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

WILSON, J.K.^{1,*}, TSENG, A.S.², POTTER, K.A.^{3,4}, DAVIDOWITZ, G.^{1,3} and J.G.
HILDEBRAND^{2,5}

¹*Department of Entomology, University of Arizona, 1140 E South Campus Drive, Forbes 410, PO Box 210036
Tucson, AZ 85721*

²*Department of Neuroscience, University of Arizona, 1040 E 4th Street, PO Box 210077, Tucson AZ 85721*

³*Department of Ecology and Evolutionary Biology, University of Arizona, 1041 E. Lowell St., PO Box 210088
Tucson AZ 85721*

⁴*Current address: School of Earth Sciences and Environmental Sustainability, Northern Arizona University, 525 S
Beaver St. PO Box 5694, Flagstaff AZ 86011*

⁵*Center for Insect Science, University of Arizona, 1007 E. Lowell Street, P.O. Box 210106, Tucson AZ 85721-0106*

*Corresponding author information: keatonwilson@email.arizona.edu, 253.301.7024

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

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50 **Key Words**-Herbivory, *Manduca*, scopolamine, *Datura*, alkaloids, *Galleria*.

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INTRODUCTION

62
63 Herbivory by insects is common and can have devastating performance and fitness consequences
64 for the plants (García and Ehrlén 2002; Lehndal and Ågren, 2015; Maron 1998; Marquis 1984;
65 Mothershead and Marquis 2000). Insect herbivory has been posited as one of the main driving
66 ecological and evolutionary forces affecting plants (Ehrlich and Raven 1964; Hairston et al.
67 1960). Plants, in response, have evolved a number of defense mechanisms (physical, chemical,
68 and indirect) to deal with herbivory by insects. Although there has been contention in developing
69 a unifying theory of plant defense (Stamp 2003), we know that many plants have an array of
70 chemical defenses at their disposal, many of which have been appropriated by humans for a
71 variety of uses (Houghton 2001). These chemicals vary widely in their composition, from
72 nitrogen-containing alkaloids such as nicotine, to polypeptides and even the incorporation of
73 silica into vegetative material (Mithöfer and Boland 2012). As plants developed defenses against
74 insect attack, some insects have in turn evolved a variety of physiological and behavioral
75 strategies to cope. Although controversial (Lawton 1978; Bernays and Graham 1988; Jermy
76 1993; Hunt et. al 2007), this co-evolutionary arms race between plants and insects has been
77 posited as the driving force behind the radiation of both insects and angiosperms (Ehrlich and
78 Raven 1964; Fritz and Simms 1992; Farrell 1998), two of the most successful groups of
79 multicellular organisms on the planet. Regardless of how common this putative mechanism of
80 speciation is, interactions among plants and the insect herbivores that feed on them are deeply
81 shaped by host plant chemistry and physiological mechanisms of tolerance in insects (Schuman
82 and Baldwin 2016).

83
84 We examined one aspect of a complex interaction between an insect herbivore and the plant on
85 which it feeds. *Manduca sexta* (Sphingidae) is a large hawkmoth with a wide distribution across
86 tropical and temperate regions of the nearctic (Hodges 1971). In the desert southwest (USA),
87 adults emerge from the ground during the monsoon (July through September) and mate, and
88 females lay eggs on their primary host-plant species (*Datura wrightii*) and less frequently on
89 secondary host-plant species such as *Proboscidea parviflora* tomato and pepper plants (Hodges
90 1971; Mechaber and Hildebrand 2000; Mira and Bernays 2002). *Manduca sexta* larvae develop
91 through five instars on plants before burrowing into the soil and undergoing pupation (Sprague
92 and Woods 2015). *D. wrightii* and other species in this genus have been studied extensively

