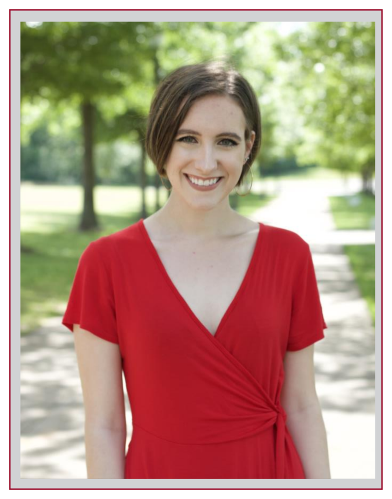

Mechanical Antibiotic Effect of Dragonfly wings on Fluorescently Transformed *E. coli*

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Abstract

Bacterial antibiotic resistance is a growing concern in the healthcare community, resulting in increased research into the development of novel chemical and mechanical bactericides. Our research examined the mechanical antibiotic effects of Blue Dasher (*Pachydiplax longipennis*), Eastern Amberwing (*Perithemis tenera*), and Eastern Pondhawk (*Erythemis simplicicollis*) dragonfly wings on fluorescently transformed *E. coli*. Wings were cut into 6 mm circles and placed in nutrient broth with transformed bacteria in a 96-well plate. Growth of bacteria was monitored for 24 hours using a fluorescence microplate reader. Dragonfly wings as a substrate for bacterial growth were compared to wells containing either no substrate, glue only, polypropylene plastic, or contact lenses. Blue Dasher and Eastern Pondhawk wings significantly inhibited bacterial growth ($p \leq 0.05$) compared to wells with no substrate, glue only, polypropylene plastic, and contact lenses. No significant difference ($p \geq 0.05$) was observed between blue dasher and eastern Pondhawk wings; however, a significant difference was observed between these two dragonfly species when compared to Eastern Amberwing bactericidal efficiency. In conclusion, we found that all substrates of study facilitate bacterial growth, with the exclusion to the Blue Dasher and Eastern Pondhawk dragonfly wings. Additionally, the differences between bacterial growth among dragonfly species, we concluded that bacterial inhibition is dependent on dragonfly species. More trials are needed before Blue Dasher, Eastern Amberwing, or Eastern Pondhawk dragonfly wings can serve as a model for mechanical bactericidal surfaces.

Figure 1: Adult *Pachydiplax longipennis* (top), *Perithemis tenera* (left), *Erythemis simplicicollis* (right). Photos: Odonata Central

Introduction

In recent years, for the development of biocidal surfaces with the ability of killing/preventing pathogenic bacterial growth. There are two primary methods employed for destroying these pathogens: use of antibiotic or chemically coated surfaces and use of natural bactericidal surfaces, with the former of the two causing significant concern. This concern comes regarding bacterial antibiotic resistance, which can lead to widespread destruction.

Antibiotic resistance can lead to the ability of bacteria to withstand the effects of the antibiotic, leading to the increasing difficulty of treating diseases such as tuberculosis and gonorrhea (Muniz and Bonilla 2009). In response to this resistance, the contact killing mechanism has been applied. A mechanism in which sharp nanostructures on biocidal surfaces pierce into the bacterial cell wall, causing it to rupture, and ultimately killing it (Tripathy et al. 2017). This method was found to be remarkably effective in killing both gram negative bacteria and gram positive bacteria when applied to dragonfly wings. Cicada wings however lack potency in the destruction of gram positive bacteria. This is because gram positive bacteria have a far more rigid, thicker cell wall, allowing it to resist more deformation (Wu et al. 2018).

For our research, we tested the bactericidal efficiency of Blue Dasher, Eastern Amberwing, and Eastern Pondhawk dragonfly wings against fluorescently transformed *E. coli*. Our null hypotheses were: (1) there will be no significant difference in the bacterial inhibition of fluorescently transformed *Escherichia coli* across the substrates of study, (2) there will be no significant difference in the bacterial inhibition of fluorescently transformed *Escherichia coli* across dragonfly species.

