable que les arbres ne soient pas en mesure de modifier leur aire de répartition, phénologie et reproduction, les laissant vulnérables au déclin (Hoffmann et Sgro, 2011). Les changements climatiques on déjà été constatés depuis un siècle en France avec une augmentation de 1°C des températures moyennes et une perturbation des régimes des pluies. L'augmentation de la pluviométrie hivernale attendue pourrait aboutir à une augmentation de la densité de l'inoculum de *P. cinnamomi* dans le sol et les sécheresses estivales prédites par les scénarios climatiques du GIEC pourraient accentuer la sévérité de la maladie. La température hivernale est un autre paramètre déterminant. L'élévation des températures se traduit par une augmentation de la période de végétation, ce qui est favorable aux pathogènes multicycliques comme le sont les *Phytophthora spp.* Par conséquent, cela pourrait conduire à une inflation de la pression de l'inoculum, ce qui leur permet non seulement de dépasser le seuil minimal pour que l'infection s'exprime (Serrano *et al.*, 2015), mais aussi de se propager davantage. D'autre part, le châtaignier est loin d'être le seul hôte de *P. cinnamomi*, les chênes (*Quercus rubra, Q. ilex, Q. suber*) le sont également. Sur *Q. rubra* notamment, *P. cinnamomi* peut survivre dans le tronc et il a été montré qu'une augmentation de 3°C en moyenne conduirait à l'expansion de l'aire de répartition du pathogène en Europe (Marcais *et al.*, 2000). Ainsi, les changements climatiques

pourraient se révéler favorables à la dissémination du pathogène mais encore faut-il connaitre sa distribution actuelle pour déterminer l'impact des changements climatiques sur cette dissémination. Les changements de conditions climatiques pourraient également expliquer les différences de répartition de *P. cinnamomi* et *P. x cambivora* que nous avons mise en évidence. En effet, puisque les deux espèces n'ont pas la même capacité à former des structures de dormance (Vannini *et al.*, 2012; Crone *et al.*, 2013), les effets de la sécheresse pourraient ne pas avoir le même impact. C'est un aspect que nous avons également abordé avec Simone Prospero lors de notre essai en serre qui vise à étudier les mécanismes expliquant le possible phénomène de remplacement de *P. x cambivora* par *P. cinnamomi*.

REFERENCES

Augusta, C., Helena, P., & Manuel, M. (2010). Analysis of spatial patterns of oak decline in cork oak woodlands in Mediterranean conditions. *Annals of Forest Science*, *67*(2), 204.

Barwell, L. J., Perez-Sierra, A., Henricot, B., Harris, A., Burgess, T. I., Hardy, G., … Purse, B. V. (2021). Evolutionary trait-based approaches for predicting future global impacts of plant pathogens in the genus *Phytophthora*. *Journal of Applied Ecology*, *58*(4), 718–730. https://doi.org/10.1111/1365-2664.13820

Beaulieu, J., Ford, B., & Balci, Y. (2017). Genotypic diversity of *Phytophthora cinnamomi* and *P. plurivora* in Maryland's nurseries and Mid-Atlantic forests. *Phytopathology*, *107*(6), 769–776. https://doi.org/10.1094/PHYTO-05-16-0215-R

Begon, M. and Bowers, R. G. (1994). Host-host-pathogen Models and microbial pest control: The effect of host self regulation. *J. theor. Biol*. 169: 275-287

Begon, M., Bowers, R. G., Kadianakis, N., Hodgkinson, D. E., The, S., Naturalist, A., … Hodgkinsont, D. E. (2016). Disease and Community Structure : The Importance of Host Self-Regulation in a Host-Host-Pathogen Model. *University of Chicago Press for American Society of Naturalists DISEASE AND COMMUNITY STRUCTURE : THE IMPORTANCE OF HOST SELF-REGULATION* . *139*(6), 1131–1150.

