Published in final edited form as:

Arthropod Plant Interact. 2018 February ; 12(1): 21–29. doi:10.1007/s11829-017-9548-y.

THE EFFECTS OF THE ALKALOID SCOPOLAMINE ON THE PERFORMANCE AND BEHAVIOR OF TWO CATERPILLAR SPECIES

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Abstract

Plants have evolved many defenses against insect herbivores, including numerous chemicals that can reduce herbivore growth, performance, and fitness. One group of chemicals, the tropane alkaloids, is commonly found in the nightshade family (Solanaceae) and has been thought to reduce performance and fitness in insects. We examined the effects of the tropane alkaloid scopolamine, the alkaloid constituent of *Datura wrightii*, which is the most frequent host plant for the abundant and widespread insect herbivore *Manduca sexta* in the southwestern United States. We exposed caterpillars of two different species to scopolamine: *M. sexta*, which has a shared evolutionary history with *Datura* and other solanaceous plants, and *Galleria mellonella*, which does not. We showed that the addition of ecologically-realistic levels of scopolamine to both the diet and the hemolymph of these two caterpillar species (*M. sexta* and *G. mellonella*) had no effect on the growth of either species. We also showed that *M. sexta* has no behavioral preference for or against scopolamine incorporated into an artificial diet. These results are contrary to other work showing marked differences in performance for other insect species when exposed to scopolamine, and provide evidence that scopolamine might not provide the broad-spectrum herbivore resistance typically attributed to it. It also helps to clarify the coevolutionary relationship between *M. sexta* and one of its main host plants, as well as the physiological mechanism of resistance against scopolamine.

Keywords

Herbivory; *Manduca*; scopolamine; *Datura*; alkaloids; *Galleria*

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INTRODUCTION

Herbivory by insects is common and can have devastating performance and fitness consequences for the plants (García and Ehrlén 2002; Lehdal and Ågren, 2015; Maron 1998; Marquis 1984; Mothershead and Marquis 2000). Insect herbivory has been posited as one of the main driving ecological and evolutionary forces affecting plants (Ehrlich and Raven 1964; Hairston et al. 1960). Plants, in response, have evolved a number of defense mechanisms (physical, chemical, and indirect) to deal with herbivory by insects. Although there has been contention in developing a unifying theory of plant defense (Stamp 2003), we know that many plants have an array of chemical defenses at their disposal, many of which have been appropriated by humans for a variety of uses (Houghton 2001). These chemicals vary widely in their composition, from nitrogen-containing alkaloids such as nicotine, to polypeptides and even the incorporation of silica into vegetative material (Mithöfer and Boland 2012). As plants developed defenses against insect attack, some insects have in turn evolved a variety of physiological and behavioral strategies to cope. Although controversial (Lawton 1978; Bernays and Graham 1988; Jermy 1993; Hunt et. al 2007), this co-evolutionary arms race between plants and insects has been posited as the driving force behind the radiation of both insects and angiosperms (Ehrlich and Raven 1964; Fritz and Simms 1992; Farrell 1998), two of the most successful groups of multicellular organisms on the planet. Regardless of how common this putative mechanism of speciation is, interactions among plants and the insect herbivores that feed on them are deeply shaped by host plant chemistry and physiological mechanisms of tolerance in insects (Schuman and Baldwin 2016).