Methods

Preparation of Substrates

- Following species selection, each of the three dragonfly's forewings were removed using sterile scissors and forceps.
- 12 6-mm circles were punched out of each wing, polypropylene, and contact lens (Acuvue Vita lenses were used for this study) using a sterile 6-mm hole punch.
- All substrates were cleaned using 75% ethanol and allowed to dry.

Microplate Preparation

- A 5-mm glue dot was placed into each well, excluding the negative control group.
- Once the substrates were completely dry, they were added to their respective wells (Figure 4).
- Using a 2:1 dilution of nutrient and bacterial broth, 80 µL of this solution was added to all 96 wells.
- The prepared plate was then inserted into the spectrophotometer a 200 rpm and 32°C for 15 hours, recording fluorescence readings every 30 minutes.
- After the incubation time elapsed, the microplate was removed, and growth charts were examined.

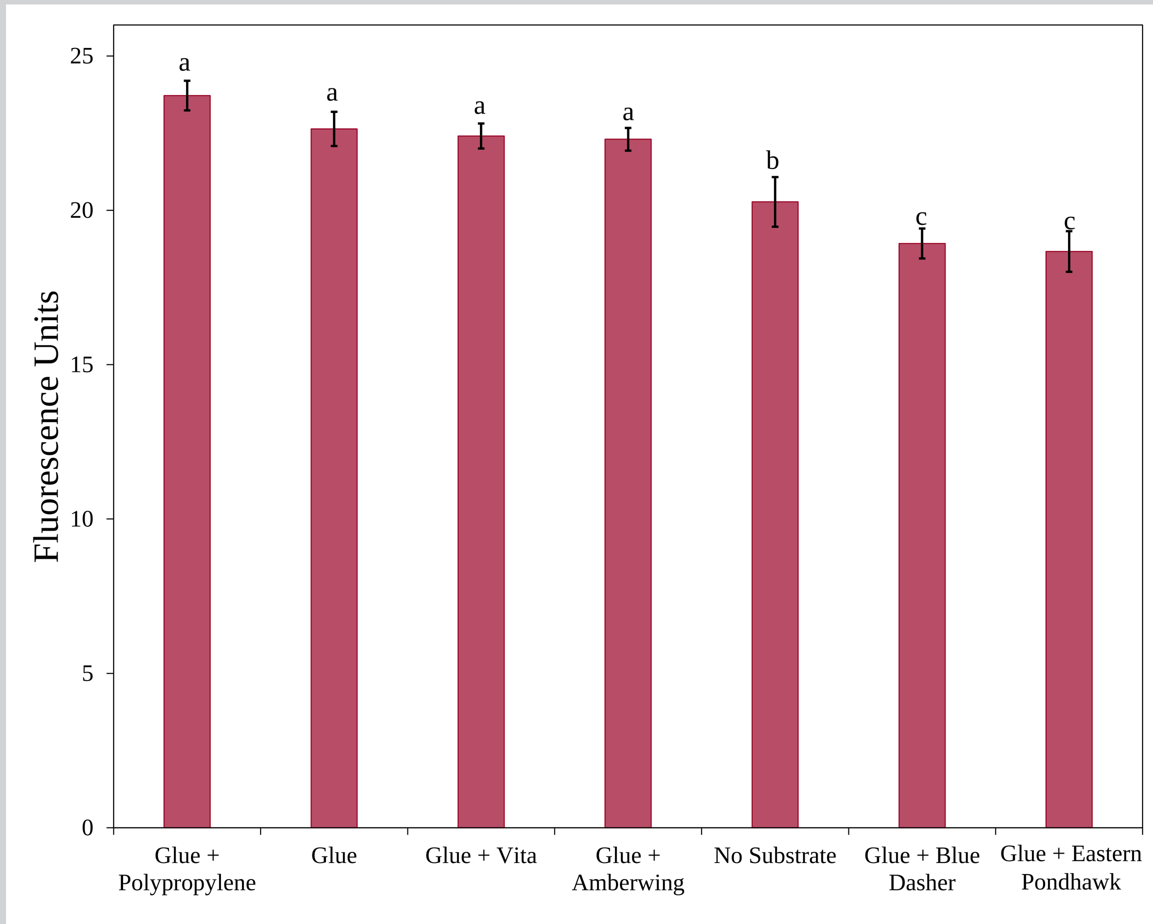

Figure 2. Bar graph displaying the peak fluorescence for each treatment. Statistical significance is indicated above each bar with an a, b, or c. The value of fluorescence units indicates the number of *E coli*, remaining on the surface of study.

