The Interaction of Milkweed Cardenolides and Pre-existing Color Preferences in Bumblebee Foraging Choices Reed Warburton, Class of 2023

Pollinator preferences for particular plants can be influenced by multiple factors including flower color, scent, shape, location, and nectar chemistry. Milkweed plants contain toxic compounds, called cardiac glycosides (or cardenolides), which deter herbivores from eating the plants' leaves. These same compounds also occur in flower nectar, which is surprising given that they are expected to be toxic to pollinators as well. The common eastern bumblebee (Bombus impatiens) has been used extensively as a model organism for research on foraging behavior and bumblebee cognition. I used a laboratory assay of bee foraging behavior in flight arenas to analyze the relationship between the expected deterrent effects of milkweed cardenolides are not commercially available, therefore I used ouabain, a cardenolide structurally similar to the ones found in milkweed nectar, at 10 ng/µl and 100 ng/µl which are the two ends of the spectrum of naturally occurring concentrations of cardenolides in nectar. I tested 63 bumblebees from six colonies, with each bee making multiple foraging bouts among blue and yellow artificial flowers in a laboratory arena.

Colonies of bees were allowed to forage within an artificial arena populated with 12 clear simulated flowers. Good foragers were tagged and later allowed to forage three times alone, with the clear flowers being replaced with blue and yellow. Each color was associated with a different nectar treatment, and the color and treatment pairing were balanced across all 63 bees. The treatments were: the control in which both flower colors were paired with 0.7 M sucrose (i.e. sucrose vs. sucrose), sucrose vs 10 ng/ μ l ouabain, sucrose vs 100 ng/ μ l ouabain and 100 ng/ μ l ouabain vs 10 ng/ μ l ouabain. Pairing colors and nectar treatments allowed me to test the strength of bee's nectar preferences in the context of their innate color preferences.

My results indicate that bumblebees are not deterred by ouabain in nectar at concentrations as high as $100 \text{ ng/}\mu$ l. The results also indicate that bees are more strongly influenced by the color of a flower than by the nectar chemistry. Blue flowers were significantly preferred over yellow flowers no matter the nectar treatment. Interestingly, the bumblebees preferred low levels of ouabain over sucrose when it was paired with yellow flowers. This result indicates the presence of a complex web of overlapping preferences, while also lending support to the theory that bees may prefer low doses of toxic compounds under certain conditions.

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