# A Chemoemitter System Mimicking Chemical Communication in Insects

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## 1. INTRODUCTION

**The pheromone communication of insects has evolved to become a complex scheme for encrypting and transferring messages. Based on this, the aim of this study was to develop an artificial communication system based on functional equivalents of biological machinery that allow eusocial insects to exchange information [1]. A key component of such a system is a chemoemitter that generates semiochemical signals. Here we present the complete design, procedure and functional tests of the first MEMS-based chemoemitter consisting of a micromachined evaporator that together with a biomicroreactor mimic the *S. littoralis* female pheromone biosynthesis.**

## 2. MICROFABRICATION

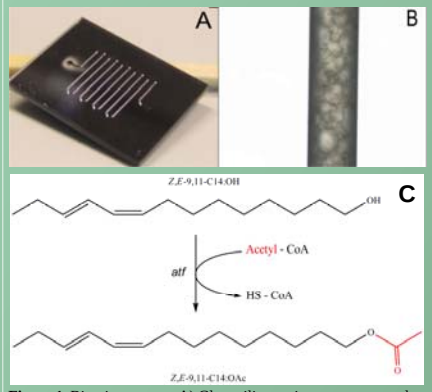


**Figure 1.** Biomicroreactor: A) Glass-silicon microreactor, meander channel with rectangular cross-section (0.25 x 0.20 x 91.26 mm); B) Agarose beads (Novagen) inside the microchannel; C) Pheromone biosynthetic reaction

**The glass-silicon microreactor (Figure 1A) was fabricated using established lithographic methods followed by deep reactive ion etching (DRIE) and anodic wafer bonding. All surfaces connecting capillaries and microchannel were coated with anchored polyelectrolyte multilayer structure (article in preparation), in order to prevent adsorption of substrate, product and enzyme. Inside the microchannel, nitrilotriacetic acid (NTA)-functionalized agarose beads were densely packed (Figure 1B). Purified $His_6$-tagged acetyl transferase (atf) was immobilized on them to transform the substrate (Z,E-9,11-C14:OH) into the pheromone (Z,E-9,11-C14:OAc) (Figure 1C).**

**The second part of the chemoemitter, the evaporator, consists of a silicon membrane (5.00 x 5.00 x 0.04 mm) perforated with ~40000 micromachined via-holes. Rectangular microfluidic channels deliver the mixture of predefined volatile compounds from two inlets to the reservoir (375 nL) located under the membrane (Figure 2). Two thin-film platinum heaters and a 4-wire resistive temperature sensor are integrated in the evaporator and work in a PID loop in order to stabilize the temperature with a variation of 30 mK. The liquid passes through the membrane and evaporates from small droplets that are formed on the outlet of every via-hole.**

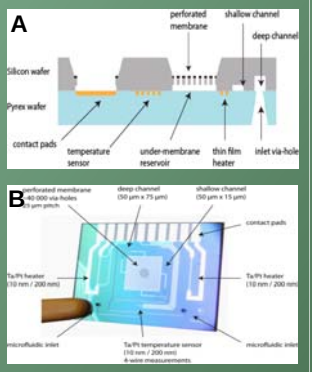


**Figure 2.** Evaporator: A) Schematic representation of the evaporator chip cross-section; B) Image of the evaporator, channel side visible

## 3. FUNCIONALITY OF THE CHEMOEMITTER

**First, the activity of the biomicroreactor was measured and compared to the activity in batch. The results from the experiment (Figure 3) for a flow rate of 10 µL/min evidenced similar conversion in the biomicroreactor and in the batch assay.**

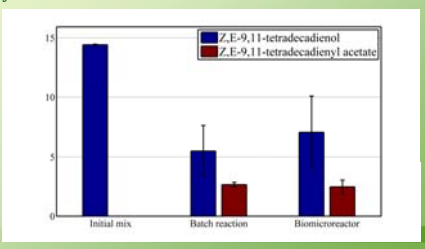


**Figure 3.** Activity test results based on GC-MS peak areas of the substrate and the pheromone relative to that of the internal standard .

