Received: 12 November 2019 | Revised: 30 April 2020 | Accepted: 21 May 2020

DOI: 10.1111/efp.12618

Forest Pathology WILEY-BLACKWELL WILEY

ORIGINAL ARTICLE

Volatile organic compounds (VOC) as biomarkers for detection of *Ceratocystis platani*

Federico Brilli[1] | **Nicola Luchi**[1] | **Marco Michelozzi**[2] | **Luca Calamai**[3] | **Gabriele Cencetti**[2] | **Francesco Pecori**[1] | **Emanuele Nigrone**[1,3] | **Alberto Santini**[1]

[1]Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Sesto Fiorentino, Italy

[2]Institute of Biosciences and Bioresources (IBBR), National Research Council of Italy (CNR), Sesto Fiorentino, Italy

[3]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Firenze, Italy

Correspondence
Federico Brilli and Nicola Luchi, Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy, (CNR), Via Madonna del Piano 10, Sesto Fiorentino, 50019, Italy.
Emails: federico.brilli@ipsp.cnr.it; nicola.luchi@ipsp.cnr.it

Funding information
European Commission H2020 Research and Innovation Programme 'HOlistic Management of Emerging forest pests and Diseases' (HOMED), Grant/Award Number: 771271

Abstract

Ceratocystis platani causes canker stain of plane trees, and it represents a serious disease of *Platanus* spp. both in the United States and Europe. Current chemical or biological controls do not effectively manage *C. platani*, so new preventive methods need to be developed in order to limit this pathogen spreading. In this work, we have characterized the main volatile organic compounds (VOC) emitted in vitro from pure cultures of *C. platani* and other common pathogenic fungal species of hosts plants growing in the same ecosystems as plane trees. We found that *C. platani* emitted a similar blend of VOC compared with phylogenetically similar species *C. populicola*. In particular, *C. platani* was characterized by emission of isoamyl acetate and isobutyl acetate while *C. populicola* by ethyl acetate and isobutyl acetate, which were not released by any of the other out-group fungal species grown on the same medium. Moreover, following a targeted approach based on the main VOC found in vitro, we have successfully validated in vivo that VOC uniquely emitted by *C. platani* (i.e. isobutyl acetate along with isoamyl alcohol) were released from the bark of plane trees following *C. platani* inoculation. Our results highlight the possibility to exploit VOC emitted specifically by *C. platani* as biomarkers to recognize *Platanus x acerifolia* plants infected by this pathogen.

KEYWORDS

biomarkers, canker stain, platanus, disease type, wilt, volatile organic compounds

1 | INTRODUCTION

Over the last decades, the globalization of markets facilitates the free circulation of goods across continents, including plant material, but inevitably favours the spreading of plant pathogens into larger geographical areas where new hosts may be found. *Ceratocystis platani* is a quarantine organism causing canker stain of plane trees, a lethal disease of *Platanus* spp. In Europe, both Oriental plane (*Platanus orientalis* L.), a native species with south eastern range, and London plane (*Platanus* × *acerifolia* (Ait.) Willd.), one of the most widely used ornamental species world-wide, are seriously threatened by the invasive fungal pathogen *Ceratocystis*

The peer review history for this article is available at https://publons.com/publon/10.1111/efp.12618

*Associate Editor: Michelle Cleary

platani (Walter) Engelbr. &T.C. Harr., the causal agent of canker stain disease (CSD) of plane trees. *C. platani* is considered to be native to North America (Engelbrecht, Harrington, Steimel, & Capretti, 2004; Walter, Rex, & Schreiber, 1952) and was unintentionally introduced into Europe during World War II (Cristinzio, Marziano, & Vernau, 1973; Panconesi, 1999) where it continues to spread clonally (Ocasio-Morales, Tsopelas, & Harrington, 2007; Santini & Capretti, 2000) towards the natural range of *P. orientalis* (Lehtijärvi et al., 2017; Tsopelas, Santini, Wingfield, & de Beer, 2017). *C. platani* can be naturally transmitted either via root anastomosis or through river water containing infectious propagules of the fungus, but the most important means of dispersal are non-sanitary cultural practises when dealing with infected wood fragment arising from human activities. In fact, contaminated pruning and cutting tools are the main pathway of both long- and short-distance dispersal, especially in urban areas. In addition, terracing machinery used in *C. platani* infected areas can also play a role, by transporting infected wood fragment and soil (Panconesi, 1999; Tsopelas et al., 2017) and contaminated sawdust can infect freshly wounded plane trees (Luchi, Ghelardini, Belbahri, Quartier, & Santini, 2013).

