Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Microfluidic Device for the Point of Need Detection of a Pathogen Infection Biomarker in Grapes

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a. Enzymatic AzA detection tests at the macroscale

Initial reaction conditions for tyrosine oxidation followed by oxidative coupling to MBTH, were optimized using a working volume of 1 mL. Reaction mediums with different concentrations of both tyrosine and MBTH were tested in order to obtain Michaelis-Menten kinetic curves. Due to the colorimetric nature of the reaction, it is possible to follow the reaction progression in real time by measuring the absorbance at λ= 480 nm, using a spectrophotometer (MultiskanGo Thermo Scientific). Once initial reaction conditions for the colored precipitate production were found, different concentrations of AzA were added to the reaction medium to quantify its inhibitory effect on the reaction.

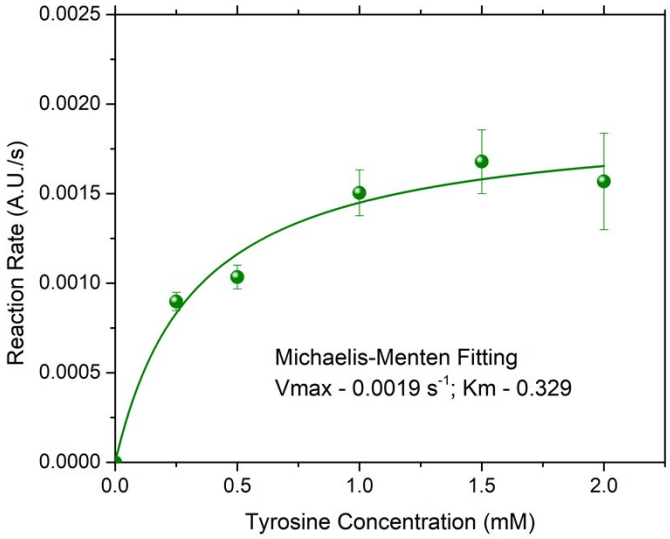

Figure S1 – Michaelis-Menten kinetics for tyrosine oxidation by tyrosinase, followed by oxidative coupling *via* MBTH. MBTH was used in excess to ensure that the limiting factor was the enzymatic activity.

b. Photosensor characterization

Throughout this work, the transducer of choice was an a-Si:H photodiode, which was fabricated using our cleanroom facilities in the same manner as shown by Santos et. al.[1] Here we show the diode characterization, which consists of obtaining a IV curve in the dark, allowing for the assessment of sensor quality. The dark current at a 0V bias is also acquired which will allow to determine the noise of the measurements mainly due to thermal

generation of charge carrier pairs. The final step consists of a photoresponse curve, the slope of this curve, *γ*, is related to recombination mechanisms between charge carriers, while the External Quantum Efficiency (EQE) is a figure of merit which relates the number of photons that leave the light source with the number of electron-hole pairs generated. The responsivity is another figure of merit, which gives us the notion of how much optical power is needed to produce a given current value.

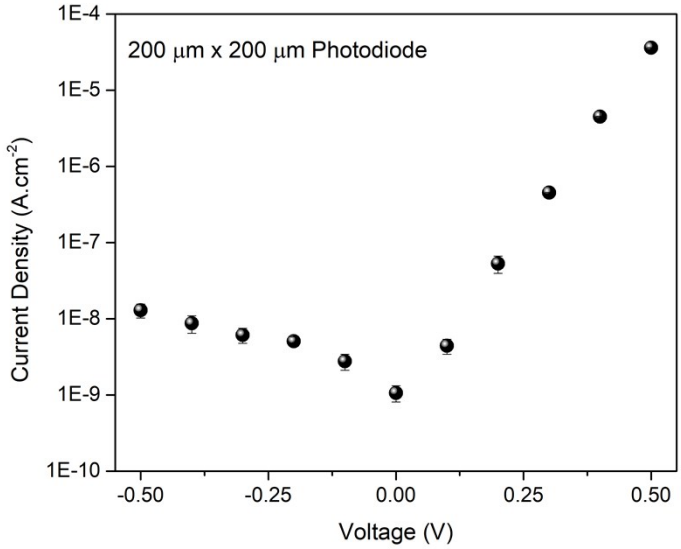

Figure S2 – IV curve in the dark for photodiode characterization.

