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Supplementary tables follow the references.

Cardenolide, Potassium, and Pyrethroid Insecticide Combinations Reduce Growth and Survival of Monarch Butterfly Caterpillars (Lepidoptera: Nymphalidae)

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Abstract
The monarch butterfly, *Danaus plexippus* L., has evolved to be insensitive to milkweed cardenolides via genetic modifications of Na^+/K^+-ATPase. There is concern for insecticide exposures near agriculture, with little information on monarch caterpillar toxicology. It is unclear how cardenolide insensitivity may affect the sensitivity of monarch caterpillars to pyrethroid insecticides. Additionally, potassium fertilizers may affect monarch caterpillar physiology and cardenolide sequestration. Here, we investigated the growth, survival, and development of caterpillars exposed to the cardenolide ouabain, bifenthrin, and potassium chloride (KCl) alone and in combination. Caterpillars were either exposed to (1) ouabain from third- to fifth-instar stage, (2) KCl at fifth-instar stage, (3) KCl and bifenthrin at fifth-instar stage, or (4) combinations of ouabain at third-instar stage + KCl + bifenthrin at fifth-instar stage. Caterpillar weight, diet consumption, frass, and survival were recorded for the duration of the experiments. It was observed that 1–3 mg ouabain/g diet increased body weight and

diet consumption, whereas 50 mg KCl/g diet decreased body weight and diet consumption. Caterpillars feeding on KCl and treated with 0.2 μg/μl bifenthrin consumed significantly less diet compared to individuals provided untreated diet. However, there was no effect on survival or body weight. Combinations of KCl + ouabain did not significantly affect caterpillar survival or body weight following treatment with 0.1 μg/μl bifenthrin. At the concentrations tested, there were no effects observed for bifenthrin sensitivity with increasing cardenolide or KCl concentrations. Further studies are warranted to understand how milkweed-specific cardenolides, at increasing concentrations, and agrochemical inputs can affect monarch caterpillar physiology near agricultural landscapes.

Keywords: monarch, agrochemical, mixture, toxicology

The monarch butterfly (*Danaus plexippus*) is a well-known specialist of milkweed (*Asclepias* sp.) (Gentianales: Apocynaceae). The reduction of milkweed stems throughout the United States is implicated in the decline of the monarch butterfly population (Pleasants and Oberhauser 2013). Throughout the U.S. Corn Belt landscape, the remaining milkweed species are primarily confined to field margins, forcing monarchs to concentrate near row-crop production (Thogmartin et al. 2017). Because of this proximity to agricultural landscapes, the U.S. Fish and Wildlife Service (USFWS) has identified agrochemicals to be one of five main stressors contributing to the decline of monarchs (USFWS 2020). The monarch is a well-known flagship for insect conservation and raising public awareness about the decline of insect populations (Oberhauser and Guiney 2009). A public survey found that U.S. households valued the conservation of the monarch butterfly at $4–6 billion USD (Diffendorfer et al. 2014). The cost of monarch butterfly conservation and habitat restoration will require substantial funding from government programs to support these actions on public and private lands (Pindilli and Casey 2015). An understanding of the potential stressors and their interactions within habitat restoration sites is critical for maximizing the benefits of this economic investment.

Monarch caterpillars and other herbivorous insects that feed on milkweed are exposed to the cardenolide defenses of milkweeds. Cardenolides are secondary plant metabolites within a subclass of cardiac glycosides derived from triterpenoids with broad-spectrum insecticidal activity (Agrawal et al. 2012). These compounds act on the nervous system targeting Na^+/K^+-ATPase, where they reversibly bind to the α-subunit locking it in a phosphorylated conformation and disrupting ion translocation and nerve function (Horisberger 2004, Dobler et al. 2011). This active site for cardenolide toxicity has been identified with ouabain, a foxglove (*Digitalis* sp.) (Lamiales: Plantaginaceae) cardenolide that selectively binds to Na^+/K^+-ATPase (Lingrel 2010). There are ca. 500 identified cardenolide derivatives with diverse structural conformations (Schönfeld et al. 1985, Agrawal et al. 2012). Of the 73 native milkweed species in North America, nearly all *Asclepias* sp. produce cardenolides, albeit at different compositions and concentrations depending on the milkweed species (Brower et al. 1982, Seiber et al. 1983, Frick and Wink 1995, Agrawal et al. 2012). The monarch is insensitive to cardenolides, resulting from their co-evolution with milkweeds, and has the capacity to sequester these compounds (Holzinger and Wink 1996).

Pyrethroid insecticides are commonly used to control insect pests in corn and soybean across the Midwestern United States (Ragsdale et al. 2011). These broad-spectrum insecticides are neurotoxic and target the voltage-gated Na^+ channel to disrupt neurological function (Clements and May 1977). Because of their acute toxicity, pyrethroids have been used to control a variety of insect pests (Kogan and Turnipseed 1987, Meinke et al. 2021). For example, the timing of soybean aphid infestations and subsequent foliar applications of pyrethroids can occur when monarch caterpillars are present on the landscape (Bradbury et al. 2017). Pyrethroid field residue and exposure modeling data show monarchs developing in field margins 0–10 m from the field may be adversely affected by foliar applications during the breeding season (Olaya-Arenas and Kaplan 2019, Krishnan et al. 2020). Krueger et al. (2021) have shown the pyrethroid insecticides bifenthrin and β-cyfluthrin, at field realistic concentrations, to significantly affect the survival, growth, and development of fifth-instar monarch caterpillars.

Potassium is an essential nutrient for plant growth and stress physiology (Pettigrew 2008). However, potassium is deficient in soils across several Midwestern states (Woodruff et al. 2015). Potassium fertilization can help increase drought tolerance and immune defense in agricultural crops and may be an important tool for mitigating the effects of climate change on crops (Wang et al. 2013). Millions of tons of potassium fertilizer are applied across the United States, with potash fertilizer applied to 63% corn, 43% soybean, and 45% cotton acreage annually (U.S. Department of Agriculture Economic Research Service [USDA ERS] 2019). Pharmacological studies have shown potassium to be an antagonist of cardenolides at the target site in several mammalian systems and, in turn, decrease cardenolide toxicity and inhibition of Na^+/K^+-ATPase by orders of magnitude (Glynn 1957, Baker and Willis 1970). These studies have shown increasing K^+ serum concentrations administered after cardenolide exposure to reverse cardenolide inhibition and recover the function of Na^+/K^+-ATPase (Glynn 1957, Baker and Willis 1970). These results demonstrate the competitive binding of potassium and antagonism of cardenolides at the Na^+/K^+-ATPase (Songu-Mize et al. 1989). Previous studies documented cardenolide-binding site modifications for Na^+/K^+-ATPase in monarchs (Vaughan and Jungreis 1977). These modifications of Na^+/K^+-ATPase are reported to decrease the toxicity of the cardenolide ouabain and, as observed in mammals, the inhibition of Na^+/K^+-ATPase by ouabain can be reversed with increasing concentrations of K^+ (Vaughan and Jungreis 1977). There are 4.6 and 3.2 bill. lbs. of potassium fertilizer applied annually to corn and soybean crops, respectively, in 18 states along the monarch flyway (USDA ERS 2019). However, there are no studies that focus on the effects of potassium on cardenolide toxicity to monarchs in agricultural landscapes. Previous studies show that other lepidopterans can cope with increased concentrations of dietary K^+ (Jungreis et al. 1973, Harvey et al. 1975, Dow and Harvey 1988), albeit with an energetic cost for the maintenance and regulation of osmolarity. The influx of potassium fertilizer applications in agricultural landscapes may affect the sequestration and protective benefits of cardenolides in developing monarchs.

This study evaluated the effects of cardenolide ouabain and potassium chloride (KCl) combinations on the sensitivity of monarch caterpillars to the pyrethroid insecticide bifenthrin. Bifenthrin was chosen as a representative pyrethroid not only because of its relevance as a crop-protection insecticide in Nebraska and Midwest agricultural landscapes but also as a continuation of our previous studies on the sublethal toxicity of pyrethroid insecticides to caterpillars (Krueger et al. 2021). First, we examined the weight and diet consumption of caterpillars exposed to ouabain, KCl, and bifenthrin. Second, we examined the weight, diet consumption, and survival of caterpillars exposed to combinations of KCl and bifenthrin. Third, we examined the weight, diet consumption, and survival of caterpillars pre-exposed to ouabain followed by exposure to combinations of KCl and bifenthrin.