C. platani can be transported long distances, even overseas, on wood used for packaging and with infected nursery stock (Tsopelas et al., 2017). Infected young plants show symptoms within a few weeks after infection and die rapidly, but during winter months *C. platani* grows slowly within plant tissues (Pilotti, Lumia, Di Lernia, & Brunetti, 2012). Disease symptoms are less evident in wintertime, since plane trees are deciduous and thus infected nursery stock can escape the attention of inspections. As there is currently no effective chemical or biological control against *C. platani*, preventive measures are paramount to contain this fungal pathogen. Therefore, non-invasive methodologies need to be developed for the rapid detection of *C. platani* infected plane trees. So far, different molecular assays able to detect even very low quantities of *C. platani*, as real-time PCR (Polymerase Chain Reaction) and LAMP (Loop Mediated Isothermal Amplification), have been set up (Aglietti et al., 2019; Luchi et al., 2013; Pilotti et al., 2012). These methods lead to both reliable and accurate results even in the absence of symptoms on the infected plants. In particular, real-time PCR showed a detection limit as low as 2 fg/µl, although it is time consuming and it needs to be performed in the laboratory. On the other hand, the LAMP assay is more rapid than PCR (an outcome can be achieved in <1 hr) and it could be performed in the field still achieving reliable results (detection limit = 0.02 pg/µl). However, since phytosanitary services have to deal with a large and increasing number of plants to be inspected at ports of entry, spending one hour to run a single analysis still remains a time constrain. Moreover, these molecular tools can sample just a few plants among an entire consignment. Therefore, finding a technique that allows to screen large plant consignment in a short period of time may effectively help to speed up the inspection work, thus avoiding the introduction of new plant pathogens.

A peculiarity of *Ceratocystis* spp. is the emission of volatile organic compound(s) (VOC) which resemble the fruity aroma of banana (Lanza, Hwee Ko, & Palmer, 1976). In this work, we have first investigated in vitro the possibility to differentiate *C. platani* among other fungal species commonly associated with hosts plants growing in the same ecosystems as plane trees through the emission of specific VOC; then, we verified in vivo whether these "targeted" VOC could be detected in plane trees inoculated with *C. platani*.

2 | MATERIALS AND METHODS

2.1 | Fungal strains

Four different *Ceratocystis platani* fungal strains obtained from infected *Platanus* sp. trees in Italy, six additional fungal species phylogenetically related to *C. platani* and five out-group species were used in this study (Table 1). These fungal species were selected because they are all pathogens commonly associated with host plants growing in the same environments in which plane trees are either planted or naturally grown. Each fungal strain was grown as pure culture at 21°C on three different 90 mm Petri dishes containing 1.5% potato dextrose agar (PDA; Difco). After 10 days, from each one of these three Petri dishes a sample of the strain was subcultured in different sterilized 20 ml glass vials (Perkin Elmer, USA) containing 7 ml 1.5% PDA for a total of three vials per strain (1 Petri dish = 1 vial); each vial was sampled only once in order to have three replicates for each one of the selected fungal strains. All the vials were gas-tight sealed with caps and incubated at 21°C for 4 weeks in the dark.

