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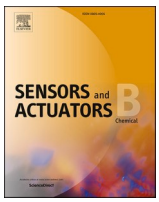

Detection of plant pathogenic fungi by a fluorescent sensor array

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ABSTRACT

Detection and identification of plant pathogenic fungi have relevant implications for both food safety and human health, and require fast and easy-to handle diagnostic techniques. In this context, we report the design and development of a fluorescent array-based sensor, containing different organic receptors, able to detect selectively *Penicillium italicum*, *Alternaria alternata* and *Fusarium sacchari*. The detection of these pathogenic fungi can be performed using a smartphone, leading to the first example of detection of microscopic fungal plant pathogens by fluorescent array using a smartphone as detector. This study represents a *proof-of-concept* for the realization of practical sensoristic devices able to detect pathogens in plant products and food or in environmental samples, in order to guarantee food and human health security.

1. Introduction

Fungi, in the broad sense of the term which includes both True Fungi (Fungi sensu stricto) and Oomycetes, are the most numerous groups of plant pathogens [1]. Many plant diseases, such as rusts, smuts, powdery mildews, downy mildews, fruit and vegetable rots, anthracnoses and scabs, are responsible for severe pre- and postharvest crop losses globally. Moreover, some plant pathogenic fungi, particularly species of *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*, produce noxious secondary metabolites collectively called mycotoxins, that may contaminate plant products and foods [2,3]. Recently, secondary metabolites produced by *Alternaria alternata* and *Penicillium digitatum* on infected orange fruits have been identified. They belong to different chemical classes and include AAL-toxin TB2, AAL-toxin TE2, AF-toxin II, Altenusin, Alternariol monomethyl ether, Alternethanoxin A, Altersolanol L, Aurasperone C, Curvularin, Dihydroaltersolanol, Erythroglaucin, Macrosporin A, Maculosin, Porriolide, Porritoxinol and Tentoxin, all produced by *A. alternata*, as well as Alantrypinone, Anacine, Asteltoxin, Atrovenetins,Fungisporin, Lichexanthone, Palitantin, Penipacid B, Penochalasin K, Rubratoxin B, Serantrypinone, Solistatin and Patulin, produced by *P. digitatum* [4]. Fusarium species, including *F. sacchari*, are known to produce several toxic secondary metabolites, the major toxins associated with *F. sacchari* and other *Fusarium* species include fumonisins (B1, B2 and B3), tricothecenes (deoxynivalenol - DON or vomitoxin and T-2), zearalenone, beauvericin, enniatins and moniliformin. These toxins frequently co-occur and contaminate important agricultural crops, posing health hazards to humans and animals [5]. A few destructive fungal plant pathogens, such as *Plenodomus tracheiphilus*, the causal agent of mal secco disease of citrus [6,7], are included in the lists of organisms of quarantine concern as they are considered a threat for agriculture and nature conservation. Restrictions are imposed to the trade of plants and plant products to prevent the spread of these quarantine organisms and a major task of the regional and national phytosanitary services is to intercept them, primarily at the borders, to prevent their introduction and spread. *Alternaria alternata* is the causal agent of several plant diseases worldwide such as Alternaria brown spot of mandarins [8] and heart rot of pomegranate [2,9,10]. *Fusarium sacchari* is one of the species of this genus of fungi responsible for

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post-harvest rot of banana fruits [10,11,12]. Detection and identification of plant pathogenic fungi, or biological hazard substances, have relevant implications for both food safety and human health [13]. Due to the perishable nature of plant products and the high number of samples to be examined crucial features of routine diagnostic methods must be rapidity and ease of application. Conventional diagnostic protocols for fungi rely on examination of morphology and DNA analyses [14–16]. In general, these techniques show high sensitivity and selectivity, but are expensive and time consuming. Moreover, they require equipped laboratories and specialized personnel.

The recent development of array sensor, also known as "chemical nose", may help in solving these problems due to the simple analysis protocol, a non-destructive sampling, fast response and ease to handle [17,18]. An optical array consists in many optical/fluorescent organic receptors/probes, which show different affinity for the target analyte. The array technology is based on the non-specific interaction between analyte and the organic receptors/probes. The presence of many receptors in the array device leads, after a proper mathematical treatment (i.e. multivariate analysis), to multiple interactions with an enhanced selectivity compared to the classical sensor-single molecule [19]. In particular, array sensing can be performed exploiting covalent reaction between the analyte and the receptor on the array, leading to the formation of a new chemical species [20]. Our strategy is to perform the sensing exploiting non-covalent interactions, such as hydrogen bonds, π−π staking and cation-π, in order to finely modulate the interactions between each probe and the analyte, thus leading to higher selectivity [21–28]. In fact, a covalent reaction between probe and analyte leads to a simple *off-on* signal, while the formation of non-covalent interactions leads to a tunable recognition signal by depending the nature and the structure of the analyte, increasing the output information of the array. In addition, the possibility to perform sensing in real field by using a simple methodology to acquire and elaborate the data of the array should be extremely useful for practical applications. In this context, modern smartphones with a high-resolution camera and specialized software for image data treatment, can be useful due to the possibility to obtain qualitative/quantitative information following the optical or colorimetric changes of the organic receptors into the array device [29–34]. Moreover, this method does not require the presence of specialized operator and cuts costs and time of the analysis.