We examined one aspect of a complex interaction between an insect herbivore and the plant on which it feeds. *Manduca sexta* (Sphingidae) is a large hawkmoth with a wide distribution across tropical and temperate regions of the nearctic (Hodges 1971). In the desert southwest (USA), adults emerge from the ground during the monsoon (July through September) and mate, and females lay eggs on their primary host-plant species (*Datura wrightii*) and less frequently on secondary host-plant species such as *Proscidea parviflora* tomato and pepper plants (Hodges 1971; Mechaber and Hildebrand 2000; Mira and Bernays 2002). *Manduca sexta* larvae develop through five instars on plants before burrowing into the soil and undergoing pupation (Sprague and Woods 2015). *D. wrightii* and other species in this genus have been studied extensively because of their potent chemical defenses, most notably the presence of tropane alkaloids (Griffin and Lin 2000; Wink and Theile 2002; Renner et al. 2005; Doncheva et al. 2006). Alkaloids are synthesized in the roots and transported and stored in vacuoles in leaf tissue (Evans and Patridge 1953; Parr et al. 1990; Shonle and Bergelson 2000). Leaf and foliar material contain mainly two alkaloids: hyoscyamine and scopolamine (Griffin and Lin 2000; Doncheva et al. 2006). These compounds affect the activity of the neurotransmitter acetylcholine (Roddick 1991) by competing for sites in muscarinic cholinergic receptors (Renner et al. 2005) and have been shown to impact many herbivorous insects negatively (Hsiao and Fraenkel 1968; Krug and Proksch 1993; Wink 1993; Shonle and Bergelson 2000). Although the lethal effects of many tropane alkaloids on *M. sexta* have been examined (Wink and Theile 2002), scopolamine, a major constituent, has not been studied, nor have more subtle sub-lethal changes to insect performance.

Nevertheless, *M. sexta* larvae must have some level of tolerance to scopolamine, given their rapid development on *D. wrightii* plants in the field (Wilson and Woods 2015). However, caterpillars show variation in performance in the field (Wilson and Woods 2015), which may be caused by differences in alkaloid content among plants. Variation in alkaloid content among individuals of closely-related *Datura* species can be substantial (Shonle and Bergelson 2000).

Manduca sexta deal with plant secondary metabolites, particularly alkaloids (Wink and Theile 2002), mainly by rapid excretion (Maddrell and Gardiner 1975; Murray et al. 1994; Self et al. 1964; Snyder et al. 1994; Wink and Theile 2002). Specifically, *M. sexta* degrade and excrete common plant-based alkaloids including nicotine and hyoscyamine (Wink and Theile 2002). Moreover, this ability to cope with alkaloids is inducible (Wink and Theile 2002) and may involve both physiological and neural mechanisms of tolerance (Morris 1984; Sattelle et al. 1980). In contrast, the ability of wax moth larvae, *Galleria mellonella* (Pyralidae) larvae to deal with allelopathic plant chemicals has been examined less frequently (Chowanski et al. 2016). In one study, exposure to another plant-derived alkaloid, α -solanine, resulted in significant reductions in performance and fitness of *G. mellonella* (Büyükgüzel et al. 2013). We used these two species of caterpillars to compare the effects of scopolamine on a species that shares an evolutionary history with that and other tropane alkaloids (*M. sexta*) with effects on a species (*G. mellonella*) that is naïve to those substances.

In this work we addressed three main questions with a series of laboratory experiments using artificial diets and injections: (1) does scopolamine have negative impacts on larval performance in an insect that has an evolutionary history with the alkaloid (*M. sexta*) compared to one that does not (*G. mellonella*); (2) what is the mechanistic basis of any tolerance to scopolamine; and (3) do *M. sexta* larvae have a behavioral preference for or against scopolamine incorporated into diet? Our work answers some questions about the interaction between an insect herbivore and a host plant and also provides a broader understanding of the ecological relationship between *M. sexta* and *D. wrightii*. Furthermore, it sheds light on tolerance and defense mechanisms of *M. sexta* against other cultivated and wild solanaceous plants, such as tobacco, tomato and chili (Madden and Chamberlin 1945).

METHODS AND MATERIALS

Larval Care

Manduca sexta larvae for this project were from a laboratory colony at the University of Arizona. Caterpillars were raised from eggs on a wheat-germ-based artificial diet (Davidowitz et al. 2003) at 26° C in a growth chamber (Environmental Growth Chambers) under a 17h L: 9h D photocycle. Scopolamine hydrobromide trihydrate (1.11 g, Sigma Aldrich) was dissolved in distilled water and added to the standard rearing diet to generate a diet containing 0.2% scopolamine by dry weight. We chose 0.2% because this value represents the upper level of concentrations that caterpillars likely experience in the field (Hare and Walling 2006; Parr et al. 1990; Shonle and Bergelson, 2000).

