PHOTOACOUSTIC SENSING AS A NON-DESTRUCTIVE OPTICAL TOOL TO DETECT SEED-BORNE PHYTOPATHOGENS

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In a context where global food demand is continuously rising, the need for innovative, highly specific, sensitive, and low cost methods for the early detection of seed-borne phytopathogens is a key step for growing healthy and safe crops. In addition to the economic losses resulted from a reduced production, seed-borne pathogens may easily spread worldwide by means of infected seeds[1], that are often asymptomatic.

Here we focus on the development of a new optical detection method for the bacterial plant pathogen *C. flaccumfaciens* pv. *flaccumfaciens* (*Cff*) (Hedges) Collins&Jones. *Cff* is a Gram positive, aerobic, nonspore-forming bacterium, and one of the most important biotic constraints affecting the production of edible legumes all over the world.[2]

As no effective chemicals against this bacterium are known, the development of specific and sensitive diagnostic tests for the rapid detection of *Cff* on plant materials is fundamental to prevent disease outbreaks. Currently, the available methods for *Cff* detection are the visual inspection of suspected seed lots[3] or molecular PCR-based methods applied also on-site, such as "loop-mediated isothermal amplification" (LAMP) method.[4-6]
The last are unable to discriminate a viable from a not-viable inoculum and are based on destructive procedures, besides to be time consuming, laborious, expensive, and difficult to automate.

For the above requirements, non-destructive methods such as optical techniques are of great interest,[7] but nowadays the few examples of optical technologies employed for plant diseases diagnosis are mainly based on Vis-NIR reflectance spectroscopy which is limited to superficial features of the sample, especially in the visible region.[7]

Sensing techniques based on photoacoustic effect, which combine spectroscopic specificity with the deep penetration of ultrasound in biological tissues, hold the potential to overcome the main limits of pure optical techniques.[8] The photoacoustic principle rests on the use of short optical pulses to excite specific chromophores to trigger a cascade of consecutive events that include the steps of optical absorbance, photothermal conversion and heat propagation, and thermoelastic conversion and acoustic propagation.[8,9] As a consequence, photoacoustic sensing is an hybrid technique which combines the best advantages of optical contrast and acoustic detection.

Photoacoustic imaging and sensing have been mainly exploited in biomedical applications.[8-9] Conversely, the huge potential of such technologies in the agri-food sector as sensitive, precise and low cost diagnostic techniques is still unexplored.

Here, we propose the use of photoacoustic sensing for the non-destructive detection of the quarantine bacterial phytopathogen *Cff*, by exploiting the optical absorbance of inner specific chromophores (likely carotenoids) it produces.

We demonstrated the feasibility to optically detect *in vitro* grown *Cff* colonies, by selecting the most suitable excitation wavelengths.

These results may open the way to innovative applications of photoacoustic sensing to non-destructively assess health and quality of plants and seeds.

References
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[7] ElMasry G., Mandour N., Al-Rejaie S., Belin E., Rousseau D., Sensors, 19, 1090, 2019.
[8] Wang L.V, Photoacoustic Imaging and Spectroscopy, (CRC Press, Boca Raton, USA, 2009)
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Monday	12th September
12h00-13h30	Registration
13h30-14h15	Opening Ceremony
Session 1	**FT-IR and Raman spectroscopies and imaging -1-**
14h15-14h55	**Keynote lecture** **Monika Szymańska-Chargot (Polish Academy of Sciences, Warsaw, Poland)** ***"Raman spectroscopy and imaging in investigation of plant biomolecules"***
14h55-15h15	Ana González Moreno (Universidad de Málaga, Spain) *"Depth profiling of tomato fruit cuticle during development using Confocal Raman Microscopy"*
15h15-15h35	Andrea Ponzecchi (University of Copenhagen, Denmark) *"Raman microspectroscopy of pristine and brown rot degraded acetylated wood at controlled relative humidity"*
15h35-15h55	Raphaël Coste (Université de Reims Champagne Ardenne, INRAE, France) *«Raman microspectroscopy and AFM-IR as tools to characterize the physicochemical properties of plant cell walls at the nanoscale"*
15h55-16h10	Flash presentations by poster authors Stefan Kolašinac, Ilinka Pećinar, Hannele Tuominen
16h10-16h40	Coffee break
16h40-17h00	Angelina D'Orlando (INRAE, Nantes, France) *"AFM/Raman micro-spectroscopy coupling to study the correlation between mechanical properties and chemical composition of plant cell walls"*
17h00-17h20	Abdallah Alayan (Université de Montpellier, France) *"Impregnation of emulsion in banana leaf for phytosanitary application by means of vibrational micro spectroscopies"*
17h20-17h40	Alexander Veber (Humboldt-Universität zu Berlin, Germany) *"In situ imaging of the local orientation of cellulose fibrils in Sorghum bicolor secondary cell walls"*
17h40-17h55	Flash presentations by poster authors Sylvie Durand, Miriam Aledda, Julien du Pasquier
18h30-19h30	**Ice breaker**

