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On-package Additively Manufactured Chemiresistive Sensors for Smart Agriculture: Detection of Airborne Sex Pheromone of Domestic Silk Moths

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***Abstract*—Domestic silk moths, *Bombyx mori*, which have been bred for centuries, are insects that play a leading role in the production of silk, an economically valuable and highly sought-after natural fiber. Female domestic silk moths produce two types of sex pheromones, named bombykol and bombykal, respectively. Even though detection of the sex pheromones of domestic silk moths has great economic and academic significance, related work has rarely been reported. In this work, flexile carbon nanotube-based chemiresistive sensors, which were amine-functionalized and additively manufactured via inkjet-printing and drop-casting, were used to target the aldehyde sex pheromone bombykal. Domestic silk moths were home-raised starting from *Bombyx mori* silkworm eggs. Female silk moths, which produce the two types of sex pheromones, were used as the analyte in this work. Custom-designed boxes were 3D-printed in-house to facilitate the pheromone building-up and their exposure to the sensors. Sensing trials were performed in air under ambient conditions with a commercial data acquisition system, automated with home-developed LabVIEW-based programs. A typical sensor showed prompt and significant response upon exposure to the airborne pheromone vapor emitted by 10 female silk moths, achieving a relative sensitivity of 1.2% after 10 minute exposure. A sensing mechanism, which involves the chemical reaction between the aldehyde group of the sex pheromone bombykal and the surface amine groups on the sensors, is proposed.**

Keywords*—Domestic silk moths, Bombyx mori, pheromones, bombykal, smart agriculture, chemiresistive sensors, carbon nanotubes

I. INTRODUCTION

Domestic silk moths, *Bombyx mori*, which have been bred for centuries, are insects that play a leading role in the production of silk, an economically valuable and highly sought-after natural fiber. These moths have a plump body and creamy-white or pale-yellow coloration. The primary goal of adult silk moths is to reproduce. They don't feed during their adult stage. The female silk moths release sex pheromones to attract males. In insects, including silk moths, intraspecific communications between opposite sex individuals is mainly mediated by sex pheromones. The first sex pheromone of silk moths, bombykol ((E, Z)-10, 12-hexadecadien-1-ol), was isolated and characterized in 1959 by Butenandt et al. [1]. Bombykol is secreted from the abdominal glands of the female silk moths and sensed by the antennae of male silk moths. It had been assumed that bombykol was the only sex pheromone through which female and male silk moths communicated, until Kasang et al. found a second sex pheromone in 1977 in the abdominal glands of female silk moths [2]. The second sex pheromone, which is the oxidized form of bombykol, was named bombykal ((E, Z)-10, 12-hexadecadienal). The chemical structures of bombykol and bombykal are shown in Figure 1.

Fig. 1. Chemical structure of the two sex pheromones (bombykol and bombykal) emitted by female silk moth *Bombyx mori***.** Bombykol and bombykal are chemically an alcohol and an aldehyde, respectively.

Detecting the sex pheromones of domestic silk moths has great significance for smart agriculture. For instance, since sex pheromones play a critical role in the mating process of silk moths, information obtained from monitoring the sex pheromones can be used to ensure that mating occurs, to screen for desirable strains, to optimize environments for mating and egg-laying, and to develop sustainable practices to ensure continued silk production without depleting natural resources or causing harm to wild silk moth species. Academically, investigating the sex pheromones of silk moths can provide valuable insights into their behavior, communication, and evolution.

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However, the work on sensing the sex pheromones of silk moths (domestic or wild) has rarely been reported. Some types of sensing methods, which have been used to sense pheromones of other insects, have not been reported to sense silk moth pheromones. Such methods include MEMS-based [2] and metal oxide-based sensors [3], and bulky equipment-based spectrometric methods (ion mobility spectrometry, mass spectrometry, nuclear magnetic resonance spectrometry *etc.*). To the best of our knowledge, only biosensors have been reportedly used to sense pheromones of *Bombyx mori* silk moths. In a typical biosensor for *Bombyx mori* pheromones, a biological recognition element is used to interact with the target pheromones. For example, *Bombyx mori* pheromone binding protein BmorPBP1 was used in a reduced graphene oxide field-effect transistors (rGO-FET) to sense both bombykol and bombykal [1], due to the fact that BmorPBP1 exhibits great and similar affinities to both sex pheromones [4]. For such a sensor, however, the target pheromones have to be dissolved in an aqueous solution, due to the fact that its sensing element, BmorPBP1, is a biological macromolecule which can keep its functionality only in an aqueous solution. In another example, an antenna removed from a male silk moth was used as the sensing element in an electroantennogram-based mobile robot [5]. In summary, these biosensors typically either lack durability or need lengthy sample preparation, and consequently, are not suitable for practical on-site use.

