

1 Running head: Soil shapes floral chemistry, bee health

2

3 From plant fungi to bee parasites: mycorrhizae and soil nutrients shape floral chemistry and bee
4 pathogens

5

6 Julie K. Davis^{1,2*}, Luis A. Aguirre³, Nicholas A. Barber⁴, Phil C. Stevenson^{5,6}, and Lynn S. Adler³

7

8 ¹ Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, USA

9 ² Department of Entomology, Cornell University, Ithaca, NY 14850, USA

10 ³ Department of Biology, University of Massachusetts, Amherst, MA 01003, USA

11 ⁴ Department of Biological Sciences, San Diego State University, San Diego, CA 92182, USA

12 ⁵ Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey TW9 3AB, UK

13 ⁶ Natural Resources Institute, University of Greenwich, Chatham, Kent ME4 4TB, UK

14

15 *corresponding author: jd982@cornell.edu, ph 413-387-3124

16

17

18

19

20

21

22

23

24 **Abstract**

25 Bee populations have experienced declines in recent years, due in part to increased disease
26 incidence. Multiple factors influence bee-pathogen interactions, including nectar and pollen
27 quality and secondary metabolites. However, we lack an understanding of how plant interactions
28 with their environment shape bee diet quality. We examined how plant interactions with the
29 belowground environment alter floral rewards and, in turn, bee-pathogen interactions. Soil-
30 dwelling mycorrhizal fungi are considered plant mutualists, although the outcome of the
31 relationship depends on environmental conditions such as nutrients. In a 2x2 factorial design, we
32 asked whether mycorrhizal fungi and nutrients affect concentrations of nectar and pollen
33 alkaloids (anabasine and nicotine) previously shown to reduce infection by the gut pathogen
34 *Crithidia* in the native bumblebee *Bombus impatiens*. To ask how plant interactions affect this
35 common bee pathogen, we fed pollen and nectar from our treatment plants, and from a
36 wildflower pollen control with artificial nectar, to bees infected with *Crithidia*. Mycorrhizal
37 fungi and fertilizer both influenced flowering phenology and floral chemistry. While we found
38 no anabasine or nicotine in nectar, high fertilizer increased anabasine and nicotine in pollen.
39 AMF decreased nicotine concentrations, but the reduction due to AMF was stronger in high than
40 low-nutrient conditions. AMF and nutrients also had interactive effects on bee pathogens via
41 changes in nectar and pollen. High fertilizer reduced *Crithidia* cell counts relative to low
42 fertilizer in AMF plants, but increased *Crithidia* in non-AMF plants. These results did not
43 correspond with effects of fertilizer and AMF on pollen alkaloid concentrations, suggesting that
44 other components of pollen or nectar were affected by treatments and shaped pathogen counts.
45 Our results indicate that soil biotic and abiotic environment can alter bee-pathogen interactions

46 via changes in floral rewards, and underscore the importance of integrative studies to predict
47 disease dynamics and ecological outcomes.

48 **Key words:** *bee decline, bee parasites, Bombus impatiens, Crithidia, mycorrhizae, multi-*
49 *trophic, trypanosome, soil, alkaloid, floral chemistry*

50 **Introduction**

51 Pollinators provide critical ecosystem services important for both natural ecosystem
52 resilience and crop yield, and bees are major contributors to these services (Klein et al. 2003).
53 Many bee species are experiencing population declines due to a range of factors, including
54 increased disease incidence (Cameron et al. 2011, Goulson et al. 2015). Host-pathogen dynamics
55 do not occur in isolation, and diet quality may act as a buffer against disease stressors. For
56 instance, protein can enhance bee immunocompetence (Alaux et al. 2010). Furthermore,
57 environmental factors, including soil conditions and species interactions, shape the quality of
58 floral resources (Adler et al. 2006). However, we lack a comprehensive understanding of how
59 biotic and abiotic factors, such as soil microbes and nutrients, shape bee-pathogen outcomes
60 mediated by diet quality. Due to the importance of pollinators for diversity in natural ecosystems
61 and yield in crop systems (Ashman et al. 2004, Klein et al. 2007), bottom-up effects of soil
62 conditions on bees could have widespread ecological and economic applications.

63 Secondary metabolites in plants can provide resistance against plant antagonists but also
64 may shape interactions with pollinators. Alkaloids in nectar can deter or attract pollinators
65 depending on concentration and pollinator taxa (Detzel and Wink 1993, Adler 2000, Kessler et
66 al. 2008), and nectar compounds could benefit pollinators by reducing bee pathogen load
67 (Manson et al. 2010, Richardson et al. 2015). In particular, consuming the alkaloids nicotine and
68 anabasine, found in Solanaceous plants, reduced infection by the gut pathogen *Crithidia* in the

69 bumblebee *Bombus impatiens* at ecologically-relevant concentrations (Richardson et al. 2015).
70 However, these effects can be dose-dependent (Anthony et al. 2015), and in some studies, no
71 effect was detected (Biller et al. 2015, Thorburn et al. 2015). Given that some bee species have
72 declined in recent years, due in part to pathogens (Potts et al. 2010), and the largely unknown
73 role of floral traits in pollinator-pathogen dynamics (McArt et al. 2014, Koch et al. 2017), it is
74 critical to explore factors that influence flower-bee-pathogen interactions.

75 Fine scale shifts in the diverse sugars, secondary compounds, and other nutrients in
76 nectar (Bentley and Elias 1983) may alter bee-pathogen interactions, and it is thus important to
77 study bee-disease dynamics in the context of real nectar. Sugar concentration and composition
78 can influence *Crithidia* growth (Conroy et al. 2016, Palmer-Young and Thursfield 2017) directly
79 or possibly synergistically with secondary metabolites in nectar (Palmer-Young et al. 2017). Few
80 studies have examined the effects of secondary compounds in real nectar on bee pathogens
81 (Tiedeken et al. 2016). To address this, we tested the effect of plant growing environment on
82 bumble bee pathogens *via* changes in floral chemistry using real nectar and pollen.

83 While some studies have examined the effect of nectar secondary chemistry on pollinator
84 pathogens, comparatively few have examined pollen secondary chemistry. Pollen is the male
85 gamete and is typically more defended than nectar with higher concentrations of secondary
86 compounds (Cook et al. 2013, Palmer-Young et al. 2019), and therefore may have a stronger
87 effect on bee pathogen loads. Furthermore, adequate dietary protein from pollen is important for
88 honeybee and bumblebee immune response, and other dietary constituents, including amino acid
89 and lipid levels, may also shape bee tolerance to infection (reviewed in Koch et al. 2017). A diet
90 including both pollen and nectar is ecologically realistic, and because pollen may have higher

91 concentrations of secondary compounds, it is crucial to understand the combined role of these
92 diet constituents on bee pathogens.

93 Plants interact with a range of organisms that can affect nectar and pollen traits, which
94 may scale up to alter bee-pathogen interactions. For example, plants widely associate with
95 arbuscular mycorrhizal fungi (AMF), which can enhance nutrient absorption (Brundrett and
96 Tedersoo 2018) and induce systemic expression of proteins and genes involved in defense in root
97 and foliar tissues (Liu et al. 2007, Campos-Soriano et al. 2010). We therefore hypothesize that
98 AMF could alter expression of secondary chemistry in floral reward tissues *via* a systemic
99 defense response. Moreover, because plant benefit from AMF depends on the relative
100 availability of soil nitrogen and phosphorus (Johnson 2010), and soil nutrients can shape nectar
101 secondary chemistry (Adler et al. 2006), AMF and nutrients may interactively influence defense
102 expression, including in floral tissue. Therefore, AMF have the potential to affect floral traits
103 including defensive chemistry *via* both changes in nutrient uptake and other pathways involved
104 in defense. To date, no study has examined the effect of AMF on floral secondary chemistry,
105 other than floral volatiles (Becklin et al. 2011), although there is evidence that AMF can increase
106 or decrease pollinator attraction via changes in floral display (Barber and Soper Gordon 2015).

