Identification of VOCs associated with plant pathogens grown in pure culture

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Introduction

Plant pathogens can severely reduce plant health and productivity. Their detection, assessment and management is critical for sustainable agriculture and balanced ecosystems. All living organisms emit volatile organic compounds (VOCs) constitutively and in response to changes in their physio-chemical environment. As these responses can be highly specific, they could serve as biomarkers to detect and identify different species under varying environmental conditions. The goal of our current study was to determine the VOC profiles of 49 plant pathogenic oomycetes, including 40 *Phytophthora* species, and two fungal plant pathogens in pure culture **to test if the VOC profiles could be used for species identification.** We focused especially on *Phytophthora ramorum,* a pathogen that is attacking multiple forest and horticultural plants. This quarantine pathogen typically geso under the radar of the current plant health system's physical controls .

Materials and Methods

The names of the 51 tested species, their respective clades and codes are given in Table 1. Each isolate was grown in clarified V8 agar until the mycelium covered a 9 cm-petri dish (Fig.1). Two Petri dishes were placed in one oven bag and securely sealed before VOC collection. VOCs were collected on Tenax filters over 3 hours using a head space collection device from Flusys with a constant airflow of 0,6 l/min. We collected VOCs from 5 replicates in parallel with a non-infected petri dish containing only clarified V8 agar (control) in each run. We collected VOCs from one isolate per species, except for *P. ramorum* (12 lineages/isolates) and *Phytopythium vexans* (two isolates).

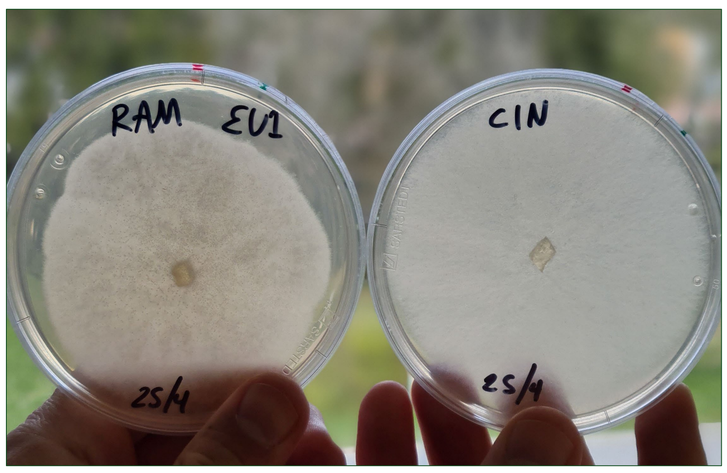

Figure 1 *Phytophthora ramorum* and *P. cinnemomi* on clarified V8 agar in 9cm petri dishes.

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). VOC's associated with one or more of the different isolates in at least four out of five replicates, and absent in the control were selected for principal component analysis (PCA). We classified each species into its respective group based on discriminant analysis, using a subset of VOCs as predictors. The 12 different *P. ramorum* lineages were classified into their different lineages group based on the same subgroup of VOCs.

Results

We found 22 VOCs that were associated with one or several of the isolates tested. Based on the PCA, we selected 20 VOCs to classify the species into their respective groups.

Table 1 Tested isolates with their respective clades, species names, codes, and percentage correct species classification (%) based on discriminant analysis selecting 20 informative VOCs.

Clade	Species	Code	Correct group (%)
1	*P. nicotianae*	NIC	100
1a	*P. ×serendipita*	SER	100
1a	*P. cactorum*	CAC	80
1c	*P. infestans*	INF	100
2a	*P. meadii*	MEA	100
2a	*P. occultans*	OCC	100
2a	*P. citrophthora*	CIP	100
2b	*P. siskiyouensis*	SIS	100
2b	*P. tropicalis*	TRO	100
2c	*P. multivora*	MUL	100
2c	*P. plurivora*	PLU	100
2d	*P. furcata*	FUR	100
2e	*P. elongata*	ELO	100
3	*P. pseudosyringae*	PSE	100
4	*P. palmivora*	PAL	100
4	*P. quercetorum*	QUT	100
5	*P. castaneae*	CAS	100
6a	*P. inundata*	INU	80
6b	*P. chlamydospora*	CHL	100
6b	*P. crassamura*	CRA	100
6c	*P. asparagi*	ASP	100
7a	*P. rubi*	RUB	100
7a	*P. ×alni*	ALN	100
7a	*P. ×cambivora*	CAM	80
7b	*P. variabilis*	VAR	100
7c	*P. cinnamomi*	CIN	100
7c	*P. parvispora*	PAR	100
7d	*P. nagai*	NAG	100
8a	*P. pseudocryptogea*	PSC	100
8c	*P. lateralis*	LAT	100
8c	*P. foliorum*	FOL	100
8c	*P. hibernalis*	HIB	100
8c	*P. ramorum*	EU-IC-NA-NP	96.7
8d	*P. syringae*	SYR	100
9a1	*P. hydropathica*	HYD	100
9b	*P. constricta*	CON	100
10c	*P. kernoviae*	KER	100
10b	*P. gallica*	GAL	100
11	*P. lilii*	LIL	100
12	*P. quercina*	QUE	100
	Phytopythium citrinum	PC	100
	Pp. litorale	PL	100
	Pp. vexans	Pva/b	100
	Pythium myriotylum	PM	100
	Py. kashmirense	PK	100
	Halophytophthora avicennae	HA	100
	Nothophytophthora amphigynosa	NA	100
	N. intricata	NI	100
	Elongisporangium anandrum	EA	100
	Fusarium oxysporum	FO	100
	Rhizoctonia solani	RS	100