This work reports the first demonstration in which a chemiresistive sensor was used to sense the airborne sex pheromone bombykal emitted by female silk moths.

II. EXPERIMENTAL DETAILS

A. Rearing of silk moths

Bombyx mori silkworm eggs were purchased from Coastal Silkworms Inc. and stored in a 4 °C refrigerator. To begin rearing silkworms, the silkworm eggs were taken out from the refrigerator and exposed to air in A. D. Fang's bedroom (with a temperature of around 20 °C) where the eggs hatched in a couple of weeks. The silkworms were fed with white mulberry leaves until they cocooned. Used egg cartons from a Kroger® supermarket were utilized for the silkworms to spin cocoons. Inside that cocoon a silkworm shed its skin and became a pupa which slowly changed into a silk moth inside the brown pupa shell. About three weeks after a full cocoon was spun, a silk moth eclosed from its cocoon. Male and female silk moths were separated right after their eclosion to prevent mating before the sensing trials. Since a silk moth can live for only about 5 days, sensing measurements were performed immediately after enough number of female silk moths were eclosed.

B. Fabrication of chemiresistive pheromone sensors

Silver-based interdigitated electrodes (IDEs) were inkjet-printed on a polyimide (PI) film with a thickness of 5 mils (Kapton® HN 500, DuPont, Wilmington, DE, USA). A drop-on-demand piezoelectric inkjet printer (DMP-2831, Fujifilm Dimatix, Inc., Santa Clara, CA, USA), a 10-picoliter cartridge (Fujifilm Dimatix, Inc.) and a commercial silver nanoparticle-based ink (EMD5730, Sun Chemical Corporation, Parsippany, NJ, USA) were used to print the IDEs. Three passes of the silver ink were printed on the PI film followed by a 150 °C/30 minute annealing process. A single wall carbon nanotube- (SWCNT-) based homogeneous suspension in dimethylformamide (DMF) solvent with a SWCNT content of 25 µl/ml was prepared with the aid of a probe sonicator (Sonicator 3000, Misonix, Farmingdale, NY, USA). Just before performing sensing trails, a 2.5 µl drop of the resulting suspension was drop-cast onto the finger area of the IDEs followed by drying on a 150 °C hotplate for 10 minutes. Finally, a 2.5 µl drop of tris (2-aminoethyl) amine in DMF was drop cast onto the resulting CNT layer followed by drying on a 150 °C hotplate for 10 minutes.

C. 3D printing of sensing chambers

In order to facilitate the building up of the pheromones and their exposure to the sensors, a sensor chamber, a sample chamber, and a lid for the sample chamber were custom-designed and 3D printed in-house with a stereolithography (SLA) printer (Form 3+, Formlabs). Clear resin (Formlabs) was utilized to print both the sensor and the sample chambers (translucent), and Black resin (Formlabs) was used to print the lid (black) of the sample chamber.

D. Setup of pheromone sensing trials

Figure 2 shows the layer-by-layer topology of the accommodation of the sensors and the silk moths. The sensor chamber (Figure 2a) was immobilized to the surface of a table with double sided tape, in order to minimize vibration during a

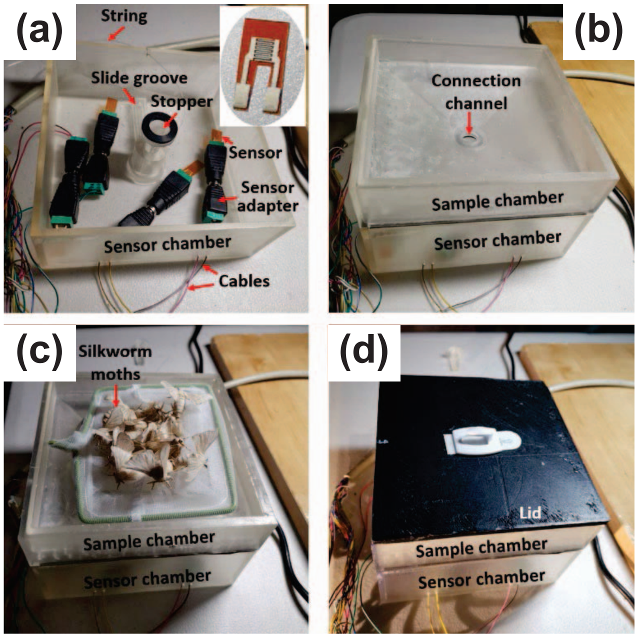

Fig. 2. Topology of the sensing setup. a). The sensor chamber and its contents (bottom layer). Inset shows a typical sensor. An area of the polyimide substrate of the sensor was cut off to fit a sensor adapter. b). The sample chamber (2nd layer) stacked on top of the sensor chamber. The hole at the bottom of the sample chamber acted as a connection channel between the two chambers. This connection channel facilitated the pheromones emitted by the silk moths to diffuse into the sensor box. c). Silk moths in the sample chamber. A home-made net was used as a carrier to transfer live female silk moths to the sample chamber. d). The lid (3rd layer) for the sample chamber.