Brasier, C. M. (2001). Rapid evolution of introduced plant pathogens via interspecific Hybridization. *BioScience*, *51*(2), 123–133. https://doi.org/10.1641/0006-3568(2001)051[0123:REOIPP]2.0.CO;2

Brasier, C. M. (1992). Evolutionary biology of *Phytophthora*. Part I: genetic system, sexuality and the generation of variation. *Annual Review of Phytopathology. Vol. 30*, (277), 153–171. https://doi.org/10.1146/annurev.phyto.30.1.153

Brasier, C. M., & Kirk, S. A. (2001). Comparative aggressiveness of standard and variant hybrid alder *Phytophthoras, Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. *Plant Pathology*, *50*(2), 218–229. https://doi.org/10.1046/j.1365-3059.2001.00553.x

Caetano, P., Ávila A., Sánchez M.E., Trapero A., Coelho A.C. (2009). *Phytophthora cinnamomi* populations on *Quercus* forests from Spain and Portugal E.M. Goheen, S.J. Frankel (Eds.), pp. 261-269 USDA Forest Service General Technical Report PSW-GTR-221, Albany

Camisón, Á., Martín, M. Á., Oliva, J., Elfstrand, M., & Solla, A. (2019). Increased tolerance to *Phytophthora cinnamomi* in offspring of ink-diseased chestnut (Castanea sativa Miller) trees. *Annals of Forest Science*, *76*(4). https://doi.org/10.1007/s13595-019-0898-8

Caron, V., Ede, F. J., & Sunnucks, P. (2014). Unravelling the paradox of loss of genetic variation during invasion: Superclones may explain the success of a clonal invader. *PLoS ONE*, *9*(6). https://doi.org/10.1371/journal.pone.0097744

Crone, M., McComb, J. A., O'Brien, P. A., & Hardy, G. E. S. J. (2013). Survival of *Phytophthora cinnamomi* as oospores, Stromata, And thick-walled chlamydospores in roots of symptomatic and

asymptomatic annual and herbaceous perennial plant species. *Fungal Biology*, *117*(2), 112–123. https://doi.org/10.1016/j.funbio.2012.12.004

Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L., & O'Brien, P. A. (2003). Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. *Phytopathology*, *93*(6), 695–704. https://doi.org/10.1094/PHYTO.2003.93.6.695

Dobson, A. (2004). Population dynamics of pathogens with multiple host species. *American Naturalist*, *164*(5 SUPPL.). https://doi.org/10.1086/424681

Dominguez-Begines J., Avila J. M., Garcia L. V., Gomez-Aparicio L. (2020). Soil-borne pathogens as determinants of regeneration patterns at community level in Mediterranean forests. *New Phytologist*. 227:588-600

Gerlach, J. P., Reich, P. B., Puettmann, K., & Baker, T. (1997). Species, diversity, and density affect tree seedling mortality from *Armillaria* root rot. *Canadian Journal of Forest Research*, *27*(9), 1509–1512. https://doi.org/10.1139/cjfr-27-9-1509

Gomez-Aparicio L., Dominguez-Begines J., Kardol P., Avila J. M., Ibanez B. and Garcia L. V. , J. P., Reich, P. B., Puettmann, K., & Baker, T. (2017). Plant-soil feedbacks in declining forests: implications for species coexistence. *Ecology*. 98(7): 1908-1921.

Hoffmann, A. A., & Sgró, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Homet, P., González, M., Matías, L., Godoy, O., Pérez-Ramos, I. M., García, L. V., & Gómez-Aparicio, L. (2019). Exploring interactive effects of climate change and exotic pathogens on *Quercus suber* performance: Damage caused by *Phytophthora cinnamomi* varies across contrasting scenarios of soil moisture. *Agricultural and Forest Meteorology*, *276–277*(May), 107605. https://doi.org/10.1016/j.agrformet.2019.06.004

Hüberli, D., Tommerup, I. C., & Hardy, G. E. S. J. (2000). False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology*, *29*(3), 164–169. https://doi.org/10.1071/AP00029

Hüberli, D., Tommerup, I. C., Dobrowolski, M. P., Calver, M. C., & Hardy, G. E. (2001). Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research*, *105*(9), 1053–1064. https://doi.org/10.1016/S0953-7562(08)61967-X

Jung, T., Colquhoun, I. J., & Hardy, G. E. S. J. (2013). New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia. *Forest Pathology*, *43*(4), 266–288. https://doi.org/10.1111/efp.12025

Levi, T., Keesing, F., Holt, R. D., Barfield, M., & Ostfeld, R. S. (2016). Quantifying dilution and amplification in a community of hosts for tick-borne pathogens. *Ecological Applications*, *26*(2), 484–498. https://doi.org/10.1890/15-0122

Liang, M., Liu, X., Gilbert, G. S., Zheng, Y., Luo, S., Huang, F., & Yu, S. (2016). Adult trees cause density-dependent mortality in conspecific seedlings by regulating the frequency of pathogenic soil fungi. *Ecology Letters*, *19*(12), 1448–1456. https://doi.org/10.1111/ele.12694