107 By examining how plant interactions with AMF and nutrients affect floral chemistry and
108 bee-pathogen dynamics, our study addresses a novel pathway by which belowground interactions
109 affect pollinator health. Specifically, we asked whether floral traits (flowering phenology, nectar
110 volume, and mean value or variation in pollen and nectar alkaloid and sugar concentrations)
111 change in response to plant nutrient or mycorrhizal status; and whether AMF and nutrient effects
112 on pollen and nectar affect pathogen levels in bumblebees infected with *Crithidia*. We
113 hypothesized that if effects of AMF on secondary metabolism depend on soil nutrients (Getman-

114 Pickering et al, unpublished data), AMF would increase alkaloids more under low than high
115 nutrient conditions. Alternatively, if AMF effects on alkaloid levels are due to up-regulation of
116 defense pathways, rather than nutrient status (Vannette and Hunter 2009), we would expect
117 plants grown with AMF to have higher floral alkaloids regardless of fertilizer treatment. We
118 expected plants grown with high fertilizer to have higher floral alkaloids, consistent with a
119 previous study of nutrient effects on nectar (Adler et al. 2006). We predicted that diet from
120 treatment groups with high alkaloid concentrations would reduce *Crithidia* cell counts.
121 Furthermore, because variation in diet quality could affect plant-pollinator interactions (Kessler
122 et al. 2012, Wetzel et al. 2016), we assessed mean value and variation in floral chemical traits.

123 **Methods**

124 *Study System*

125 We selected *Nicotiana tabacum* L. (cultivated tobacco; Solanaceae) as a model system
126 because it is colonized by AMF (Andrade et al. 2013) and produces plentiful nectar containing
127 the alkaloids nicotine and anabasine (Detzel and Wink 1993, Adler et al. 2006) that can reduce
128 *Crithidia bombi* in *Bombus impatiens* (Richardson et al. 2015). *Rhizophagus irregularis*
129 (previously *Glomus intraradices*) is a commercially available species of AMF used in agriculture
130 and restoration. It increases alkaloid concentrations in root and leaf tissues of *N. tabacum*
131 (Andrade et al. 2013), but its effect on floral chemistry is unknown. *Bombus impatiens* (common
132 eastern bumblebee) is native to the eastern US (Williams et al. 2014). While *B. impatiens* is not
133 in decline, it is related to several bee species that are in decline, associated with pathogen
134 incidence (Cameron et al. 2011). It is therefore a useful model species to study pollinator-
135 pathogen dynamics. *Bombus* spp. are infected by the hind gut pathogen *Crithidia bombi*, which
136 is found in up to 80% of bees at some sites (Gillespie 2010). *Crithidia* reduces queen colony

137 founding success, size and fitness (Brown et al. 2003), and worker foraging and learning abilities
138 (Gegeer et al. 2006, Otterstatter and Thomson 2006).

139

140 *Experimental Design*

141 Starting in November 2016, we grew 200 tobacco plants in the greenhouse in a 2x2
142 blocked factorial design, manipulating presence and absence of AMF, and high and low levels of
143 fertilizer. Each block contained four plants, one from each treatment group. We collected pollen
144 and nectar and analyzed nicotine and anabasine concentrations. We also collected and pooled
145 pollen and nectar from plants for laboratory bioassays with bees. We fed *Crithidia*-infected bees
146 pollen and nectar from plants in each treatment combination, and included a control group with a
147 diet of wildflower pollen blend and artificial nectar to determine whether tobacco nectar and
148 pollen in general reduced *Crithidia* counts relative to a typical laboratory experimental diet.

149 We broadcast *N. tabacum* seeds into sterilized potting soil/sand mixture and kept
150 seedlings fertilized uniformly throughout early development. When seedlings were ready for
151 transplant in April 2017, we transferred them to 10 cm bleach-sterilized pots with sterilized
152 potting soil/sand mixture. At this point, plants were randomly assigned AMF treatments (~50
153 plants/treatment) and inoculated with either 1.71 g of 500 spores/g inoculum *Rhizophagus*
154 *irregularis* (Premier Tech, Quebec, Canada) in perlite carrier, or the same AMF and perlite
155 mixture that had been autoclaved. In May, we added high (4.02 g \pm 0.05 g) or low (1.005 g \pm -
156 0.05 g) NPK fertilizer, as in Adler et al. (2006) to plants assigned high and low fertilizer
157 treatments (for details see Appendix S1, *Plant propagation and administering treatments*).

158 In early April, after administering AMF/fertilizer treatments, we noticed an aphid
159 outbreak in the greenhouse. We quantified (scored 0-3) and manually removed aphids on two
160 sampling dates in April, and estimated proportion of leaf area that had mold resulting from aphid

161 honeydew (see Appendix S1, *Aphid and mold quantification*). Because aphid incidence and mold
162 were associated with AMF or fertilizer treatments (Appendix S1, *Aphid and mold*
163 *quantification*), we included these as separate predictors in our models to account for potential
164 effects of aphids and mold on responses. Collinearity was low in all cases ($VIF < 2.0$),
165 suggesting that we could separate effects of aphids and mold from treatment effects.

166

167 *Quantifying Mycorrhizal Colonization*

168 We sampled roots after all nectar and pollen collection was complete, between August
169 25th-31st, 2017. Samples were taken from the center of the root ball, washed in tap water, then
170 stained with trypan blue and mounted on microscope slides (Appendix S1, *Mycorrhizal*
171 *inoculation quantification and validation*). Colonization was confirmed using a compound
172 microscope at 400 magnification and the grid intersect method outlined in McGonigle et al.
173 (1990). AMF treatment increased the likelihood a plant would have arbuscules by 79% (residual
174 deviance = 795.12 on 79 df, $p < 0.001$), and quadrupled mean arbuscular colonization (residual
175 deviance = 868.16 on 87 df, $p < 0.001$), indicating treatments were effective. Colonization was
176 not affected by the fertilizer treatment ($p > 0.09$), but colonized plants were more likely to have
177 aphids (Appendix S1, *Mycorrhizal inoculation, quantification and validation*).

178

179 *Measuring Plant Traits*

180 *Plant and floral traits*. The date of first flowering (first open flower with five dehisced anthers)
181 was recorded for each plant, ranging from April 27th until the experiment ended on August 31st,
182 2017. During the last week of the experiment we measured plant height and leaf number to
183 estimate treatment effect on size. From the first two flowers, we measured nectar volume using
184 50 μ L glass micro-capillary tubes (Fisher Scientific, Hampton, New Hampshire, USA) and a

185 digital caliper and nectar sugar concentration using a refractometer. To determine treatment
186 effect on nectar and pollen alkaloids, pollen (from the first several flowers a plant produced until
187 minimum amount for analysis was obtained) and nectar (from the first 2-4 flowers as needed for
188 a volume of 25 μ L) were collected between 0700 and 1300 and stored at -80°C until chemical
189 analysis. To collect pollen, we plucked dehiscing anthers from the filament, holding flowers
190 upside-down to reduce the risk of contaminating nectar. After we removed pollen, we separated
191 sepals and ovaries from the floral tube so that nectar remained in the floral tube. By holding the
192 flower at an angle, we were able to prevent the nectar from contacting the site of phloem
193 exposure. Nectar contaminated with pollen or other fluids, or that made contact with the damage
194 site was discarded. After completing collection of pollen and nectar for chemical analysis, we
195 collected nectar (using a 200 μ L micropipetter) and pollen for the bee bioassay from subsequent
196 flowers. For 52 plants, we analyzed a separate set of pollen from late-season sampling dates to
197 test for correlations between early and late-season alkaloids (Appendix S1, *Pollen and Nectar*
198 *Chemistry*).