sensing trial. In order to fit a sensor to a commercial sensor adapter (Figure 2a), the interdigitated electrodes of a sensor was designed to be Y-shaped and an area of the polyimide substrate was cut off (Figure 2a inset). Sensors were hooked up in the sensor box and connected to the data acquisition system (NI Corporation, Austin, TX, USA) via cables and adapters. A custom-designed 3D-printed cylinder-shaped stopper was used to block the connection channel between the sensor chamber and the sample chamber, in order for the pheromones emitted by female silk moths to build up in the sample chamber.

After the female silk moths were placed in the sample chamber (Figure 2c) and its lid was in place (Figure 2d), the pheromones emitted by the moths began to build up inside the sample box. After 10 minute building-up, the string was pulled from outside the sensor box to open the connection channel between the sensor chamber and the sample chamber, thus exposing the sensors in the sensor chamber to the pheromones.

The pheromone launching process can be described more clearly with Figure 3 which shows more details of our custom-designed and 3D printed sensor box. During the pheromone building-up period, the stopper (translucent and solid), which rested on the stopper stand (rectangular cuboid-shaped), was in a position that made it tightly block the connection channel between the sample and sensor chambers. One end of a string was attached to the stopper stand, and the other end of the string went outside the sensor box through a tiny hole in one wall of the sensor chamber. When it was time to expose sensors to the pheromones, the string was pulled from outside the sensor chamber, causing the stand to move along the slide groove towards the string end that was pulled. Consequently, the stopper fell to the bottom of the sensor chamber and the connection channel was unblocked, enabling the diffusion of pheromone vapor from the sample chamber down to the sensor chamber. Indeed, since the molecular weights of bombykol ($C_{16}H_{30}O$), bombykal ($C_{16}H_{28}O$) and air are 238.41, 236.39 and 28.96 g/ml, respectively, both pheromones are much heavier than air, which is beneficial for them to diffuse downwards to the sensor chamber.

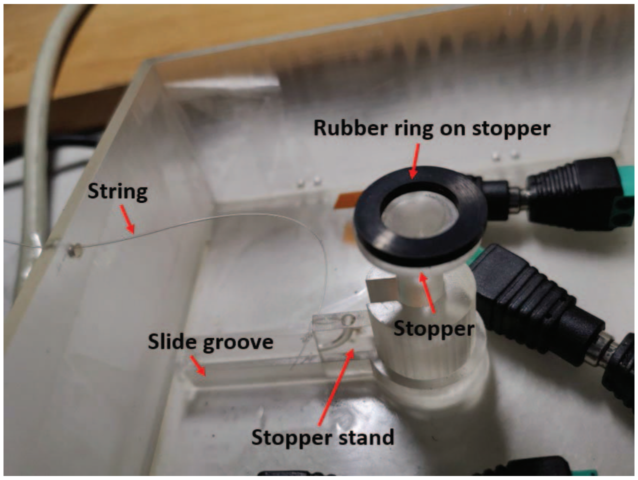

Fig 3. Custom-designed 3D-printed sensor chamber with details of the components for blocking/unblocking the connection channel between the sample and sensor chambers. In the configuration shown in this image, the stopper was resting on the stopper stand.

During a sensing trial, the electric resistance changes of a sensor were recorded by a data acquisition system (NI Corporation) automated with home-developed LabVIEW-based programs.

III. RESULTS AND DISCUSSIONS

Of the two sex pheromones emitted by female silk moths (Figure 1), bombykol is chemically an alcohol while bombykal is an aldehyde. Since the sensors used in this work were functionalized with amino groups, the sex pheromone detected by the sensors must be bombykal. Amine groups don't react with the alcohol bombykol but readily react with the aldehyde bombykal forming an imine derivative or a Schiff base (Figure 4) [6], which might be the mechanism responsible for the observations reported in this work.

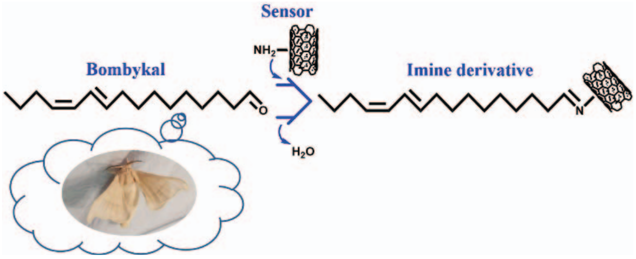

Fig 4. Reaction between a sensor and sex pheromone bombykal emitted by a female silk moth.