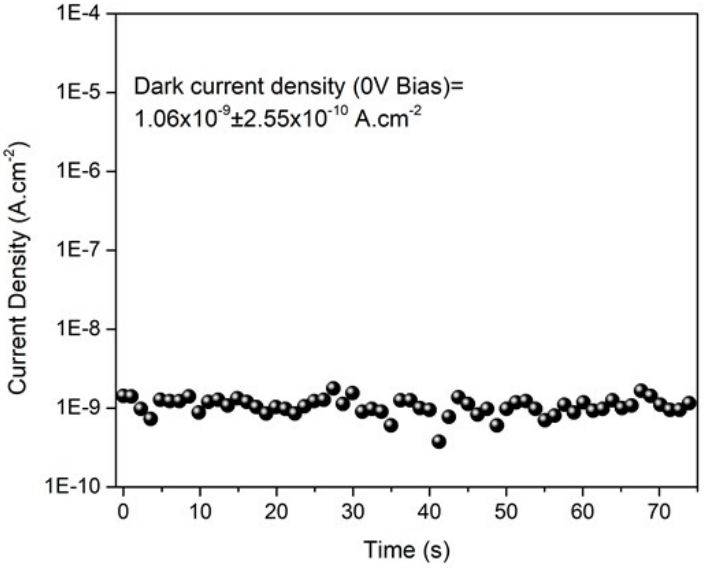

Figure S3 – Dark current measurement at 0V bias. Measurement noise can be extracted from these results, which will be used for further characterization.

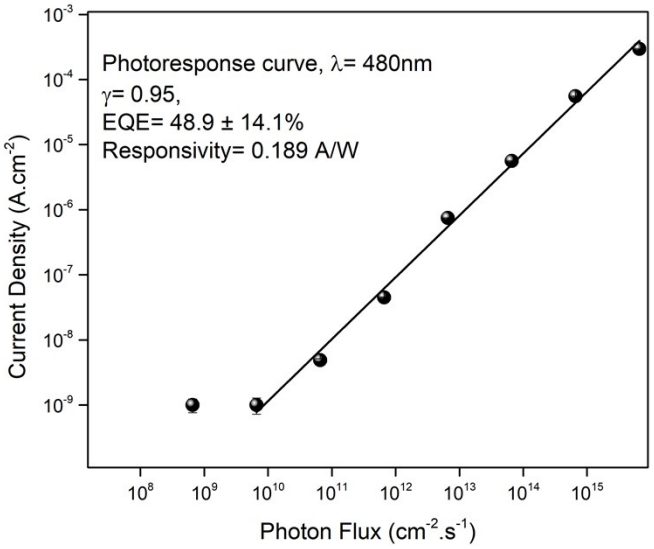

Figure S4 – Photoresponse curve obtained for the photodiode.

c. Phenol Quantification

For the quantification of the phenol content in the grape juice samples, the Folin-Ciocalteau method was employed. As a standard, gallic acid was chosen and the standard calibration curve is presented below. Results obtained by this method will hence be presented as Gallic Acid Equivalents (GAE).

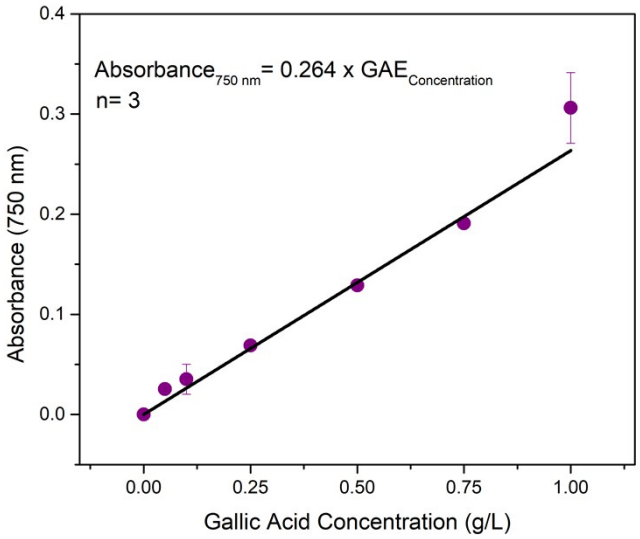

Figure S5 – Gallic acid standard calibration curve for phenol quantification via the Folin-Ciocalteau method.

