Toward pest control via mass production of realistic decoys of insects

Drew P. Pulsifer[a], Akhlesh Lakhtakia[a,*], Jayant Kumar[b], Thomas C. Baker[c], and Raúl J. Martín-Palma[d]

[a]Materials Research Institute and Department of Engineering Science and Mechanics,
The Pennsylvania State University, University Park, PA 16802, USA

[b]Center for Advanced Materials and Department of Physics and Applied Physics,
University of Massachusetts Lowell, Lowell, MA 01854, USA

[c]Department of Entomology,
The Pennsylvania State University, University Park, PA 16802, USA

[d]Materials Research Institute and Department of Materials Science and Engineering,
The Pennsylvania State University, University Park, PA 16802, USA

ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis*, is an invasive species of beetles threatening the ash trees of North America. The species exhibits a mating behavior in which a flying male will first spot a stationary female at rest and then execute a pouncing maneuver to dive sharply onto her. The pouncing behavior appears to be cued by some visual signal from the top surface of the female's body. We have adopted bioreplication techniques to fabricate artificial visual decoys that could be used to detect, monitor, and slow the spread of EAB populations across North America. Using a negative die made of nickel and a positive die made of a hard polymer, we have stamped a polymer sheet to produce these decoys. Our bioreplication procedure is industrially scalable.

Keywords: Artificial visual decoy, Bioreplication, Conformal-evaporated-film-by-rotation method, Electroforming, Emerald ash borer, Negative die, Positive die, Stamping

1. INTRODUCTION

Bioreplication is the direct replication of a biological structure to realize at least one functionality [1]. In many cases, biological structures of interest have complicated geometries and often include micro- and nano-scaled features that are difficult to produce by conventional fabrication techniques. Technoscientists have begun to adapt templating techniques to exactly replicate these structures [1]. Bioreplication techniques currently include the sol-gel method [2], atomic layer deposition [3, 4], imprinting & casting/stamping techniques [5, 6, 7], and the conformal-evaporated-film-by-rotation (CEFR) method [8].

A bioreplication project undertaken by us aims at the possible eradication of a pest recently introduced to North America. The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is an invasive beetle species that is an increasingly serious threat to North America's native ash trees [9]. This species attacks all North American ash tree species in the genus *Fraxinus* and is likely to cause mortality of nearly all ash trees in areas into which it spreads [10]. Early detection is critical to controlling its spread and recently every year more than 10,000 poorly effective, quite non-selective large purple traps have been deployed by the US Department of Agriculture to try to monitor and arrest this pest's spread. Several seasons of field observations of feral EAB mate-finding behavior by Penn State entomologists showed that the location of a mate is dominated by males responding visually to a female sitting in bright sunlight on an ash leaflet [11, 12]. Males in flight patrolling the tree canopy rapidly descend to land directly onto a female on a leaflet, dropping from a height of between

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[*] Corresponding author. E-mail: akhlesh@psu.edu Tel:+1 814 863 4319

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∼0.3 and 1 m [11, 12]. Knowledge of this behavior has produced new avenues for designing more effective traps. The males' descending flights can be evoked readily by dead EAB individuals that have been pinned to leaves [11], and removal of any possible semiochemicals from the dead *visual decoys* by washing with a solvent does not reduce the attraction of the males at all. Furthermore, when a dead visual decoy is coated with a spray-on sticky material, it becomes a highly effective trap [12].

In order to produce artificial visual decoys of the emerald ash borer (EAB) *en masse*, we need three items: (i) a sheet of a soft moldable polymer that has the same color as the EAB cuticle, (ii) a negative die made of a metal, and (iii) a positive die made of a hard polymer. Our basic idea is to first interpose the polymer sheet between the negative and the positive dies and then to apply pressure so that the polymer sheet is molded into a shape that looks like an EAB specimen from the top. This process to make visual decoys is industrially scalable.