Sensing trials in the absence and presence of 10 female moths were conducted similarly except that in the former case the net in the sample chamber (Figure 2c) was empty. In comparison, the net was accommodated with 10 female moths in the latter case.. A sensor was used for a sensing trial in the absence of silk moths, followed by another in the presence of 10 female silk moths. A typical sensing trial took 35 minutes. With female silk moths present in the sample chamber, the first 25 minutes in the sensing trials were intended to serve two purposes: one was to stabilize the background resistance of the sensor and the other to build up pheromones in the sample chamber. After 25 minutes (1500 s), the stopper blocking the channel between sample and sensor chambers was removed (by gently pulling the string from outside the chamber box), allowing the pheromones built up in the sample chamber to diffuse downwards into the sensor chamber. After 10 minutes (600 s) exposure of the pheromones to the sensor, the lid and the net was taken away to remove the pheromone source. After another 30 minutes, data recording was stopped.

Figure 5a shows the real-time resistance changes, as a function of time, of a sensor in the absence (Figure 5a I) and presence (Figure 5a II) of 10 female silk moths. In the absence of silk moths, the electrical resistance of the sense smoothly drifted upwards during the entire sensing trial, with no observable changes taken place when the pheromone exposure was launched or stopped (Figure 5a I). The cause of the baseline drift remains uncertain, yet this phenomenon is frequently observed in polymer-based sensors and documented in various studies [7, 8]. In our particular case in which the sensors were amine-functionalized, there exists another possibility: the amine groups (which are Lewis acids) on the surface of our sensors might react with the CO_2 gas (which is a Lewis base) in the atmosphere, causing the electrical resistance of the carbon nanotubes to increase. In the presence of 10 female silk moths in the sample chamber, the resistance of the sensor also drifted upwards at the early stage of the sensing process (0 – 25 minutes). In spite of this baseline drift, the sensor still exhibited a significant increase in resistance when exposed to the pheromones. Accordingly, even if CO_2 gas in the atmosphere was an interferent for our pheromone analyte, it did not impact our sensors' ability to trigger alerts when the pheromone analyte was present. The sensor was exposed to the pheromones for a duration of 10 minutes (spanning from 1500 to 2100 s), during which the sensor's resistance continued to rise. Upon removing the pheromone source, the resistance of the sensor immediately began to drop. Figure 5b shows the sensor's relative sensitivity changes, as a function of time, in the presence of 10 female silk moths. A relative sensitivity of 1.2% was achieved after the sensor was exposed to the pheromones for 10 minutes. The relative sensitivity (S) is defined by Equation (1):

$$S = (R - R_0) / R_0 \tag{1}$$

where R and R_0 are the resistance values of a sensor at a particular time after and right before, respectively, the sensor was exposed to the target vapor.

Even though the resistance baseline drift negatively affected, our observations of sensor recovery after the pheromone exposure had been stopped, it was still clear that the recovery of the sensor was slow under our experimental conditions (i.e., ambient conditions) (Figure 5a I and 5b). External efforts are needed to recover the sensor to its fresh state.

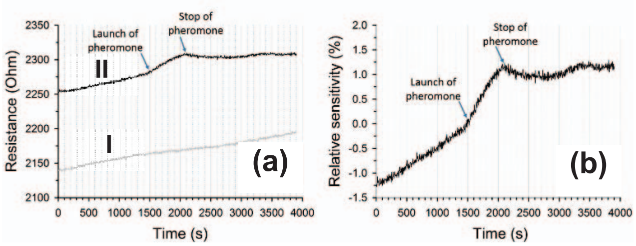

Fig. 5. Sensing profiles of a sensor in the absence and presence of 10 female silk moths. a). Real time resistance profiles of a sensor in the absence (I) and presence (II) of 10 female silk moths. b). Relative sensitivity profile corresponding to the resistance profile in a) II.

IV. CONCLUSIONS

In summary, this study presents the inaugural instance of utilizing a chemiresistive sensor to detect the airborne sex pheromone bombykal emitted by female silk moths. Flexible carbon nanotube-based chemiresistive sensors, functionalized with amines and fabricated using inkjet printing and drop-casting, were employed to target the aldehyde sex pheromone bombykal. Silk moths were bred domestically from Bombyx mori silkworm eggs, with female moths serving as the analyte source for this research due to their production of the relevant pheromones. Custom-designed boxes were internally 3D-printed to aid in pheromone accumulation and exposure to the sensors. Sensing experiments were conducted in ambient air conditions using a commercial data acquisition system, controlled by custom LabVIEW programs. Upon exposure to vapor emitted by 10 female silk moths, a typical sensor exhibited rapid and substantial response, achieving a relative sensitivity of 1.2% after 10 minutes. A proposed sensing mechanism involves a chemical reaction between the aldehyde group of bombykal and the surface amine groups on the sensors.

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