All *G. mellonella* larvae (from moths originally obtained from Carolina Biological Supply) were reared from eggs on a standard artificial diet comprising 15mL honey, 1mL distilled water, 20mL glycerol, 10g milk powder, and 40g wheat germ. The larvae were raised under constant darkness at 26° C. To create the scopolamine diet, 0.191 g of scopolamine hydrobromide trihydrate (Sigma Aldrich) was dissolved into the distilled water of the standard diet recipe (above), generating a diet containing 0.2% scopolamine by dry weight, as above.

Caterpillar Growth in Response to Dietary Scopolamine Fifty *M. sexta* larvae were randomly assigned to each treatment group (standard control diet or diet containing 0.2% scopolamine). In order to ensure that all larvae in a given treatment group had roughly the same hatch time, we picked newly hatched larvae from our colony within an hour-long window for a particular treatment group. The time halfway between when the first and last larva was selected was used as the hatch time for all larvae within a treatment group. We measured mass, developmental stage and mortality at 18, 42, 66, 90, 114, 186, 258, 330 and 402 hours after hatch time. To maintain hygiene and food quality, the cups and diet were replaced every 3 days. Caterpillars were given a constant supply of food and allowed to feed *ad libitum*. Upon reaching the third instar, the larvae were placed into larger individually-labeled (414mL) cups to allow sufficient space for growth. All cups had perforated lids for gas exchange.

Fifth-instar *G. mellonella*, weighing 35mg (± 5 mg) were selected from the colony and placed into individual 2mL plastic cups with either control diet or 0.2% dry-weight scopolamine-laced diet. To maintain hygiene and food quality, the cups and diet were replaced every 3 days. As with *M. sexta* trials, caterpillars were given a constant supply of food, and allowed to feed *ad libitum*. To prevent over handling, the larvae were weighed before they were given diet treatments and after pupation.

Injections In addition to feeding larvae scopolamine, we injected a different set of larvae with scopolamine solution. One of our goals with this work was to determine the physiological mechanism by which caterpillars tolerate scopolamine in *Datura spp.* leaves. By bypassing filtering mechanisms in the gut, we could determine the effects that scopolamine had once it entered the hemolymph.

Forty-five *M. sexta* caterpillars were injected with 70 μ L of a 0.114 M solution of scopolamine hydrobromide trihydrate in saline, and 38 caterpillars were injected with 70 μ L of saline solution (as a control). The saline solution included 149.9mM NaCl, 3.0mM KCl, 3.0mM CaCl₂, and 10mM TES, adjusted with 1M NaOH to pH 6.9. We used a 0.114 M solution of scopolamine because it was similar to levels that we predicted might be present when *M. sexta* consume *D. wrightii* in the wild (Hare and Walling 2006; Mechler and Kohlenbach 1978) and because a trial experiment showed that lower concentrations had little effect on growth (Tseng, unpublished). The dosage we gave caterpillars is approximately 30% of the LD₅₀ in rats (3800 mg/kg) (Stockhaus and Wick 1969) at 1100 mg/kg of caterpillar mass.

Manduca sexta caterpillars for injection experiments were reared on scopolamine-free diet. Larvae were selected during the fourth instar and weighed, on average, 1.5g (± 0.1 g). The selected larvae were placed into individually-labeled cups, and we followed feeding protocols described above. Forty-eight hours after isolation, the larvae were starved for 90 min. The larvae were then placed on ice for 30 minutes. A 30.5-gauge needle with 1mL syringe with 10 μ L gradations (Becton, Dickinson and Company, Franklin Lakes NJ, USA) was used to deliver the 70 μ L injections to the hindmost left proleg. After injection, the wound was sealed with VetBond (3M). The larvae were replaced into their respective cups and given an excess of fresh diet. Upon pupation, the mass and sex of the pupae were recorded. The reported results include only those individuals that survived to pupation.