Materials and Methods

Chemicals

Bifenthrin (CAS# 82657-04-3, 99.5%) was purchased from Chem Service Inc. (West Chester, PA) and stored at room temperature. Ouabain (CAS# 11018-89-6) and KCl (CAS#7447-40-7, 99.0%) were purchased from Sigma-Aldrich (St. Louis, Missouri) and stored at room temperature. Stock solutions of bifenthrin were prepared in acetone. Ouabain and KCl solutions were dissolved in deionized water for incorporation into the diet. Deionized water was used as a solvent control for ouabain and KCl treatments, and acetone was used as a solvent control for bifenthrin treatments.

Insects and Artificial Diet

Monarch caterpillars were sourced from a laboratory colony in the Department of Entomology at the University of Nebraska–Lincoln and maintained as described in Krueger et al. (2021). Briefly, eggs were collected daily and stored at 16°C for up to 14 d. The eggs were moved to room temperature and hatched within 2–3 d. Neonates were then placed on an artificial diet within 24 h of eclosion and maintained on the diet through the third- and fifth-instar stages for the experiments. The number of caterpillars per treatment and replications were different for each experiment due to asynchrony of development in the third- to fifth-instar stages. The number of caterpillars tested per treatment and replications are shown in Supplementary Table 1.

The monarch caterpillar diet was prepared following methods outlined in Krueger et al. (2021) with the following modifications. Diet was prepared using Southland multi-species Lepidoptera diet (Southland Products Incorporated, Lake Village, Arkansas) with the addition of 15% (w/w) lyophilized common milkweed (*Asclepias syrica*) leaf powder. Milkweed leaves were collected from garden spaces or field sites receiving no insecticide application, washed in a 10% (v:v) bleach solution, and stored at −80°C. Leaves were freeze dried, ground to a powder, and stored at −20°C.

Monarch caterpillars on artificial diet have been shown to develop significantly slower than caterpillars feeding on milkweed leaves (Greiner et al. 2019). Newly hatched caterpillars take approximately 4–5 d to develop and molt to a third-instar caterpillar, 8–10 d to develop and molt to a fifth-instar stage caterpillar, and 15–17 d to pupation. All experiments used caterpillars that had molted approximately 24 h prior to the start of the experiment to avoid confounding effects from molting.

Single and Combination Chemical Treatments

Single bifenthrin treatments were reported in Krueger et al. (2021). Briefly, stock solutions were prepared in acetone at 0.025, 0.05, 0.1, 0.2, and 0.4 μg/μl and 1 μl was applied topically with a pipette to the dorsal prothorax of each fifth-instar caterpillar to determine effect thresholds. Stock solutions of ouabain were prepared in deionized water and mixed with the diet to achieve concentrations of 0.03, 0.1, 0.3, 1, and 3 mg ouabain/g diet. Concentrations were selected to mimic a range of total cardenolide concentrations documented across *Asclepias* sp. (Rasmann and Agrawal 2011). Artificial diet was prepared in a single batch and cooled to 15–17°C before aliquots of diet were removed to prepare individual diet treatments. Individual diet treatments were dispensed using a 60 ml syringe (BD Biosciences, San Jose, California) in 2.5 ml aliquots into 1 oz. condiment cups (Dart, Mason, Michigan). Diet was either fully consumed or beginning to dry out after 6 days. If the diets were observed to dry out, then the diet was prepared again on day 6. Treated diet that was prepared again on day 6 was prepared following the same methods as described for day 0; however, diet was dispensed in 4 ml aliquots into 2 oz. condiment cups (Dart) to provision caterpillars through the conclusion of the study. KCl was dissolved in deionized water at 0.4, 2, 10, and 50 mg KCl/g diet and treated diets were prepared in separate batches. Since the effects of KCl on caterpillar growth and diet consumption were unknown, KCl concentrations were selected to span a wide range, with the highest concentration approaching limits of solubility in water. KCl was weighed for each treatment, dissolved into deionized water, and boiled before the addition of artificial diet mix and milkweed powder. Diets were dispensed in 4 ml aliquots into 2 oz. condiment cups (Dart) using a 60 ml syringe (BD Biosciences). The number of caterpillars tested per treatment and replications are shown in Supplementary Table 1.

Fifth-instar caterpillars were exposed to either 0 or 10 mg/g KCl and 0, 0.1, 0.2, or 0.4 μg/μl bifenthrin to yield eight treatment groups. KCl concentration was selected to mimic a realistic, environmental exposure and the threshold for significant effects on caterpillar growth and diet consumption. Bifenthrin concentrations were selected based on effects documented by Krueger et al. (2021), which were shown to affect the growth, development, and survivorship of fifth-instar caterpillars. KCl-treated diet was prepared as previously described for the single-compound treatments. Bifenthrin was dissolved in acetone at 0.1, 0.2, and 0.4 μg/μl and topically applied to the dorsal prothorax as described by Krueger et al. (2021). The number of caterpillars tested per treatment and replications are shown in Supplementary Table 1.

On day 0, 100–120 third-instar caterpillars were weighed, and 50–60 individuals were placed on 2.5 ml of untreated diet. Another 50–60 individuals were placed on 2.5 ml diet treated with 1 mg ouabain/g diet. Ouabain diets were prepared following the same method previously described for the single ouabain treatment. On day 6 of the experiment, KCl and bifenthrin treatments were started when caterpillars reached the fifth-instar stage. For each untreated and ouabain-treated group, caterpillars were randomly assigned to 1 of 4 different KCl-bifenthrin treatment groups: (1) no KCl + no bifenthrin, (2) no KCl + 0.1 μg/μl bifenthrin, (3) 10 mg/g KCl + no bifenthrin, or (4) 10 mg/g KCl + 0.1 μg/μl bifenthrin. There was a total of eight different treatment combinations of ouabain, KCl, and bifenthrin. KCl concentrations were selected based on results from the KCl + bifenthrin experiments, and bifenthrin concentrations were selected as a more field-realistic exposure that consistently affected caterpillar growth and diet consumption with minimal effects on survival. KCl and bifenthrin were prepared on day 6, as previously described for the single-compound treatments. The number of caterpillars tested per treatment and replications are shown in Supplementary Table 1.

Diet Consumption and Growth Experiments

Third-instar caterpillars were used to mimic a subchronic ouabain exposure, and fifth-instar caterpillars were used for KCl and bifenthrin treatments as reported by Krueger et al. (2021). First-instar caterpillars were reared in a 128-well bioassay tray (Frontier Agricultural Sciences, Newark, Delaware) to the second-instar stage on 0.5 g untreated artificial diet. For ouabain treatments, third-instar caterpillars were stratified by weight on day 0, and 10 individuals were randomly assigned to each treatment group after molting to the third-instar stage to ensure equal size distribution across treatments. For KCl + bifenthrin treatments, third-instar caterpillars were placed on 2.5 g untreated diet in 32-well bioassay trays (Frontier Agricultural Sciences) to continue developing to the fifth-instar stage. After molting, fifth-instar caterpillars were stratified by weight, and individuals were randomly assigned to the treatment groups. For ouabain + KCl + bifenthrin, caterpillars were randomly assigned to ouabain treatment groups on day 0 and stratified by weight on day 6 for KCl and bifenthrin treatment groups. Caterpillars, diet, and frass were weighed daily for single-compound treatments with ouabain and KCl, and 72-h KCl + bifenthrin treatments. Caterpillars, diet, and frass were weighed every other day on days 0–6 for ouabain + KCl + bifenthrin experiments and weighed every day from day 6 to 10 after administering the KCl and bifenthrin treatments. Mortality was recorded daily for each experiment. To quantify diet consumption, three evaporative control containers were set up for each experiment to quantify loss of diet weight from evaporation. Diet consumption is reported as the difference in diet weight minus the difference in evaporative controls over the same time frame. Ouabain, KCl + bifenthrin, and ouabain + KCl + bifenthrin experiments were repeated in triplicate and KCl experiments were repeated in quadruplicate using caterpillars from three different generations. The number of caterpillars tested per treatment and replications are shown in Supplementary Table 1.

