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Smell the Disease - Developing Rapid, High-throughput and Non-destructive Screening Methods for Early Detection of Alien Invasive Forest Pathogens and Pests Featuring Next-Generation Technologies

Ida Nordström[1,*], Patrick Sherwood[1], Björn Bohman[1], Julio Javier Diez[2], Michelle Cleary[1,**]

[1] Swedish University of Agricultural Sciences, Alnarp, Sweden [2] University of Valladolid, Palencia, Spain * : ida.nordstrom@slu.se **: michelle.cleary@slu.se

Abstract

Global forests are increasingly threatened by alien invasive pathogens and pests. The magnitude of this threat is expected to further increase in the future, due to the warmer climate and more extensive global transports and trade of plants. Pests and pathogens are often introduced to new areas by trade with ornamental plants as intermediate hosts, and there is a great need to modernize the tools for detection of alien species in imported plants and in monitoring of those that are already established in our forests. To achieve this goal, research in forest pathology is focused on combining recent technological advances in robotics, next generation sequencing, and mass spectroscopic methods with knowledge about the specific metabolic responses in the pests and pathogens and the trees that they infest. Gas Chromatography (GC) Analysis of Volatile Organic Compounds (VOCs) adsorbed on Solid Phase Micro-Extraction (SPME) fibers is one promising method with potential for high-throughput detection of larger plant shipments. By the establishment of a library of chemical fingerprints characterizing specific pests and pathogens, one could non-destructively scan a large number of plants in ports or nurseries to eliminate presence of disease. The species-specific combination of VOCs can be utilized to prevent introduction of harmful pests and pathogens to new markets. One pathogen considered as a quarantine species and a serious threat on-the-horizon for coniferous forests is Pine Pitch Canker (PPC), a fungal pathogen affecting a variety of pine species with devastating economical and biological consequences, especially if it were to be established in a country like Sweden where about 38% of the standing forest volume consist of pine. Pathogens like this one are already introduced in several European countries and need to be monitored and identified early to prevent further forest damage – a challenge that Forest pathologists have accepted.

Methods

VOCs emitted during I) *in vitro* fungal growth and II) *in vivo* plant infection were analyzed to distinguish unique chemical signatures of *F. circinatum*. Sampling took place at 3 infection stages; asymptomatic (7D), mild (14D) and severe symptoms (28D) using SPME fibers and thermal desorption in GC-MS.

I) in vitro

- Four *Fusarium* species: *F. circinatum, F. bulbicola, F. oxysporum spp. pini* and *F. graminearum*
- Sterile inoculation with inserted plugs
- Grown on a defined media in sealed headspace vials

II) in vivo

- 1-year-old pine seedlings
- *Three Pinus species: P. radiata (susceptible), P. sylvestris (intermediate)* and *P. pinea (resistant)*
- *A*rtificial stem- and soil inoculation with *F. circinatum spore solution*
- Static headspace sampling

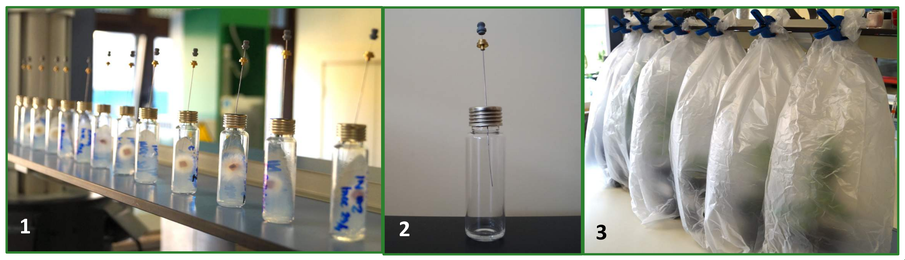

Fig. 1. 1 & 2: *In vitro* experimental setup; fungal cultures grown on EMA media and sampled with SPME through the permeable septa. 3: *In vivo* experimental setup; seedlings enclosed in autoclave bags using SPME fibers. Photo: Ida Nordström

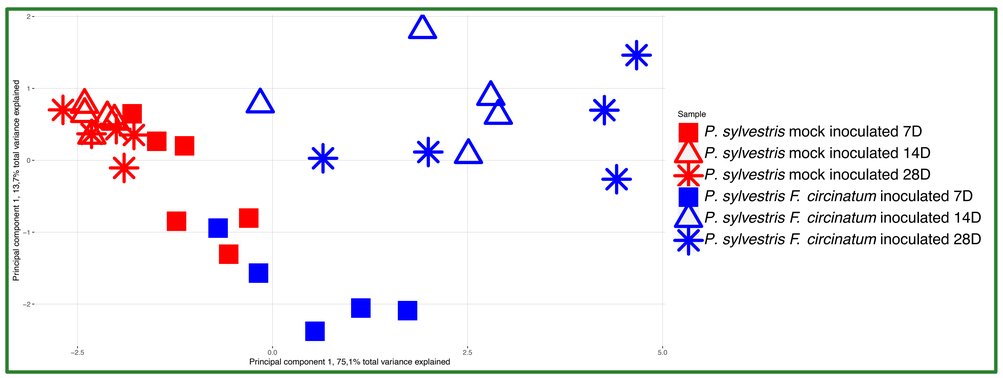

Fig. 2. PCA plot of stem inoculated *Pinus sylvestris* based on 7 VOCs. The *F. circinatum* inoculated seedlings diverge from the mock inoculated seedlings some time before the first symptoms are visible.

Results

Preliminary analysis of chromatograms suggests that the four *Fusarium* species are distinguishable based solely on *in vitro* VOC profiles. *F. circinatum* inoculated seedlings were distinguishable through PCA based on 7 VOCs (*Fig. 2*), tentatively identified as different kinds of monoterpenes and alcohols. *P. radiata* and *P. sylvestris* were symptomatic within about 2 weeks following inoculation by *F. circinatum*, while *P. pinea* remained asymptomatic during the observation period (4 months). The soil inoculation did not result in any symptoms in any of the pine species, the presence of *F. circinatum* in seedling roots is yet to be confirmed. Further data analysis regarding both *in vitro* and *in vivo* results is currently ongoing.

Fig. 3. 1 year-old *P. sylvestris* seedlings inoculated on stem with PDB (1) and *F. circinatum* spore solution (2, 3) 28 days post inoculation. The Pine Pitch Canker affected seedlings show characteristic symptoms such as wilting and yellowing needles – a fast onset of severe symptoms. Photo: Ida Nordström

Conclusion & future applications

- *Fusarium* spp. are difficult to identify by morphology or sequencing, but their **VOC profiles provide a rapid and inexpensive method of distinguishment.**
- The Spanish provenance of ***P. sylvestris*** **used in this study suggests similar susceptibility to *F. circinatum as P. radiata***
  - Previous literature suggests intermediate susceptibility of this species **[1, 2, 3]**. Supporting previous studies, *P. radiata* proved susceptible and *P. pinea* resistant **[1].**
- As little as **7 VOCs sufficed to differentiate between the *F. circinatum*** **and mock inoculated *P. sylvestris*** **seedlings**
- Given the results presented in this study, **VOC analysis can complement other novel techniques like NGS sequencing in pathogen detection**.

Funders

FORMAS, Kungliga Skogs- och Lantbruks-Akademien: C.F. Lundströms stiftelse, SLU: Fonden för skogsvetenskaplig forskning, ERASMUS+, Anna-Britta & Vadim Söderström and Nordgen

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