93 because of their potent chemical defenses, most notably the presence of tropane alkaloids
94 (Griffin and Lin 2000; Wink and Theile 2002; Renner et al. 2005; Doncheva et al. 2006).
95 Alkaloids are synthesized in the roots and transported and stored in vacuoles in leaf tissue (Evans
96 and Patridge 1953; Parr et al. 1990; Shonle and Bergelson 2000). Leaf and foliar material contain
97 mainly two alkaloids: hyoscyamine and scopolamine (Griffin and Lin 2000; Doncheva et al.
98 2006). These compounds affect the activity of the neurotransmitter acetylcholine (Roddick 1991)
99 by competing for sites in muscarinic cholinergic receptors (Renner et al. 2005) and have been
100 shown to impact many herbivorous insects negatively (Hsiao and Fraenkel 1968; Krug and
101 Proksch 1993; Wink 1993; Shonle and Bergelson 2000). Although the lethal effects of many
102 tropane alkaloids on *M. sexta* have been examined (Wink and Theile 2002), scopolamine, a
103 major constituent, has not been studied, nor have more subtle sub-lethal changes to insect
104 performance. Nevertheless, *M. sexta* larvae must have some level of tolerance to scopolamine,
105 given their rapid development on *D. wrightii* plants in the field (Wilson and Woods 2015).
106 However, caterpillars show variation in performance in the field (Wilson and Woods 2015),
107 which may be caused by differences in alkaloid content among plants. Variation in alkaloid
108 content among individuals of closely-related *Datura* species can be substantial (Shonle and
109 Bergelson 2000).

110

111 *Manduca sexta* deal with plant secondary metabolites, particularly alkaloids (Wink and Theile
112 2002), mainly by rapid excretion (Maddrell and Gardiner 1975; Murray et al. 1994; Self et al.
113 1964; Snyder et al. 1994; Wink and Theile 2002). Specifically, *M. sexta* degrade and excrete
114 common plant-based alkaloids including nicotine and hyoscyamine (Wink and Theile 2002).
115 Moreover, this ability to cope with alkaloids is inducible (Wink and Theile 2002) and may
116 involve both physiological and neural mechanisms of tolerance (Morris 1984; Sattelle et al.
117 1980). In contrast, the ability of wax moth larvae, *Galleria mellonella* (Pyralidae) larvae to deal
118 with allelopathic plant chemicals has been examined less frequently (Chowański et al. 2016). In
119 one study, exposure to another plant-derived alkaloid, α -solanine, resulted in significant
120 reductions in performance and fitness of *G. mellonella* (Büyükgüzel et al. 2013). We used these
121 two species of caterpillars to compare the effects of scopolamine on a species that shares an
122 evolutionary history with that and other tropane alkaloids (*M. sexta*) with effects on a species
123 (*G. mellonella*) that is naïve to those substances.

124

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

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METHODS AND MATERIALS

138

Larval Care

140 *Manduca sexta* larvae for this project were from a laboratory colony at the University of
141 Arizona. Caterpillars were raised from eggs on a wheat-germ-based artificial diet (Davidowitz et
142 al. 2003) at 26°C in a growth chamber (Environmental Growth Chambers) under a 17h L: 9h D
143 photocycle. Scopolamine hydrobromide trihydrate (1.11g, Sigma Aldrich) was dissolved in
144 distilled water and added to the standard rearing diet to generate a diet containing 0.2%
145 scopolamine by dry weight. We chose 0.2% because this value represents the upper level of
146 concentrations that caterpillars likely experience in the field (Hare and Walling 2006; Parr et al.
147 1990; Shonle and Bergelson, 2000).

148

149 All *G. mellonella* larvae (from moths originally obtained from Carolina Biological Supply) were
150 reared from eggs on a standard artificial diet comprising 15mL honey, 1mL distilled water,
151 20mL glycerol, 10g milk powder, and 40g wheat germ. The larvae were raised under constant
152 darkness at 26°C. To create the scopolamine diet, 0.191 g of scopolamine hydrobromide
153 trihydrate (Sigma Aldrich) was dissolved into the distilled water of the standard diet recipe
154 (above), generating a diet containing 0.2% scopolamine by dry weight, as above.

155

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

167

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

174

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

180

181 Forty-five *M. sexta* caterpillars were injected with 70 μ L of a 0.114 M solution of scopolamine
182 hydrobromide trihydrate in saline, and 38 caterpillars were injected with 70 μ L of saline solution
183 (as a control). The saline solution included 149.9mM NaCl, 3.0mM KCl, 3.0mM CaCl₂, and
184 10mM TES, adjusted with 1M NaOH to pH 6.9. We used a 0.114 M solution of scopolamine
185 because it was similar to levels that we predicted might be present when *M. sexta* consume *D.*

186 *wrightii* in the wild (Hare and Walling 2006; Mechler and Kohlenbach 1978) and because a trial
187 experiment showed that lower concentrations had little effect on growth (Tseng, unpublished).
188 The dosage we gave caterpillars is approximately 30% of the LD₅₀ in rats (3800 mg/kg)
189 (Stockhaus and Wick 1969) at 1100 mg/kg of caterpillar mass.

