SIAMOIS

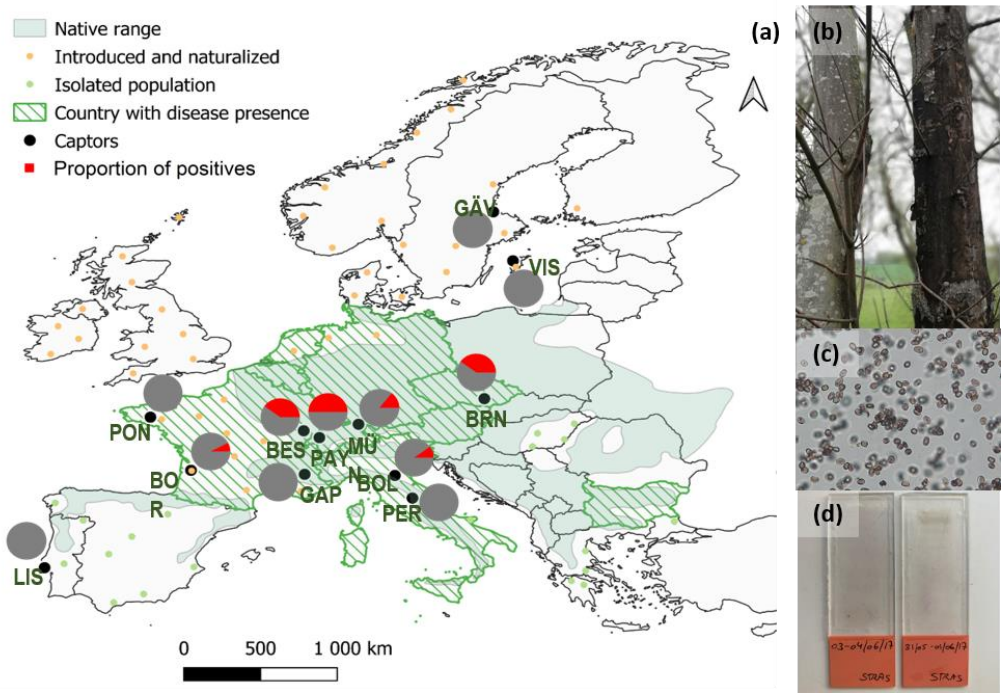

Figure 1. *Proportion of positive aerobiological samples for Cryptostroma corticale (May to September 2018, n = 10) from different European captors following the natural distribution area of its host (Acer pseudoplatanus) and the disease distribution (a); diseases maple tree by C. corticale (b); spores of C. corticale; and microscopic slides from aerobiological samples (d).*

Smart and Innovative monitoring of airborne fungal invaders by molecular methods

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Work package: *WP2*

Context — Emergent and invasive diseases are an increasing threat to forest ecosystems. On the one hand, diseases can emerge following the introduction of exotic pathogens to new environments. On the other hand, pathogens can expand or change their distribution through climate-driven processes. In both cases, epidemiological surveillance is crucial, as forest disease management largely relies on prevention. There is a need to improve surveillance to cover large spatial scales and to detect pathogens before diseases are established. We suggest the use of aerobiological networks which monitor pollen levels in many cities in Europe to monitor forest pathogens and early detect quarantine pathogens.

Objectives — We aimed at (*i*) exploring the potential of pollen aerobiological networks to monitor the spatial and temporal dynamic of forest fungal diseases (such as *Hymenoscyphus fraxineus and Cryptostroma corticale* and species of the *Heterobasidion annosum sensu lato)*, in France and Europe; and (*ii*) evaluating the capacity of these networks to detect quarantine and/or regulated pathogens at low frequencies, such as *Melampsora medusae* or *Phytophthora lateralis*.

Approaches — The starting material consisted of microscopic slides (Fig 1d) used by the pollen aerobiological network. DNA was extracted and analysed by real-time PCR (qPCR) targeting specific pathogens. To assess the spatio-temporal dynamic of forest fungal pathogens at a large scale, we performed two types of studies for each for the four selected pathogens (objective *i*): (1) regional studies in France, where detailed epidemiological data on those pathogens were available by the French Forest Health Department; and (2) continental studies in Europe, including a total of 12 aerobiological captors from six countries within a large longitudinal and latitudinal range (Italy, Czech Republic, Switzerland, Sweden, France and Portugal). The regional studies included a selection of captors across the French territory following a gradient of disease presence, and covering 2 to 4 years, depending on the availability of slides and the pathogen distribution. The continental studies allowed to align the aerobiological data with the presence-absence of the specific pathogen in each country. Finally, to assess the capacity to detect quarantine pathogens by the aerobiological networks, we selected captors close to the records of those pathogens in France, and we tested the digital droplet PCR to increase the capacity to detect potentially low rates of quarantine pathogens.

Key results — (presented as separated bullet points)

- We detected *H. fraxineus* spores in all the captors located within the diseased area. Out of the diseased area, spores were detected within a distance of ca. 300 km from the disease front. In the continental study, the number of *H. fraxineus* spores reached a peak at 10 years of disease presence.
- Spores of *C. corticale* decreased with distance to the disease outbreak, with averages of 12 (8-20, 90% confidence interval) and 3 spores (1-8, 90% confidence interval) at 10 and 310 km, respectively.
- Aerobiology of *C. corticale* followed the disease distribution in Europe, with high detection in Central Europe (host native area and disease presence) and absence of spores in Portugal and Sweden where the disease is not present yet (Fig 1a).
- We failed to detect *M. medusae* and *P. lateralis* near the disease records, probably due to the low presence for the former, and to the predominance of soilborne (over aerial) propagules for the latter.
- ddPCR did not improve our detection rates for *P. lateralis* and the *H. annosum* species complex.

Main conclusions including key points of discussion —

- Aerobiological surveillance is promising for diseases driven by airborne pathogens, such as *H. fraxineus, C. corticale,* and *Melampsora larici-populina*.
- Aerobiological surveillance can inform the temporal dynamic of an invasive disease with the peak of spores paralleling the peak of host mortality (ash dieback disease).
- Invasive pathogens can be detected out of the already-invaded area (up to 300 km from the disease front, and before the onset of symptoms) by aerobiology, being a great tool to monitor disease progression in a large geographical area.
- Single copy genes are not appropriate for pathogen detection in aerobiological samples, while qPCR tests based on the ITS region yielded good results. In this sense, we were able to detect *H. annosum* s.l. (ITS-based test), but not any of the four species of this complex present in Europe (monocopy-gene-based test), which limits the use of aerobiology to study the epidemiology of the Annosum root rot disease.

Perspectives — DNA extracts are further used for the development of a rapid molecular diagnostics protocol using third generation high throughput sequencing MinIon (work in progress).

Valorization — Two scientific publications are in preparation: One on the aerobiology of *C. corticale*, to be submitted to a special issue in the journal Neobiota; another one on the aerobiology of *H. fraxineus*.

Leveraging effect of the project— The SIAMOIS project is linked to the European project HOMED. The results of both projects are going to be published together. A policy brief for the European Union is in preparation to guide and drive the future use of pollen aerobiological European networks as a surveillance tool in forest pathology.