ACS SENSORS

Letter

Cite This: ACS Sens. 2019, 4, 1180−1184

pubs.acs.org/acssensors

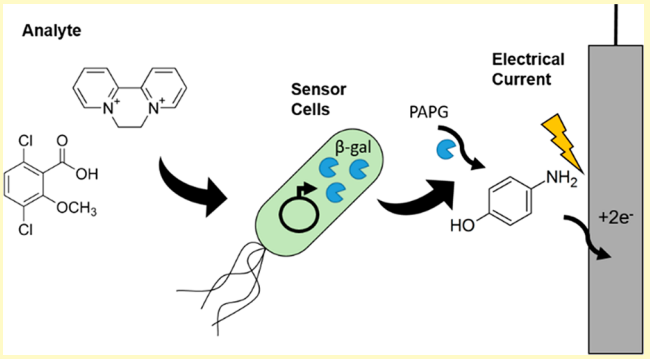
Redox-Based Synthetic Biology Enables Electrochemical Detection of the Herbicides Dicamba and Roundup via Rewired *Escherichia coli*

Eric VanArsdale,[†,‡] Chen-yu Tsao,[†,‡] Yi Liu,[‡] Chen-yu Chen,[†,‡] Gregory F. Payne,[‡] and William E. Bentley*[,†,‡,§]

[†]Fischell Department of Bioengineering, [‡]Institute for Bioscience and Biotechnology Research, and [§]Robert E. Fischell Institute for Biomedical Devices, University of Maryland, College Park, Maryland 20742, United States

Supporting Information

ABSTRACT: Synthetic biology is typically exploited to endow bacterial cells with new biosynthetic capabilities. It can also serve to create "smart" bacteria such as probiotics that detect and treat disease. Here, we show how minimally rewiring the genetic regulation of bacterial cells can enable their ability to recognize and report on chemical herbicides, including those routinely used to clear weeds from gardens and crops. In so doing, we demonstrate how constructs of synthetic biology, in this case redox-based synthetic biology, can serve as a vector for information flow mediating molecular communication between biochemical systems and microelectronics. We coupled the common genetic reporter, β-galactosidase, with the *E. coli* superoxide response regulon promoter pSoxS, for detection of the herbicides dicamba and Roundup. Both herbicides activated our genetic construct in a concentration dependent manner. Results indicate robust detection using spectrophotometry, via the Miller assay, and electrochemistry using the enzymatic cleavage of 4-aminophenyl β-D-galactopyranoside into the redox active molecule *p*-aminophenol. We found that environmental components, in particular, the availability of glucose, are important factors for the cellular detection of dicamba. Importantly, both herbicides were detected at concentrations relevant for aquatic toxicity.



KEYWORDS: *synthetic biology, biofabrication, redox, dicamba, diquat, Roundup*

Reporter cells have been developed over the last 20 years for sensing specific molecular cues in medical and environmental diagnostics.[1−8] These cellular "devices" have typically converted the molecular cues into fluorescent or chemiluminescent read-outs which can require bulky or sophisticated apparatus for integrated measurement.[9−11] Our group has sought to use synthetic biology as a means to convert molecular information into a readily recorded electrical format.[12−16] Our methods revolve around converting information into a redox reaction which can be monitored by the donation or retrieval of electrons from a charged electrode. In this way, one avoids bulky and sophisticated instrumentation. Instead, we use the cell's natural recognition and computing power to transduce information from the environment to a simple electrochemical format.

While cell-based biosensing is prevalent in environmental diagnostics, their use for the detection of herbicide contamination has been limited. In particular, with the new generation of herbicide resistant plants, such as those engineered to contain bacterial-origin dicamba-monooxygenase, the use of volatile herbicides has become problematic, with reported incidents of gaseous drift to neighboring fields.[17−19] Dicamba, an auxin-mimetic herbicide, has been found to cause environmental contamination due to airborne-dispersal practices and its high water solubility.[18,20−22] It has also been reported as a domestic water supply contaminant in both the United States and Canada.[23−26] However, rapid and facile detection of dicamba can be difficult as it has few easily accessed molecular features (e.g., it has neither a fluorescence nor electrochemical signature). It is commonly detected, therefore, using classical analytical chemistry techniques such as mass spectroscopy.[27−30] We have sought to develop a simple cell-based sensing platform that could process herbicidal molecular information and store it in a format that could be readily accessed.