d. Grape Infection and collection

Field experiments were conducted in an experimental vineyard with 15-year-old grapevines (*Vitis vinifera* L. cv. Trincadeira) at the Instituto Superior de Agronomia, University of Lisbon, Portugal. The *B. cinerea* isolate used was obtained from diseased grapevine plants and maintained in potato dextrose agar (Difco, Detroit, MI, USA), at 5 °C. Conidia production was achieved by exposing inoculated Petri dishes with potato dextrose agar to continuous fluorescent light, at 24 °C. Conidia were harvested from 14- to 20-day-old cultures and collected by rubbing with phosphate buffer (0.03 M KH_2PO_4), filtered through cheesecloth to remove mycelia, and the concentration adjusted to 10^5 conidia ml^{-1}. Infections were made on berries by spraying the conidial suspension at the developmental stage of peppercorn size (EL29). Control clusters were sprayed with phosphate buffer. Following inoculation, the clusters were incubated by enclosing them in individual plastic bags for a period of 1 week. Collection of samples was performed at pre-veraison (EL32), veraison (EL35) and harvest (EL38) [2]. For each time point and each treatment (infected and mock), four biological replicates were harvested around 10 a.m., immediately frozen in liquid nitrogen, and transported to the laboratory in dry ice and kept at –80 °C. Prior to extraction, the seeds were removed. The fungal infection was confirmed through the use of qPCR, which is summarized in Table S1.

Table S1 - qPCR results of the infected grape samples. The Actin gene was used as a reference, as its expression was not impacted by the presence of the fungal infection. Fungal biomass accumulation and infection level was determined by qPCR amplification of the B. cinerea polygalacturonase 1 (PG1).

Sample ID	**$Ct_{CDSBcPG1}$**	**Ct_{Actin}**	**ΔCt (Ct_{target}- Ct_{Actin})**	**ΔΔCt (Healthy samples values were subtracted)**	**$2^{-\Delta\Delta Ct}$**
EL 32	29.00±2.41	22.00±0.86	7.00±1.56	-4.44±1.56	30.17±25.55
EL 35	27.16±0.56	21.49±0.12	5.67±0.50	-5.03±0.50	33.88±10.72
EL 38	27.01±1.93	23.62±2.04	3.40±1.56	-6.23±1.56	101.93±69.46

e. rp-HPLC Quantification of Azelaic Acid

Reverse phase HPLC was performed in order to assess the results obtained in the microfluidic platform. AzA standards were prepared in the mobile phase used for the chromatography process. The following table provides the results obtained, in terms of retention time and peak area, for both the standards and the grape samples under analysis.

Table S2- Results obtained for the determination of AzA in both infected and healthy grape sample, by rp-HPLC. Peak area and retention time obtained for the AzA standards is also presented.

Sample	Retention Time (min)	Peak Area (A.U.)	Retention Time (min)	Peak Area (A.U.)	Average Retention Time (min)	Average Peak Area (A.U.)
20 mM AzA	11.01	556206	10.17	559413	10.59	562809.5±4803
10 mM AzA	11.05	294843	10.18	300217	10.62	297530±3800
4 mM AzA	10.98	119215	10.33	126116	10.66	122664±4877
2 mM AzA	10.89	50833	10.44	70803	10.67	60818±14120
0.2 mM AzA	10.54	7443	10.50	8544	10.52	7993.5±778
0.04 mM AzA	10.47	2512	10.15	2256	10.31	2384±181
0.02 mM AzA	10.63	2136	10.81	2501	10.72	2318.5±258
EL 32 Control	-	-	-	-	11.00	3239
El 32 Infected	-	-	-	-	10.62	10246
EL 35 Control	-	-	-	-	10.76	3103
El 35	-	-	-	-	10.83	4933

Infected						

f. References

1. Santos DR, Soares RRG, Chu V, Conde JP. Performance of hydrogenated amorphous silicon thin film photosensors at ultra-low light levels: towards attomole sensitivities in labon- chip biosensing applications. *IEEE Sens J*. 2017:1-1. doi:10.1109/JSEN.2017.2751253.

2. Coombe BG. Growth Stages of the Grapevine: Adoption of a system for identifying grapevine growth stages. *Aust J Grape Wine Res*. 1995;1(2):104-110. doi:10.1111/j.1755-0238.1995.tb00086.x.