190
191 *Manduca sexta* caterpillars for injection experiments were reared on scopolamine-free diet.
192 Larvae were selected during the fourth instar and weighed, on average, 1.5g (± 0.1 g). The
193 selected larvae were placed into individually-labeled cups, and we followed feeding protocols
194 described above. Forty-eight hours after isolation, the larvae were starved for 90 min. The larvae
195 were then placed on ice for 30 minutes. A 30.5-gauge needle with 1mL syringe with 10 μ L
196 gradations (Becton, Dickinson and Company, Franklin Lakes NJ, USA) was used to deliver the
197 70 μ L injections to the hindmost left proleg. After injection, the wound was sealed with VetBond
198 (3M). The larvae were replaced into their respective cups and given an excess of fresh diet. Upon
199 pupation, the mass and sex of the pupae were recorded. The reported results include only those
200 individuals that survived to pupation.

201
202 *Galleria mellonella* caterpillars weighing approximately 170mg (± 20 mg) were selected to be
203 used in the injection experiments. The selected larvae were reared on control diet since
204 emergence from the egg, starved for 30 minutes prior to trials, then placed on ice for 10 minutes.
205 We scaled scopolamine amounts to the body size of *G. mellonella* caterpillars based on doses
206 given to *M. sexta*. Doses ranged from 7 to 8.9 μ L of 0.0114 M to 0.014 M solutions of
207 scopolamine, based on caterpillar weight. This volume and concentration provided a dosage level
208 by body weight similar to that provided by the *M. sexta* injections. After injection, the wound
209 was sealed with VetBond. The larvae were placed in individual wells in a 24-well plate with
210 control diet, and allowed to feed *ad libidum*. To prevent over-handling, the larvae were weighed
211 only after pupation. Pupal mass and development time were also recorded.

212 *Food Preference*

213 *Manduca sexta* caterpillars were raised from eggs on either scopolamine-free or 0.2%
214 scopolamine diet. Caterpillars from early in the third, fourth, and fifth instars were selected for
215 the food-preference experiment (n = 582 total). Two 1.25 x 1.25 x 0.63 cm cubes of each type of
216

217 diet were placed into clear circular dishes with gridlines. The selected larvae were then placed
218 individually into separate dishes such that the larvae were parallel with the center gridline and
219 equidistant from the two cubes. After 24h, the positions of the larvae were noted.

220

221 *Analysis and Statistics*

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

227

228

RESULTS

229 *Dietary Scopolamine M. sexta*

230 Larvae fed diet containing 0.2% scopolamine grew similarly to larvae fed the control diet (Fig.
231 1a; $p = 0.4957$). There appeared to be a slight effect of scopolamine on early development (the
232 first 100 h), when larvae on the scopolamine diet had slightly elevated growth rates compared to
233 larvae raised on the control diet (Fig. 1b; $p = 0.0506$), resulting in an average weight difference
234 of 2.44 mg after the first 100 hours.

235

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

238

239 *Injected Scopolamine*

240 Injected scopolamine had no effect on the growth of *M. sexta* larvae (Fig. 3; $p = 0.1290$).

241 Although the average weight of caterpillars injected with scopolamine was slightly lower at
242 wandering, this difference was not significant and was probably representative of the same group
243 having a slightly lower average weight pre-injection.

244

245 Injected scopolamine also had no effect on growth of *G. mellonella* larvae. An ANOVA showed
246 no difference between the pre-injection weight and pupal weight in both injected and control

247 groups ($p = 0.872$) and that there was no change in weight between pre-injection and
248 measurement at pupation ($p = 0.809$).