A recent report Kurenbach et al. (2015)[31] indicated that microbes, such as *Escherichia coli*, respond to dicamba through the SoxRS regulon that coordinates cell responses to oxidative stress.[32−39] In this study, we have repurposed this response circuit for the detection of dicamba through SoxRS-mediated expression of the *lacZ* gene encoding β-galactosidase. We further discovered that diquat, a broad-spectrum active ingredient of Roundup, can also trigger SoxRS-mediated β-galactosidase expression. This enzyme can then be used in

Received: January 11, 2019
Accepted: April 16, 2019
Published: April 16, 2019

DOI: 10.1021/acssensors.9b00085
ACS Sens. 2019, 4, 1180−1184

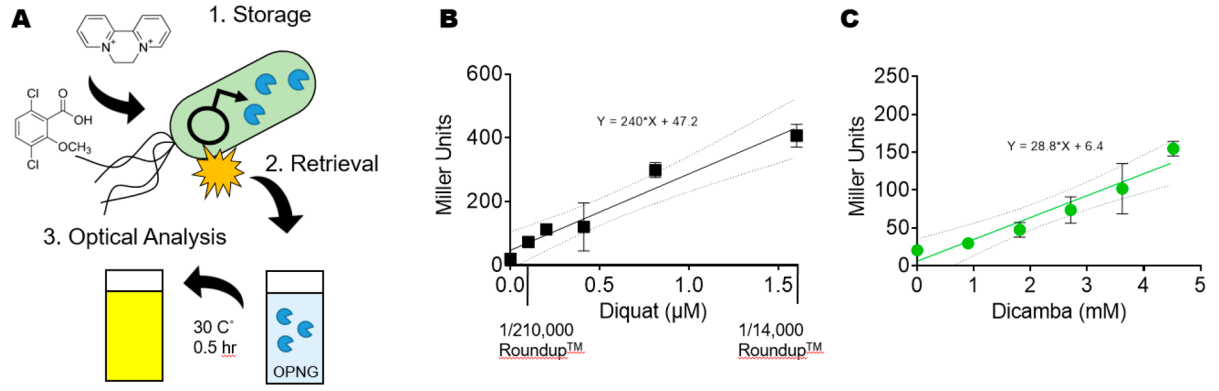


Figure 1. Spectrochemical detection of herbicides using the Miller Assay. (a) Schematic overview of the technique. First, cells are exposed to the herbicides and "store" the molecular information in the form of SoxR-dependent *lacZ* expression. Next, the cells are lysed to "retrieve" the information—the transcribed β-galactosidase enzyme. Finally, the retrieved information is read out using a spectrochemical analysis of enzymatically cleaved nitrophenol relative to the optical density of cells lysed (i.e., "Miller unit"). The measured Miller unit increased linearly with the concentration of diquat in Roundup between 0 and 1.6 μM (b). Similarly, the measured Miller unit increased linearly with the concentration of dicamba between 0 and 4.5 mM (c). The R^2 for the linear regression of the Roundup and dicamba assays were found to be 0.95 and 0.94, respectively. The Miller assay data beyond the linear range can be found in Figure S1.