Based on these premises, we report the design and fabrication of an array-based sensor, containing different fluorescent organic receptors that were demonstrated to be able to detect selectively *P. italicum*, *A. alternata* and *F. sacchari*. These fungi cause postharvest fruit rots of major fruit crops. In particular, *P. italicum* and the congeneric *P. digitatum* are the two most common causal agents of Penicillium mold, a very damaging postharvest disease of citrus fruits globally [35–39]. In particular, *P. digitatum* and *P. italicum* are responsible for green mold and blue mold of citrus fruits, respectively [36]. Green mold is the most common Penicillium rot of citrus fruits; however, blue mold is more insidious because it spreads in the box and may infect healthy fruits directly, regardless of injury [35]. Consequently, it may be regarded a nesting-type pathogen.

We based our array sensor on four different class of organic fluorophores, in detail Bodipy's, cavitands, rhodamines and naphthylamides, properly functionalized with functional groups able to interact by non-covalent interactions with the target analyte. We selected these probes to cover a wide range of emission spectra. In particular, quinoxaline cavitands show an emission band from 350 to 500 nm, naphthylamides have a characteristic emission from 400 to 600 nm, and Bodipy's lead to an emission in the range 500–700 nm. In addition, our approach is to exploit formation of hydrogen bonds (due to the presence of phenolic, alcoholic and amino groups in the probe), ion-dipole (by alkyl-pyridinium group), $\pi-\pi$ and hydrophobic effect (by aromatic rings and aliphatic chains, respectively).

We demonstrated the ability of the new device to detect and discriminate these fungi from other fungal plant pathogens, also at low concentrations. To the best of our knowledge, this is the first example of detection of fungi by array sensors, using a smartphone as a detector.

2. Results and discussion

2.1. Array design

The array was designed to contain molecular probes with different excitation/emission ranges, and the possibility to establish different non-covalent interactions with the analytes. In particular, we selected four different classes of chromophores (highlighted in red in Fig. 1): rhodamines, bodipy's, quinoxaline and naphthylamides. These probes contain many recognition sites (highlighted in blue in Fig. 1), able to interact through non-covalent interactions with the analytes: hydrogen bonds, ion-dipole, dipole-dipole, $\pi-\pi$ and hydrophobic effect. Syntheses of these probes were performed using simple synthetic protocols, detailed in the Supporting Information.

2.2. Sensing by array

In a polyamide filter paper, with a diameter of 5 cm, 1.5 μl of the probe solution (1 mM in $CHCl_3$) has been dropped and then the solvent was evaporated. Images of the array under UV lamp irradiation (365 nm) in a dark chamber have been acquired by a commercial smartphone (with a 12 megapixel camera resolution), before and after the exposure to selected water-suspensions of fungi. In particular, conidium or zoospore suspensions in sterile distilled water (s.d.w.) (1.7–2.0 $\times 10^6$ conidia, or 1.0–1.5 $\times 10^5$ zoozpores mL^{-1}) of six different fungi (*Plenodomus tracheiphilus*, *Phytophthora nicotianae*, *Phytophthora citrophthora*, *Penicillium italicum*, *Alternaria alternata* and *Fusarium sacchari,* see Supporting Information, Fig. S21), were dropped onto each probe of the array sensors. After evaporation of the solvent, the array was further photographed, and the images before and after exposure to the fungal conidia solutions were processed by Fiji. This software converts the images into RGB channel values, which are then converted to Gray channel (G) by using the formula $G = (R_{value} + G_{value} + B_{value})/3$, thus obtaining a single value for each pixel. The emission intensities of this G scale for each probe have been compared to phenanthrene (Ctrl in Fig. 1), and these normalized values (ratio between the intensity of each probe and the intensity of the control) have been tabulated for statistical treatment using the Excel software (Microsoft 365, a schematic representation of the elaboration process is provided in the Supplementary Material, Fig. S22). Fig. 2 shows the array image obtained by smartphone, under 365 nm UV lamp, before and after the exposure to *P. italicum* (1.7–2.0 $\times$ 10^6 conidia mL^{-1}).

In particular, Fig. 3 shows the array response, in terms of change of the emission of each probe, to different fungal conidial or zoospore suspensions.