249
250 *Behavioral Response for Scopolamine* *M. sexta* larvae showed no preference for or aversion to
251 diet containing 0.2% scopolamine (Table 1). Caterpillar instar did not affect behavioral
252 preference or aversion, nor did the relative naivety of caterpillars to scopolamine (Table 1).

253 254 DISCUSSION

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

268
269 First, we may not have subjected caterpillars to sufficiently high levels of scopolamine in their
270 diets or injections to affect growth. We reject this possibility, however, based on our survey of
271 literature examining concentrations of alkaloids in different members of the genus *Datura*. We
272 found that alkaloids generally, and scopolamine specifically, occur at concentrations between
273 0.1-0.5% by dry weight in leaves (Brewer and Hiner 1950; Doncheva et al. 2006; Eby et al.
274 1996; Hare and Walling 2006). We used 0.2% to mimic a reasonable upper level for what
275 caterpillars would likely experience in the field; 0.2% is well within the range of concentrations
276 used in other studies that showed effects on performance (Krug and Proksch 1993). Our
277 expectation that scopolamine would have some negative impact on growth was twofold: (1) We

278 see variance in wild growth rates of *M. sexta* larvae in the field (Kingsolver 2007; Wilson and
279 Woods 2015), which may be in part due to differences in host-plant chemistry among
280 individuals. (2) Because we used *M. sexta* from an established laboratory colony, we assumed
281 that the insects would be more susceptible to the effects of scopolamine than would wild
282 individuals, but given the lack of effect, it seems reasonable that the performance of wild
283 caterpillars probably would be little affected by scopolamine. Our goal with this study was not to
284 examine the toxicology of scopolamine for caterpillars but simply to evaluate if ecologically-
285 realistic levels have any measureable effect on caterpillar performance. Winke and Theile (2002)
286 estimated an LD₅₀ of greater than 1200 mg per kg of body weight of injected hyoscyamine (a
287 tropane alkaloid closely related to scopolamine that also acts as an anticholinergic) in *M. sexta*.
288 The concentration of scopolamine injected into caterpillars in the experiments described here
289 (1100 mg per kg body weight) represents the relatively high value tested by Wink and Theile
290 (2002), but is still below their estimated LD₅₀ for hyoscyamine. Thus, our treatment used a high,
291 but ecologically-realistic level of scopolamine. Nevertheless, it would be beneficial for future
292 work to determine the upper limit of *M. sexta*'s tolerance for scopolamine and other associated
293 tropane alkaloids.

294
295 Second, it is possible that scopolamine has little negative impact on the performance of insects in
296 general but evolved in response to herbivory by mammals. This idea is consistent with our
297 finding that scopolamine had very little effect on either of the insect species we tested. Although
298 scopolamine was shown by Krug and Proksch (1993) to decrease the pupal weight (often used as
299 a measure of performance) of *Spodoptera littoralis* (a generalist noctuid moth) larvae by up to
300 50%, a higher dose of scopolamine than that used in our work resulted in little effect on survival.
301 In comparison, the effect of scopolamine on mammals is striking. In humans, scopolamine
302 poisoning results in respiratory difficulty, partial body paralysis and muscular weakness,
303 hallucinations, and coma (Nogué et al. 1991; Smith et al. 1991). Though we used a dietary
304 concentration that was significantly lower (1100 mg per kg body weight) than the LD₅₀ in rats
305 (3800 mg per kg body weight; Stockhaus 1969) it may be that scopolamine simply has stronger
306 effects in mammals than it does in insects. *M. sexta* and other insects mostly attack *Datura*
307 *wrightii* leaf tissue (Hare and Elle, 2002; unpublished observation), but mammalian seed
308 predators utilize *D. wrightii* frequently in the Sonoran desert, often at the expense of seed

309 dispersal by ants (Ness and Bressmer 2005). Seed predators can have particularly strong effects
310 on plant fitness and population growth rates (Maron and Crone 2006). It is noteworthy that
311 compared to other plant parts, levels of scopolamine and atropine are highest in the fruit of
312 mature *D. stramonium*, but highest in leaves and stems in immature plants (Miraldi et al. 2001).
313 This pattern suggests that mature *Datura* plants direct scopolamine to seeds and fruits in order to
314 deter consumption by mammalian seed predators and not to protect themselves from herbivory
315 from *M. sexta*. Instead, *D. wrightii* plants may rely on tolerance or indirect defenses (Wilson and
316 Woods 2015; Wilson unpublished) to deter herbivory from *M. sexta*.