Tuesday	13th September
Session 2	**Chemometrics**
8h30-9h10	**Keynote lecture** **Ana de Juan (Universidad de Barcelona, Spain)** **"Image fusion to investigate the structure of vegetal tissues"**
9h10-9h30	Anne-Sophie Blervacq (Université de Lille, France) *«Proprioception in plants? How flax faces up to gravitropic adversity"*
9h30-9h50	Oriane Morel (Université de Lille, France) *"Component specific Raman imaging of Arabidopsis cell walls by pixel resolved reference spectra fitting"*
9h50-10h05	Flash presentations by poster authors Daniela Antonova, Kevin Vidot, Ivana.Grubor
10h05-10h35	Coffee break
10h35-10h55	Lisbeth Garbrecht Thygesen (University of Copenhagen, Denmark) *"Role and location of extractives during incipient brown rot decay of Norway spruce heartwood explored using Raman Microspectroscopy"*
10h55-11h15	Edouard Pesquet (Stockholm University, Sweden) *"Unravelling the function and spatial control of specific lignin chemistries during the development of different wood cell types and morphotypes"*
11h15-11h35	Els Bobelyn (Flanders Centre of Postharvest Technology, Belgium) *"Reverse calibration transfer between desktop and handheld spectrofotometer for prediction of optimal picking date for Conference pears"*
11h35-11h50	Flash presentations by poster authors Eduard Pesquet, Johannes Wellmann, Patrycja Pękala
11h50-12h00	Jeol presentation
12h00-12h30	Poster session =P1-P10
12h30-14h00	Lunch break
Session 3	**NMR spectroscopy and Imaging -1-**
14h00-14h40	**Keynote lecture** **John Ralph (University of Wisconsin-Madison, USA)** ***"NMR of lignins and whole plant cell wall materials: Improved assignments, and discovery of new lignin components"***
14h40-15h00	Céline Moreau (INRAE, Nantes, France) *"Contribution of ^{13}C CP/MAS NMR to the comprehension of enzyme action on cellulose fibers"*
Session 4	**Mass, X-ray and photoacoustic spectroscopies -1-**
15h00-15h05	Introduction
15h05-15h25	Mathieu Fanuel (INRAE, Nantes, France) *«Spatial correlation of water distribution and fine structure of arabinoxylans in developing wheat grain"*
15h25-15h45	Alexis Pirat (Université de Toulouse, INP-PURPAN, France) *"Global metabolomic analysis of steviol glycosides"*
15h45-16h05	Flash presentations by poster authors Cécile Barron, Camille Alvarado, Edwige Audibert, Kanchan Vishwakarma,
16h05-16h35	Coffee break
16h35-18h05	Technical Workshops