It can be observed that each probe changed emission differently upon exposure to different fungal suspensions. In particular, in the case of *P. italicum* (yellow bars), *A. alternata* (green bars) and *F. sacchari* (orange bars) a clear different response could be detected. This indicates the possibility to discriminate these three fungi from the others. In addition, *P. italicum* primed a general quenching of the fluorescence emission, while *F. sacchari* and *A. alternata* induced an enhancement of the emission. With the other fungi tested, *Pl. tracheiphilus*, *Ph. citrophthora* and *Ph. nicotianae*, the emission response was almost similar to each other, thus precluding the possibility of discrimination. The selectivity here showed is typical of the array technology, due to the presence of multiple receptor response. Probably, the differential luminescent responses were primed by specific volatile organic compounds (VOCs) generated by the tested fungi, with a functional mechanism similar to that of biosensors [39,40,41]. Fungi, including those associated to citrus fruits, produce a wide array of VOCs but not all are species- or genus-specific [8,42–45].

Partial Least Squares (PLS) analysis is a multivariate data treatment

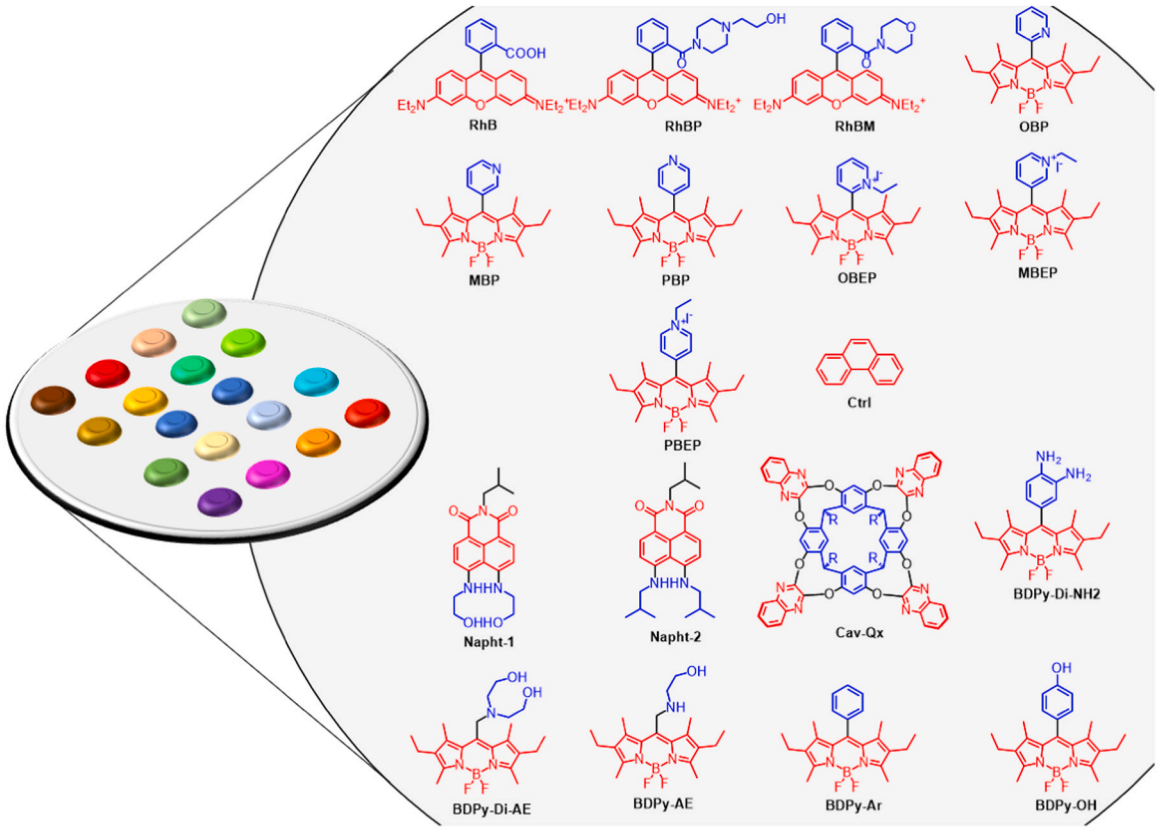

Fig. 1. Representation of the array and chemical structures of the organic probes used in the device (chromophores are represented in red, recognition sites are represented in blue).

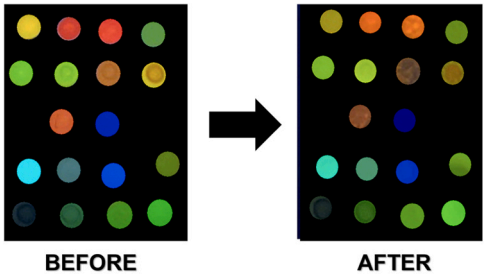

Fig. 2. Real images acquired by smartphone under UV excitation (365 nm) of array before (left) and after (right) the exposure to *P. italicum*.