The iridescent green hue of the EAB cuticle is produced by quarter-wave stacking of sub-cuticular layers, which is known from many beetle species to produce strong structural colors without the use of pigments [13]. Accordingly, the polymer sheet would have to be periodically multilayered and function as a polarization insensitive Bragg reflector of green color [13]. The method of electrostatic layer-by-layer deposition [14] has been used to make such reflectors [15]. The unit cell of the polymer sheet comprises two layers which differ substantially in refractive index. Usually the polymers are colloidal suspension of polymers, such as polystyrene or polyvinyl cinnamate (PVCN), stabilized by surface charges. The low-refractive-index polymer typically used is polymethyl methacrylate acid. The polymer colloidal suspensions are adsorbed sequentially with the help of cationic and anionic polymers in solution. Plasticization of these films from solvent vapors can yield the required period (i.e., thickness of the unit cell) for appropriate color reflectivity. An alternate approach which has also been tried is to spin coat polymers of high and low refarctive index on a desired substrate, with the optical thickness of each layer carefully controlled. While carrying out this procedure, solvents for the polymers must be suitably chosen as they must not re-dissolve the previous polymer layer. The polymers PVCN and polyacrylic acid have been used and show Bragg phenomenon in the desired part of the visible regime.

For the production of the negative die, a combination of the CEFR method with stamping [7] is attractive. The CEFR method is a thin-film deposition technique that combines physical vapor deposition [16] with tailored substrate motion. It has been used to produce conformal coatings on non-planar substrates such as fly eyes [17], butterfly wings [8], and finger prints [18, 19]. Quite recently, Pulsifer *et al.* [7] strengthened a CEFR-produced conformal nickel coating with nickel electroforming [20] in order to fabricate a negative die that captured the features of the compound eyes of a blow fly and used it in a casting scheme to produce many positive replicas of the original biotemplate.

For the production of the positive die, two mutually immiscible polymers can be used in a casting scheme. The biotemplate has to be encased in one polymer which can be cured and removed as a thin mold that has to be filled with the second polymer. After curing, the second polymer is a positive replica to be used as the positive die. The second polymer must harden significantly after curing.

This bioreplication project commenced in late 2011. Here we present our preliminary results on the production of a visual decoy made of poly(ethylene terephthalate) (PET) instead of the structurally colored polymer sheet.

2. FABRICATION OF NEGATIVE DIE

2.1. Substrate preparation

A whole EAB specimen was mounted in polydimethylsiloxane (PDMS) on a glass slide which had a layer of kapton tape to serve later as a delamination layer. The mounting was done by placing the EAB specimen on the slide and then pouring the PDMS over it. This step produced a smooth transition between the EAB specimen and the slide. Such a transition is desirable to eliminate areas that would be shadowed during the CEFR process or produce overhangs in the electroformed molds. After curing, the PDMS was peeled away from the upper surface of the specimen's elytrons and head to expose the surface of interest. Finally, the slide-mounted EAB specimen was sonicated in ethanol to remove any residue. Figure 1 shows an EAB specimen mounted on a glass slide with PDMS. This structure serves as a non-planar substrate for the deposition of a thin film of nickel on the biotemplate.

Figure 1. Photograph of an EAB specimen mounted on a glass slide with PDMS. The PDMS mounting allows for a smooth transition between the surface of interest and the glass slide.

2.2. Conformal coating of nickel

Next, a modified CEFR method [21] was used to uniformly coat the slide-mounted EAB specimen with a ~ 500-nm-thick nickel layer to serve as the working face of the negative die and the seed layer for the electroforming step to follow. Figure 2 shows the slide-mounted EAB specimen after being coated with nickel.

The modified CEFR method is implemented in a vacuum chamber containing both the substrate to be coated and a boat containing the material for the coating. A vapor flux generated from the material is obliquely directed towards the substrate. Two programmable stepper motors allow a platform holding the slide-mounted biotemplate to be manipulated in order to produce a uniform coating on the biotemplate.

For coating the EAB specimen, nickel was evaporated from a long and narrow boat made of tungsten. During deposition, the pressure in the vacuum chamber was maintained at $\sim 10^{-5}$ Torr and a current of ~90 A was passed through the boat to maintain a deposition rate of 1 nm/s. The substrate-holding platform was periodically rocked about a fixed axis in the platform plane through angles between 10° and 90° with respect to that plane. Simultaneously, the platform was rotated about its surface normal at 120 rpm. The ~500-nm-thick film was deposited in two successive CEFR runs, each run depositing ~250 nm of nickel.

2.3. Electroforming

Electroforming is necessary to build up the nickel coating on the biotemplate in order to produce a negative die able to withstand the forces exerted when stamping a polymer sheet.

Electroforming of the nickel-coated EAB specimen was carried out in three stages of successively increasing electric current. Three different electroforming stages were found to minimize grain size at the working surface of the die while also allowing acceptable growth rates. During all three stages, the electroforming bath was maintained at ~ 50 °C for 2 days. The current was set at 20 mA for the first stage, 40 mA for the second, and 60 mA for the last stage. The electroformed layer produced was $\sim 100\ \mu$m in thickness.