199
200 *Nectar and pollen alkaloids.* Because some plants died or never flowered, our analysis of floral
201 chemistry included 120 plants; 33 AMF-/high fertilizer, 27 AMF-/low fertilizer, 31 AMF+/ high
202 fertilizer, and 28 AMF+/low fertilizer. We weighed approximately 6 mg pollen samples for
203 extraction but unfortunately did not record exact weights. After extraction, we confirmed that
204 pollen weights approximated this target value and did not differ between treatments (Appendix
205 S1, *Pollen and Nectar Chemistry*). We therefore used the target value of 6 mg of pollen to
206 calculate anabasine and nicotine in $\mu\text{g}/\text{mg}$. We also analyzed our data using post-extraction
207 weights to calculate alkaloids/mg pollen, but chose to present results using an estimate of 6 mg
208 due to high variability in post-extraction weights (Appendix S1, *Pollen and Nectar Chemistry*).

209

210 *Bee-Crithidia Bioassays*211 *Preparing nectar and pollen.* We used nectar and pollen from treatment plants to determine how

212 AMF and fertilizer affected bee interactions with a gut pathogen. During collection, we pooled

213 nectar and pollen within plants in 1.5 mL micro-centrifuge tubes and stored in a -20°C freezer.

214 We ultimately pooled pollen and nectar samples within treatment group for use in

215 bioassays. When we pooled pollen, we separated it from anthers using a modified insect vacuum

216 (BioQuip Products, Inc. Compton, California, USA) outfitted with a 25- μ m filter

217 (CellMicroSieves, BioDesign Inc. of NY, Carmel, New York, USA) to catch pollen, overlaid

218 with a piece of mesh to remove anthers.

219

220 *Treatment Diets.* We conducted week-long bioassays from early January through early February221 2018. To assess the effect of diet from plants grown under different soil conditions on *Crithidia-*222 infected *B. impatiens* (BioBest LTD, Leamington, Ontario, Canada), infected bees were provided

223 with pollen and nectar from one of the four treatment combinations, or a control diet. The control

224 diet contained a wildflower pollen blend collected by honeybees from an organic farm in North

225 Dakota, USA and artificial sugar water mimicking the 12:15:10 glucose:fructose:sucrose ratios

226 in *N. tabacum* nectar (Tiedge and Lohaus 2017) and the 16% overall sugar concentration

227 observed in our nectar. We note that the control diet is not intended to be interpreted as non-

228 mycorrhizal, but rather a comparison of tobacco nectar and pollen to a more standardized lab diet

229 typically used in experiments. Because preliminary trials suggested pure tobacco pollen may be

230 toxic to bees (data not shown), we mixed tobacco pollen from each treatment group in a 1:1 ratio

231 with the control wildflower pollen. To prepare pollen for bee consumption, we mixed 1 g of each

232 pollen treatment with 1 mL of distilled water, adding small amounts of water to reach the

233 consistency of moist paste. We stored this in a -20°C freezer until use, adding small amounts of
234 water as needed when pollen dried out. For concentrations of anabasine and nicotine in bee
235 diets, see Table S2. Bees received 330 μ L of nectar and $0.013 \pm \text{SE } 0.006$ g of pollen on the first
236 day of the experiment. Nectar was topped off to 330 μ L each day. Bees received fresh pollen
237 each day, unless they still had adequate amounts that had not dried out.

238

239 *Infecting bees.* Experimental worker bees were infected using inoculum made from bee colonies
240 maintained in the laboratory that had been initially infected with *Crithidia* from wild *B.*
241 *impatiens* (Stone Soup Farm, Hadley, Massachusetts, USA, 42.363911 N, -72.567747 W) in fall
242 2014, transferring to new colonies as needed. Following methods outlined in Richardson et al.
243 (2015), we made inoculum from a *Crithidia*-infected colony (Appendix S1, *Inoculating bees*),
244 and infected experimental bees with 15-20 μ L of inoculum so that they received 9-12,000
245 *Crithidia* cells; all bees on a single day were given only one inoculum volume depending on
246 availability, such that variation due to quantity of cells provided was part of variation due to
247 inoculation date. Both concentrations are well within natural variation in feces (Otterstatter and
248 Thomson 2006). Bees that did not consume inoculum were excluded from the trial. We housed
249 experimental bees in a deli cup modified for pollen and nectar feeding (Appendix S1, *Bee*
250 *containers*) in a dark incubator at 28°C. We monitored and recorded bee death daily. Surviving
251 bees were dissected seven days after inoculation and *Crithidia* cells were assessed with the same
252 methods used to make inoculum. We measured the marginal cell of the right forewing as an
253 estimate of body size. Sample sizes were limited by food availability in the low-fertilizer
254 treatments, which produced far fewer flowers (and therefore less nectar and pollen). Initial and
255 final sample sizes (due to deaths and escapes) were: AMF+/high fertilizer (55 initial, 33 final

256 bees), AMF+/low fertilizer (10 initial, 3 final), AMF-/high fertilizer (60 initial, 41 final), AMF-
257 /low fertilizer plants (14 initial, 9 final), and control diet (58 initial, 30 final).

258
259 *Measuring Consumption.* To measure pollen and nectar consumption, we weighed pollen and
260 nectar dispensers with their rations before placing them in deli cups on the fourth day post-
261 inoculation. The next day, we weighed them again and used the difference to estimate
262 consumption. To account for evaporation, we simultaneously ran control consumption trials
263 every time we measured consumption (10 replicates of control pollen and nectar, 10 replicates of
264 tobacco nectar), which mimicked the experimental feeding setup without bees.

265
266 *Statistical Analyses*

267 All analyses were performed using R version 3.4.1 (R Core Team, 2019), and plots were made
268 using ggplot2 (Wickham 2016). For all analyses we fit a set of candidate models and used the
269 *AICcmodavg* package to perform model selection (shown in Table S3). We compared top models
270 to one that excluded each term of interest (e. g., AMF, fertilizer), using ANOVA to test for a
271 significant effect on the response. To assess pairwise differences between treatments, we used
272 the emmeans package, adjusting for multiple comparisons using false discovery rate (FDR). To
273 assess treatment effects on plant size and nectar volume, we fit linear models using AMF,
274 fertilizer, their interaction, block, aphids and mold ($VIF < 2$) in the top models. We used number
275 of leaves/cm to represent plant size (Appendix S1 *Plant Size*).

276 We used the *coxme* package to conduct a Cox Proportional Hazards test of treatment
277 effect on flowering date. The global model included fertilizer, AMF, their interaction, block,
278 mold and aphid level. Survival analysis estimates differences in the time to an event (flowering),
279 while accounting for censored values (plants that failed to flower).