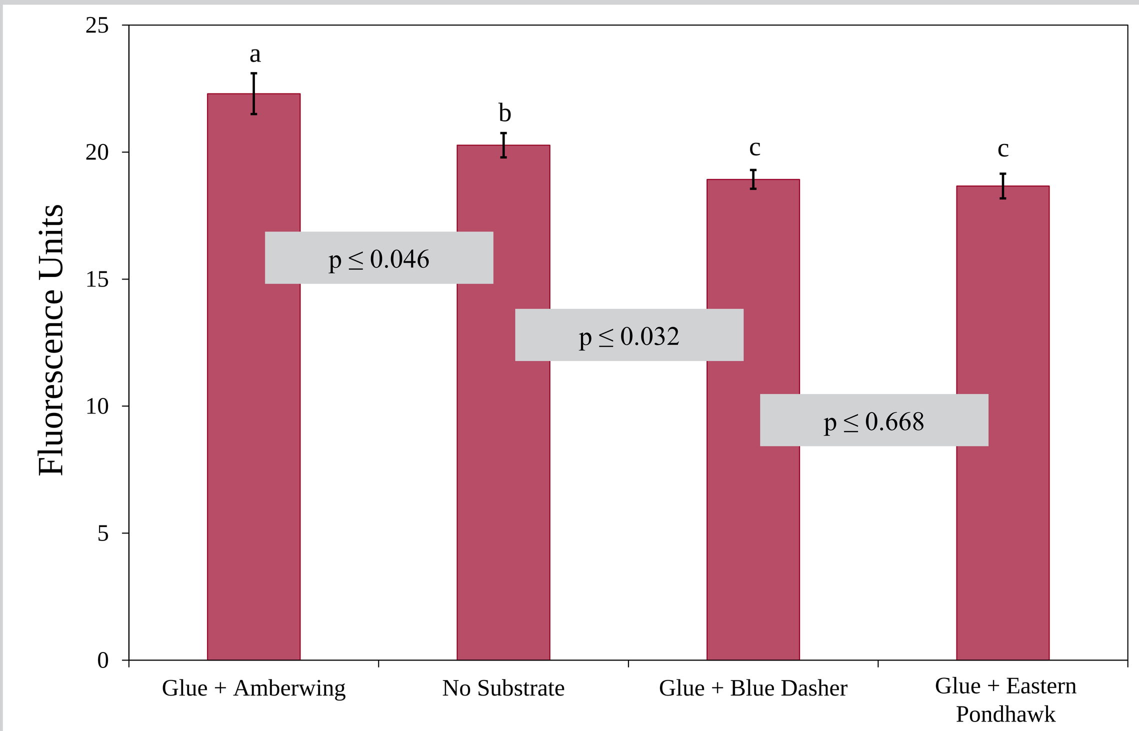

Figure 3. Bar graph displaying the peak fluorescence for each treatment. Statistical significance is indicated above each bar with an a, b, or c. The value of fluorescence units indicates the number of *E coli*, remaining on the surface of study.

Results

Data shows that the Blue Dasher and Eastern Pondhawk dragonflies significantly inhibit bacterial growth when compared to the controls. However, it was apparent that the Amberwing dragonflies did not significantly inhibit bacterial growth when compared to the controls; in fact, this species facilitated bacterial growth. In comparing the 3 dragonfly species, a statistical significance ($p \leq \sim 0.0025$) between their bactericidal abilities was observed. Growth curves indicate the number of fluorescing *E. coli* over 15.5 hours, with the value of fluorescence units indicating the number of *E coli*, remaining on the surface of study.