Galleria mellonella caterpillars weighing approximately 170mg (± 20 mg) were selected to be used in the injection experiments. The selected larvae were reared on control diet since emergence from the egg, starved for 30 minutes prior to trials, then placed on ice for 10 minutes. We scaled scopolamine amounts to the body size of *G. mellonella* caterpillars based on doses given to *M. sexta*. Doses ranged from 7 to 8.9 μ L of 0.0114 M to 0.014 M solutions of scopolamine, based on caterpillar weight. This volume and concentration provided a dosage level by body weight similar to that provided by the *M. sexta* injections. After injection, the wound was sealed with VetBond. The larvae were placed in individual wells in a 24-well plate with control diet, and allowed to feed *ad libitum*. To prevent over-handling, the larvae were weighed only after pupation. Pupal mass and development time were also recorded.

Food Preference

Manduca sexta caterpillars were raised from eggs on either scopolamine-free or 0.2% scopolamine diet. Caterpillars from early in the third, fourth, and fifth instars were selected for the food-preference experiment (n = 582 total). Two 1.25 $\times$ 1.25 $\times$ 0.63 cm cubes of each type of diet were placed into clear circular dishes with gridlines. The selected larvae were then placed individually into separate dishes such that the larvae were parallel with the center gridline and equidistant from the two cubes. After 24h, the positions of the larvae were noted.

Analysis and Statistics

All statistical analyses were performed in R (v.3.2.4, www.R-project.org). We used a linear mixed-effects modeling approach to analyze the effects of dietary scopolamine on the growth of both species of caterpillars (nlme package; R Version 3.2.4) and Analysis of Variance (ANOVA) to examine the effects of injected scopolamine. Exact two-tailed binomial tests were used to determine whether caterpillars had a preference for a given diet (McDonald 2014).

RESULTS

Dietary Scopolamine *M. sexta*

Larvae fed diet containing 0.2% scopolamine grew similarly to larvae fed the control diet (Fig. 1a; p = 0.4957). There appeared to be a slight effect of scopolamine on early

development (the first 100 h), when larvae on the scopolamine diet had slightly elevated growth rates compared to larvae raised on the control diet (Fig. 1b; $p = 0.0506$), resulting in an average weight difference of 2.44 mg after the first 100 hours.

Galleria mellonella caterpillars fared similarly, with growth that was unaffected by the addition of scopolamine to their diet (Fig. 2; $p = 0.453$).

Injected Scopolamine

Injected scopolamine had no effect on the growth of *M. sexta* larvae (Fig. 3; $p = 0.1290$). Although the average weight of caterpillars injected with scopolamine was slightly lower at wandering, this difference was not significant and was probably representative of the same group having a slightly lower average weight pre-injection.

Injected scopolamine also had no effect on growth of *G. mellonella* larvae. An ANOVA showed no difference between the pre-injection weight and pupal weight in both injected and control groups ($p = 0.872$) and that there was no change in weight between pre-injection and measurement at pupation ($p = 0.809$).

Behavioral Response for Scopolamine *M. sexta* larvae showed no preference for or aversion to diet containing 0.2% scopolamine (Table 1). Caterpillar instar did not affect behavioral preference or aversion, nor did the relative naivety of caterpillars to scopolamine (Table 1).