Data Analysis

Caterpillar weight, diet consumption, and survival for each treatment were analyzed in R 4.0.1 (R Core Team 2020). For each treatment, a repeated measures analysis was conducted for weight and diet consumption on individual caterpillars over time, assuming a Gaussian distribution with an AR-1 covariance structure to account for correlation between days. The linear mixed model repeated measures analyses were conducted using the nlme package (Pinheiro et al. 2021) with the lme function. Proportion survival was analyzed for both the KCl + bifenthrin and ouabain + KCl + bifenthrin treatments using a generalized linear mixed model assuming a binomial distribution with a PROBIT link function using the glmer function in the lme4 package (Bates et al. 2015). There was no mortality observed in solvent control treatments (i.e., SD = 0) so survival was analyzed only for treatment groups exposed to bifenthrin for KCl + bifenthrin and ouabain + KCl + bifenthrin experiments. Each treatment was replicated on three or four separate occasions, with experimental replicate treated as a fixed block across all analyses. Baseline caterpillar weight (i.e., weight on day 0) was used as a covariate for all analyses. For both ouabain and the ouabain + KCl

+ bifenthrin treatments, caterpillar growth was exponential over the 9- to 10-d treatment period and, thus, the caterpillar weight response variable and the baseline caterpillar weight covariate were both log-transformed to satisfy assumptions of normality. For the ouabain-only and KCl-only treatments, respective concentrations were log-transformed in the analysis for equal spacing of treatment levels. The Akaike Information Criterion (AIC) was then used as model selection criteria to fit the best polynomial regression. Final reduced model fit for each analysis is provided in Supplementary Tables 2–5. The assessment estimates for each treatment level were compared to the control group at each time point using Dunnett's multiple comparison procedure and reported at the $\alpha = 0.05$ significance level. Caterpillar weight and diet consumption analyses for ouabain-only and KCl-only concentrations were log transformed and treated as quantitative treatment variables since at least five concentrations were used in these experiments. In addition to the treatment comparisons, a threshold concentration was determined for each analysis as the lowest predicted concentration where caterpillar weight or diet consumption significantly differed ($P < 0.05$) from the control. For KCl + bifenthrin and ouabain + KCl + bifenthrin treatments, KCl, ouabain, and bifenthrin treatment variables were treated as qualitative since both experiments had fewer than five concentrations. Therefore, treatment comparisons were tested between the treatments and the control. Conditional residual plots were used to assess model fit. All figures were generated using the estimates obtained using the estimated marginal means (emmeans) package (Lenth et al. 2020) from the model outputs at the average base caterpillar weight for that treatment experiment and the ggplot2 package (Wickham 2016) for plotting.

Results

Single and Combination Chemical Treatments

The results of caterpillar weights after receiving an ouabain diet are shown in Figure 1A. There was no significant caterpillar mortality observed after each treatment for the duration of the experiment. There was a significant increase in body weight (36–57%) for caterpillars feeding on the 3 mg ouabain/g diet for 3–10 d compared to the individuals receiving the untreated diet ($P < 0.05$) (Fig. 1A). Similarly, the caterpillars feeding on the 1 mg ouabain/g diet for 4–10 d had significantly higher body weight (25–30%) compared to the individuals receiving the untreated diet ($P < 0.05$) (Fig. 1A). The final generalized linear mixed model showed a significant linear relationship between log(ouabain) and caterpillar weight ($F = 13.29$; df = 1, 174; $P < 0.001$) as well as log(ouabain) × day and caterpillar weight ($F = 3.92$; df = 9, 1580; $P < 0.0001$). Day, experimental replicate, and starting caterpillar weight also had a significant effect on caterpillar weight ($P < 0.0001$). The quadratic log(ouabain) ($F = 0.47$; df = 1, 174; $P = 0.45$) and quadratic log(ouabain) × day ($F = 1.76$; df = 9, 1580; $P = 0.072$) terms were kept in the model based on AIC. Using the model to estimate caterpillar weight across ouabain concentrations, after 3 d of feeding on an ouabain diet, the model estimated concentrations above 1.25 mg ouabain/g diet will significantly ($P < 0.05$) increase caterpillar weight over the course of the 10-d exposure period. The exact concentration threshold for significance varies from 1.26 to 2.50 mg ouabain/g diet between 3 and 10 d and is shown as the dotted vertical line each day in Figure 1A.

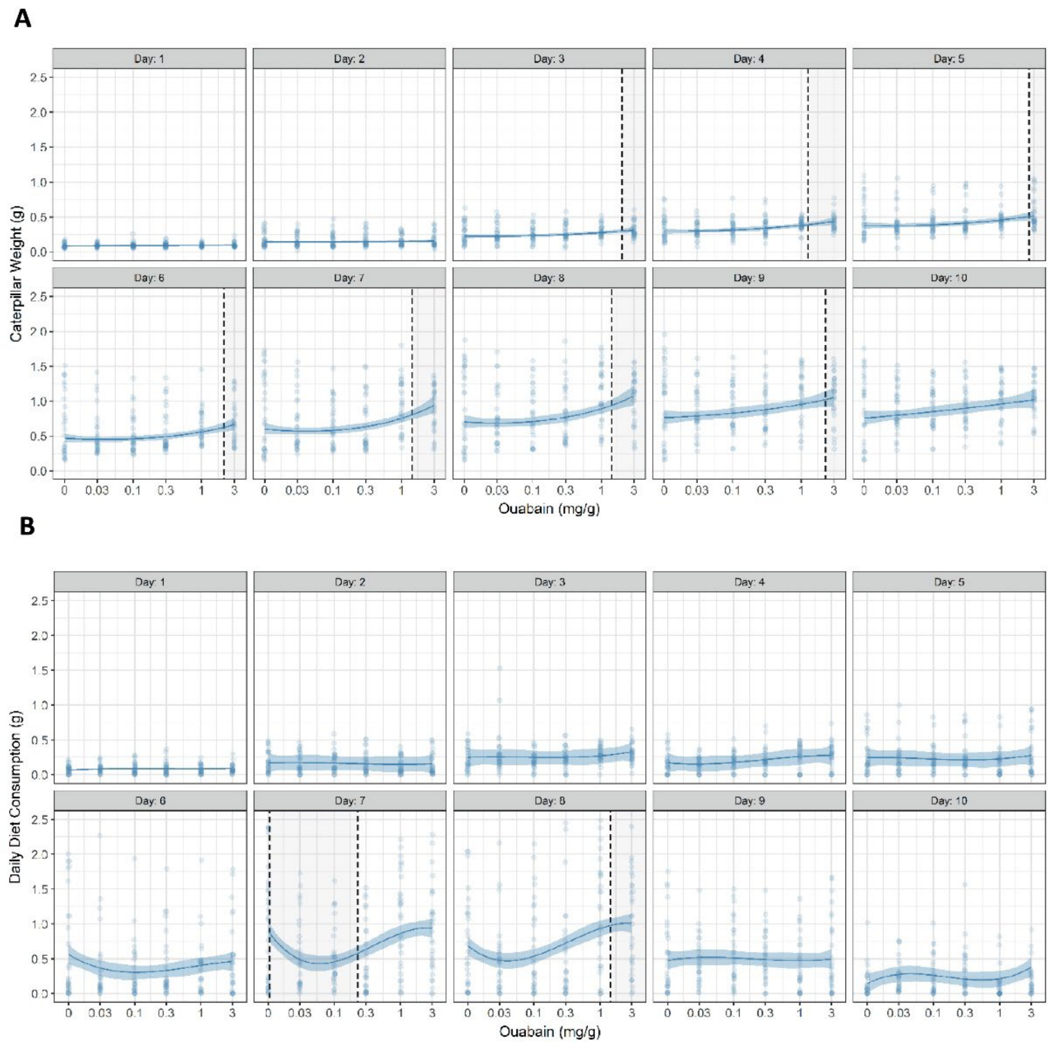

Figure 1. Caterpillar weight (A) and daily diet consumption (B) throughout ouabain exposure. Estimates of the linear mixed model output are represented by the connecting line, and 95% confidence intervals are shown as shading around the line. Response is plotted on a logarithmic scale on the ouabain axis, but axis labels are converted to linear scale. Black dotted line represents lowest concentration where the response significantly differs ($P < 0.05$) from the control. Gray shading indicates range of concentrations with significant differences ($P < 0.05$) from the control.