technique allowing comparison between multiple response and explanatory variables. PLS is a regression method and allows the identification of underlying factors, which are a linear combination of the explanatory variables or X (also known as latent variables) which best model the response or Y variables. PLS has been widely applied to predict Y variables from X variables, and then it has evolved into a classification method that is well known as PLS-discriminant analysis (PLS-DA). PLS-DA combines dimensionality reduction and discriminant analysis into one algorithm and is especially applicable to modelling data in which the number of features (variables observed) are close to or larger than the number of observations (or data points). We have applied PLS-DA to provide discrimination capabilities to the multiarray-based sensing of fungi, and PLS for the quantification of the most relevant fungus. A detailed description of the Partial least squares for discrimination was reported by Barker [46]. Fig. 4a displays the cumulative R2 and Q2 Y for all the groups after each component. That is how much of the differences between the groups is explained by the model (R2Y) and how well new observations can be classified into the proper group

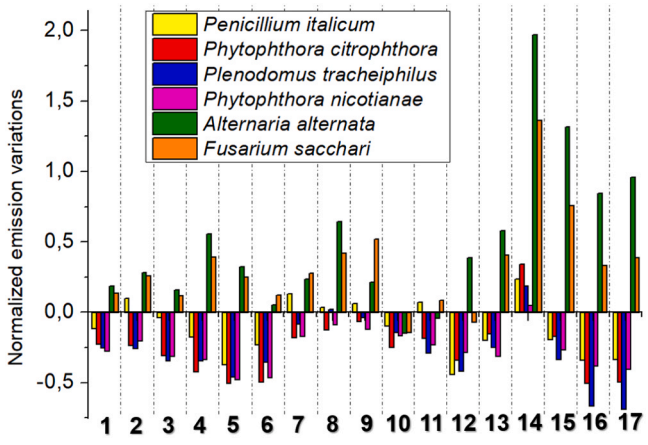

Fig. 3. Normalized emission responses of G channel ($[I_{sample}-I_{water}]/I_0$ where I_{sample} is the emission of probes after the exposition to the fungus suspension, I_{water} is the emission of probes to sterile distilled water and I_0 is the emission of probes before the exposition to the fungus suspension). Probes are numbered 1 (RHB), 2 (RHBP), 3 (RHBM), 4 (OBP), 5 (MPB), 6 (PBP), 7 (OBEP), 8 (MBEP), 9 (PBEP), 10 (Naph-1), 11 (Naph-2), 12 (CavQx), 13 (BDPy-Di-NH2), 14 (BDPy-Di-AE), 15 (BDPy-AE), 16 (BDPy-Ar), 17 (BDPy-OH).

(Q2Y). This shows how well the variation of each group is explained by the model and how well each group can be classified and predicted. In particular, Q2Y accounts the cumulative percent of the variation of the response predicted by the model, after the last component, computed by cross validation. Q2 tells us how well the model predicts new data. The larger is Q2, the more useful model will be. Considering the first 3 components 90 % Q2 is reached. Fig. 4c-d report the scores plot of the t1