DISCUSSION

We predicted that the addition of scopolamine to diet would affect the performance of *G. mellonella*, given that natural populations of *G. mellonella* feed on honeycomb (Warren and Huddleston 1962) and lack a shared evolutionary history with the group of plants (*Datura* spp.) that synthesize scopolamine and related compounds. As most alkaloids are excreted in the frass, we also predicted that scopolamine-injected larvae would show larger effects relative to control larvae because the injections bypass any gut mechanisms that may be present to prevent scopolamine absorption (Maddrell and Gardiner 1975; Murray et al. 1994; Self et al. 1964; Snyder et al. 1994; Wink and Theile, 2002). Contrary to predictions, our results show that scopolamine had no measurable effect on the performance of *M. sexta* or *G. mellonella* larvae (Figures 1–3). The results for injection experiments with *G. mellonella* are more complicated, as larvae in control treatments showed no weight gain. At the very least, however, we found that injections of scopolamine resulted in no weight loss at this developmental stage. We propose three possible explanations for this lack of effects.

First, we may not have subjected caterpillars to sufficiently high levels of scopolamine in their diets or injections to affect growth. We reject this possibility, however, based on our survey of literature examining concentrations of alkaloids in different members of the genus *Datura*. We found that alkaloids generally, and scopolamine specifically, occur at concentrations between 0.1–0.5% by dry weight in leaves (Brewer and Hiner 1950; Doncheva et al. 2006; Eby et al. 1996; Hare and Walling 2006). We used 0.2% to mimic a reasonable upper level for what caterpillars would likely experience in the field; 0.2% is well within the range of concentrations used in other studies that showed effects on performance

(Krug and Proksch 1993). Our expectation that scopolamine would have some negative impact on growth was twofold: (1) We see variance in wild growth rates of *M. sexta* larvae in the field (Kingsolver 2007; Wilson and Woods 2015), which may be in part due to differences in host-plant chemistry among individuals. (2) Because we used *M. sexta* from an established laboratory colony, we assumed that the insects would be more susceptible to the effects of scopolamine than would wild individuals, but given the lack of effect, it seems reasonable that the performance of wild caterpillars probably would be little affected by scopolamine. Our goal with this study was not to examine the toxicology of scopolamine for caterpillars but simply to evaluate if ecologically-realistic levels have any measureable effect on caterpillar performance. Winke and Theile (2002) estimated an LD₅₀ of greater than 1200 mg per kg of body weight of injected hyoscyamine (a tropane alkaloid closely related to scopolamine that also acts as an anticholinergic) in *M. sexta*. The concentration of scopolamine injected into caterpillars in the experiments described here (1100 mg per kg body weight) represents the relatively high value tested by Wink and Theile (2002), but is still below their estimated LD₅₀ for hyoscyamine. Thus, our treatment used a high, but ecologically-realistic level of scopolamine. Nevertheless, it would be beneficial for future work to determine the upper limit of *M. sexta*'s tolerance for scopolamine and other associated tropane alkaloids.

Second, it is possible that scopolamine has little negative impact on the performance of insects in general but evolved in response to herbivory by mammals. This idea is consistent with our finding that scopolamine had very little effect on either of the insect species we tested. Although scopolamine was shown by Krug and Proksch (1993) to decrease the pupal weight (often used as a measure of performance) of *Spodoptera littoralis* (a generalist noctuid moth) larvae by up to 50%, a higher dose of scopolamine than that used in our work resulted in little effect on survival. In comparison, the effect of scopolamine on mammals is striking. In humans, scopolamine poisoning results in respiratory difficulty, partial body paralysis and muscular weakness, hallucinations, and coma (Nogu et al. 1991; Smith et al. 1991). Though we used a dietary concentration that was significantly lower (1100 mg per kg body weight) than the LD₅₀ in rats (3800 mg per kg body weight; Stockhaus 1969) it may be that scopolamine simply has stronger effects in mammals than it does in insects. *M. sexta* and other insects mostly attack *Datura wrightii* leaf tissue (Hare and Elle, 2002; unpublished observation), but mammalian seed predators utilize *D. wrightii* frequently in the Sonoran desert, often at the expense of seed dispersal by ants (Ness and Bressmer 2005). Seed predators can have particularly strong effects on plant fitness and population growth rates (Maron and Crone 2006). It is noteworthy that compared to other plant parts, levels of scopolamine and atropine are highest in the fruit of mature *D. stramonium*, but highest in leaves and stems in immature plants (Miraldi et al. 2001). This pattern suggests that mature *Datura* plants direct scopolamine to seeds and fruits in order to deter consumption by mammalian seed predators and not to protect themselves from herbivory from *M. sexta*. Instead, *D. wrightii* plants may rely on tolerance or indirect defenses (Wilson and Woods 2015; Wilson unpublished) to deter herbivory from *M. sexta*.