280 To assess plant treatment effects on pollen anabasine and nicotine concentrations
281 (ng/mg), we performed two analyses. Overall, 21% and 14% of plants had no pollen nicotine and
282 anabasine, respectively, so we analyzed likelihood of having the compound using a binomial
283 model and the *MASS* package. We then fit a generalized linear model with a negative binomial
284 distribution to test treatment effects on concentration of pollen nicotine/anabasine using all
285 plants, including those with zero values. We added a squared term of sampling date to test for
286 quadratic effect of sampling date, due to patterns observed in exploratory analyses. We used the
287 *car* package to test for collinearity among multiple predictors. There was not strong collinearity
288 between sampling date and treatment (VIF < 2 in both cases), indicating that the effect of
289 treatment was not confounded with that of sampling date. The global models included AMF,
290 fertilizer, their interaction, block, sampling date, squared sampling date, mold and aphid level.
291 The top model for anabasine included AMF, fertilizer, sampling date and squared sampling date;
292 it did not include aphids, mold, block, or the AMF by fertilizer interaction. The top model for
293 nicotine included AMF, fertilizer, their interaction, sampling date and squared sampling date,
294 and did not include aphids, mold or block. During data exploration, we noticed that variation in
295 chemical concentration differed between treatments. Because inter-plant variation in pollen
296 chemistry may be ecologically significant, we used a Levene test to evaluate whether variance
297 differed by treatment. We evaluated the correlation between anabasine and nicotine
298 concentrations using a Kendall rank correlation test.

299 To assess effects of AMF and fertilizer on *Crithidia* counts, we used two approaches.
300 First, we analyzed treatments excluding the control diet to compare the effect of AMF, fertilizer
301 and their interactions on pathogen load. We note that all four treatments incorporated equal ratios
302 of tobacco to wildflower pollen; we hoped that mixing tobacco and wildflower pollen would

303 facilitate bee survival but note that this also means our diets are a conservative evaluation of the
304 strength of treatment effects. We conducted this analysis using a generalized linear model with
305 AMF, fertilizer, their interaction, colony, inoculation date, bee size (estimated as wing marginal
306 cell length), pollen and nectar consumption, and anabasine and nicotine consumption (calculated
307 as mg pollen consumed * concentration of each compound in diet treatment) in the global model,
308 with a negative binomial distribution. Our top model included all covariates except inoculation
309 date, and measures of pollen/nectar and nicotine/anabasine consumption. We used a *post hoc*
310 chi-squared test to test residual deviance. Next, we analyzed differences in counts between all
311 diets, treating diet as a single five-level factor. The goal of this additional analysis was to ask if
312 tobacco diets differed from the control diet. The global models had diet, colony, inoculation date,
313 bee size, nicotine consumption, anabasine consumption and either pollen or nectar consumption
314 as covariates, and a negative binomial distribution. Replication was too low to include both
315 pollen and nectar consumption as covariates in the same global model. The top model included
316 diet treatment (5 levels), bee size and colony, but not nectar or pollen consumption. To assess
317 differences between tobacco and control diets, we performed a *post hoc* generalized linear
318 hypothesis test using the *multcomp* package, adjusting for multiple comparisons using FDR. For
319 both analyses testing the effect of diet on *Crithidia* counts, we ran models excluding the diet
320 treatment with low replication (AMF+, low fertilizer), and found that fertilizer and AMF still
321 significantly affected *Crithidia* in similar directions (Appendix S1, *Crithidia counts*). Separately,
322 we tested whether diet treatment affected consumption of pollen or nectar using a linear model
323 (Appendix S1, *Consumption*). We conducted Cox Proportional Hazards tests to assess diet effect
324 on bee survival. Because replication was too low to use the five-level factor of diet as a
325 predictor, we excluded the control diet and tested the effects of fertilizer and AMF separately.

326

327 **Results**328 *Treatment effects on plant size and floral traits*

329 High fertilizer increased plant size by 22% ($F_{9,96} = 6.6, p < 0.001$) and likelihood of
330 flowering by a factor of 2.1 relative to low fertilizer (Figure S1; log likelihood = -713.33, $p <$
331 0.001). AMF increased plant size by about 15% ($F_{9,96} = 6.6, p = 0.002$) but reduced the
332 likelihood of flowering by a factor of -0.7 (Appendix S1, *AMF and nutrients influence flowering*
333 *phenology*, Figure S1; log likelihood = -671.7, $p < 0.001$). Aphids did not affect flowering time
334 (log likelihood = -663.75, $p = 0.094$) or size ($F_{9,96} = 6.6, p = 0.094$). We concluded that AMF
335 and fertilizer treatments had no effect on nectar sugar concentration after finding no variation in
336 the first 40 plants sampled (10 per treatment combination; all plants had 16% sucrose
337 equivalents). In contrast with previous work on *N. tabacum* nectar ([Adler et al. 2006](#)), we did not
338 find anabasine or nicotine in any of our nectar samples. While this finding was unexpected, it is
339 possible that differences in abiotic factors between our study site and that of previous work
340 account for this result (Appendix S1, *Pollen and Nectar Chemistry*).

341 AMF had no effect on the likelihood of anabasine in pollen ($\chi^2 = 57.789, N=115, p =$
342 0.092), while plants sampled at later dates were more likely to have anabasine ($\chi^2 = 85.839,$
343 $N=115, p < 0.001$). Fertilizer had no effect on the likelihood of anabasine in pollen and was not
344 in the top model. However, high fertilizer increased anabasine concentration by 484% compared
345 to low fertilizer (Figure 1; $\chi^2 = 94.949, N = 115, p < 0.001$). AMF had a non-significant trend to
346 increase pollen anabasine concentration by 56% (Figure 1; $\chi^2 = 85.888, N=115, p = 0.072$).
347 Sampling date had a positive quadratic effect on pollen anabasine concentration ($\chi^2 = 92.165, N$
348 $= 115, p < 0.001$), such that anabasine concentrations peaked mid-season. AMF and fertilizer did

349 not interact, and this term was not in the top model. We found similar results using post-
350 extraction weights, except that the effect of AMF became significant (Appendix S1, *Pollen and*
351 *Nectar Chemistry*).

352 Neither AMF nor fertilizer affected the likelihood pollen would have nicotine ($\chi^2 > 77$, p
353 > 0.21 for both), but plants that flowered later were more likely to have nicotine ($\chi^2 = 147.83$, $p <$
354 0.001). Plants with high fertilizer had 173% more pollen nicotine than those with low fertilizer
355 (log likelihood = -614.884 on 111 df, $p = 0.003$). However, these effects depended on the
356 interaction between fertilizer and AMF, such that AMF plants had reduced nicotine
357 concentration under high, but not low fertilizer conditions (Figure 1; log likelihood = -607.242
358 on 110 df, $p = 0.044$). Alone, AMF did not significantly affect nicotine concentration (log
359 likelihood = -608.183, $p = 0.082$). Sampling date had a positive quadratic effect on nicotine
360 concentration (log likelihood = -68.624 on 109 df, $p = 0.02$), such that nicotine concentrations
361 peaked mid-season. We found similar results using post-extraction weights, except that the
362 interaction between AMF and fertilizer was no longer significant (Appendix S1 *Pollen and*
363 *Nectar Chemistry*).

364 High fertilizer increased pollen anabasine ($F_{1,113} = 0.828$, $p < 0.001$) and nicotine ($F_{1,113} =$
365 10.97 , $p = 0.001$) concentration variance compared to low fertilizer. AMF did not affect
366 anabasine concentration variance ($F_{1,112} = 1.20$, $p = 0.276$), but marginally decreased nicotine
367 concentration variance ($F_{1,113} = 3.68$, $p = 0.058$). Anabasine and nicotine concentration were not
368 correlated (coefficient = 0.154, $p = 0.099$), and there was no correlation between early and late-
369 season anabasine or nicotine concentrations (Appendix S1, *Pollen and Nectar Chemistry*).