2.2 | Plant material and fungal inoculation

In early spring 2019, 10 five-year-old potted plane trees (*Platanus* x *acerifolia*) were used for this study. Five plants were inoculated with the *C. platani* strain and the other five used as control plants (mock-inoculation). Stem inoculations with *C. platani* were carried out by using a 20-day-old fungal culture (isolate Cp23, Bibbona) growing on 1.5% potato dextrose agar (PDA; Difco) at 21°C. A disk of bark and phloem of plane tree was removed 40 cm above the soil line with a 6 mm diameter cork borer, previously dipped in 95% ethanol and rinsed in sterile water, and replaced with a colonized plug of agar collected from the margins of the actively growing cultures. Agar plugs were placed mycelium side directly against the sapwood. For each plant, two opposite inoculations were produced on the same inoculation site. In mock-inoculated plants, non-colonized sterile plugs of PDA were applied. All the inoculation sites were firmly wrapped with Parafilm (American National Can Co) to retain the inoculum plug and limit both contamination and desiccation. Since *C. platani* enters the host through mechanical injury, colonizing the xylem tissues following penetration into plane trees, artificial inoculation by wounding well simulates the infection that occurs naturally.

In order to confirm the presence of the pathogens in inoculated plants, immediately after the last VOC sampling, 15 small pieces of plant tissue (5x3 mm) were removed at the same time close the

TABLE 1 Fungal species investigated in this study

Fungal species	Strain	Collector	Geographic origin
Ceratocystis platani	Cp20	IPSP-CNR	Italy
Ceratocystis platani	Cp21	IPSP-CNR	Italy
Ceratocystis platani	Cp22	IPSP-CNR	Italy
Closely related species			
Ceratocystis populicola	CBS119.78	CBS	
Ophiostoma ulmi	H2310	C. Brasier	The Netherlands
Ophiostoma ulmi	E2	C. Brasier	Spain
Ophiostoma quercus	RZ/2-S	T. Kirisits	Austria
Ophiostoma clavatum	AC/1/I/1	T. Kirisits	Austria
Grosmannia penicillata	KW/4/2/6/2	T. Kirisits	Austria
Outgroup species			
Diplodia sapinea	DS1	IPSP-CNR	Italy
Heterobasidion annosum	Pd3	P. Gonthier	Italy
Heterobasidion irregulare	45SE	P. Gonthier	Italy
Caliciopsis pinea	CLS10	IPSP-CNR	Italy
Seiridium cardinale	Sc-16	R. Danti	Italy
Geosmithia langdonii	1645	M. Kolarik	Czech Republic
Hymenoschypus fraxineus	Gro/2	T. Kirisits	Austria

necrotic areas, and then placed in 90 mm Petri dishes containing 1.5% PDA. Plates were incubated in the dark for 10-days at 21°C.

2.3 | Analysis of volatile organic compounds

Sampling of VOC was performed in vitro through exposure of carboxen/PDMS/DVB 65 um Solid Phase Micro Extraction (SPME) fibres, (Supelco, USA) for 1 hr to the headspace of vials where pathogenic fungal isolates were grown. The same type of SPME fibres was inserted for 1 hr into Nalophan® bags wrapped around the stems of plane trees where *C. platani* were inoculated to collect VOC released in vivo from the bark at different times: before inoculation (unwounded) and 1, 4, 8, 10, 17, 32 days post-inoculation (dpi). In particular, *C. platani* stems were wrapped and the same bark area was covered for both uninfected and infected plane trees immediately before inserting the SPME fibres; then the fibres were removed after 1 hr, as soon as VOC collection has completed.