After the electroforming step, the EAB specimen was removed. Figure 3 is a photograph of the negative die.

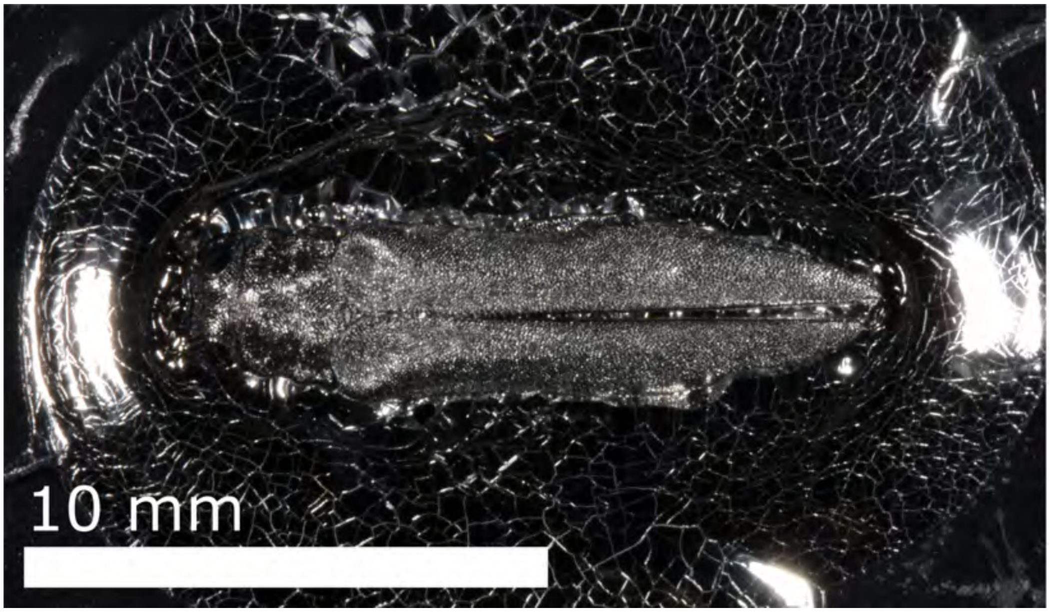

Figure 2. Photograph of the slide-mounted EAB specimen from Fig. 1 after being coated with ∼ 500 nm of nickel in two successive CEFR runs.

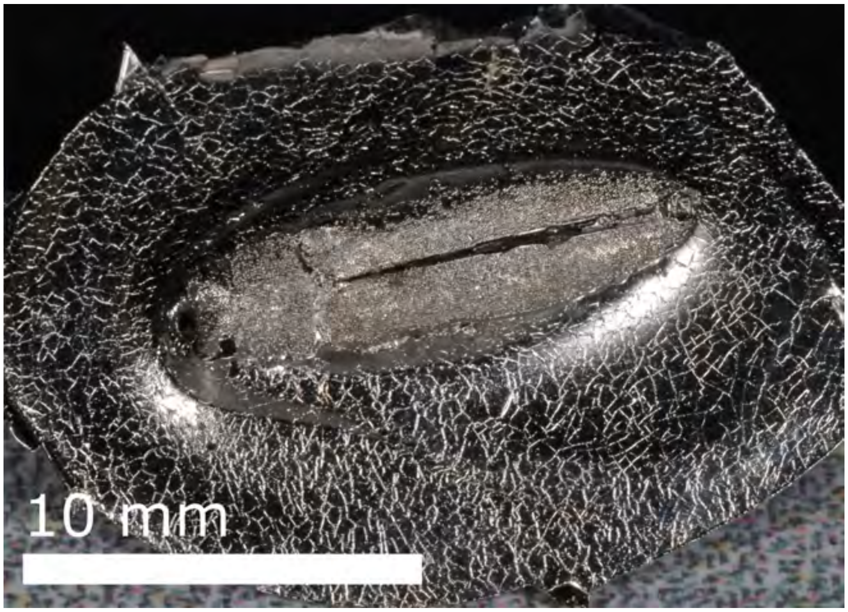

Figure 3. Photograph of the ∼100-μm-thick negative die of the EAB specimen of Fig. 1. The sunken region in the photograph had housed the biotemplate during electroforming.