370

371 *Treatment effects on pathogen counts and bee survival via changes in nectar and pollen*

372 When we excluded the control diet and tested the interaction of AMF and fertilizer on
373 *Crithidia* counts, AMF and fertilizer interacted such that diet from plants grown without AMF
374 and low fertilizer resulted in the lowest *Crithidia* counts (Figure 2; interaction term log
375 likelihood = -508.07 on 81 df, $p = 0.04$). Alone, neither AMF (log likelihood = -509.63 on 82 df,
376 $p = 0.211$), nor fertilizer (log likelihood = -510.72 on 82 df, $p = 0.104$) affected *Crithidia*.
377 Colony significantly affected counts (log likelihood = -514.82 on 81 df, $p = 0.001$), and larger
378 bees had lower infection (log likelihood = -508.73 on 81 df, $p = 0.028$). Pairwise comparisons
379 revealed that for plants grown without AMF, diet from high fertilizer treatments increased
380 *Crithidia* counts compared to low fertilizer ($p = 0.010$). In contrast, for plants grown with AMF,
381 diet from high fertilizer treatments had no effect on *Crithidia* counts compared to those grown
382 with low fertilizer ($p = 0.975$). For plants grown with high fertilizer, there was a trend for AMF
383 to result in lower *Crithidia* counts than those grown without AMF ($p = 0.053$).

384 Diet had a significant effect on *Crithidia* counts when we included the control diet
385 (wildflower pollen and sugar solution) in the analysis (log likelihood = -711.64 on 118 df, $p =$
386 0.040). When we compared the control diet to the four tobacco diets using non-orthogonal
387 contrasts, the control did not differ from plants grown with AMF regardless of fertilizer
388 treatment ($p > 0.46$). Of plants without AMF, diet from plants with high fertilizer did not differ
389 from the control ($p = 0.328$), and plants grown with low fertilizer had lower *Crithidia* counts
390 than the control ($p = 0.035$). Colony affected *Crithidia* counts (log likelihood = -711.88, $p <$
391 0.001), and larger bees had lower infection (log likelihood = -707.80, $p = 0.013$). There was no
392 effect of AMF (Wald test = 1.18, df = 1, $p = 0.277$) or fertilizer (Wald statistic = 1.36, df = 2, $p =$
393 0.2443) on survival. Pollen consumption and nectar consumption did not differ between diet

394 treatments, and diet treatment was not included in the top model of nectar consumption
395 (Appendix 1, *Consumption*).

396

397 **Discussion**

398 *AMF and nutrients affect pollen and nectar secondary chemistry*

399 We found that soil nutrients and mycorrhizal fungi affect pollen alkaloid concentrations.
400 High nutrients increased pollen nicotine and anabasine concentrations (Figure 1). This is
401 consistent with previous work showing that fertilizer increased nectar secondary compounds
402 (Adler et al. 2006), but to our knowledge is the first study to examine effects of belowground
403 environment on pollen secondary chemistry. Secondary metabolite production can be costly
404 (Züst et al. 2011), and high fertilizer plants may have more resources for defense production. The
405 effect of AMF on pollen alkaloids was more subtle. No study to our knowledge has researched
406 the effect of mycorrhizal fungi on nectar or pollen secondary chemistry, although one study
407 found that higher AMF colonization was negatively correlated with floral volatile compound
408 emission rate and diversity (Becklin et al. 2011). In our study, AMF modified effects of fertilizer
409 on nicotine in pollen (Figure 1). The increased suppressive effect of AMF on nicotine under high
410 fertilizer could be due to AMF-plant competition for nitrogen, an important ingredient in alkaloid
411 synthesis (Xi et al. 2008); under high resource conditions, plants and AMF may compete for,
412 rather than equally share, resources (Walder and van der Heijden 2015). This finding supports
413 our hypothesis that the effect of AMF on alkaloids is conditional on soil nutrients. On the other
414 hand, we did not find support for the hypothesis that AMF effect on alkaloids is due to defense
415 up-regulation alone, because AMF had inconsistent effects on alkaloids across fertilizer
416 treatments (Figure 1).

417 AMF and nutrients affected pollen alkaloid concentrations, but we did not detect any
418 anabasine or nicotine in nectar. Other work also found higher and more diverse secondary
419 compounds in pollen than nectar ([Cook et al. 2013](#), [Palmer-Young et al. 2019](#)), consistent with
420 Optimal Defense Theory, which predicts that plants invest more defense in tissues more directly
421 related to fitness. Since many plants face pollen theft by ineffective pollinators ([Solís-Montero et](#)
422 [al. 2015](#)), and floral alkaloids can reduce floral larceny ([Barlow et al. 2017](#)), reduced nicotine in
423 plants with AMF and high fertilizer suggests a potential cost of the AMF-plant mutualism that
424 depends on nutrient availability. While this hypothesis would need to be tested in the presence of
425 floral antagonists, it is consistent with other studies showing that AMF exists along a mutualist-
426 parasite continuum ([reviewed in Johnson 2010](#)).

427 In addition to having higher mean anabasine and nicotine concentrations, plants grown
428 with high fertilizer had more variable anabasine and nicotine concentrations than those grown
429 with low fertilizer. Unpredictable nectar quality can alter pollinator behavior and increase
430 outcrossing in wild *Nicotiana* species ([Kessler et al. 2012](#)). If soil conditions increase variability
431 of floral resources, there could be important implications for plant population dynamics or crop
432 yields. Future studies should also examine whether soil conditions affect variance of pollen
433 macronutrients; a recent meta-analysis showed that variation in, rather than the mean, of plant
434 nutrient traits is an important limiting factor for herbivorous insect pest performance ([Wetzel et](#)
435 [al. 2016](#)). If belowground conditions cause variation in nutritive quality of floral rewards, and if
436 pollinators, which can be thought of as a specialized type of herbivore, respond to nutrient
437 variation as insect pests do, then increased variation in floral resource nutritive quality could
438 have a detrimental effect on pollinator growth and survival.

439

440 *AMF and nutrients affect bee pathogens via changes in floral rewards*

441 We found that plant interactions with the belowground environment can affect pathogen
442 cell counts in pollinators via changes in nectar and/or pollen. Although other work has shown
443 that pollen and nectar quality can affect bee resistance to pathogens (Brunner et al. 2014,
444 Richardson et al. 2015), this is the first study to demonstrate that plant interactions with their
445 abiotic and biotic environment can shape bee-pathogen interactions. Plants have a well-
446 established ability to structure communities. This can happen directly, such as when plant
447 diversity drives arthropod diversity (Potts et al. 2003), or indirectly when plants act as
448 intermediaries of species interactions (Strauss 1997). Our results demonstrate the potential for
449 multitrophic consequences of plant-environment interactions on pathogens of pollinators.