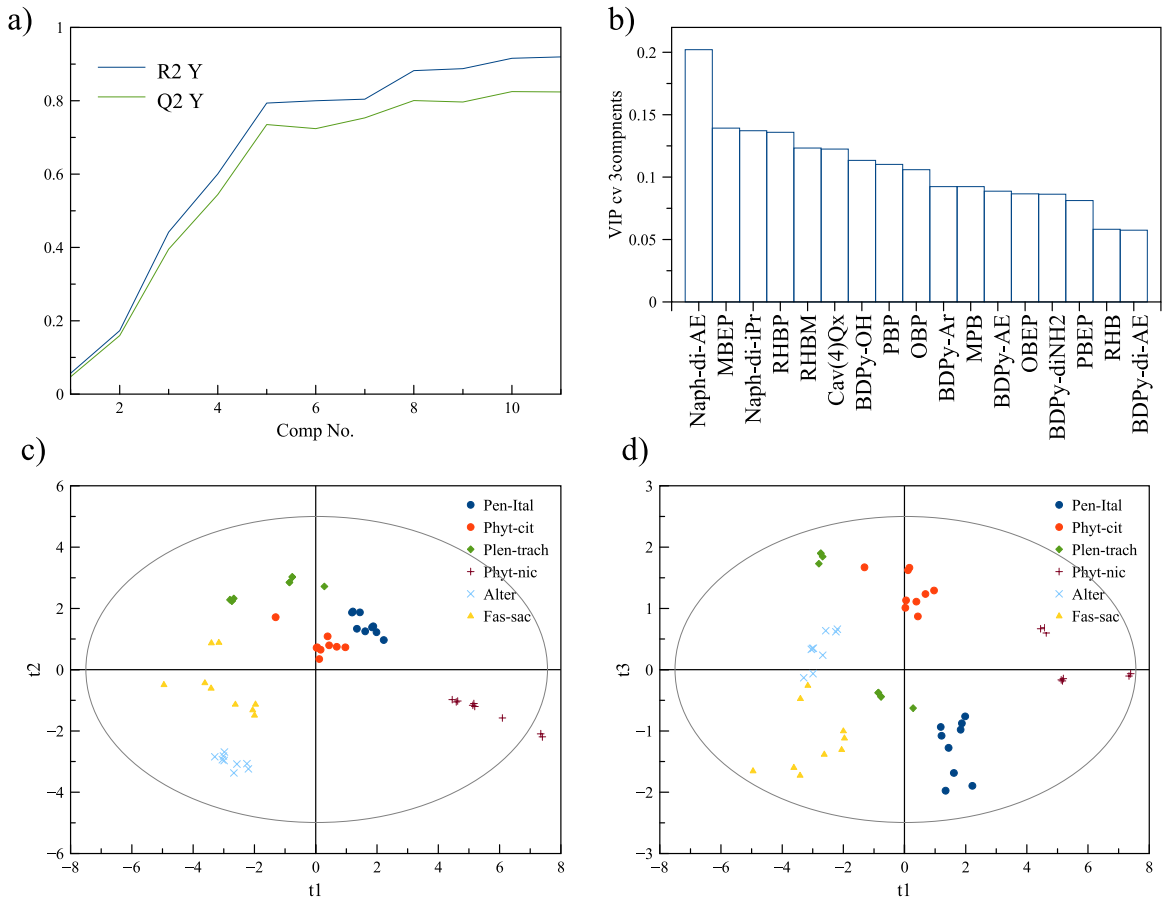

Fig. 4. a) R2 Y and Q2 Y of the PLS-DA model as a function on the calculated components, b) ordered 3 components VIP variables cross validated, c) t1 vs t2 scores plot, d) t1 vs t3 scores plot. Ellipses represent T^2 Hotelling at 0.95 confidence.

vs t2 and t1 vs t3 of the X scores. It can be interpreted as a point of view into the X space. The observations are colored by groups. This shows how well the groups are separated. We found a very good clustering and discrimination of the several fungi under investigation. Plots also reveal the presence of outliers representing observations outside the Hotelling T^2 ellipses at 95 % of confidence. A couple of samples related to *Ph. nicotianae* are located outside the ellipses. We have tried to remove them but the model in its whole substance does not significantly change. From the PLS model we computed PLS regression coefficients for each Y variable arbitrary assigned to numerically represent each group of fungi. These express the relation between the Y variables (fungi group) and all the terms in the model. The regression coefficients relate to the centered and scaled data and are computed from all extracted components. These coefficients are not independent as the variables are not independent. The coefficients are displayed with a confidence interval computed by jackknife cross-validation [47]. Fig. 4b displays the variables sorted by order of their importance in separating the groups. For each group the coefficients plot shows which variables are the most important to separate this group from the others. We noted that the most important probes to discriminate fungi by means of a 3 components model are PBEP and Naph-di-AE. BDPy-di-AE and BDPy-AR are the less important. Ultimately, the model possesses a highly discriminating capacity, which gives our method a very good ability to classify fungi.

To verify the capabilities of the method in quantitative respects, we applied it to different solutions of *P. italicum* that were diluted up to 5 log of dilution factor. We applied multivariate PLD regression. Fig. 5 shows the results. It shows the log of the dilution factor predicted by the model as the true value is changed. The model using 7 components can quantify *P. italicum* with extremely satisfactory agreement with the expected value.

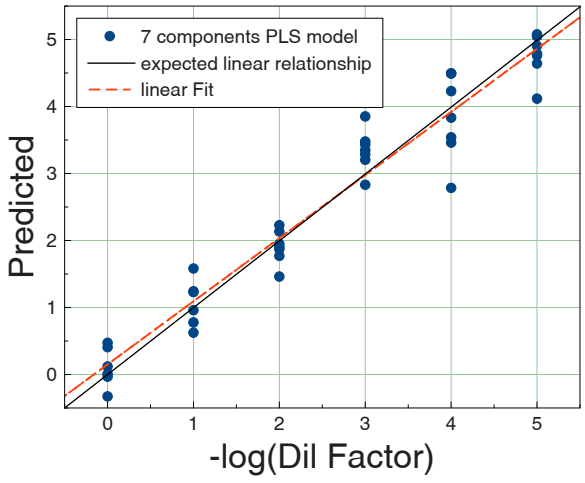

Fig. 5. Actual vs Predicted dilution factor calculated by PLS model with 7 components.

Then, the detection limit of *P. italicum* by the array was tested. In particular, starting from a mother solution of 1.7–2.0 × 10^6 conidia mL^{-1}, a serial dilution (1/10, $1/10^3$ and $1/10^4$) was prepared and the array response to each dilution was tested. Fig. 6 shows the results of the test.