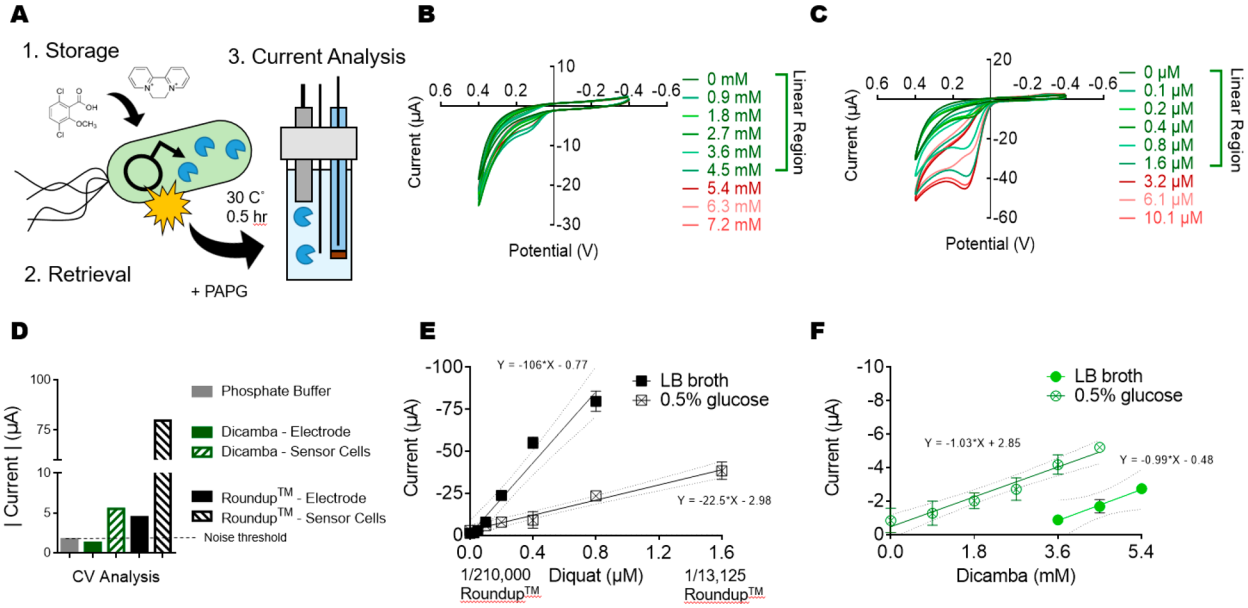


Figure 2. Electrochemical interrogation of molecular information transduced by sensor cells. (a) Schematic overview of the electrochemical measurement. The data "storage" and "retrieval" concepts are identical to the Miller assay (see Figure 1 legend). The electrochemical technique differs in that the data acquisition step is performed by cyclic voltammetry (CV) and peak current analysis of the enzymatically cleaved *p*-aminophenol. The peak current intensity was found to directly relate to the concentration of diquat in Roundup and dicamba, respectively, when analyzed by CV (scan −0.4 to 0.4 V, scan rate 50 mV/s). Dicamba could be detected from 3.6 to 7.2 mM and 0 to 4.5 mM for LB broth and LB glucose broth cultures respectively (b). We could detect Roundup diquat between 0 and 1.6 μM for LB broth cultures and 0 and 3.2 μM for LB glucose broth cultures (c). CVs for LB broth detection can be found in Figures S6–S7. The absolute value of the peak current for both diquat and dicamba greatly increased when using sensor cells relative to GCE electrode detection of untreated herbicide samples (d). The current was found to vary linearly between 0 and 0.8 μM and 0 and 1.6 μM for diquat tests (e), and for LB glucose cultures for dicamba between 0 and 4.5 mM (R^2 > 0.95 for all analyses) (f). The measured current for LB broth cultures for dicamba varied linearly between 3.6 and 5.4 mM with lower confidence (R^2 = 0.89) (f). The error bars in all plots represent SEM from 3 technical replicates and 3 biological replicates. The presented CVs are the average of 3 separate samples.

conjunction with ortho-Nitrophenyl-β-galactoside (OPNG) or 4-Aminophenyl β-D-galactopyranoside (PAPG) to produce either an optical or electrical signal revealing a concentration dependent response to both compounds.

We chose to first demonstrate the utility of our cellular detection system using the common Miller assay. This β-gal assay is an accepted standard as a transcriptional promoter probe in *E. coli*; here, we examine the *soxS* promoter response to both molecules (Figure 1a). In Figure 1b,c, we found that the cell responses to both dicamba and Roundup were linear (to 1.5 uM diquat in Roundup and 4.5 mM for dicamba). Importantly, these levels coincide with ranges previously reported to cause acute aquatic toxicity to (i) fish for dicamba (e.g., >0.45 mM)[19,22] and (ii) algae and protozoans for diquat (e.g., >0.8 μM)—the broad-leaf component of Roundup.[40,41] At concentrations above these linear ranges, optical measure-