450 Treatment effects on *Crithidia* counts could not be explained by changes in floral
451 alkaloid concentrations. Because anabasine- and nicotine-containing sucrose solutions reduced
452 *Crithidia* pathogen counts in bumblebees previously (Richardson et al. 2015) and fertilizer
453 increased nectar anabasine concentrations in *N. tabacum* (Adler et al. 2006), we hypothesized
454 that high fertilizer would reduce *Crithidia* counts by increasing nectar or pollen anabasine and
455 possibly nicotine. However, pollen with low mean anabasine reduced *Crithidia* most, and pollen
456 with high anabasine resulted in intermediate *Crithidia* counts (Figure 1, Figure 2). In one study,
457 nectar nicotine and anabasine reduced *Crithidia* independently but not in tandem (Thorburn et al.
458 2015), which could explain some of our results; nicotine and anabasine could have either neutral,
459 synergistic or antagonistic interactions at varying concentrations. However, we cannot rule out
460 the possibility of a mechanism other than alkaloids affecting *Crithidia* counts.

461 Other components of pollen or nectar could act with or independent of alkaloids to reduce
462 *Crithidia* counts. For example, excessive soil nutrients can decrease amino acid concentrations in

463 pollen, with consequences for bumblebee larval survival (Ceulemans et al. 2017). Bumblebees
464 consuming a pollen-limited diet have reduced expression of immune genes (Brunner et al. 2014),
465 which could be due to lack of protein or other pollen constituents. Alternatively, diets rich in
466 certain constituents could have promoted *Crithidia* growth by providing the pathogen with a
467 more beneficial food source. Future studies should examine ecological factors that shape pollen
468 and nectar constituents other than alkaloids, and manipulate presence and concentrations in bee
469 diet to identify mechanisms mediating belowground effects on bee-pathogen interactions.

470 In conclusion, our results demonstrate that abiotic and biotic soil components change
471 floral defensive chemistry and traits that affect bumblebee pathogens. These results suggest
472 potential novel costs of the mycorrhizae-plant mutualism *via* changes in floral reward chemistry,
473 and pose exciting directions for studying context dependency of mutualisms in communities.

474 **Acknowledgements**

475 This research took place on occupied lands of the Wbanaki Confederacy and Nipmuc nation. We
476 thank E. Stone, E. Palmer-Young, E. Amponsah, B. Joyce, G. LoCascio, R. Pasquale, D. Farman
477 and K. Urban-Mead for feedback and help with data collection, Z. Getman-Pickering, J. Thaler
478 and two reviewers for comments on the manuscript, BioBest (Ontario, Canada) for bee colonies,
479 Premier Tech Biotechnologies (Quebec, Canada) for AMF inoculum, and H. Grab, C.
480 Sutherland, the UMass Amherst Quantitative Statistics Group and Cornell Statistical Consulting
481 Unit for advice on data analysis. Funding was provided by the UMass Amherst Honors College
482 Research Fellowships, Torrey Plant Biology Fellowship, Nauset Garden Club, a PGAV
483 Pollinator Research Grant, NIH 1 R01 GM122062-01, NSF-DEB-1258096, and USDA-AFRI
484 2013-02536. Opinions, findings, conclusions or recommendations expressed in this material are
485 those of the authors and do not necessarily reflect the views of the funding agency.

486 **References**

- 487 Adler, L. S. 2000. The ecological significance of toxic nectar. *Oikos* 91:409–420.
- 488 Adler, L. S., M. Wink, M. Distl, and A. J. Lentz. 2006. Leaf herbivory and nutrients increase
489 nectar alkaloids. *Ecology Letters* 9:960–967.
- 490 Alaux, C., F. Ducloz, D. Crauser, and Y. L. Conte. 2010. Diet effects on honeybee
491 immunocompetence. *Biology Letters*.
- 492 Andrade, S. a. L., S. Malik, A. C. H. F. Sawaya, A. Bottcher, and P. Mazzafera. 2013.
493 Association with arbuscular mycorrhizal fungi influences alkaloid synthesis and accumulation in
494 *Catharanthus roseus* and *Nicotiana tabacum* plants. *Acta Physiologiae Plantarum*
495 35:867–880.
- 496 Anthony, W. E., E. C. Palmer-Young, A. S. Leonard, R. E. Irwin, and L. S. Adler. 2015. Testing
497 dose-dependent effects of the nectar alkaloid anabasine on trypanosome parasite loads in
498 adult bumblebees. *PLOS ONE* 10:e0142496.
- 499 Ashman, T.-L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R.
500 Dudash, M. O. Johnston, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen
501 limitation of plant reproduction: ecological and evolutionary causes and consequences.
502 *Ecology* 85:2408–2421.
- 503 Barber, N. A., and N. L. Soper Gordon. 2015. How do belowground organisms influence plant–
504 pollinator interactions? *Journal of Plant Ecology* 8:1–11.
- 505 Barlow, S. E., G. A. Wright, C. Ma, M. Barberis, I. W. Farrell, E. C. Marr, A. Brankin, B. M.
506 Pavlik, and P. C. Stevenson. 2017. Distasteful nectar deters floral robbery. *Current Biology*
507 27:2552–2558.e3.
- 508 Becklin, K. M., G. Gamez, B. Uelk, R. A. Raguso, and C. Galen. 2011. Soil fungal effects on
509 floral signals, rewards, and aboveground interactions in an alpine pollination web.

- 510 American Journal of Botany 98:1299–1308.
- 511 Bentley, B., and T. S. Elias. 1983. The Biology of Nectaries. Columbia University Press.
- 512 Biller, O. M., L. S. Adler, R. E. Irwin, C. McAllister, and E. C. Palmer-Young. 2015. Possible
513 synergistic effects of thymol and nicotine against *Crithidia bombi* parasitism in bumble
514 bees. PLOS ONE 10:e0144668.
- 515 Brown, M. J. F., R. Schmid-Hempel, and P. Schmid-Hempel. 2003. Strong context-dependent
516 virulence in a host-parasite system: reconciling genetic evidence with theory. Journal of
517 Animal Ecology 72:994–1002.
- 518 Brundrett, M. C., and L. Tedersoo. 2018. Evolutionary history of mycorrhizal symbioses and
519 global host plant diversity. New Phytologist 220:1108–1115.
- 520 Brunner, F. S., P. Schmid-Hempel, and S. M. Barribeau. 2014. Protein-poor diet reduces host-
521 specific immune gene expression in *Bombus terrestris*. Proc. R. Soc. B 281:20140128.
- 522 Cameron, S. A., J. D. Lozier, J. P. Strange, J. B. Koch, N. Cordes, L. F. Solter, and T. L.
523 Griswold. 2011. Patterns of widespread decline in North American bumble bees.
524 Proceedings of the National Academy of Sciences 108:662–667.
- 525 Campos-Soriano, L., J. M. García-Garrido, and B. S. Segundo. 2010. Activation of basal defense
526 mechanisms of rice plants by *Glomus intraradices* does not affect the arbuscular
527 mycorrhizal symbiosis. New Phytologist 188:597–614.
- 528 Ceulemans, T., E. Hulsmans, W. V. Ende, and O. Honnay. 2017. Nutrient enrichment is
529 associated with altered nectar and pollen chemical composition in *Succisa pratensis*
530 Moench and increased larval mortality of its pollinator *Bombus terrestris* L. PLOS ONE
531 12:e0175160.
- 532 Conroy, T. J., E. C. Palmer-Young, R. E. Irwin, and L. S. Adler. 2016. Food limitation affects