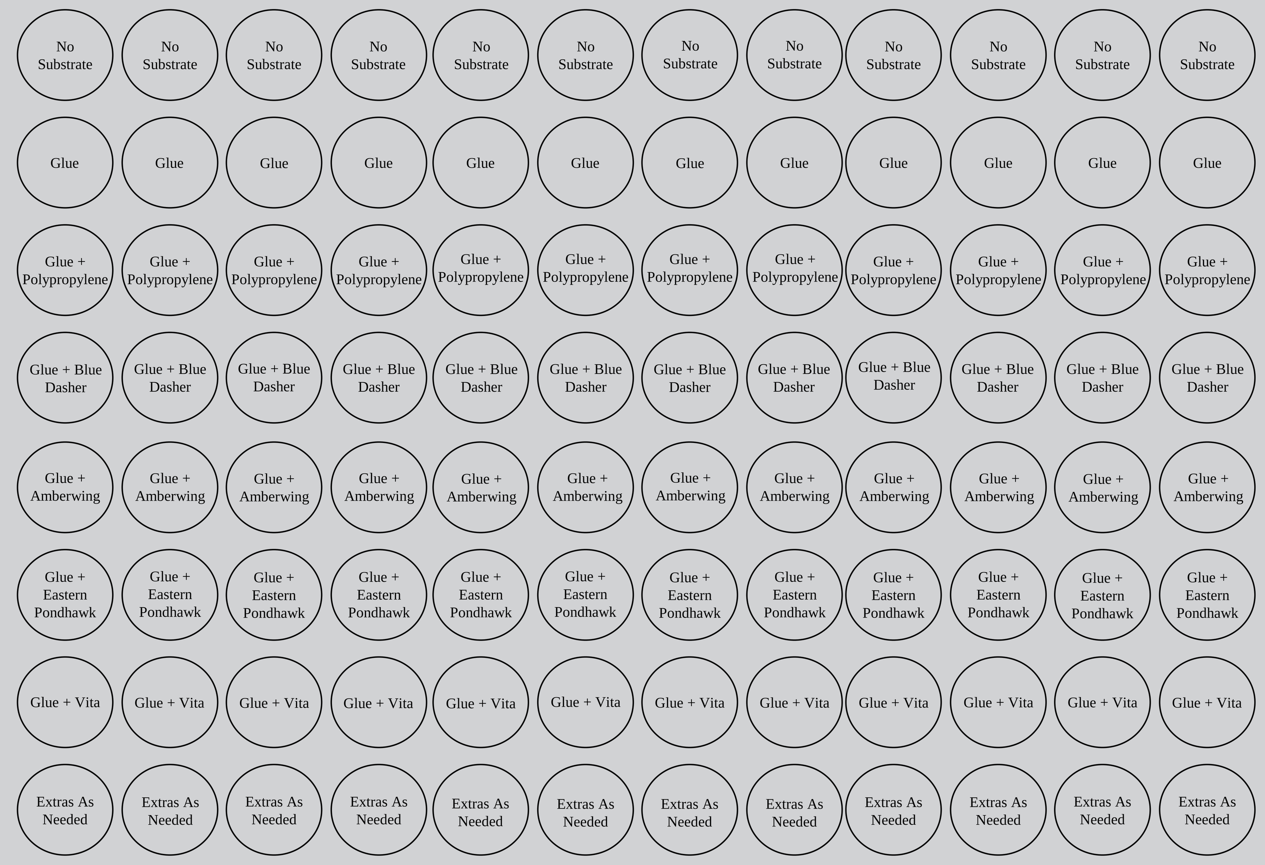

Figure 4: Example microplate containing no substrate, glue, glue + polypropylene, glue + Blue Dasher, glue + Eastern Amberwing, glue + Eastern Pondhawk, and glue + Vita.

Discussion

There was a significant difference among the substrates as well as between the dragonfly species (Figures 2 and 3). There was a significant difference in bacterial inhibition between the Amberwings and control containing no substrate as well as between this control and other dragonfly species ($p \leq 0.05$).

The Blue Dasher and Eastern Pondhawk significantly affected the bactericidal nature of the *E. coli*. (Figure 3). In contrast, the Amberwings appeared to facilitate bacterial growth. These observations were likely due to differing nanostructure properties across wing species such as height and spacing. As determined through our results, these structural differences lead to varying degrees of bactericidal activity between dragonfly species. The culmination of these observations led us to conclude that bacterial inhibition does depend on dragonfly species, contrasting to previous studies.

Literature Cited

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