At dilutions between 0 (corresponding to 1.7–2.0 × 10^6 conidia mL^{-1}) and $1/10^3$ (corresponding to 1.7–2.0 × 10^3 conidia mL^{-1}), an inverse relationship between the emission level and concentration of

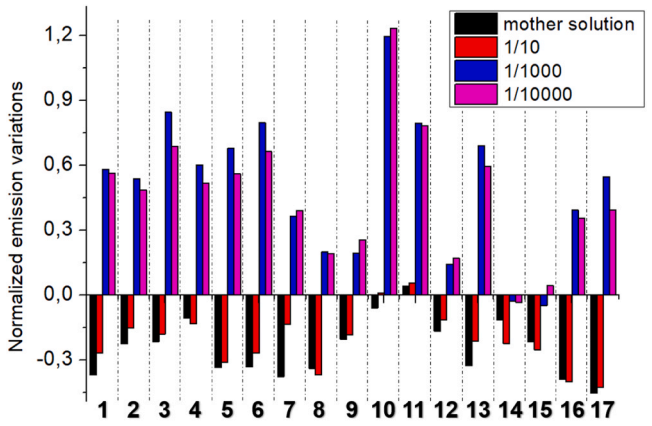

Fig. 6. Normalized emission responses of gray channel to a different dilutions of a suspension of *Penicillium italicum* conidia ($[I_{sample}\text{-}I_{water}]$ where I_{sample} is the emission of probes after the fungal exposition and I_{water} is the emission of probes to sterile distilled water). Probes are numbered 1 (RHB), 2 (RHBP), 3 (RHBM), 4 (OBP), 5 (MPB), 6 (PBP), 7 (OBEP), 8 (MBEP), 9 (PBEP), 10 (Naph-1), 11 (Naph-2), 12 (CavQx), 13 (BDPy-Di-NH2), 14 (BDPy-Di-AE), 15 (BDPy-AE), 16 (BDPy-Ar), 17 (BDPy-OH).

P. italicum conidia in the solution was observed. Conversely, the emission levels at concentrations of $1/10^3$ and $1/10^4$ conidia mL^{-1} did not differ significantly between each other, indicating a detection limit of less than 1.7–2.0 $\times$ 10^3 conidia mL^{-1}.

Finally, the sensitivity of the array was assessed in real sample. In particular, it was determined on washing waters from lemon fruits artificially inoculated with *P. italicum*. In detail, tests were carried out on water samples from markedly symptomatic fruits (7 days post inoculation - dpi). Fig. 7 shows the comparison between results obtained with the washing waters (red bars) and *P. italicum* mother solution (black bars, 1.7–2.0 $\times$ 106 conidia mL^{-1}) previously reported in Fig. 3. In general, probes show the same trend (in terms of quenching or enhancement of emission) both with laboratory and real samples. The difference of intensities can be ascribed to the washing water, which

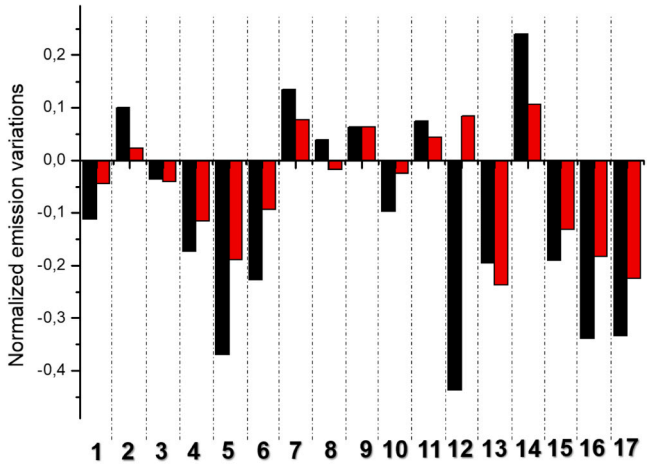

Fig. 7. Normalized emission responses of the array to laboratory solutions (black bars, *P. italicum* mother solution, 1.7–2.0 $\times$ 10^6 conidia mL^{-1}) and real samples of washing waters (red bars), in terms of G channel ($[I_{sample}\text{-}I_{water}]/I_0$ where I_{sample} is the emission of probes after the exposition to the fungus suspension, I_{water} is the emission of probes to sterile distilled water (in the case of laboratory solutions), washing water (in the case of real sample) and I_0 is the emission of probes before the exposition to the fungus suspension). Probes are numbered 1 (RHB), 2 (RHBP), 3 (RHBM), 4 (OBP), 5 (MPB), 6 (PBP), 7 (OBEP), 8 (MBEP), 9 (PBEP), 10 (Naph-1), 11 (Naph-2), 12 (CavQx), 13 (BDPy-Di-NH2), 14 (BDPy-Di-AE), 15 (BDPy-AE), 16 (BDPy-Ar), 17 (BDPy-OH).

contains chemical and biological substances produced by the fruit or originating from the pathogen/fruit peel interaction that can interfere with the response respect to the laboratory *P. italicum* solution.

As for molecular diagnostic methods based on quantitative PCR (qPCR), which is regarded as the most sensitive diagnostic method currently available, different detection limits have been reported in literature (most between 10 pg and 10 fg of target DNA), which make it possible the detection of single propagules (e.g. single unicellular conidia or zoospores) of fungal plant pathogens [8]. Consequently, the detection limit of the array-based sensor device developed in this study is by far higher than qPCR. However, the array-based method is very fast and easy to handle, moreover it does not require a destructive sampling, equipped laboratories and specialized personnel. Finally, for the detection of fungal propagules in a liquid medium, such as washing waters from citrus packinghouses, its sensitivity can be increased through filtration or centrifugation.