The results of daily diet consumed by caterpillars after receiving an ouabain-treated diet are presented in Figure 1B. A significant 37% ($t = 2.88$; df = 173; $P = 0.020$) and 47% ($t = 3.08$; df = 173; $P = 0.011$) increase in diet consumption was observed for caterpillars exposed to a 1 and 3 mg ouabain/g diet, respectively, after 8 d compared to the individuals receiving an untreated diet (Fig. 1B). However, the caterpillars exposed to a 0.1 mg ouabain/g diet exhibited a significant 46% reduction ($t = -2.70$; df = 173; $P = 0.034$) in diet consumption after 6 d compared to the caterpillars receiving an untreated diet (Fig. 1B). After 7 d, the caterpillars exposed to 0.03, 0.10, and 0.30 mg ouabain/g diet were observed to exhibit a significant 45% ($t = -4.85$; df = 173; $P < 0.0001$), 49% ($t = -460$; df = 173; $P < 0.0001$), and 41% ($t = -2.89$; df = 173; $P = 0.019$) reduction in diet consumption, respectively, compared to the individuals receiving an untreated diet (Fig. 1B). The final generalized linear mixed model used a significant quadratic log(ouabain) ($F = 5.09$; df = 1, 173; $P = 0.025$) term as well as linear ($F = 3.96$; df = 9, 1572; $P < 0.0001$), quadratic ($F = 2.72$; df = 9, 1572; $P = 0.0032$), and cubic log(ouabain) × day ($F = 1.99$; df = 9, 1572; $P = 0.037$) interaction terms. Day and starting caterpillar weight also had a significant effect on diet consumption in the model ($P < 0.01$). Given the significance of the cubic and quadratic interaction terms and limited significant comparisons, model predictions were not determined.

The results of caterpillar weights after receiving a KCl diet are shown in Figure 2A. A significant 39% ($t = -8.16$; df = 143; $P < 0.0001$), 51% ($t = -13.2$; df = 143; $P < 0.0001$), and 55% ($t = -14.5$; df = 143; $P < 0.0001$) decrease in body weight was observed for caterpillars exposed to a 50 mg KCl/g diet at 24, 48, and 72 h, respectively, relative to the untreated individuals. The final generalized linear mixed model used significant linear, quadratic, cubic, and quartic log(KCl) and log(KCl) × day interaction terms ($P < 0.05$). Day, experimental replicate, and starting caterpillar weight also had a significant effect on caterpillar weight ($P < 0.01$). Using the model to estimate caterpillar weight across KCl concentrations, the model estimated concentrations between 21 and 26 mg KCl/g diet will decrease caterpillar weight relative to controls over the 72-h exposure period.

The results of daily diet consumed by caterpillars after receiving a KCl-treated diet are presented in Figure 2B. A significant 88% ($t = -8.00$; df = 143; $P < 0.0001$), 90% ($t = -9.38$; df = 143; $P < 0.0001$), and 91% ($t = -6.79$; df = 143; $P < 0.0001$) decrease in diet consumption was observed at 24, 48, and 72 h, respectively, for caterpillars exposed to 50 mg KCl/g diet compared to individuals receiving the untreated diet. The final generalized linear mixed model used significant linear, quadratic, cubic, and quartic log(KCl) terms ($P < 0.05$). Day, experiment, and starting caterpillar weight also had a significant effect on caterpillar weight ($P < 0.01$). Using the model to estimate diet consumption across KCl concentrations, the model estimated concentrations between 22 and 30 mg KCl/g diet will decrease diet consumption relative to untreated individuals.

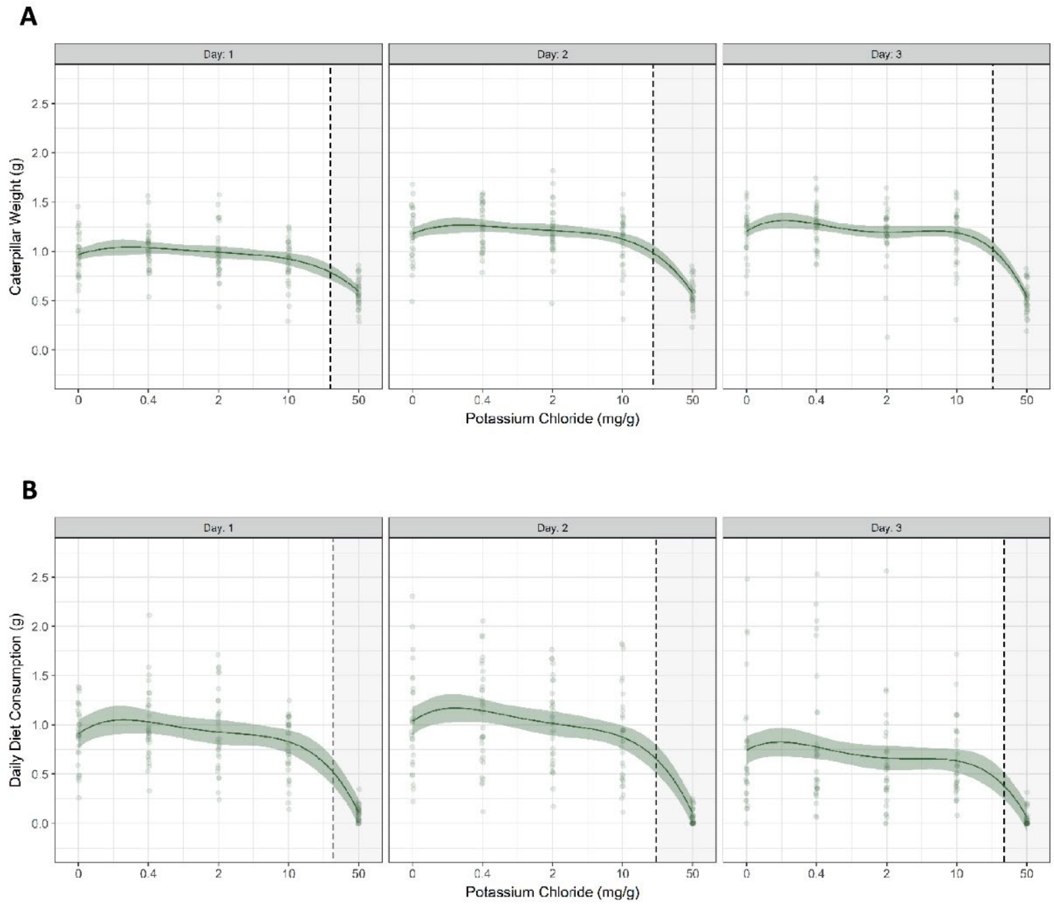

Figure 2. Caterpillar weight (A) and daily diet consumption (B) throughout potassium chloride exposure. Estimates of the linear mixed model output are represented by the connecting line, and 95% confidence intervals are shown as shading around the line. Response is plotted on a logarithmic scale on the potassium chloride axis, but axis labels are converted to linear scale. Black dotted line represents lowest concentration where the response significantly differs ($P < 0.05$) from the control. Gray shading indicates range of concentrations with significant differences ($P < 0.05$) from the control.

The results of caterpillar weight after receiving a KCl-treated diet and bifenthrin treatment are presented in Figure 3A. There were no significant differences in weight between caterpillars that received a KCl-treated diet relative to caterpillars receiving an untreated diet at any time point during the 72-h exposure period. The results of daily diet consumed by caterpillars receiving a KCl-treated diet and treated with bifenthrin are presented in Figure 3B. A significant 41% ($t = -1.99$; df = 146; $P = 0.048$) and 52% ($t = -2.77$; df = 146; $P = 0.0063$) decrease in daily diet consumption was observed at 48 and 72 h, respectively, for caterpillars receiving a KCl-treated diet and treated with 0.2 μg/μl bifenthrin compared to caterpillars receiving an untreated diet and treated with 0.2 μg/μl bifenthrin. Caterpillars receiving only KCl-treated diet and not treated with bifenthrin exhibited a significant 43%

(t = −5.08; df = 146; P < 0.0001) reduction in diet consumption after 48 h compared to caterpillars receiving an untreated diet. The results of survival for caterpillars receiving a KCl-treated diet and treated with bifenthrin are presented in Figure 4. There were no significant differences in survival between untreated caterpillars and caterpillars receiving a KCl-treated diet and treated with 0.1 (z = 0.85; P = 0.39), 0.2 (z = 1.55; P = 0.12), and 0.4 (z = −1.71; P = 0.088) μg/μl bifenthrin. On the untreated diet, the binomial model predicts 75, 72, and 25% of caterpillars will survive 0.1, 0.2, and 0.4 μg/μl bifenthrin, respectively, 72 h after treatment. On the KCl-treated diet, the model predicts 86, 91, and 4% of caterpillars will survive 0.1, 0.2, and 0.4 μg/μl bifenthrin, respectively, 72 h after treatment.