After VOC collection, all the SPME fibres were analysed by an Agilent 7820A Gas-Chromatograph (GC) coupled with a 5977E mass selective detector (MSD) (Palo Alto, USA). The GC was equipped with a split/splitless injector operating in splitless mode and a J&W innovax capillary column (50 m, 0.20 mm i.d., 0.4 μm *df*) which was kept at 40°C for 1 min, then increased by 2°C/min until 80°C, 5°C/min until 150°C, 10°C/min until 220°C, 30°C/min until 260°C, with a hold time of 5 min. The MSD was operating in scan mode at 5.0 Hz acquisition rate in the 30–300 m/z range. Peaks integration parameters were set for: area threshold = 10.000; peak width = 5 s; and signal-to-noise ratio = 10. Tentative identification of VOC was performed by comparison of the peak spectra with those reported in NIST14 and by matching of their Kovats retention indices with those reported in the NIST chemistry webbook database. An Agilent Chemstation software version 2.01.00 was used for data elaboration (Agilent). Background of VOC was measured both in the headspace of vials containing only media and from empty Nalophan® bags and then subtracted to the measurements taken from vials containing the fungal cultures and from the stems of *C. platani* infected plane trees, respectively. Both for in vitro and in vivo analysis, results were expressed as percentage of single VOC of the total amount of the six targeted VOC which have been identified. In addition, for each single VOC, uncertainty was calculated as percentage of standard error on the averaged values of the measurements replicated three times.

3 | RESULTS

Analysis of the VOC emitted by different pure fungal strains grown in vitro on the same media and under the same conditions (Table 1) allowed us to identify six major VOC (Table 2). In particular, all the different *C. platani* isolates emit a similar blend of VOC mostly comprising of esters such as isoamyl acetate, isobutyl acetate, ethyl acetate and n-propyl acetate consistent with the previous report of Sprecher and Hanssen (1983) (Figure 1; Table 2). These VOC have a fruity type aroma that imparts a banana flavour to *C. platani* cultures (Saha, Bucknallb, Arcota, & Driscolla, 2018). None of the other examined fungal species had a similar blend of VOC, except *C. populicola* (Table 2).

TABLE 2 Percentages of single VOC out of the total sum of the most abundant VOC measured in the headspace of vials where pathogenic fungal isolates of different species were grown (values are means of three replicated measurements ± percentage uncertainty)

Fungal species	Strain	ethyl acetate (%)	*n*-propyl acetate (%)	isobutyl acetate (%)	isobutyl alcohol (%)	isoamyl acetate (%)	isoamyl alcohol (%)
Ceratocystis platani	Cp20	23.6 ± 2.4	0.4 ± 0.2	60.4 ± 2.1	–	15.6 ± 1.6	–
	Cp21	28.1 ± 4.5	3.4 ± 0.3	28.0 ± 3.8	–	40.5 ± 5.6	–
	Cp22	36.3 ± 4.0	1.3 ± 0.5	45.0 ± 4.9	–	18.1 ± 1.2	–
Ceratocystis populicola	CBS119.78	44.0 ± 10.0	2.2 ± 0.8	42.1 ± 7.7	–	11.7 ± 3.8	–
Ophiostoma ulmi	H2310	11.7 ± 3.2	–	–	37.3 ± 0.7	–	51.1 ± 2.5
	E2	3.7 ± 1.7	–	–	43.7 ± 4.8	–	53.1 ± 6.3
Ophiostoma quercus	RZ/2-S	8.7 ± 3.5	–	–	37.8 ± 1.5	–	53.5 ± 4.7
Ophiostoma clavatum	AC/1/I/1	6.0 ± 1.0	–	–	29.2 ± 4.0	–	64.8 ± 3.4
Grosmannia penicillata	KW/4/2/6/2	2.8 ± 0.2	–	–	5.2 ± 1.3	–	92.0 ± 1.1
Geosmithia langdonii	1645	–	–	–	58.0 ± 12.2	–	42.0 ± 12.2
Diplodia sapinea	DS1	–	–	–	–	–	100.0 ± 0.0
Hymenoschypus fraxineus	Gro2	–	–	–	–	–	–
Heterobasidion annosum	Pd3	–	–	–	–	–	–
Heterobasidion irregulare	45SA	–	–	–	–	–	–
Seiridium cardinale	Sc-16	–	–	–	–	–	–
Caliciopsis pinea	CLS10	–	–	–	–	–	–

Abbreviation: –, not present.

