Identification of VOCs emitted by Rhododendron or Larch infected with different *Phytophthora* species.

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Introduction

Every day, plant material is entering and moving throughout Europe harboring plant pathogens invisible to the unaided eye. Pathogens threaten plant health and productivity as their European host plants are often poorly adapted to these invasive species. Routine plant inspections with high accuracy and throughput are needed to limit this threat. In the HEU project **Purpest**, we are developing handheld sensors of volatile organic compounds (VOCs) emitted by infected plants to support phytosanitary measures (see box below).

Figure 1 A) *Rhododendron repens* 'Red carpet' B) Larch (*Larix kaempferi*).

The oomycete genus '*Phytophthora*' holds some of the most destructive plant pathogens worldwide. The objective of our study was to determine if the VOC profiles from rhododendron (*R. repens*) and larch (*Larix kaempferi*) infected with different *Phytophthora* species could be used to classify the plant-pathogens into their respective groups correctly.

Materials and methods

Rhododendron (*R. repens*) 'Red Carpet' (Fig. 1A) and larch (*Larix kaempferi*) (Fig. 1B) were inoculated by adding inoculum to the substrate and flooding it to enable zoospore production and infections. Inoculation preparation is described in Jung et al. 1996. Flooding was repeated after 3, 5, 7 and 9 wk or until plants died. Control and infected plants were enclosed in oven bags and connected with a head space collection device (Flusys) to ensure that a coal filtered air stream entered the bag, pass over the plant and then through a Tenax filter tube (Fig. 2) at 0,6L/min for 3 hours to collect the VOCs.

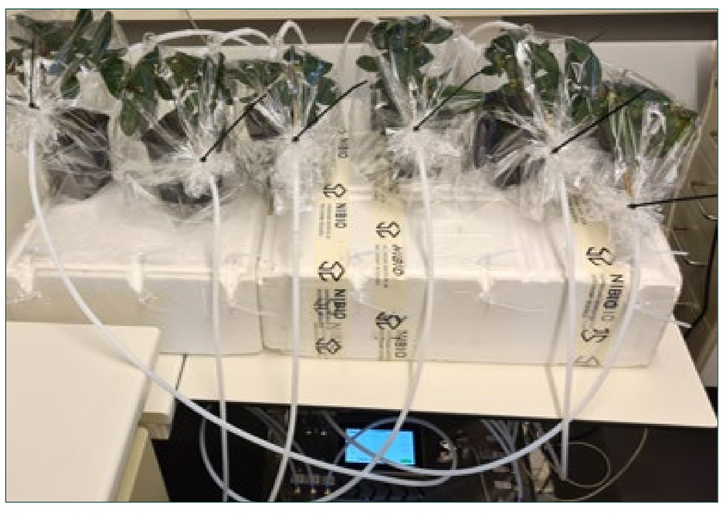

Figure 2 Head space collection from *Rhododendron repens* plants.

Tenax tubes were desorbed on a Markes TD 100-xr Automated Thermal Desorber, and compounds separated and detected with an Agilent 8890 GC- 5977B MS system.

The column was a 30m HP 5MS-UI with 0.25mm inner diameter and 0.25 µm film thickness. Agilent Mass Hunter Unknown Analysis software (version 10.2) was used for compound identification, which includes deconvolution, search by NIST23 library (match factor >90) and Retention Indexes (Kovats). We used VOC data collected 7 and 12 wk after inoculation for rhododendron and larch, respectively. VOCs associated with infected plants at least in four out of five replicates, and absent from the control, were selected for discriminant analysis. The discriminant analysis was run on a subset of data from 5 rhododendron- and 4 larch-pathogen combinations. Host plant, *Phytophthora* species and isolate codes are given in Table 1. Successful inoculation was confirmed by re-isolating the pathogens through soil baiting or root isolation.

Results

In our study, we identified 12 VOCs that were associated with rhododendron-pathogen infections and 10 VOCs associated with larch-pathogen infections. Rhododendron and larch infected with different isolates emitted distinct VOC profiles. However, some of the same VOCs appeared in several plant-pathogen combinations. In larch trees infected either with *P. ramorum* lineages NP2 or IC1, α, α-dimethyl Benzenemethanol was emitted (see Table 1)

Table 1 Host plants, pathogen species, codes and VOCs associated with host-pathogen combinations.