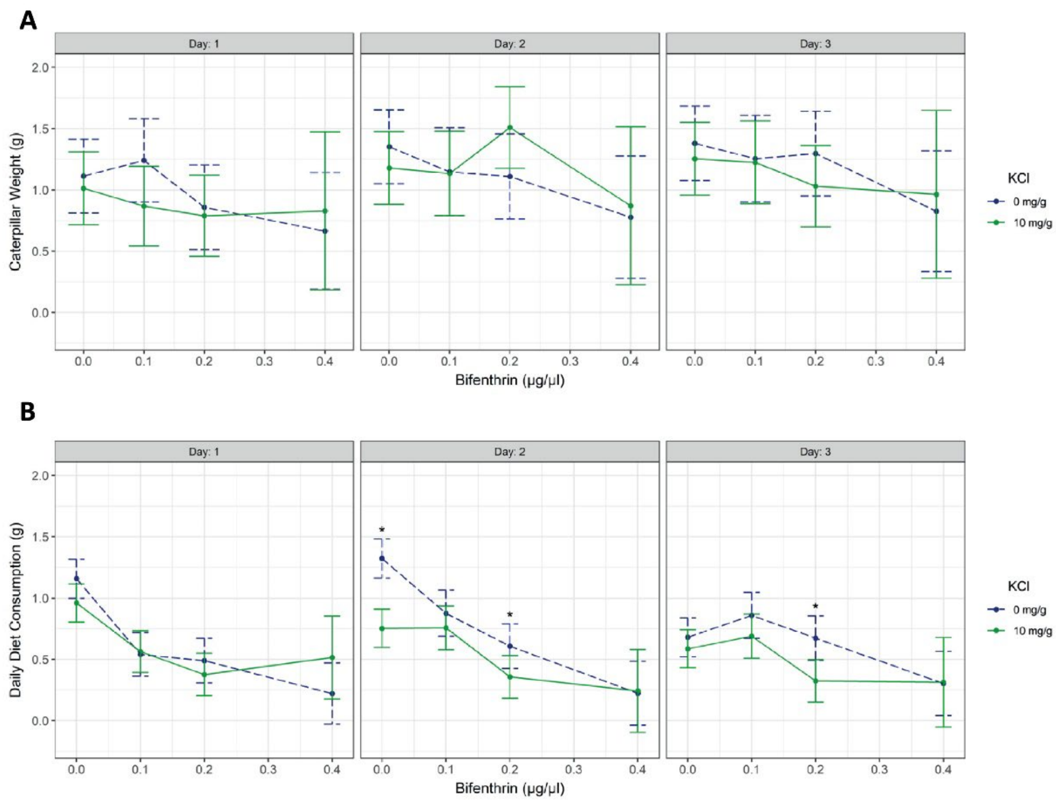

Figure 3. Caterpillar weight (A) and diet consumption (B) with KCl and bifenthrin exposure. Symbols and depict average with upper and lower 95% confidence intervals. Asterisks denote significant (P < 0.05) differences between 0 KCl and 10 mg/g KCl.

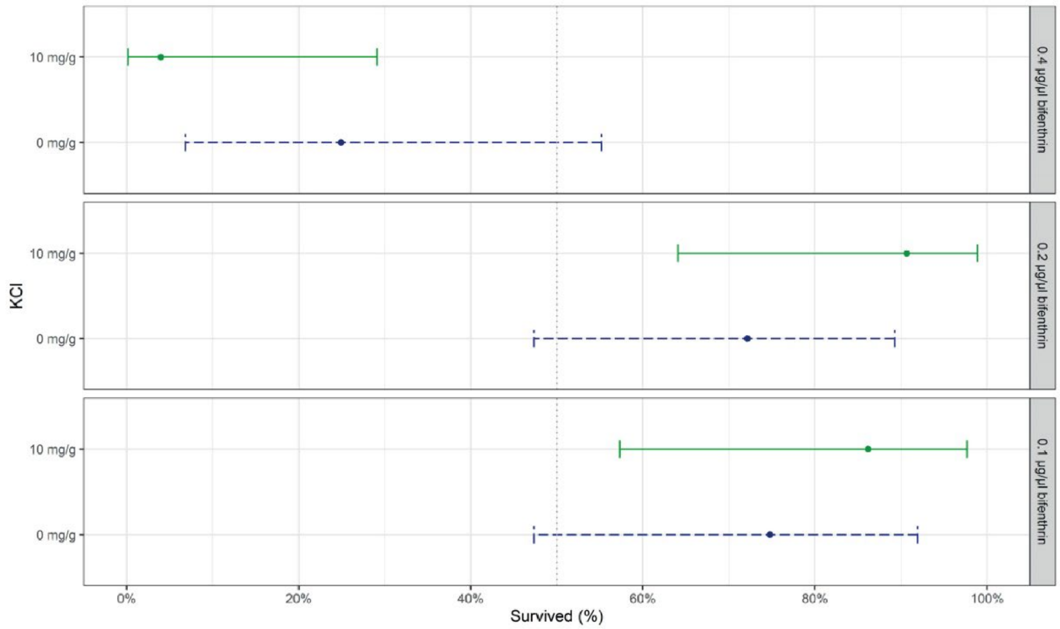

Figure 4. Survival of fifth-instar caterpillars 72 h following topical bifenthrin treatment with and without KCl exposure. Symbols depict average with upper and lower 95% confidence intervals. No significant ($P < 0.05$) differences in survival between caterpillars exposed to 0 KCl or 10 mg/g KCl were observed.

The results of caterpillar weight after receiving a KCl- and ouabain-treated diet and treated with bifenthrin are presented in Figure 5A. There were no significant ($P > 0.05$) differences in growth between any treatments on day 2, 4, or 6 prior to bifenthrin or KCl treatments. Ouabain had no significant effect ($P > 0.05$) on caterpillar growth or diet consumption for any combination of KCl and bifenthrin. On day 7, 24 h after the KCl and bifenthrin treatments, a significant 32% ($t = -5.14$; df = 208; $P < 0.0001$), 28% ($t = -4.35$; df = 208; $P < 0.0001$), 26% ($t = -3.94$; df = 208; $P < 0.0001$), and 29% ($t = -4.54$; df = 208; $P < 0.0001$) decrease in body weight was observed for caterpillars receiving an untreated diet, KCl-treated diet, ouabain-treated diet, and ouabain plus KCl-treated diet, respectively, relative to individuals that were treated with acetone. Similarly, on day 8, 48 h after treatment with bifenthrin, a significant 27% ($t = -4.07$; df = 208; $P < 0.0001$), 23% ($t = -3.38$; df = 208; $P = 0.0009$), 17.1% ($t = -2.42$; df = 208; $P = 0.0163$), and 26% ($t = -3.82$; df = 208; $P = 0.0002$) decrease in body weight was observed for caterpillars receiving an untreated diet, KCl-treated diet, ouabain-treated diet, and ouabain plus KCl-treated diet, respectively. However, on day 9, a significant 23% ($t = -3.25$; df = 208; $P = 0.0014$) and 13% ($t = -2.25$; df = 208; $P = 0.0257$) decrease in body weight was only observed for caterpillars receiving a KCl-treated diet and an ouabain plus KCl-treated diet, respectively. The final categorical model for the effect of ouabain, KCl, and bifenthrin on caterpillar weight used significant day × ouabain × KCl ($F = 0.77$; df = 5, 954; $P = 0.0421$), day × KCl ($F = 2.83$; df = 5, 954; $P = 0.0151$), and day × bifenthrin ($F = 8.37$, df = 5, 954, $P < 0.0001$) interaction terms. Day, experimental replicate, and caterpillar starting weight also had a significant effect on caterpillar weight ($P < 0.0001$). The ouabain × KCl ($F = 2.15$; df = 1, 208; $P = 0.14$), ouabain × KCl × bifenthrin ($F = 0.201$; df = 1, 208; $P = 0.65$), and day × ouabain × KCl × bifenthrin ($F = 0.774$; df = 5, 954; $P = 0.0151$) interaction terms were not significant in the model.