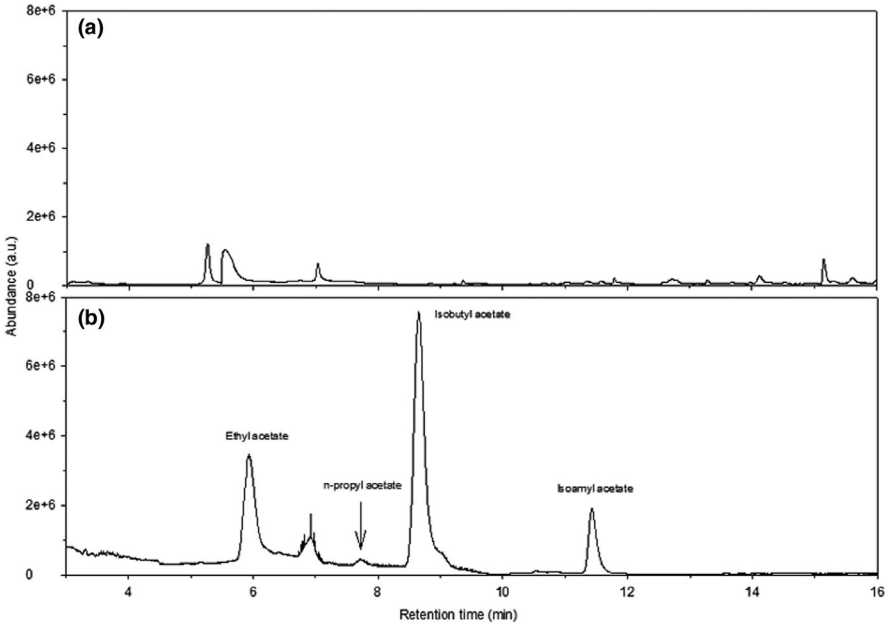

FIGURE 1 Representative chromatograms showing the VOC emitted in the headspace of the vials containing: (a) only PDA medium; (b) pure cultures of *Ceratocystis platani* grown on PDA medium

On the basis of the six major VOC emitted in vitro from the investigated pathogenic fungal species (Table 2), we have further targeted our analysis in vivo on plane trees following *C. platani* infection (Table 3). One-day post-artificial inoculation of *C. platani* within the cambium of plane trees, we did not detect any of these targeted VOC (Table 3). This demonstrated that our artificial inoculation was able to realistically simulate *C. platani* infection. Four days post-*C. platani* inoculation in plane trees, despite visible symptoms not yet observed on the host, a blend of VOC characterized by isobutyl acetate (92.5 ± 1.4%), isobutyl alcohol (6.5 ± 1.3%) and isoamyl acetate (1.0 ± 0.1%) was detected (Figure 2; Table 3). Then from 4 to 10 dpi, when symptoms were still not clearly visible, isobutyl acetate and isobutyl alcohol were released from the bark of infected planes. Whereas isobutyl acetate was the only VOC detected 17 dpi, ethyl acetate was the only VOC emitted from infected plants after 32 dpi (Table 3). No similar VOC were detected before inoculation or from mock-inoculated plants. At the end of the experiment, the presence of *C. platani* within the symptomatic tissues of infected plane trees was confirmed by re-isolation, while samples collected from mock-inoculated trees did not yield any pathogen.

4 | DISCUSSION AND CONCLUSIONS

This study provides two main results. Firstly, it shows that *Ceratocystis* spp. grown in vitro emit a unique blend of VOC (Sánchez, Sánchez, Christen, & Revah, 2004) that clearly differentiate this species from other fungal pathogens. Emission of specific VOC by *Ceratocystis* spp. is likely a gene-determined trait that characterizes the metabolism of this fungal species and, demonstrating biological activity, may have an ecological role in conferring *Ceratocystis* spp. an advantage to compete with other microbial species (Li et al., 2015). Secondly,

TABLE 3 Percentages of single VOC out of the total sum of the six targeted VOC measured over time in the plane trees stem where *C. platani* has been inoculated (values are means of three replicated measurements ± percentage uncertainty). Symptoms were detected in all the investigate seedlings at the same dpi