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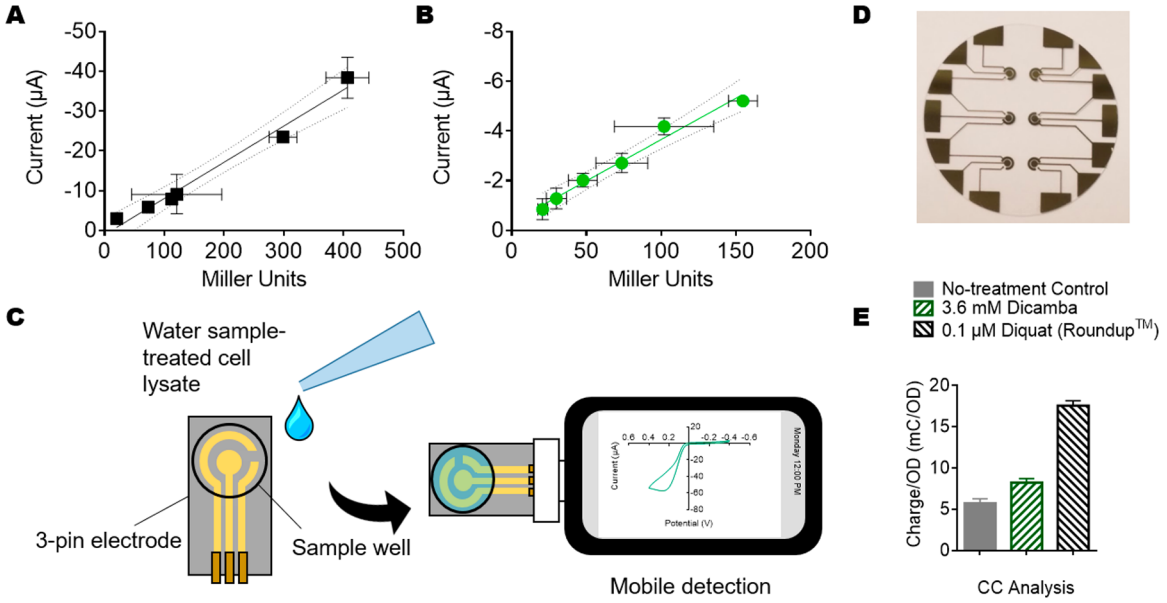

Figure 3. Comparison of the Miller Assay to the electrochemical method. The peak current measured by the electrochemical method linearly correlated (R^2 > 0.95) with measured Miller Unit for detection of both Roundup (a) and dicamba (b). An illustration of future applications of electrochemical cell-based detection using disposable electrodes (c). 3-pin electrodes would contain a measurement well for application of a mixture of water samples with sensor cells. This sensor could then be plugged into an adaptor to interface with any mobile electronic, such as the pictured cell phone, for a simple detection paradigm. A sample 3-pin electrode array was patterned onto a glass wafer (d) and used to test sensor cells incubated with water from the Anacostia River that was or was not spiked with each herbicide (e). The error bars in a,b represent SEM from 3 technical replicates and 3 biological replicates. The error bars in e represent SEM from 3 biological replicates. The dotted lines represent the 95% confidence interval of the linear regression fit.

ments either saturated for Roundup, or dropped precipitously for dicamba (Figure S1a,b). We suspected the latter was due to cell toxicity. We confirmed this hypothesis by analyzing the growth kinetics of cells exposed to dicamba with and without the sensor plasmid. We discuss these results in the Supporting Information (Figures S2 and S3). In sum, our results clearly indicated that the Miller assay represents a convenient method to assess Roundup and dicamba levels in laboratory samples.

While in Figure 1b we reported the response to Roundup as a function of one of its ingredients, diquat, our results do not support that this specific ingredient was responsible for actuating *soxS* gene expression. Thus, we subsequently screened the various Roundup constituents and found that diquat, and not glyphosate (i.e., the major component within Roundup formulations), was responsible for activating our gene construct (see Figure S4a,b; Figure S5a). We also found that glucose supplementation (e.g., 0.5% w/v) reduced the cell's sensitivity to diquat, as noted by a reduced slope of the cell sensor's linear region. In Figures S4a and S5a, we found that the attenuation in β-gal expression was independent of the genomic SoxRS regulon, as the results in the *soxS* mutant and its isogenic parent were identical. Also, because glucose does not interfere with the Miller assay, we conclude that the attenuated *soxS* response was due to glucose-mediated metabolic changes. We did not further investigate these changes.

Interestingly, when applying this same test for dicamba, we found that additional glucose also decreased the slope of the linear range, and further shifted the linear range to lower concentrations—the latter being more useful. We also noted that reporter signals were greater in a genomic *soxS* knockout compared to the isogenic parent, indicating a confounding relationship with the cell's normal oxidative stress response (Figure S4c and Figure S5b compare the Δ*soxS* strain vs the isogenic parent). Upon further inquiry, we found that the measured changes due to glucose supplementation corresponded to retarded cell growth rates at lower concentrations of dicamba relative to LB grown sensor cells (Figure S4d). The shift in growth rate upon glucose supplementation suggests that glucose sensitizes *E. coli* to dicamba toxicity. We concluded from these tests that environmental conditions are significant for cell based sensors, and glucose, in particular, is a key regulator of our sensor's performance.