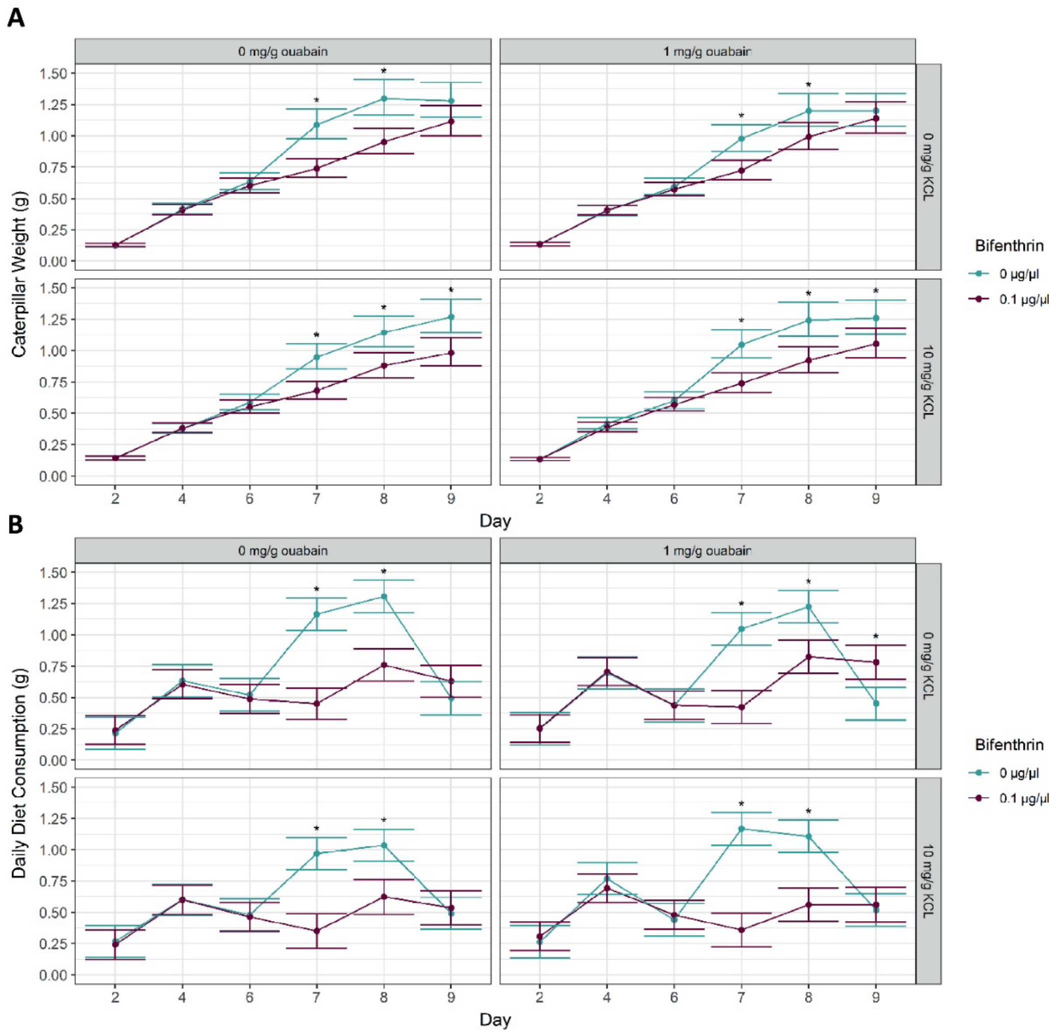

Figure 5. Caterpillar weight (A) and diet consumption (B) with combinations of ouabain, KCl and bifenthrin. Symbols depict average with upper and lower 95% confidence intervals. Asterisks denote significant ($P < 0.05$) differences between 0 bifenthrin and 0.1 µg/µl bifenthrin. Caterpillars were exposed to ouabain from the third-instar stage (day 0) through the duration of the experiment. Caterpillars were exposed to KCl at the fifth-instar stage on day 6-10 and treated with bifenthrin on day 6.

The results of daily diet consumed by caterpillars receiving a KCl- and ouabain-treated diet and treated with bifenthrin are presented in Figure 5B. Caterpillars receiving ouabain and KCl exhibited a significant 32% ($t = -3.25$; df = 208; $P = 0.0014$) and 28% ($t = -3.25$; df = 208; $P = 0.0014$) reduction in diet consumed on day 7 and 8, respectively, 24 and 48 h after treatment with bifenthrin relative to caterpillars receiving the same diet treated with acetone. Caterpillars received ouabain, KCl, and bifenthrin exhibited a significant 32% (t = −2.74; df = 208; P = 0.0067) and 28% (t = −2.26; df = 208; P = 0.0243) reduction in diet consumption on day 8 and 9, respectively, relative to individuals receiving only ouabain and bifenthrin. A significant 17% (t = −2.14; df = 208; P = 0.0334) and 21% (t = −2.96; df = 208; P = 0.0035) reduction in diet consumption was observed for caterpillars receiving a KCl-treated diet on day 8 and 9, respectively, relative to caterpillars receiving an untreated diet. The final categorical model for the effect of ouabain, KCl, and bifenthrin on diet consumption used significant bifenthrin (F = 43.7; df =1, 208; P < 0.0001), day × bifenthrin (F = 54.1; df = 5, 952; P < 0.0001), and day × KCl (F = 3.23; df = 5, 952; P = 0.0068) interaction terms. Day, experimental replicate, and caterpillar starting weight also had a significant effect on diet consumption (P < 0.0001). No other ouabain, KCl, bifenthrin, or day interactions were significant (P > 0.05). The results of caterpillar survival after receiving a KCl- and ouabain-treated diet and treated with bifenthrin are presented in Figure 6. Survival did not significantly differ (P < 0.05) following treatment with 0.1 µg/µl bifenthrin on any ouabain or KCl diet. The binomial model predicts 89, 84, 83, and 83% of caterpillars will survive treatment with 0.1 µg/µl bifenthrin on untreated diet, 1 mg/g ouabain diet, 10 mg/g KCl diet, and 1 mg/g ouabain + 10 mg/g KCl diet, respectively.

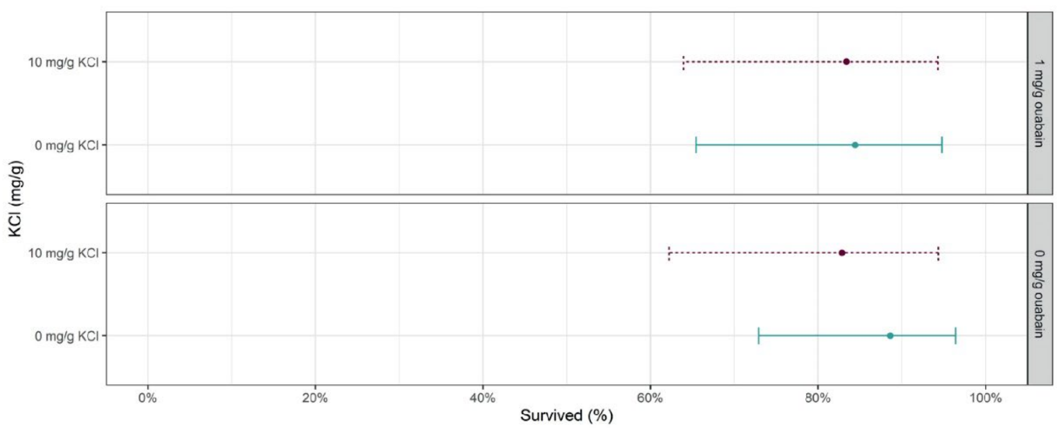

Figure 6. Survival of fifth-instar caterpillars 72 h after treatment with 0.1 µg/µl bifenthrin and combinations of ouabain and KCl. Symbols depict average with upper and lower 95% confidence intervals. Caterpillars were exposed to ouabain from the third-instar stage (day 0) through the duration of the experiment. Caterpillars were exposed to KCl at the fifth-instar stage on day 6–10 and treated with bifenthrin on day 6.

Discussion

This study provides the first report of potassium to affect the growth and development of monarch caterpillars. Here, we also show a concentration-dependent increase in the body weight of caterpillars exposed to the polar *Digitalis*-derived cardenolide ouabain. We observed a significant interaction of KCl + bifenthrin on caterpillar diet consumption and a significant interaction of ouabain + KCl + bifenthrin on caterpillar weight. While these interaction terms were significant in the mixed model analyses, there was no significant interaction observed for KCl and ouabain on the sensitivity of caterpillars to bifenthrin.