Days post-inoculation (dpi)	Presence of symptoms on inoculated plants	ethyl acetate (%)	*n*-propyl acetate (%)	isobutyl acetate (%)	isobutyl alcohol (%)	isoamyl acetate (%)	isoamyl alcohol (%)
1	No visible symptoms	–	–	–	–	–	–
4	No visible symptoms	–	–	92.5 ± 1.4	6.5 ± 1.3	1.0 ± 0.1	–
8	No visible symptoms	–	–	92.2 ± 1.4	7.7 ± 1.4	–	–
10	Wilting of apical leaves	–	–	89.8 ± 2.9	10.2 ± 2.9	–	–
17	Wilting and chlorosis of leaves; necrosis on bark	–	–	100.0 ± 0.0	–	–	–
32	Extensive chlorosis of the leaves and longitudinal necrosis on stem; plant death	100.0 ± 0.0	–	–	–	–	–

Abbreviation: –, not present

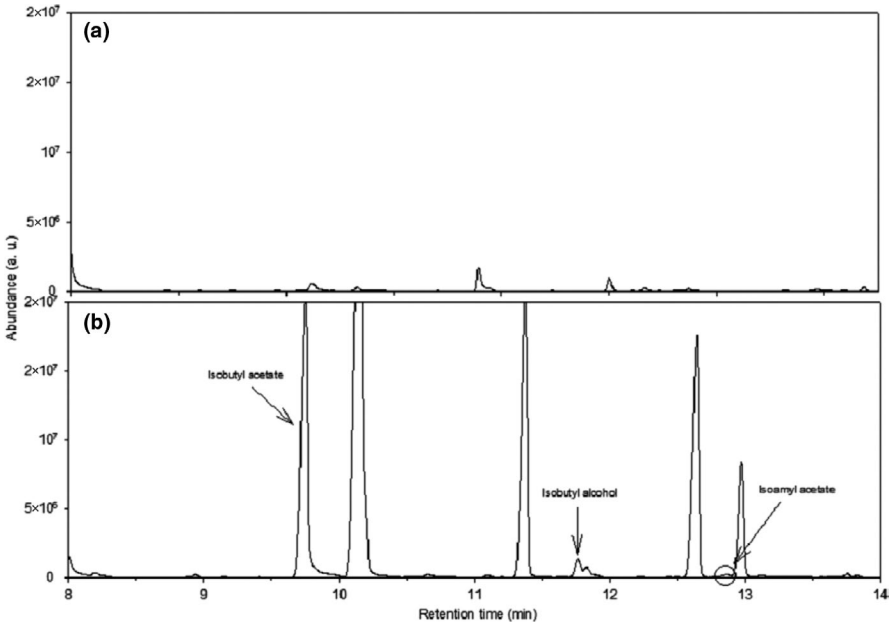

FIGURE 2 Representative chromatograms showing the VOC emitted from: (a) empty bag; (b) bark of plane trees infected with *Ceratocystis platani*

this study highlights the possibility to use "targeted" VOC uniquely emitted by *C. platani* to detect in vivo infected plants. Indeed, our results showed that specific VOC emitted in vitro by pure culture of *C. platani* (i.e. isobutyl acetate, isobutyl alcohol and isoamyl acetate) were also released in vivo from the bark of infected asymptomatic plane trees already four dpi, and continued to be detectable even after 32 days (Table 3). Since production of VOC from microbial species may strongly depend on culture conditions (Blom et al., 2011), our results confirm that *C. platani* emit the same blend of VOC both when grown on media and on living tissues of plane trees. In particular, we have focused our analysis only on this blend of VOC emitted by *C. platani* without considering other VOC (i.e. monoterpenes) that were constitutively released by the bark of plane trees (Table S1), because these VOC are also emitted from the leaves of many tree species (Kesselmeiert & Staudt, 1999) and thus cannot be considered unambiguous indicators of *C. platani* infection.