Finally, scopolamine may have had no measureable effect on the growth and development of *M. sexta* and *G. mellonella* because it was taken out of chemical context. That is, scopolamine might synergize with other alkaloids and/or other secondary compounds

present in *Datura* to negatively affect insect growth. Such non-additive effects are common (Rasmann and Agrawal 2009) and can occur across wide chemical spectra within plants (Duffey and Stout 1996). *M. sexta* raised in the laboratory on artificial diet can have different growth rates from those in the field on host plants (Kingsolver 2007). This difference may be due in part to the synergistic effects of multiple plant secondary compounds, but also might reflect population-level differences, and unintended artificial selection in the historic laboratory populations for large size and rapid growth rates (D'Amico et al. 2001). Further work could involve combining scopolamine with other commonly found alkaloids (e.g. hyoscyamine or atropine), as well as with other resistance constituents found in *Datura* species, such as proteinase inhibitor 1, peroxidase, or polyphenol oxidase (Hare and Walling 2006).

We were also interested in the physiological mechanism(s) by which *M. sexta* copes with scopolamine. Generally, herbivores employ four different mechanisms for dealing with toxic secondary compounds produced by plants: detoxification, excretion, storage and non-absorption (Barbehenn 2001). Typically, nicotine and many other alkaloids are absorbed by the midgut, pass into the haemolymph, are reabsorbed by the malpighian tubules, and then are mixed with feces and excreted from the rectum, although some portion (30–83%, depending on the type of alkaloid) may be metabolized after absorption (Maddrell and Gardiner 1975; Wink and Theile 2002). We asked if scopolamine followed this route or if absorption was reduced or absent in the gut by testing whether there were differences in survival and growth when scopolamine was administered in diet, or injected directly into the haemolymph. Because there was no difference between treatments, we could not discern exactly how *M. sexta* caterpillars cope with scopolamine. Nevertheless, the lack of effect when scopolamine was injected into the haemolymph suggests that non-absorption is not the mechanism of tolerance. Other studies have followed the metabolic fate of alkaloids more closely by examining the presence of alkaloids and metabolites in frass and in caterpillars after feeding (Maddrell and Gardiner 1975; Wink and Theile 2002). This approach would be useful in future work to determine more precisely how *M. sexta* processes scopolamine.

M. sexta is considered to be an extreme specialist that feeds across its range on solanaceous plants (Hodges 1971), with the one exception of feeding on *Prohiscidea parviflora* (Martyniaceae) in the desert southwest (Mechaber and Hildebrand 2000). *M. sexta* caterpillars can discriminate host plants through olfaction and gustation (Hanson and Dethier 1973) and have strong behavioral preferences for many of the secondary compounds present in host plants (Städler and Hanson 1978). Here, we show that *M. sexta* has little preference for diets containing scopolamine over control diets, regardless of experience with either diet (Table 1). This suggests either that *M. sexta* larvae cannot sense scopolamine or that they can detect the alkaloid but it plays little role in determining dietary preference.

In summary, we found that a major chemical constituent of plants in the genus *Datura*, often assumed to play a role as a constitutive defense against insect herbivores, had no effect on the performance of a specialized herbivore with a shared evolutionary relationship with *Datura*. Even more surprising, the addition of scopolamine had little effect on an insect herbivore with no evolutionary history with scopolamine or *Datura*. This indicates that the production of scopolamine may not be the broad-spectrum herbivore resistance trait that it is

often assumed to be. Instead it might have evolved in response to mammalian seed predation or may function as a defensive compound only when in the presence of synergists in the host plant.