- 533 parasite load and survival of *Bombus impatiens* (Hymenoptera: Apidae) infected with
534 *Crithidia* (Trypanosomatida: Trypanosomatidae). *Environmental Entomology* 45:1212–
535 1219.
- 536 Cook, D., J. S. Manson, D. R. Gardner, K. D. Welch, and R. E. Irwin. 2013. Norditerpene
537 alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on
538 pollinators. *Biochemical Systematics and Ecology* 48:123–131.
- 539 Detzel, A., and M. Wink. 1993. Attraction, deterrence or intoxication of bees (*Apis mellifera*) by
540 plant allelochemicals. *Chemoecology* 4:8–18.
- 541 Gegeer, R. J., M. C. Otterstatter, and J. D. Thomson. 2006. Bumble-bee foragers infected by a
542 gut parasite have an impaired ability to utilize floral information. *Proceedings of the*
543 *Royal Society of London B: Biological Sciences* 273:1073–1078.
- 544 Gillespie, S. 2010. Factors affecting parasite prevalence among wild bumblebees. *Ecological*
545 *Entomology* 35:737–747.
- 546 Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined
547 stress from parasites, pesticides, and lack of flowers. *Science* 347:1255–1257.
- 548 Johnson, N. C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular
549 mycorrhizas across scales. *New Phytologist* 185:631–647.
- 550 Kessler, D., S. Bhattacharya, C. Diezel, E. Rothe, K. Gase, M. Schöttner, and Baldwin. 2012.
551 Unpredictability of nectar nicotine promotes outcrossing by hummingbirds in *Nicotiana*
552 *attenuata*. *The Plant Journal* 71:529–538.
- 553 Kessler, D., K. Gase, and I. T. Baldwin. 2008. Field experiments with transformed plants reveal
554 the sense of floral scents. *Science* 321:1200–1202.
- 555 Klein, A., I. Steffan–Dewenter, and T. Tschardt. 2003. Fruit set of highland coffee increases

- 556 with the diversity of pollinating bees. *Proceedings of the Royal Society of London. Series*
557 *B: Biological Sciences* 270:955–961.
- 558 Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen,
559 and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world
560 crops. *Proceedings of the Royal Society of London B: Biological Sciences* 274:303–313.
- 561 Koch, H., M. J. Brown, and P. C. Stevenson. 2017. The role of disease in bee foraging ecology.
562 *Current Opinion in Insect Science* 21:60–67.
- 563 Liu, J., I. Maldonado-Mendoza, M. Lopez-Meyer, F. Cheung, C. D. Town, and M. J. Harrison.
564 2007. Arbuscular mycorrhizal symbiosis is accompanied by local and systemic
565 alterations in gene expression and an increase in disease resistance in the shoots. *The*
566 *Plant Journal* 50:529–544.
- 567 Manson, J. S., M. C. Otterstatter, and J. D. Thomson. 2010. Consumption of a nectar alkaloid
568 reduces pathogen load in bumble bees. *Oecologia* 162:81–89.
- 569 McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease:
570 floral traits and the transmission of plant and animal pathogens. *Ecology Letters* 17:624–
571 636.
- 572 McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and Swan. 1990. A new method
573 which gives an objective measure of colonization of roots by vesicular—arbuscular
574 mycorrhizal fungi. *New Phytologist* 115:495–501.
- 575 Otterstatter, M. C., and J. D. Thomson. 2006. Within-host dynamics of an intestinal pathogen of
576 bumble bees. *Parasitology* 133:749–61.
- 577 Palmer-Young, E. C., I. W. Farrell, L. S. Adler, N. J. Milano, P. A. Egan, R. R. Junker, R. E.
578 Irwin, and P. C. Stevenson. 2019. Chemistry of floral rewards: intra- and interspecific

- 579 variability of nectar and pollen secondary metabolites across taxa. *Ecological*
580 *Monographs* 89:e01335.
- 581 Palmer-Young, E. C., B. M. Sadd, R. E. Irwin, and L. S. Adler. 2017. Synergistic effects of floral
582 phytochemicals against a bumble bee parasite. *Ecology and Evolution* 7:1836–1849.
- 583 Palmer-Young, E. C., and L. Thursfield. 2017. Pollen extracts and constituent sugars increase
584 growth of a trypanosomatid parasite of bumble bees. *PeerJ* 5:e3297.
- 585 Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010.
586 Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*
587 25:345–353.
- 588 Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and
589 flowers: how do floral communities structure pollinator communities? *Ecology* 84:2628–
590 2642.
- 591 R Core Team (2019). R: A language and environment for statistical computing. R Foundation for
592 statistical computing, Vienna, Austria. URL <https://www.R-project.org>
- 593 Richardson, L. L., L. S. Adler, A. S. Leonard, J. Andicoechea, K. H. Regan, W. E. Anthony, J. S.
594 Manson, and R. E. Irwin. 2015. Secondary metabolites in floral nectar reduce parasite infections
595 in bumblebees. *Proceedings of the Royal Society B: Biological Sciences* 282.
- 596 Solís-Montero, L., C. H. Vergara, and M. Vallejo-Marín. 2015. High incidence of pollen theft in
597 natural populations of a buzz-pollinated plant. *Arthropod-Plant Interactions* 9:599–611.
- 598 Strauss, S. Y. 1997. Floral characters link herbivores, pollinators and plant fitness. *Ecology*
599 78:1640–1645.
- 600 Thorburn, L. P., L. Adler, R. Irwin, and E. C. Palmer-Young. 2015. Variable effects of nicotine,
601 anabasine, and their interactions on parasitized bumble bees. *F1000Research* 4.

- 602 Tiedeken, E. J., P. A. Egan, P. C. Stevenson, G. A. Wright, M. J. F. Brown, E. F. Power, I.
603 Farrell, S. M. Matthews, and J. C. Stout. 2016. Nectar chemistry modulates the impact of
604 an invasive plant on native pollinators. *Functional Ecology* 30:885–893.
- 605 Tiedge, K., and G. Lohaus. 2017. Nectar sugars and amino acids in day- and night-flowering
606 *Nicotiana* species are more strongly shaped by pollinators' preferences than organic acids
607 and inorganic ions. *PLoS ONE* 12.
- 608 Tihelka, E. 2017. The immunological dependence of plant-feeding animals on their host's
609 medical properties may explain part of honey bee colony losses. *Arthropod-Plant*
610 *Interactions*:1–8.
- 611 Vannette, R. L., and M. D. Hunter. 2009. Mycorrhizal fungi as mediators of defence against
612 insect pests in agricultural systems. *Agricultural and Forest Entomology* 11:351–358.
- 613 Walder, F., and M. G. A. van der Heijden. 2015. Regulation of resource exchange in the
614 arbuscular mycorrhizal symbiosis. *Nature Plants* 1:15159.
- 615 Wetzel, W. C., H. M. Kharouba, M. Robinson, M. Holyoak, and R. Karban. 2016. Variability in
616 plant nutrients reduces insect herbivore performance. *Nature* 539:425–427.
- 617 Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2016.
- 618 Williams, P. H., R. W. Thorp, L. L. Richardson, and S. R. Colla. 2014. *Bumble bees of North*
619 *America: an identification guide*. Princeton University Press.
- 620 Xi, X., C. Li, and F. Zhang. 2008. Tobacco plants can use nitrogen taken up before mechanical
621 wounding to synthesize nicotine afterwards. *Plant Signaling & Behavior* 3:87–90.
- 622 Züst, T., B. Joseph, K. K. Shimizu, D. J. Kliebenstein, and L. A. Turnbull. 2011. Using knockout
623 mutants to reveal the growth costs of defensive traits. *Proceedings of the Royal Society*
624 *of London B: Biological Sciences* 278:2598–2603

625 **Figure Legends**

626

627 **Figure 1. Effects of AMF and fertilizer treatments on pollen anabasine and nicotine**
628 **concentrations.** Effect of AMF and fertilizer on anabasine and nicotine concentrations. Error
629 bars represent +/- SE. Results shown are back-transformed model estimates.