## 4. PHEROMONE QUANTIFICATION BY ELECTROANTENNOGRAPHY

**For quantification purposes, a calibration curve was made in base to the amount of pheromone evaporated from a 10 ng/µl aq. soln. of Z,E-9,11-14:OAc, containing 4% DMSO, at different flow rates (0.01-2 µl/min) (Figure 4). Substitution in the curve of the mean EAG response obtained with the microreactor (see below) minus blank provided a concentration of pheromone of 5 ng/µl, consistent with the GC-MS analysis of an aliquot of the solution coming out from the microreactor (3.2 ng/µl). EAG detection proved to be more sensitive than GC-MS because whereas this technique was in the detection threshold for this concentration, the EAG can yet detect lower concentrations, as shown in Figure 4.**

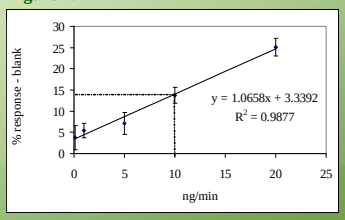


**Figure 4.** Calibration curve of flow rate *vs* EAG response to Z,E-9,11-14:OAc evaporated from an aq. soln.

## 5. MICROREACTOR AND EVAPORATOR TESTS BY ELECTROANTENNOGRAPHIC DETECTION

**The second step involved integration of the whole chemoemitter system and its test by electroantennographic detection. Electroantennography, the selected technique for pheromone detection, records and quantifies the depolarization response of an insect antenna to a chemical stimulus [2]. Figure 5A shows detail of a *S. littoralis* male antenna fixed on two tungsten electrodes.**

**The mean response of 10 male antennae to the sample emerging from the microreactor after partial conversion of Z,E-9,11-14:OH into Z,E-9,11-14:OAc and a blank (320 µM aq. soln. of Z,E-9,11-14:OH containing 4% of DMSO) at a flow rate of 2 µl/min were recorded (Figure 5C). The difference of the mean responses was significant (Student *t* test, $P < 0.01$). EAG trace of the blank (left) *vs* that of the microreactor (right) is shown in Figure 5B.**

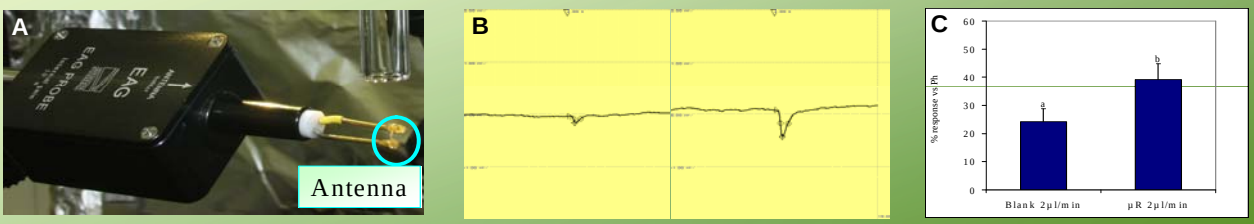


**Figure 5.** Electroantennographic detection of the pheromone produced by the microreactor and emitted by the evaporator.

## 6. CONCLUSIONS

These results proved that the microreactor and the evaporator worked reasonably well and that the EAG is a suitable technique for the detection and quantification of the pheromone produced by the chemoemitter system.

## 7. REFERENCES

[1] Cole, M., Gardner, J. W., Rácz, Z., Pathak, S., Guerrero, A., Muñoz, L., Carot, G., Pearce, T. C., Challiss, J., Markovic, D., Hansson, B. S., Olsson, S., Kübler, L., Gardeniers, J. G. E., Dimov, N. and Bula, W. 2009. Biomimetic insect infochemical communication system. Proceedings 8th Annual IEEE Conference on Sensors, Christchurch, New Zealand.

[2] Guerrero, A., Murgó, R., and Martorell, X. 1986. Physiol. Entomol. 11, 273-77.