We have observed significant increases in caterpillar body mass starting on day 3 and continuing over the 10-day period when caterpillars were exposed to 1 and 3 mg ouabain/g diet. In addition to increased caterpillar weight, we observed an accelerated development time of caterpillars that were exposed to 1 and 3 mg ouabain/g diet compared to lower concentrations. There were more caterpillars that developed to the fifth-instar stage on day 5 and 6 when feeding on the elevated concentrations of ouabain compared to those that fed on lower concentrations. The 1 mg ouabain/g diet used in the interaction experiments was chosen to mimic total cardenolide concentrations of approximately 1 mg/g reported in *Asclepias curassavica* (Rasmann and Agrawal 2011, Tan et al. 2019). Higher cardenolide concentrations in milkweed species have been associated with reduced growth and survival, particularly with early-instar caterpillars (Zalucki et al. 1990, 2001; Pocius et al. 2017). However, polar cardenolides, such as ouabain, are less toxic to and readily sequestered by monarch caterpillars (Frick and Wink 1995) compared to nonpolar cardenolides (Jones et al. 2019). The prevalence of polar cardenolides compared to nonpolar cardenolides is variable between milkweed species (Agrawal et al. 2012). It has been reported that milkweed species with higher cardenolide defenses also contain higher amounts of nonpolar cardenolides (Rasmann and Agrawal 2011). It is challenging to extrapolate our findings with ouabain to native milkweed species. However, it has been reported that tropical milkweed (*A. curassavica*), white swamp milkweed (*Asclepias perennis*), spider milkweed (*Asclepias viridis)*, tall green milkweed (*Asclepias hirtella*), and broadleaf milkweed (*Asclepias latifolia*) all have a higher total concentration and proportion of polar cardenolides (i.e., low polarity score) (Rasmann and Agrawal 2011, Jones et al. 2019). Our data suggest that caterpillars grow faster and have higher body weight when feeding on milkweeds with high levels of polar cardenolides.

We have observed significant effects on caterpillar growth and diet consumption after exposure to 50 mg KCl/g diet with the final model analysis predicting adverse effects to caterpillars at concentrations exceeding 21 mg KCl/g diet. Interestingly, the final models for the effect of KCl on caterpillar weight and diet consumption show a significant quartic relationship with KCl. This quartic relationship estimates increased caterpillar growth and diet consumption after exposure to low concentrations of KCl and before the onset of adverse effects elicited from higher KCl concentrations. Lepidopterans require higher concentrations of salt in artificial diets (Beck et al. 1968, Islam et al. 2004, Han et al. 2012), which is due to the higher K^+:Na^+ ratio maintained in their hemolymph (Harvey et al. 1975). The salt requirements in artificial diets suggest there are elevated concentrations of salts already present in the diet before the addition of potassium. An incremental increase of KCl might adjust the overall concentration to an optimal concentration range of KCl in the diet for the caterpillars. As a result, it is also unclear how much potassium caterpillars receive during the exposure period. Potassium fertilizer exposure is dynamic in the field and the bioavailability to caterpillars is unknown following the application of the fertilizer. Future studies are warranted to estimate the bioavailability of KCl to caterpillars in the field.

Caterpillar weight and diet consumption were used as metrics of sublethal toxicity for each experiment as described by Krueger et al. (2021). These metrics provide similar results for effect thresholds when exposure is limited to a single instar stage of caterpillars (i.e., 72-h treatment for fifth-instar caterpillars). This is evident with the KCl data and the congruent model predictions of effect thresholds for caterpillar diet consumption and weight. However, caterpillar diet consumption can be highly variable across multiple instar stages and longer exposure periods. Caterpillars are observed to stop feeding on the diet as they approach the next molting stage. During these premolt stages, any treatment effects reducing consumption will be confounded by a naturally lower consumption of diet. Additionally, caterpillars within a treatment group will be variable in the molting time, which can lead to differences in daily consumed diet (Fig. 1B). The results from the chronic ouabain exposure suggest caterpillar weight can capture delayed development effects and have reduced margins of error (Fig. 1A).

We observed a significant interaction of KCl × bifenthrin on diet consumption. However, there were no significant interactions observed for caterpillar weight or survival. Caterpillars exposed to 10 mg KCl/g diet and treated with 0.2 μg/μl bifenthrin consumed significantly less diet compared to those provided an untreated diet. Despite the differences in diet consumption, there were no significant differences in caterpillar weight for individuals provided an untreated and KCl-treated diet and caterpillars treated with 0.2 μg/μl bifenthrin. While not statistically significant, there was a trend of increased survival on the KCl-treated diet at 0.1 and 0.2 μg/μl bifenthrin and reduced survival on the KCl-treated diet at 0.4 μg/μl bifenthrin. Padhy et al. (2014) report a reduction in carbamate toxicity to cyanobacterium when co-exposed to potash fertilizers. For the caterpillars treated with combinations of ouabain + KCl + bifenthrin, there was a significant day × KCl × ouabain interaction on caterpillar individual weights, but there was no significant interaction on diet consumption or survival. Overall, the interactions observed do not show KCl or ouabain to affect the sensitivity of caterpillars to bifenthrin. Herbivorous insects feeding on chemically defended host plants often have developed metabolic detoxification resistance to cope with phytotoxins (Després et al. 2007). The overproduction of detoxification proteins, such as esterases and cytochrome P450 monooxygenases, has been documented for a number of insect species (Kasai et al. 1998). This phenomenon has prompted the exploration of cross-resistance between plant allelochemicals and insecticides. For example, swallowtail butterflies, *Papilio glaucus canadensis* [Lepidoptera: Nymphalidae], have evolved resistance to phenolic glycosides in the leaves of host plants via elevated esterase activity, but when challenged with pyrethroid insecticides, the increased activity had no effect on pyrethroid toxicity (Lindroth 1989). Recent work has shown changes in expression of some detoxification genes in monarch caterpillars after feeding on different milkweed species (Tan et al. 2019). We did not observe any evidence for cross-resistance with ouabain and bifenthrin at the concentrations tested in this study.

The bifenthrin concentrations used in this study have been shown to be field-relevant following aerial applications of the formulated product Brigade-2EC (Krueger et al. 2021). We mimicked a KCl exposure where bifenthrin and KCl would be applied simultaneously. In cotton, where potassium fertilization is imperative, tank mixes of pyrethroids and potassium have been shown to be compatible and not interfere with pyrethroid efficacy

(Oosterhuis 2002). Fertilizer applications may increase in the future to counteract nutrient limitations and mitigate drought resiliency in the face of increasing temperatures and eroding soils. The habitat requirements for restoring the monarch population (i.e., 1.8 billion stems) can only be met if milkweed stems are planted on agricultural working lands (Thogmartin et al. 2017). Therefore, it is imperative to understand the implications of increased potassium, and other agricultural product, inputs to build monarch habitat for 50-yr candidate conservation agreements (USFWS 2020).

Here, we report no significant interactions of ouabain and KCl on bifenthrin sensitivity at the concentrations tested in this study. An understanding of interacting agricultural inputs on monarch growth and survival is important for managing their critical habitats in the Midwest United States. To assess these interactions, we first need to understand the effects of milkweed-specific cardenolides on monarch physiology and, in turn, the implications for monarch insecticide toxicity. Future studies should explore these exposure combinations to provide a better understanding of monarch resiliency, such as oviposition, foraging, and fecundity, in changing landscapes when faced with multiple stressors.