Acknowledgments

The authors are grateful to the late Alice Stone for her valuable assistance with experimental design and logistics. This study was supported in part by NSF grant IOS-1053318 to G.D. Also, we'd like to thank the Center for Insect Science's NIH PERT (Postdoctoral Excellence in Research and Teaching: K12GM000708) program for its support.

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Figure 1.

(a) Weight of *Manduca sexta* caterpillars on two experimental diets. Dashed lines are growth trajectories of caterpillars fed a diet containing 0.2% scopolamine by dry weight, and solid lines are growth trajectories of caterpillars fed the control diet. Weight data were natural-log transformed. The dark black line represents the best fit linear-mixed effects model, which modeled caterpillar weight as a function of time with random slopes and intercepts for individual caterpillar growth trajectories. A comparison of models showed no difference in the slope ($p = 0.07$) or intercept ($p = 0.4957$) between diet-treatment groups. Gray rectangle outlines area magnified in panel b. (b) Weight of *Manduca sexta* caterpillars fed the two experimental diets over the first 100 hours of development. Dashed lines are growth trajectories of caterpillars fed a diet containing 0.2% scopolamine by dry weight, and solid lines are growth trajectories of caterpillars fed the control diet. Weight data were natural-log transformed. The dark lines represent the best fit linear-mixed effects model, which modeled caterpillar weight as a function of time with random slopes and intercepts for individual caterpillar growth trajectories. The dashed line depicts the trend line for caterpillars on the 0.2% scopolamine diet, whereas the solid line depicts the trend-line for caterpillars on the control diet. A comparison of models showed that caterpillars fed the 0.2% scopolamine diet

had slightly higher average weights ($0.97 \text{ mg} \pm 0.47 \text{ mg SD}$) during this developmental period ($p = 0.013$).

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Figure 2.

Pre-diet larval and pupal weights of *Galleria mellonella* fed the experimental and control diets. Open boxes show the 0.2% scopolamine by dry weight diet, and gray boxes show the control diet. Black horizontal bars within boxes represent the median for each group.

Whiskers extend to the highest and lowest values within 1.5 units of the inter-quartile range. Boxes represent the first and third quartiles. Data within each group are represented by light gray points, with outliers shown in black. Weight is plotted on a log scale for better data visualization for both groups.

Figure 3.

Growth trajectories of *Manduca sexta* caterpillars injected with 70 μ L of a 0.114M solution of scopolamine and caterpillars in the control group injected with saline. Weights are shown at four developmental periods: molting into the fifth instar, pre-injection, weight at wandering, and pupal weight. Caterpillars injected with the scopolamine solution are shown with dotted lines and white boxes and caterpillars injected with saline are shown in gray boxes and solid lines. Boxes at each time period show the group median, the first and third quartiles and whiskers that represent the highest and lowest value within 1.5 units of the interquartile range. Outliers are plotted as solid points and weight values are plotted with gray points. Significant overlap between experimental and control groups demonstrates that scopolamine injections had little effect on *Manduca sexta* weights and growth.

Table 1

Scopolamine diet preference of *Manduca sexta* raised on artificial diets containing scopolamine and control diets after 24 hours.

	Number of caterpillars on 0 % Diet	Number of caterpillars on 0.2 % Diet	Exact two-tailed Binomial Test <i>p</i>
Raised on 0% scopolamine diet			
3 rd Instar	53	44	0.4168
4 th Instar	52	45	0.5426
5 th Instar	45	52	0.5426
Raised on 0.2% Scopolamine diet			
3 rd Instar	48	49	1
4 th Instar	51	46	0.6849
5 th Instar	39	58	0.0671