630

631 **Figure 2. Effects of diet on *Crithidia* cell counts in bees.** Effect of AMF and nutrients on
632 *Crithidia* counts. Black bar shows control diet, and error bars show +/- SE. Cell counts are back-
633 transformed estimates from the full model comparing control diet to all four diet types.

634

635

636

637

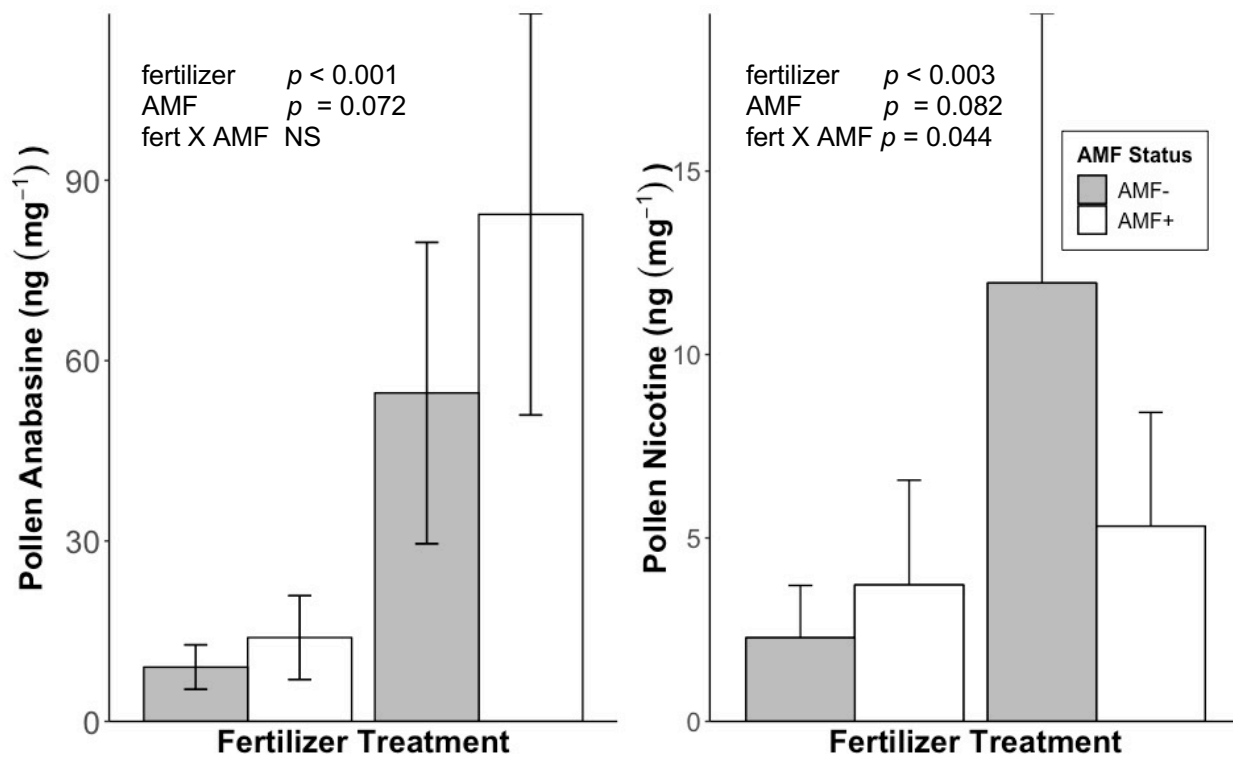
638

639

640

641

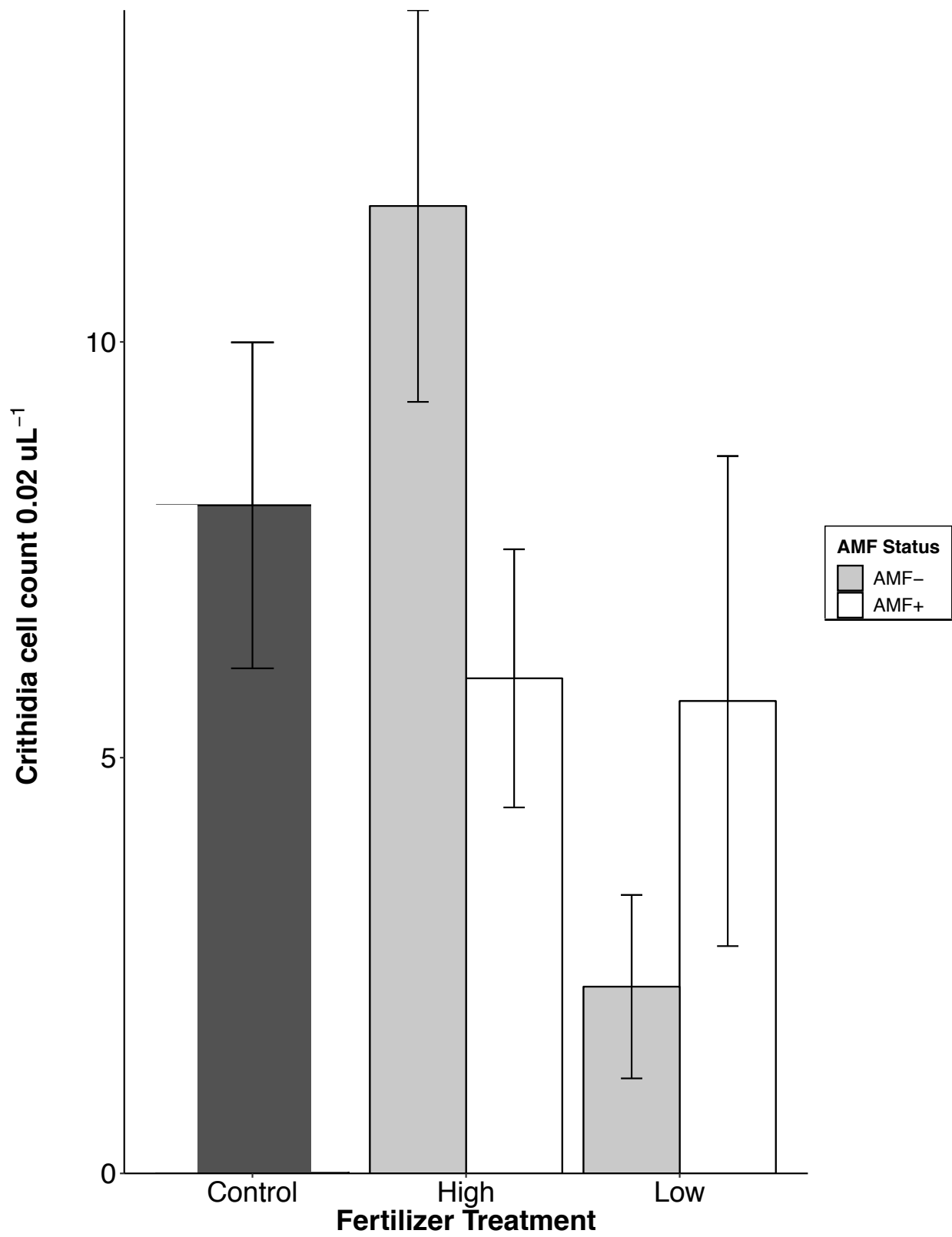
642



643

644

Figure 1.



645

646 **Figure 2**