The efficacy of VOC as biomarkers of infected plants depends on the plant-pathogen combination (Jansen et al., 2011). By following a qualitative approach, in this study we highlight that *C. platani* could be identified through detection of specific VOC, such as isobutyl acetate along with isoamyl alcohol, emitted both in vitro and in vivo. Thus, this blend of VOC could be potentially exploited as a non-invasive early diagnostic marker of *C. platani* infection, even before symptoms are visible. Nevertheless, more experiments are needed to thoroughly estimate the sensitivity of VOC as biomarkers of *C. platani* by comparing the flux rates of VOC emitted from the tissues of infected plants with the development of the symptoms assessed by molecular methods (i.e. quantitative PCR; Luchi et al., 2013). In addition, the specificity of the VOC blend emitted by *C. platani* should be further validated by screening the VOC emitted by endo- and epiphytic fungal species associated with plane trees.

In conclusion, this is the first report on the use of a VOC blend for the detection of a quarantine plant pathogen, showing that VOC fingerprinting could represent an innovative method to help phytosanitary services in rapid surveying of plant consignments at ports of entry by sampling the air within a container where plants have been stored. Previous measurements already demonstrated in real-time detection of isoamyl acetate, and isobutyl acetate at very low concentrations in air (Mayr, Tilmann, Lindinger, Hugues, & Chahan, 2003). However, since VOC production and emission depend on both plant ontogenetic cycle and environmental conditions (Laothawornkitkul, Taylor, Paul, & Hewitt, 2009), future investigations should test the possibility of recognizing naturally infected young and adult plane trees through detection of the targeted VOC uniquely emitted by *C. platani*.

ACKNOWLEDGEMENTS

This study was funded by European Commission Horizon 2020 Research and Innovation Programme "HOlistic Management of Emerging forest pests and Diseases" (HOMED) (grant No 771271).

ORCID

Federico Brilli https://orcid.org/0000-0002-2563-6086
Alberto Santini https://orcid.org/0000-0002-7955-9207

REFERENCES

Aglietti, C., Luchi, N., Pepori, A. L., Bartolini, P., Pecori, F., Raio, A., … Santini, A. (2019). Real-time loop-mediated isothermal amplification: An early-warning tool for quarantine plant pathogen detection. *AMB Express*, *9*, 50. https://doi.org/10.1186/s13568-019-0774-9

Blom, D., Fabbri, E., Connor, E. C., Schiestl, F. P., Klauser, D. R., Boller, T., … Weisskopf, L. (2011). Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. *Environmental Microbiology*, *13*, 3047–3058. https://doi.org/10.1111/j.1462-2920.2011.02582.x

Cristinzio, M., Marziano, F., & Vernau, R. (1973). La moria del platano in Campania. *Rivista di Patologia Vegetale*, *9*, 189–214.

Engelbrecht, C. J., Harrington, T. C., Steimel, J., & Capretti, P. (2004). Genetic variation in Eastern North American and putatively introduced

populations of Ceratocystis fimbriata f. platani. *Molecular Ecology*, *13*, 2995–3005. https://doi.org/10.1111/j.1365-294X.2004.02312.x

Jansen, R. M. C., Bouwmeester, H. J., van Henten, E. J., Wildt, J., Kappers, I. F., & Hofstee, J. W. (2011). Detection of diseased plants by analysis of volatile organic compound emission. *Annual Review of Phytopathology*, *49*, 157–174. https://doi.org/10.1146/annurev-phyto-072910-095227

Kesselmeiert, J., & Staudt, M. (1999). Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. *Journal of Atmospheric Chemistry*, *33*, 23–88.