Host plant	Pathogen species	Isolate Code	VOCs found
Rhododendron	*P. ramorum* NP2	RP2	4,5-dimethyl-Nonane
			1-(2-butoxyethoxy)-Ethanol,
			Benzonitrile
Larch	*P. ramorum* NP2	RP2	**α, α-dimethyl Benzenemethanol**
Rhododendron	*P. ramorum* IC1	RI1	Phenylethylalcohol
			dimethyl-Silanediol
			Undecane
Larch	*P. ramorum* IC1	RI1	**α, α-dimethyl Benzenemethanol**
			Benzene
			Nonanal
			Trichlormethane
			3-methyl-Tridecane
Rhododendron	*P. ×cambivora*	CAM	Copaene
Larch	*P. ×cambivora*	CAM	1,2-difluoro-4-(trifluoromethyl)-Benzene,
			1-methyl-4-(1-methylethyl)-1,3-Cyclohexadiene,
Rhododendron	*P. plurivora*	PLU	Methylal,
			Benzylalcohol
Larch	*P. plurivora*	PLU	1,3-dimethyl-Benzene
Rhododendron	*P. cinnamomi*	CIN	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
			4,6-dimethyl-Dodecane
			1,1'-(1,4-phenylene)bis-Ethanone
Larch	*Phytopythium litorale*	PL	Dodecane
			4,6-dimethyl-Dodecane
			Hexadecane
Larch	*P. cactorum*	CAC	**1-methyl-4-(1-methylethyl)-1,3-Cyclohexadiene,**
			α, α-dimethyl Benzenemethanol

Larch infected with *P. ×cambivora* or *P. cactorum* both emitted 1-methyl-4-(1-methylethyl)-1,3-Cyclohexadiene. Rhododendron plants infected with *P. cinnamomi,* and larch infected with *Pp. litorale* both produced 4,6-dimethyl-Dodecane. (see Table 1).

Discriminant analysis of a VOC data set from rhododendron plants infected with *P.×cambivora, P. cinnamomi, P. plurivora*, *P. pseudosyringae* and *P. ramorum* NP2 using 4,5-dimethyl-Nonane; 1-(2-butoxyethoxy)-Ethanol; Benzonitrile; Phenylethylalcohol; dimethyl-Silanediol; Undecane; Copaene; Benzylalcohol; 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester; 4,6-dimethyl-Dodecane and 1,1'-(1,4-phenylene)bis-Ethanone as predictors, grouped the host-pathogen combinations correctly in 72% of the cases (Table 2A).

Table 2A Classification summary for rhododendron-pathogen combinations based on selected VOCs.

	Contr-Rho	CAM-Rho	CIN-Rho	PLU-Rho	PSE-Rho	RP2-Rho
Contr-Rho	5	0	0	0	0	1
CAM-Rho	0	3	0	0	0	1
CIN-Rho	0	1	4	0	0	0
PLU-Rho	0	1	0	5	0	0
PSE-Rho	0	0	0	0	4	0
RP2-Rho	1	0	1	0	0	3
Total N	6	5	5	5	5	5
N correct	5	3	4	5	4	3
Proportion	0,833	0,600	0,800	1,000	0,800	0,600

Discriminant analysis of a VOC data set from larch plants infected with *P. cinnamomi, Pp. litorale, P. ramorum* NP2, and *P. ramorum* IC1, using α, α-dimethyl Benzenemethanol; Benzene, Nonanal, Trichlormethane, 3-methyl-Tridecane; 1,2-difluoro-4-(trifluoromethyl)- Benzene; 1-methyl-4-(1-methylethyl)- 1,3-Cyclohexadiene, 1,3-dimethyl-Benzene, Dodecane, 4,6-dimethyl-Dodecane; Hexadecane as predictors, grouped the host-pathogen combinations correctly in 96% of the tested cases (Table 2B).

Table 2B Classification summary for larch-pathogen combinations based on selected VOCs.

	Contr-LAR	CIN-Lar	PL-Lar	RI1-Lar	RP2-Lar
Contr-Lar	6	0	0	0	0
CIN-Lar	0	5	0	0	0
PL-Lar	0	0	5	1	0
RI1-Lar	0	0	0	4	0
RP2-Lar	0	0	0	0	4
Total N	6	5	5	5	4
N correct	6	5	5	4	4
Proportion	1,000	1,000	1,000	0,800	1,000

Discussion

Our study showed that VOCs could be used to identify different plant-pathogen combinations. However, accuracy was too low for reliable detection in rhododendron, and we will need to test the identified VOCs on a larger data set to confirm our results. We also need to confirm compound identity to make sure some of the VOC candidates are not background contamination. As VOC production can be highly variable depending on the plant's physiological status, we will repeat the experiments to further confirm the reliability of our results.

PurPest is a multidisciplinary project funded by the European Union under their Horizon Europe program. The goal of **Purpest** is to develop a sensor platform that can detect plant pathogens on plant material, facilitating phytosanitary control during import.

Jung, T.; Blaschke, H.; Neumann, P. 1996. Isolation, identification and pathogenicity of Phytophthora species from declining oak stands. European Journal of Forest Pathology, 26.5: 253-272.

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