To the best of our knowledge, no threshold limit values (TLVs) of concentration of *P. digitatum* and *P. italicum* conidia in the environment have been determined to forecast the risk of Penicillium molds infections on citrus fruits in packinghouses. Conversely, even though no official standards or TLVs, usually expressed in terms of colony forming units (CFU), have been so far set for airborne concentrations of allergenic mold spores by public agencies (https://www.osha.gov/shib; https://www.epa.gov/mold/mold-testing-or-sampling), several studies tried to determine a quantitative relationship between the concentration of spores of allergenic molds in the environment and the effects on human health [46,48–53]. As fungal genera detected using the array device developed in the present study, such as *Alternaria*, *Fusarium* and *Penicillium*, are reported among the most common airborne allergenic fungi [48–53], it can be hypothesized that this array could complement the instruments used currently to detect allergenic fungi for sanitary purposes. Interestingly, very recently a smartphone-based digital system was applied to read automatically and improve the performance of a lateral flow assay widely used for the diagnosis of cryptococcosis, an invasive fungal disease affecting immunocompromised people [54]. Moreover, as for Penicillium molds of citrus fruits, it can be envisaged that the array device developed in the present study can be used to monitor the inoculum of *Penicillium* species in the environment, as a decision support tool for the application of sanitation measures in packinghouses to reduce the risk of infections during fruit handling and storing.

3. Conclusions

This is the first example of detection of microscopic fungal plant pathogens by fluorescent array using a smartphone as detector. The identification of *Penicillium*, *Alternaria* and *Fusarium* species was demonstrated by analyzing the fluorescence intensities emitted by the probes. Classification was implemented by means of a supervised classification method based on the PLS algorithm. *P. italicum*, in particular, could be also detected at a low concentration ($1/10^4$ conidia mL^{-1}). This study represents a proof-of-concept for the realization of practical sensoristic devices able to detect pathogens in plant products and food samples or in the environment, in order to guarantee food and human health security. Although, the test showed selectivity for some fungal genera before application it should be tested on mixtures of diverse fungi to verify if it can discriminate target fungal pathogens from congeneric closely related species and other fungi in the complex context of microbiome associated to fruits and food matrices or to equipment's and room walls in packinghouses. Compared with molecular diagnostic methods, this fluorescence array sensor has a lower detection limit, but offers a rapid response, portability, multiple capabilities, and low cost. These significant advantages could enable many new applications for the detection of phytopathogenic fungi that other methods cannot achieve. Work is in progress to detect fungi in real samples, improve reproducibility with the results obtained with fungal standard solutions

and quantify the amount of these pathogens, resulting in a practical analytical protocol. An additional potential application of the newly developed array device could be the detection of allergenic fungi in the air. To these aims, appropriate sampling procedures should be conceived and validated.

4. Experimental section

4.1. General experimental methods

The NMR experiments were carried out at 27 °C on a Varian UNITY Inova 500 MHz spectrometer (^{1}H at 499.88 MHz, ^{13}C NMR at 125.7 MHz) equipped with a pulse field gradient module (Z axis) and a tunable 5 mm Varian inverse detection probe (ID-PFG). ESI mass spectra were acquired on an API 2000- ABSciex using CH_3CN or CH_3OH (positive or negative ion mode). A JASCO V-560 UV−vis spectrophotometer equipped with a 1 cm path-length cell was used for the UV−vis measurements (resolution 0.1 nm). Luminescence measurements were carried out using a Cary Eclipse Fluorescence spectrophotometer with resolution of 0.5 nm, at room temperature. The emission was recorded at 90°with respect to the exciting line beam using 5:5 slit-widths for all measurements. All chemicals were reagent grade and were used without further purification.

4.2. Preparation of conidial or zoospore suspensions

Spore suspensions (fungal conidia and oomycetes zoospores) were prepared according to Aloi et al., 2021. The True Fungi (*Pl. tracheiphilus, P. italicum, A. alternata* and *F. sacchari*) were grown on potato dextrose agar (PDA) at 25 °C in the dark for seven days until the mycelium covered at least 90 % of the Petri dish (see Supporting Information). Five pieces of mycelium (5 mm diameter) were grown on potato dextrose broth (PDB) for at least 24 h. Conidia were then recovered by centrifugation, rinsed and resuspended in sterile distilled water (s.d.w.). The final conidial concentration was adjusted to 10^6 conidia/mL using a hemocytometer. Oomycetes (*Phytophthora nicotianae, Ph. citrophthora*) were first grown on V8 juice agar (V8A) at 20 °C in the dark for seven days. For the zoospore production, mycelium plugs from these colonies were flooded in sterile distilled water (s.d.w.) with autoclaved soil extract and incubated for 2–3 days at 20–22 °C with 16/8 h photoperiod. Sporangia formation was monitored during this incubation period and once mature sporangia were observed, the plates were cold shocked by incubation at 4 °C for 45 min after which they were removed and left at room temperature for 1 h to stimulate zoospore release. The zoospores were removed from the plates, pooled together and the concentration was determined by using a hemocytometer and standardized to 10^5 zoospores/mL.