Lanza, E., Hwee Ko, K., & Palmer, J. K. (1976). Aroma production by cultures of *Ceratocystis moniliformis*. *Journal of Agriculture and Food Chemistry*, *24*, 1247–1250. https://doi.org/10.1021/jf60208a019

Laothawornkitkul, J., Taylor, J. E., Paul, N. D., & Hewitt, C. N. (2009). Biogenic volatile organic compounds in the Earth system. *New Phytologist*, *183*, 27–51. https://doi.org/10.1111/j.1469-8137.2009.02859.x

Lehtijärvi, A., Oskay, F., Doğmuş Lehtijärvi, H. T., Aday Kaya, A. G., Pecori, F., Santini, A., & Woodward, S. (2017). *Ceratocystis platani* is killing plane trees in Istanbul (Turkey). *Forest Pathology*, *48*, e12375. https://doi.org/10.1111/efp.12375

Li, Q., Wu, L., Hao, J., Luo, L., Cao, Y., & Li, J. (2015). Biofumigation on Post-Harvest Diseases of Fruits Using a New Volatile-Producing Fungus of *Ceratocystis fimbriata*. *PLoS One*, *10*(7), e0132009. https://doi.org/10.1371/journal.pone.0132009

Luchi, N., Ghelardini, L., Belbahri, L., Quartier, M., & Santini, A. (2013). Rapid Detection of Ceratocystis platani Inoculum by Quantitative Real-Time PCR Assay. *Applied and Environmental Microbiology*, *79*, 5394-5404.

Mayr, D., Tilmann, M., Lindinger, W., Hugues, B., & Chahan, Y. (2003). Breath-by-breath analysis of banana aroma by proton transfer reaction mass spectrometry. *International Journal of Mass Spectrometry*, *223–224*, 743–756. https://doi.org/10.1016/S1387-3806(02)00967-3

Ocasio-Morales, R. G., Tsopelas, P., & Harrington, T. C. (2007). Origin of *Ceratocystis platani* on native *Platanus orientalis* in Greece and its impact on natural forests. *Plant Disease*, *91*, 901–904.

Panconesi, A. (1999). Canker stain of plane tree: A serious danger to urban plantings in Europe. *Journal of Plant Pathology*, *81*, 3–15.

Pilotti, M., Lumia, V., Di Lernia, G., & Brunetti, A. (2012). Development of real-time PCR for in wood-detection of *Ceratocystis platani*, the agent of canker stain of *Platanus* spp. *European Journal of Plant Pathology*, *134*, 61–79. https://doi.org/10.1007/s10658-012-0022-5

Saha, B., Bucknallb, M. P., Arcota, J., & Driscolla, R. (2018). Profile changes in banana flavour volatiles during low temperature drying. *Food Research International*, *106*, 992–998. https://doi.org/10.1016/j.foodres.2018.01.047

Sánchez, L., Sánchez, F., Christen, P., & Revah, S. (2004). Effects of water activity, leucine and thiamine on production of aroma compounds by *Ceratocystis fimbriata*. *World Journal of Microbiology and Biotechnology*, *20*, 151–160. https://doi.org/10.1023/B:WIBI.0000021751.82008.30

Santini, A., & Capretti, P. (2000). Analysis of the Italian population of *Ceratocystis fimbriata* f. sp. *platani* using RAPD and mini-satellite markers. *Plant Pathology*, *49*, 461–467. https://doi.org/10.1046/j.1365-3059.2000.00470.x

Sprecher, E., & Hanssen, H.-P. (1983). Distribution and strain-dependent formation of volatile metabolites in the genus *Ceratocystis*. *Antonie Leeuwenhoek*, *49*, 494–499.

Tsopelas, P., Santini, A., Wingfield, M. J., & de Beer, Z. W. (2017). Canker stain: A lethal disease destroying iconic plane trees. *Plant Disease*, *101*, 645–658. https://doi.org/10.1094/PDIS-09-16-1235-FE

Walter, J. M., Rex, E. G., & Schreiber, R. (1952). The rate of progress and destructiveness of canker stain of plane-trees. *Phytopathology*, *42*, 236–239.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Brilli F, Luchi N, Michelozzi M, et al. Volatile organic compounds (VOC) as biomarkers for detection of *Ceratocystis platani*. *For. Path.*. 2020;00:e12618. https://doi.org/10.1111/efp.12618