

Functional Ecology

Appendix S1. Author details, cardenolide extraction protocol, and the generalized linear mixed model (GLMM) used to determine the distribution of plants that produced undetectable cardenolides.

Author and Title Section:

Phytochemical changes in milkweed induced by elevated CO₂ alter wing morphology but not toxin sequestration in monarch butterflies

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Cardenolide Analysis:

Samples were ground, extracted in methanol, spiked with 0.15 mg/mL digitoxin internal standard, and compounds of interest were separated using ultra performance liquid chromatography (UPLC, Waters Inc., Milford, MA, USA) with an Acquity BEH C18 column (1.7 μ m, 2.1 x 50 mm, Waters Inc.). Peaks were detected by absorption at 218 nm using a diode array detector. Peaks with symmetric absorption maxima between 216-222 nm were considered cardenolides. We calculated cardenolide concentration as the sums of all separated peak areas, corrected by the concentration of the internal digitoxin standard.

GLMM:

To address the large occurrence of zeros in our chemistry dataset, we performed an analysis investigating whether or not CO₂ treatment or milkweed species influenced the probability that a plant would produce undetectable foliar cardenolides. We ran a generalized linear model with a binomial distribution (link=logit) where the probability that a plant produced no detectible levels of cardenolides was the dependent variable, CO₂ treatment, milkweed species and their interaction were fixed effects and chamber was a random effect.

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