Wednesday	14th September
Session 5	**Autofluorescence based techniques and Imaging**
8h30-9h10	**Keynote lecture** **Giovanni Agati (Istituto di Fisica Applicata "Nello Carrara",** **Florence, Italy)** **"Talking about *in situ* fluorescence spectroscopy** **and plant phenolic compounds"**
9h10-9h30	Marie Berger (INRAE, Nantes, France) *« Fluorescence macrovision to assess chemical tissue variability in whole cross-sections of maize stems"*
9h30-9h50	Anne-Laure Chateigner-Boutin (INRAE, Nantes, France) *"Following autofluorescent compounds of developing wheat grain by autofluorescence multispectral macroscopic imaging"*
9h50-10h10	William Gardi (Stockholm University, Sweden) *"Fluorescent dyes discriminating cell wall glycans for quantitative glycomic imaging with subcellular resolutions"*
10h10-10h40	Coffee break
Session 6	**Mass, X-ray and photoacoustic spectroscopies -2-**
10h40-11h00	Lucia Cavigli (istituto di Fisica Applicata "Nello Carrara », Sesto Fiorentino Italy) *"Photoacoustic sensing as a non-destructive optical tool to detect seed-borne phytopathogens"*
11h00-11h20	Camille Rivard (SOLEIL Synchrotron, Gif sur Yvette, France) *"X-Ray fluorescence imaging and X-ray absorption for plant studies on LUCIA beamline at SOLEIL synchrotron"*
11h20-11h40	Marie-Laure Pons (INRAE, Aix en Provence, France) *"Contribution of X-ray absorption spectroscopy techniques to the understanding of cadmium fate in plants: evidence of sulfur-bound CD in the CD-hyperaccumulator Solanum nigrum and the non-accumulator Solanum Melongena"*
11h40-11h55	Flash presentations by poster authors Sally Ralph, Pierre-Nicolas Boulc'h, Catherine Deborde
11h55-12h30	Poster session P11-P19
12h30-14h00	Lunch break
Session 7	**FT-IR and Raman spectroscopies and imaging -2-**
14h00-14h40	**Keynote lecture** **Andras Gorzsás (Umeå university, Sweden)** ***"Fourier-transform infrared (FTIR) spectroscopic imaging for plant sciences"***
14h40-15h00	Nikolay Kotov (KTH Royal Institute of Technology, Stockholm, Sweden) *"Characterization of nanocellulosic materials at ultrahigh lateral resolution with nano-infrared spectroscopy"*
15h00-15h20	Aude Lereu (Aix Marseille Univ, France) *"Physical and chemical properties of plant cell walls by multimodal near field optical microscopy"*
15h20-15h40	Mustafa Kansiz (Photothermal Spectroscopy Corp, Santa Barbara, USA) *"Enhanced tri-modal optical-photothermal infrared (O-PTIR) spectroscopy-Advances in spatial resolution, sensitivity & tri modality (IR, Raman Fluorescence)"*
15h40-16h00	Sabrina Diehn (The Hebreu University of Jerulasem, Rehovot, Israel) *"Structural and chemical properties underlying lodging resistance in TEF "*

16h00-16h30	Coffee break
16h30-18h00	Technical Workshops
	Social dinner

Thursday	**15th September**
Session 8	**NMR spectroscopy and imaging -2-**
9h00-9h40	**Keynote lecture** **Annick Moing (INRAE Bordeaux, IBVM Villenave Dornon, France** **« *NMR-based metabolomics of fruits and crops"***
9h40-10h00	Guilhem Pagés (INRAE, St Genès-Champanelle, France) *"Can metabolic MRI be useful to image tomato metabolism ?"*
10h00-10h20	Maja Musse (INRAE, Rennes, France) *"Tissue characterisation of potato tubers using localised MRI T2 relaxometry"*
10h20-10h40	Nuixe M (INRAE, St Genès-Champanelle, France) *« Low-field MRI: a useful tool to characterize water in intact plants"*
10h40-11h10	Coffee break
11h10-11h50	Feedback on the technical workshops
11h50-11h55	« Biomolecules 2022 Best Poster Awards »
11h55-12h05	Closing Remarks
12h00-12h30	Meal basket