Antimicrobial Properties of Colored Nectar in the Pepper Plant *Capsicum pubescens*

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Background

- *Capsicum pubescens* produces a yellow-colored nectar. Most documented nectar is non-colored; few studies have been conducted on colored nectar.
- LC-MS data shows that this yellow color is due to the presence of riboflavin.
- Nectar is the host of many microbes and as a result angiosperms have evolved mechanisms to defend against microbial invasion.
- Riboflavin has been shown to have some antimicrobial properties.
- **Here we test if the concentration of riboflavin present in *Capsicum pubescens* could serve as an antimicrobial agent against nectar microbes and explain the occurrence of this pigment in the nectar.**

Capsicum pubescens flower

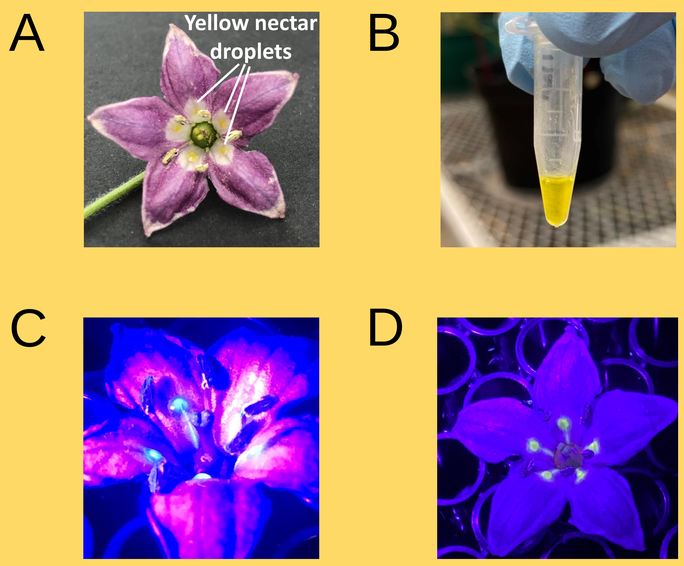

Figure 1. *Capsicum pubescens* flowers and nectar.
A) *C.pubescens* flower in anthesis. **B)** 50 µL nectar collected with micropipettes and combined from many flowers.
C/D) Nectar fluorescing under UV light.

Nectar riboflavin characterization

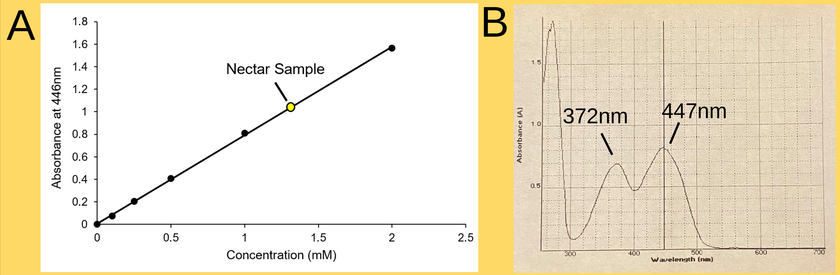

Figure 2. Identifying riboflavin in *C. pubescens* nectar and measuring concentration.
A) Standard curve for riboflavin. Equation is Absorbance=0.79(Concentration (mM)) + 0.006. Calculated nectar concentration = 1.35mM.
B) Absorbance plot from 250-700nm for 1mM riboflavin diluted in M9 media. Peaks occurred at 372nm and 447nm.
C) pH strip readings from *C.pubescens* nectar. pH appears to be between 5.0 and 5.5.

Synthetic nectar antimicrobial assay

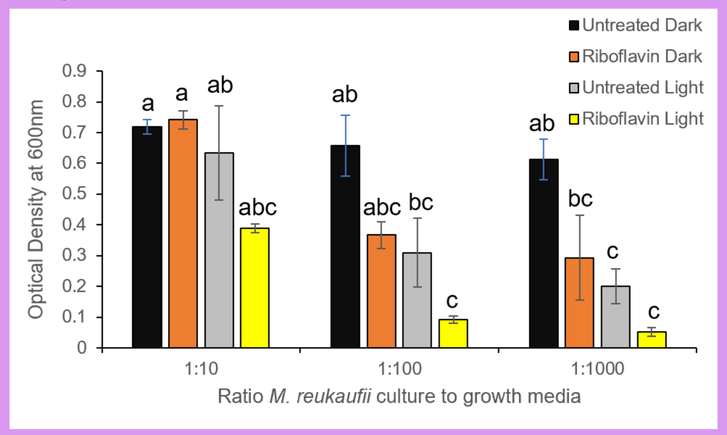

Figure 3. Light/Dark synthetic nectar antimicrobial assay.
A synthetic nectar was created using M9 minimal media to test riboflavin's antimicrobial effect. A microplate was prepared with pure M9 wells and wells treated with a mixture of M9 and 1 mM riboflavin. *Metschnikowia reukaufii* was cultured overnight in M9 media then added in proportions of 1:10, 1:100, and 1:1000 overnight culture to media. The data in the figure represents optical density of the samples measured at t = +96 h. Error bars represent SE (N=3). Bars that share a letter are not significantly different from one another based on one-way ANOVA ($p<0.05$).

Effect of light on nectar coloration

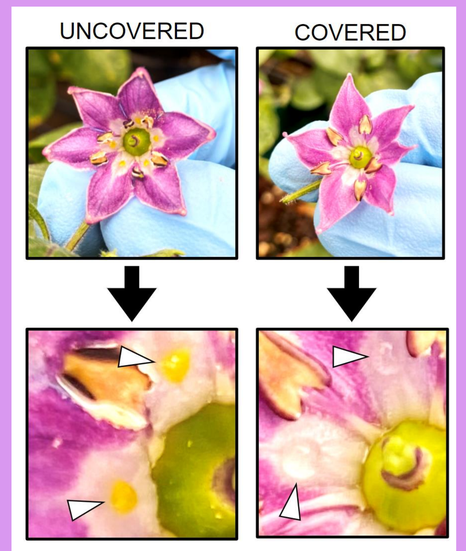

Figure 4. Light impacts the accumulation of riboflavin in *Capsicum* nectar. Prior to nectar secretion, flowers were covered with foil for 24 h to block sunlight then foil was carefully removed. A flower exposed to full sunlight (left) accumulates ~1 mM riboflavin in the nectar, whereas a flower covered in tinfoil (right) still produces nectar but does not accumulate riboflavin.

Summary

- It appears that riboflavin does have some antimicrobial effects against the nectar bacteria *M.reukaufii and Acinetobacter* at the 1mM concentration.
- The antimicrobial effect of riboflavin might be enhanced when in light conditions as compared to dark, but it could be the case that light and riboflavin have additive rather than synergistic effects.
- Unanswered questions:
  - How does riboflavin impact growth?
  - How is riboflavin made and secreted by the flower? Could it depend on light?
  - Does the color of riboflavin help to attract pollinators?