2.4. Mounting of negative die

Although ∼100-μm thick, the electroformed nickel die has an irregular shape with the negative of the EAB specimen protruding out of the plane of the majority of the die area. To facilitate the application of pressure that would be required to stamp polymer sheets, the negative nickel die was mounted on a brass sheet with an epoxy. Thus the negative die was recessed into the epoxy, thereby allowing pressure to be applied later without inducing a large degree of deformation.

Figure 4 shows the electroformed negative die embedded in a layer of epoxy and mounted on a glass slide (instead of a brass plate).

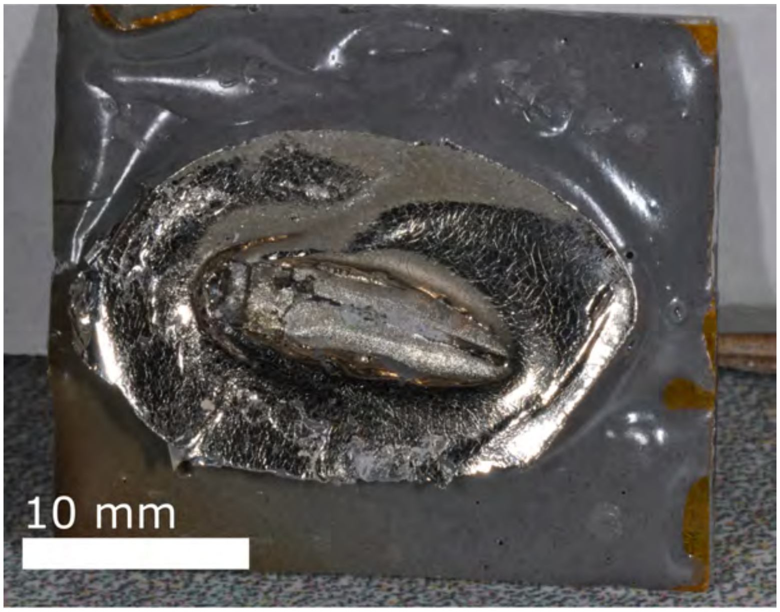

Figure 4. Photograph of the electroformed negative die—from Fig. 3—embedded in a layer of epoxy and mounted on a glass slide.

Figure 5. Photograph of the positive epoxy die fabricated.

3. FABRICATION OF POSITIVE DIE

An EAB specimen was attached to a glass slide covered with a layer of kapton tape and then submerged in PDMS. After curing the PDMS was pulled away from the biotemplate to produce a negative casting of it. This negative casting made of PDMS was then filled with an epoxy. Once the epoxy was cured, the PDMS negative casting was peeled away to leave behind a positive epoxy replica of the EAB. This positive replica was attached to a brass sheet to allow it to be handled and heated. The epoxy in the positive die thus fabricated was trimmed to remove excess material. Figure 5 shows the positive epoxy die which can be mated with the negative nickel die.

4. FABRICATION OF "DECOYS"

In the absence of colored polymer sheets described in Sec. 1, artificial decoys—of neither the right color nor the right materials—were made of poly(ethylene terephthalate) by interposing a PET sheet between the positive and negative dies. Both dies were heated on a hot plate above the melting temperature (about 250 °C) of PET. After sufficient time had elapsed for the dies to reach the desired temperature, a PET sheet was melted into the negative die. With a pool of molten PET in the negative die, the positive die was pressed into the negative by hand. The positive and negative dies were removed from the hot plate together and set aside to cool. Once cooled, the dies were separated. Then the PET replica was removed from the negative die. Figure 6 shows a PET replica which is a visual "decoy"—but only in part because it does not have the right color.

Figure 6. Photograph of a partial decoy made of PET.

5. CONCLUSIONS AND FUTURE WORK

Here we have presented a bioreplication procedure to make artificial visual decoys. This procedure involves the stamping of a polymer sheet with two dies directly replicated from specimen of the emerald ash borer. We have also provided a partial proof of the underlying concept. Our procedure is industrially scalable.

We plan to use this procedure to pattern colored polymer sheets which function as a Bragg reflector, in order to produce decoys that can be used to either further study the behavior of the emerald ash borer or to trap the beetles and thus halt the ecological damage being inflicted by them in North America. In doing so, we have to ensure that the polymer sheets display structural color and are not to be melted during the stamping process.

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