Using the following VOCs as predictors, we grouped 96.9% of the isolates correctly (see Table 1): (R)-(+)-3-Methylcyclopentanone; 2,3-Butanedione; 2,3-Pentanedione; 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-; 1-methoxy-2-Propanol; 3,5-di-tert-Butyl-4-hydroxybenzaldehyde; 2-methyl-3-Hexanol; 3-Hexen-2-one; 3-Octanol; 2-methyl-6-Hepten-1-ol; Acetoin; 4-hydroxy- Butanoic acid; Ethylbenzene; 2-methoxy- Furan; Methyl salicylate; p-Cymen-9-ol; 2-methoxy-Phenol; 4-ethyl-2-methoxy-Phenol; Phenylethyl Alcohol and Styrene.

The only species not correctly classified for all replicates, were *P. cactorum* (one out of five classified as *P. ×serendipita), P. inundata* (one out of five classified as *P. constricta*), *P. ramorum* (two out of 60 classified as *P. variablis),* and *P. ×cambivora* (one out of five classified as *P. constricta*).

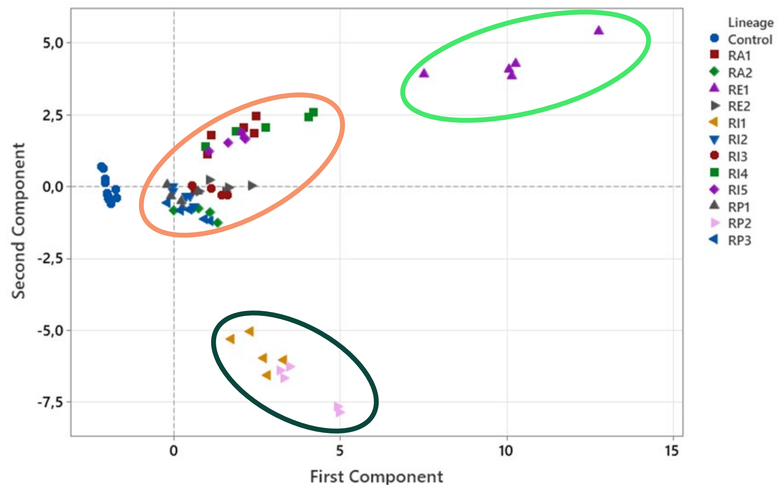

Figure 2 Score plot of PCA for 12 different lineages of *P. ramorum*, using 20 VOCs as variables

Using the same VOCs as predictors, we correctly classified each of the different *P. ramorum* isolates into their respective lineage group. The Score plot of the PCA showed that *P. ramorum* lineages EU1; IC3 and NP2; and EU2, IC2, IC2, IC4, IC5, NA1, NA2, NP1 and NP3 fell into 3 distinct groups (Fig.2).

Discussion

Our study showed that each of the tested isolates was associated with distinct VOC profiles that could be used to classify each isolate into their correct species group. One or two samples of four species could not be correctly classified, indicating that some of the species-specific VOC profiles were very similiar. As our dataset was dominated by oomycetes of the genus *Phytophthora*, this is not surprising. However, even though some of the related species produced similar VOC profiles, VOC analysis could be used to distinguish between the 12 different lineages of *P. ramorum* and classify them into their respective lineage group. All isolates in pure culture emitted distinct VOC profiles, but VOC profiles emitted by the pathogens in different media, under different growth conditions or on their host plant might be very different, as VOC production changes with the pathogen's metabolism. In planta experiments are needed to check the validity our results from pure culture.

Summary:

- VOCs from 63 isolates, including 49 oomycete and two fungal pathogens were analyzed
- VOC profile distinct for each isolate.
- 20 VOCs used to discriminate between different species and classify them in their respective groups
- Correct classification of species was 96.9%
- The 12 lineages of *P. ramorum* were correctly classified based on 20 selected VOCs.

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