Marcais B., Douhot-Delduc L., Le Tacon F. (2000). Effets possibles des changements globaux sur les micro-organismes symbiotiques et pathogènes et les insectes ravageurs des forêts. *Revue Forestière Française*, (sp), 99. https://doi.org/10.4267/2042/5409

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, *40*, 349–379. https://doi.org/10.1146/annurev.phyto.40.120501.101443

Moreira, A. C., & Martins, J. M. S. (2005). Influence of site factors on the impact of *Phytophthora cinnamomi* in cork oak stands in Portugal. *Forest Pathology*, *35*(3), 145–162. https://doi.org/10.1111/j.1439-0329.2005.00397.x

O'Regan, S. M., Vinson, J. E., & Park, A. W. (2015). Interspecific contact and competition may affect the strength and direction of disease-diversity relationships for directly transmitted microparasites. *American Naturalist*, *186*(4), 480–494. https://doi.org/10.1086/682721

Rauschert, E. S. J., & Shea, K. (2017). Competition between similar invasive species: modeling invasional interference across a landscape. *Population Ecology*, *59*(1), 79–88. https://doi.org/10.1007/s10144-016-0569-7

Redondo, M. A., Boberg, J., Stenlid, J., & Oliva, J. (2018). Contrasting distribution patterns between aquatic and terrestrial *Phytophthora* species along a climatic gradient are linked to functional traits. *ISME Journal*, *12*(12), 2967–2980. https://doi.org/10.1038/s41396-018-0229-3

Roberts, M. G., & Heesterbeek, J. A. P. (2018). Quantifying the dilution effect for models in ecological epidemiology. *Journal of the Royal Society Interface*, *15*(140). https://doi.org/10.1098/rsif.2017.0791

Robin, C., & Desprez-Loustau, M. L. (1998). Testing variability in pathogenicity of *Phytophthora cinnamomi*. *European Journal of Plant Pathology*, *104*(5), 465–475. https://doi.org/10.1023/A:1008649806620

Rudolf, V. H. W., & Antonovics, J. (2005). Species coexistence and pathogens with frequency-dependent transmission. *American Naturalist*, *166*(1), 112–118. https://doi.org/10.1086/430674

Russell, J. C., Sataruddin, N. S., & Heard, A. D. (2014). Over-invasion by functionally equivalent invasive species. *Ecology*, *95*(8), 2268–2276. https://doi.org/10.1890/13-1672.1

Searle, C. L., Cortez, M. H., Hunsberger, K. K., Grippi, D. C., Oleksy, I. A., Shaw, C. L., … Duffy, M. A. (2016). Population density, not host competence, drives patterns of disease in an invaded community. *American Naturalist*, *188*(5), 554–566. https://doi.org/10.1086/688402

Serrano M. S., Rios P., Gonzalez M. and Sanchez M. E. (2015). Experimental minimum threshold for *Phytophthora cinnamomi* root disease expression on *Quercus suber*. *Phytopathologia Mediterranea.* 13-16.

Tommerup, I. C., Dobrowolski, M. P., Hüberli D., O'Brien P. A., Hardy G. E. St. J. and Shearer, B. L. (1999). Risks and Threats Due To Genetic Variation in *Phytophthora cinnamomi* for disease management in natural vegetation ecosystems. In: First International Meeting on Phytophthoras in Forest and Wildland Ecosystems - *Phytophthora* Diseases of Forest Trees, IUFRO Working Party 7.02.09, August 30 – September 3, 1999, Grants Pass, Oregon USA pp. 55-63.

Vannini, A., Breccia, M., Bruni, N., Tomassini, A., & Vettraino, A. M. (2012). Behaviour and survival of *Phytophthora cambivora* inoculum in soil-like substrate under different water regimes. *Forest Pathology*, *42*(5), 362–370. https://doi.org/10.1111/j.1439-0329.2012.00768.x

Vivas, M., Hernández, J., Corcobado, T., Cubera, E., & Solla, A. (2021). Transgenerational induction of resistance to *Phytophthora cinnamomi* in holm oak. *Forests*, *12*(1), 1–13. https://doi.org/10.3390/f12010100

Whyte, G., Howard, K., Hardy, G. E. S. J., & Burgess, T. I. (2016). The Tree Decline Recovery Seesaw; a conceptual model of the decline and recovery of drought stressed plantation trees. *Forest Ecology and Management*, *370*, 102–113. https://doi.org/10.1016/j.foreco.2016.03.041

Zeyl, C., & Bell, G. (1997). The advantage of sex in evolving yeast populations. *Nature*, *388*(6641), 465–468. https://doi.org/10.1038/41312