Armed with this knowledge, we evaluated electrochemical detection of each of the herbicides using the identical β-gal produced in the cell-based sensors. We used a similar scheme as the Miller assay for direct comparison, but instead of using the colorimetric precursor OPNG, we used the redox "silent" substrate PAPG, which can be cleaved into a redox-active component, *p*-aminophenol (Figure 2a). We found that dicamba and Roundup could both be detected by measuring the peak-current of *p*-aminophenol using cyclic voltammetry (Figure 2b,c). We also confirmed that the peak-current for dicamba control samples without the sensor cells was indistinguishable from background, indicating the signal was due to the expression level of β-gal (Figure 2d; see Figure S6a for CV data without sensor cells, see Figure S7a,c for cell-based detection without the sensor plasmid). Importantly, in Figure 2e,f, we found that the cell sensor responses to Roundup and dicamba, in both range and sensitivity, as measured electrochemically were nearly identical to the analogous measurements using the Miller assay. The peak-current for Roundup, increased dramatically (~17×, Figure 2d) compared to controls without the sensor cells (Figure S6a-c) and without the *soxS*-actuator plasmid (Figure S7b,c). An advantage of an enzymatic reporter is that it can amplify signals; accordingly,

DOI: 10.1021/acssensors.9b00085
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these results indicated the sensor cells enzymatically amplified the relevant redox signals by cleaving additional substrate.

As in the case of the Miller assay, we found that the linear-range of detection could be manipulated by glucose supplementation (Figure 2e,f; Figures S8−S10). For Roundup, we found the sensitivity decreased dramatically upon glucose addition (Figure 2e). Dicamba detection, however, was improved by glucose supplementation in that the linear range again shifted to lower concentrations (Figure 2f). We have no mechanistic explanation for this observation, however. Importantly, we repeated all cyclic voltametry measurements with chronocoulometry and found identical trends (Figure S11).

The concordance between the gold-standard Miller assay and our electrochemical methodology using PAPG for quantifying β-galactosidase activity was expected and is highly accurate. That is, in Figure 3a,b, we replotted the electrochemical results as a function of the Miller units. In both cases, diquat (Figure 3a) and dicamba (Figure 3b), the responses were linear over the tested ranges. Both of these assays transform molecular information from diquat and dicamba to readily accessible forms. In the case of the Miller assay, measurements are mediated by spectrophotometry and measurements are made by quantification of yellow color. In the case of the electrochemical measurements, no such instrumentation is needed. Instead, the molecular cues carried by diquat and dicamba are converted directly to electrochemical mediators, ultimately to electrical current as measured by simple electrode systems.[42,43]

Finally, in Figure 3c−e, we demonstrate a potential application of this electrochemically motivated synthetic biology approach by suggesting a miniaturized 3-pin electrode system with a small sample chamber connected to a microelectronic controller.[43] We supplemented water taken from Paint Branch Creek, a local tributary of the Anacostia River, with both diquat and Roundup at similar concentrations as above. Results indicated significant SoxS activation from diquat (Roundup) and much less, but statistically significant activation, from dicamba relative to the fresh water samples. We also noticed the background CV results from creek water versus Z-buffer were significantly different—the peak current from 50 μM PAP was far weaker and occurred at a less-defined potential (Figure S12). These results are not optimized for either cell number, background water supply, or electrode design,[12,13] but readily show that the herbicide-specific sensor cell can provide an indication of these contaminants.[44]

In conclusion, our results demonstrate that dicamba and Roundup can be detected using genetically modified *E. coli* and by simple electrochemical analysis using PAP. Importantly, the levels tested were relevant for toxicity in aquatic systems.[22,40,41] These results represent a step forward in developing rapid, inexpensive, and simple measurement techniques for evaluating contamination of various aquatic samples from toxic herbicides.[31,45−51] We envision future applications will focus on disposable, prepackaged sensors that can easily integrate with basic electronics for mobile detection.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.9b00085.

Supporting figures S1−S11, materials and methods, and a discussion of the influence of dicamba toxicity on sensor performance (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: bentley@umd.edu. Phone: 301-405-4321.

ORCID

Gregory F. Payne: 0000-0001-6638-9459

William E. Bentley: 0000-0002-4855-7866

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Partial support of this work was provided by DTRA (HDTRA1-13-0037), NSF (DMREF #1435957, ECCS#1807604, CBET#1805274), and the National Institutes of Health (R21EB024102).

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DOI: 10.1021/acssensors.9b00085
ACS Sens. 2019, 4, 1180−1184