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Supplementary Table 1. Sample size overview for experimental replicates for each exposure

1. Ouabain Treatments						
	NC	0.03	0.1	0.3	1	3
R1	10	10	10	10	10	10
R2	10	10	10	10	10	10
R3	10	10	10	10	10	10
TOTAL	**30**	**30**	**30**	**30**	**30**	**30**

2. KCl Treatments					
	NC	0.4	2	10	50
R1	9	9	9	9	9
R2	7	7	7	7	7
R3	5	5	5	5	5
R4	7	10	10	10	10
TOTAL	**28**	**31**	**31**	**31**	**31**

3. KCl + Bifenthrin								
	Untreated Diet				KCl Diet			
	SC	0.1	0.2	0.4	SC	0.1	0.2	0.4
R1	10	10	10	10	10	10	10	10
R2	8	8	8	8	9	9	9	9
R3	8	9	10	10	10	10	10	10
TOTAL	**26**	**27**	**28**	**28**	**29**	**29**	**29**	**29**

4. Ouabain + KCl + Bifenthrin								
	Untreated Diet		KCl Diet		Ouabain Diet		Oua + KCl Diet	
	SC	BIF	SC	BIF	SC	BIF	SC	BIF
R1	8	8	8	8	10	12	10	10
R2	10	12	10	12	9	10	9	10
R3	6	10	7	10	4	10	5	10
TOTAL	**24**	**30**	**25**	**30**	**23**	**32**	**24**	**30**

Supplementary Table 2. ANOVA tables from caterpillar weight and diet consumption analyses for ouabain experiments

Caterpillar Weight: Model Selection				
Model	df	AIC	BIC	logLik
Full	37	162.3478	365.4147	-44.1739
Reduced	36	160.4659	358.0445	-44.233

Caterpillar Weight: Final Model				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	1580	2570.873	**0.00000**
experiment	2	174	23.70158	**0.00000**
logouabain_x	1	174	13.29347	**0.00035**
day	9	1580	457.7591	**0.00000**
I(logouabain_x^2)	1	174	0.467727	0.49494
basecat0	1	174	43.60872	**0.00000**
logouabain_x:day	9	1580	3.915577	**0.00006**
day:I(logouabain_x^2)	9	1580	1.757951	0.07167

Diet Consumption: Model Selection				
Model	df	AIC	BIC	logLik
Full	66	1603.575	1965.839	-735.788
Reduced	46	1598.179	1850.666	-753.089

Diet Consumption: Final Model				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	1572	558.4225	**0.00000**
experiment	2	173	0.393011	0.67562
logouabain_x	1	173	3.711473	0.05568
day	9	1572	40.83835	**0.00000**
I(logouabain_x^2)	1	173	5.088425	**0.02534**
I(logouabain_x^3)	1	173	0.194462	0.65978
basecat0	1	173	10.61339	**0.00135**
logouabain_x:day	9	1572	3.96228	**0.00005**
day:I(logouabain_x^2)	9	1572	2.723375	**0.00375**
day:I(logouabain_x^3)	9	1572	1.987918	**0.03720**

Supplementary Table 3. ANOVA tables from caterpillar weight and diet consumption analyses for KCl experiments

Caterpillar Weight: Model Selection				
Model	df	AIC	BIC	logLik
Full	22	-496.495	-405.8	270.2474

Caterpillar Weight: Final Model				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	294	6336.728	**0.00000**
experiment	3	143	5.11628	**0.00216**
logkcl_x	1	143	182.7989	**0.00000**
day	2	294	129.3693	**0.00000**
I(logkcl_x^2)	1	143	119.9775	**0.00000**
I(logkcl_x^3)	1	143	10.92353	**0.00120**
I(logkcl_x^4)	1	143	6.530348	**0.01165**
basecat0	1	143	135.3774	**0.00000**
logkcl_x:day	2	294	22.87283	**0.00000**
day:I(logkcl_x^2)	2	294	16.13276	**0.00000**
day:I(logkcl_x^3)	2	294	6.329494	**0.00204**
day:I(logkcl_x^4)	2	294	3.449106	**0.03307**

Diet Consumption: Model Selection				
Model	df	AIC	BIC	logLik
Full	22	471.2009	561.8958	-213.6
Reduced	18	465.5392	539.7441	-214.77

Diet Consumption: Final Model				
Column1	numDF	denDF	F-value	p-value
(Intercept)	1	298	938.1506	**0.00000**
experiment	3	143	4.240271	**0.00664**
logkcl_x	1	143	126.5118	**0.00000**
day	2	298	21.44136	**0.00000**
I(logkcl_x^2)	1	143	62.80727	**0.00000**
I(logkcl_x^3)	1	143	5.894955	**0.01643**
I(logkcl_x^4)	1	143	3.949137	**0.04881**
basecat0	1	143	5.373216	**0.02187**
logkcl_x:day	2	298	2.896143	0.05679
day:I(logkcl_x^2)	2	298	2.020786	0.13436

Supplementary Table 4. ANOVA Tables for KCL+Bifenthrin analyses

Caterpillar Weight				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	284	6.622185	**0.01058**
experiment	2	146	0.918053	0.40159
kcl	1	146	0.215271	0.64336
bifenthrin	3	146	1.713606	0.16677
day	2	284	1.939332	0.14570
basecat0	1	146	13.24515	**0.00038**
kcl:bifenthrin	3	146	0.563393	0.64001
kcl:day	2	284	0.034017	0.96656
bifenthrin:day	6	284	0.676921	0.66841
kcl:bifenthrin:day	6	284	0.955563	0.45573

Diet Consumption				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	284	95.06509	**0.00000**
experiment	2	146	14.21049	**0.00000**
kcl	1	146	3.12167	0.07935
bifenthrin	3	146	18.15681	**0.00000**
day	2	284	26.6295	**0.00000**
basecat0	1	146	1.559517	0.21373
kcl:bifenthrin	3	146	1.69148	0.17141
kcl:day	2	284	9.306582	**0.00012**
bifenthrin:day	6	284	7.977917	**0.00000**
kcl:bifenthrin:day	6	284	2.1478	**0.04821**

*p-values < 0.05 are shown in bold

Survival				
Model Terms	npar	Sum Sq	Mean Sq	F value
experiment	2	6.64576	3.32288	3.32288
bifenthrin	2	11.51811	5.759057	5.759057
kcl	1	0.007135	0.007135	0.007135
basecat0	1	21.65864	21.65864	21.65864
bifenthrin:kcl	2	6.915922	3.457961	3.457961

Supplementary Table 5. ANOVA Tables for Ouabain+KCL+Bifenthrin analyses

Caterpillar Weight				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	954	4261.256	**0.00000**
experiment	2	208	73.90893	**0.00000**
bifenthrin	1	208	42.79901	**0.00000**
day	5	954	1662.43	**0.00000**
ouabain	1	208	0.002932	0.95687
kcl	1	208	1.074022	0.30124
lnbasecat0_1000	1	208	105.6034	**0.00000**
bifenthrin:day	5	954	22.40271	**0.00000**
bifenthrin:ouabain	1	208	0.140182	0.70848
day:ouabain	5	954	0.435984	0.82360
bifenthrin:kcl	1	208	0.560787	0.45479
day:kcl	5	954	1.708305	0.12996
ouabain:kcl	1	208	0.095412	0.75772
bifenthrin:day:ouabain	5	954	0.270122	0.92952
bifenthrin:day:kcl	5	954	0.629798	0.67707
bifenthrin:ouabain:kcl	1	208	0.056686	0.81205
day:ouabain:kcl	5	954	2.275633	**0.04524**
bifenthrin:day:ouabain:kcl	5	954	0.774928	0.56786

*p-values < 0.05 are shown in bold

Supplementary Table 5, *continued next page*

Supplementary Table 5, *continued*

Diet Consumption				
Model Terms	numDF	denDF	F-value	p-value
(Intercept)	1	952	3034.777	**0.00000**
experiment	2	208	23.67556	**0.00000**
bifenthrin	1	208	43.65991	**0.00000**
day	5	952	112.5098	**0.00000**
ouabain	1	208	1.912352	0.16818
kcl	1	208	1.369778	0.24319
basecat0_1000	1	208	5.716545	**0.01770**
bifenthrin:day	5	952	54.10652	**0.00000**
bifenthrin:ouabain	1	208	0.161868	0.68786
day:ouabain	5	952	1.265813	0.27649
bifenthrin:kcl	1	208	0.445791	0.50508
day:kcl	5	952	3.226955	**0.00677**
ouabain:kcl	1	208	1.017513	0.31428
bifenthrin:day:ouabain	5	952	0.350651	0.88198
bifenthrin:day:kcl	5	952	0.773483	0.56892
bifenthrin:ouabain:kcl	1	208	1.397297	0.23853
day:ouabain:kcl	5	952	0.647748	0.66330
bifenthrin:day:ouabain:kcl	5	952	0.736792	0.59595

*p-values < 0.05 are shown in bold

Survival				
Model Terms	npar	Sum Sq	Mean Sq	F value
experiment	2	2.863647	1.431824	1.431824
ouabain	1	0.065627	0.065627	0.065627
kcl	1	0.112816	0.112816	0.112816
basecat6_1000	1	22.17215	22.17215	22.17215
ouabain:kcl	1	0.143299	0.143299	0.143299