317
318 Finally, scopolamine may have had no measureable effect on the growth and development of *M.*
319 *sexta* and *G. mellonella* because it was taken out of chemical context. That is, scopolamine might
320 synergize with other alkaloids and/or other secondary compounds present in *Datura* to
321 negatively affect insect growth. Such non-additive effects are common (Rasmann and Agrawal
322 2009) and can occur across wide chemical spectra within plants (Duffey and Stout 1996). *M.*
323 *sexta* raised in the laboratory on artificial diet can have different growth rates from those in the
324 field on host plants (Kingsolver 2007). This difference may be due in part to the synergistic
325 effects of multiple plant secondary compounds, but also might reflect population-level
326 differences, and unintended artificial selection in the historic laboratory populations for large
327 size and rapid growth rates (D'Amico et al. 2001). Further work could involve combining
328 scopolamine with other commonly found alkaloids (e.g. hyoscyamine or atropine), as well as
329 with other resistance constituents found in *Datura* species, such as proteinase inhibitor 1,
330 peroxidase, or polyphenol oxidase (Hare and Walling 2006).

331
332 We were also interested in the physiological mechanism(s) by which *M. sexta* copes with
333 scopolamine. Generally, herbivores employ four different mechanisms for dealing with toxic
334 secondary compounds produced by plants: detoxification, excretion, storage and non-absorption
335 (Barbehenn 2001). Typically, nicotine and many other alkaloids are absorbed by the midgut, pass
336 into the haemolymph, are reabsorbed by the malpighian tubules, and then are mixed with feces
337 and excreted from the rectum, although some portion (30-83%, depending on the type of
338 alkaloid) may be metabolized after absorption (Maddrell and Gardiner 1975; Wink and Theile
339 2002). We asked if scopolamine followed this route or if absorption was reduced or absent in the

340 gut by testing whether there were differences in survival and growth when scopolamine was
341 administered in diet, or injected directly into the haemolymph. Because there was no difference
342 between treatments, we could not discern exactly how *M. sexta* caterpillars cope with
343 scopolamine. Nevertheless, the lack of effect when scopolamine was injected into the
344 haemolymph suggests that non-absorption is not the mechanism of tolerance. Other studies have
345 followed the metabolic fate of alkaloids more closely by examining the presence of alkaloids and
346 metabolites in frass and in caterpillars after feeding (Maddrell and Gardiner 1975; Wink and
347 Theile 2002). This approach would be useful in future work to determine more precisely how *M.*
348 *sexta* processes scopolamine.

349

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

359

360 In summary, we found that a major chemical constituent of plants in the genus *Datura*, often
361 assumed to play a role as a constitutive defense against insect herbivores, had no effect on the
362 performance of a specialized herbivore with a shared evolutionary relationship with *Datura*.
363 Even more surprising, the addition of scopolamine had little effect on an insect herbivore with no
364 evolutionary history with scopolamine or *Datura*. This indicates that the production of
365 scopolamine may not be the broad-spectrum herbivore resistance trait that it is often assumed to
366 be. Instead it might have evolved in response to mammalian seed predation or may function as a
367 defensive compound only when in the presence of synergists in the host plant.

368

369

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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

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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 ($p = 0.013$).

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.

451 **Figure 3.** Growth trajectories of *Manduca sexta* caterpillars injected with 70 μ L of a 0.114M
452 solution of scopolamine and caterpillars in the control group injected with saline. Weights are
453 shown at four developmental periods: molting into the fifth instar, pre-injection, weight at
454 wandering, and pupal weight. Caterpillars injected with the scopolamine solution are shown with
455 dotted lines and white boxes and caterpillars injected with saline are shown in gray boxes and
456 solid lines. Boxes at each time period show the group median, the first and third quartiles and
457 whiskers that represent the highest and lowest value within 1.5 units of the interquartile range.
458 Outliers are plotted as solid points and weight values are plotted with gray points. Significant
459 overlap between experimental and control groups demonstrates that scopolamine injections had
460 little effect on *Manduca sexta* weights and growth.

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