4.3. Procedure for sensing by array

Experimental Setup. The UV−Vis lamp power 6 W, and the excitation wavelength was 365 nm. The position of the array device into the dark chamber can be modified, due to the presence of the control probe. In fact, the possible variations of the irradiation are normalized by the comparison with the control. The array device is located at 20 cm from the smartphone and UV source. Into several circular polyamide supports (0.2 μm pore size, 5 cm of diameter), after UV/O_3 treatment, were dropped on different positions 1.5 μL of each probes (1×10^{-3} M in $CHCl_3$) and 1.5 μL of the phenanthrene (1 M in $CHCl_3$). The solid sensors were illuminated with a UV lamp (365 nm) in a dark chamber and the visible emission image was acquired with a smartphone (iPhone 13, 24 Mpixel). Then, six different fungal conidial or zoospore suspensions (*P. tracheiphilus, P. italicum, A. alternata, F. sacchari*, 1.7–2.0 × 10^6 conidia mL^{-1} and *P. nicotianae, P. citrophthora,* 1.0–1.5 × 10^5 or zoozpores mL^{-1}) in distilled sterile water were dropped by a cellulose filter onto the different sensors and they were dried at air. The solid sensors were further photographed. The photos revealed the changing in fluorescence of the probes. Fluorescence variations, if any, were instantaneous and stable in time for hours. The images before and after exposure of the fungal conidia suspension were processed by Fiji [55]. In particular, the software converts the images into RGB channel values and then converted to Gray (G) by using the formula G= (Rvalue + Gvalue +Bvalue)/3, thus obtaining a single value for each pixel. The emission intensities of this scale for each probe have been compared to the control (phenanthrene), and these normalized values (ratio between the intensity of each probes and the intensity of the control) have been reported, this process was repeated for each fungal. The resulting values were tabulated for statistical treatment using the Excel software (Microsoft 365).

In particular, the following formula was applied: $[I_{sample}\text{-}I_{water}]/I_0$ where I_{sample} is the emission of probes after the fungal exposition, I_{water} is the emission of probes to distilled sterile water and I_0 is the emission of probes before the fungal exposition.

Then we tested the possibility to quantify *Penicillium italicum* in solutions with different concentrations, from 1.7 to 2.0×10^6 mL^{-1} to 17–20.

4.4. Synthesis of probes

RhB has been acquired by Merck. RhBP, RhBM [56], OBP, MBP, PBP, OBEP, MBEP, PBEP, BDPy-Ar, BDPy-OH [57], Napht-1 and Napht-2 [58], Cav-Qx (Santonocito et al., 2022) [34], BDPy-Di-AE and BDPy-AE [59], BDPy-Di-NH2 [21] have been synthetized following a modified synthetic procedures and detailed in the Supporting Information.

4.5. PLS-DA classification

Multivariate analysis of dataset was performed by means of SIMCA-P 11 (Umetrics). Dataset was centred and unity scaled.

4.6. Preparation of washing waters from artificially inoculated lemon fruits

Lemon fruits cv. Femminello Siracusano, collected from an organic citrus commercial orchard in the Siracusa province (Sicily, Italy), were preliminarily surface-sterilized with a 1 % NaClO solution for 2 min, rinsed with tap water, and air-dried at room temperature. For the inoculation, fruits were wounded with a 2-mm-diameter plastic tip (3 wounds per fruit) without injuring the juice sacks below the albedo; then, 20 μl of *P. italicum* conidial suspension (concentration 10^6 conidia/mL) were placed into the wound. Inoculated fruits were placed in a plastic container and maintained at 20 °C and 80 % of relative humidity. After 7 days incubation (dpi), symptomatic inoculated fruits were washed in sterile distilled water (sdw) (ratio: 200 mL of sdw / 100 mL of fruits) for 1 min, under magnetic stirring, Finally, washing water was aseptically collected and tested by the array.

CRediT authorship contribution statement

Rossella Santonocito: Data curation, Formal analysis. **Rossana Parlascino**: Data curation, Formal analysis. **Alessia Cavallaro**: Data curation. **Roberta Puglisi**: Formal analysis. **Andrea Pappalardo**: Formal analysis. **Francesco Aloi**: Formal analysis. **Antonino Licciardello**: Methodology. **Nunzio Tuccitto**: Data curation, Software, Validation. **Santa Olga Cacciola**: Conceptualization, Writing - original draft. **Giuseppe Trusso Sfrazzetto**: Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2023.134305.

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